# CCLXXXIII. OXIDATION OF AMINES BY ANIMAL TISSUES

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It has been shown by Pugh & Quastel [1937, 1] that an amine oxidase exists in brain and other tissues capable of oxidizing aliphatic amines with the liberation of ammonia. Butylamine, amylamine, *iso*amylamine and heptylamine were shown to be attacked whilst the lower amines were not appreciably affected. It is of interest that the higher amines which inhibit the normal respiration of brain cortex in presence of glucose are, themselves, attacked by this organ. In view of the possible clinical significance of the inhibitory effects of certain amines on brain respiration [Quastel & Wheatley, 1933, 1], a further study has been made of the oxidizing powers of brain and other tissues on amines.

## *Technique*

The following paper resolves itself into a study of the oxidation of a number of amines by intact tissue slices and by tissue (chiefly liver) extracts.

Respiration of the tissue in the presence and in the absence of amines was measured either with the Warburg or the Barcroft manometric apparatus. Ammonia estimations on the fluids in the manometric vessels at the termination of the experiments were made, in the case of volatile amines, by the procedure of Pugh & Quastel [1937, 2]. In the case of non-volatile amines, ammonia was estimated in the ordinary way by distillation in a current of ammonia-free air at the temperature of the boiling water-bath into standard acid solution; this was then titrated with standard alkali. When an appreciable amount of protein was present, as in the case of liver extracts, the protein was precipitated with trichloroacetic acid and ammonia estimated in the filtrate.

Tissue slices were cut from organs freshly dissected from the animals and their thicknesses usually lay between 0·2 and 0·4 mm. They were placed in manometric vessels containing phosphate-glucose-Locke solution[1] as basal medium, the amount of tissue slices in each vessel having a total dry weight varying from 15 to 30 mg. The manometer vessels were filled with oxygen and determinations of oxygen uptake were carried out at 37° usually over an experimental period of 2 hr.

The results quoted were obtained from experiments carried out with the slices of the organ of one animal, although in the case of brain it was occasionally necessary to mix the slices from the brains of two animals. The results are typical of a number of duplicate experiments.

The solutions in which tissue slices were immersed were made up to have an osmotic pressure approximately equal to 0·16 *M* NaCl. All amines were used as their hydrochlorides, a final concentration of *M*/150 usually being employed. The hydrogen ion concentration was maintained at *p*H 7·4 in all experiments.

Details of the preparation of tissue extracts will be given later.

[1] Phosphate buffer 0·022 *M*; *p*H 7·4; glucose 0·08 %.

*Respiration and ammonia production of tissue slices in presence of various amines*

*Tyramine.* As shown in an earlier paper [Quastel & Wheatley, 1933, 1], tyramine has a large inhibitory effect on the normal respiration of brain cortex in a glucose medium. This is shown in the case of the rat in Table I, which gives

Table I

| Organ | Amine | $Q_{O_2}$ (over 1st hr.) | | $Q_{O_2}$ (over 2nd hr.) | | $Q_{NH_3}$ | |
|---|---|---|---|---|---|---|---|
| | | Amine absent | Amine present | Amine absent | Amine present | Amine absent | Amine present |
| Rat brain | Tyramine 0·0052 *M* | 15·3 | 11·7 | 11·8 | 3·7 | 0·2 | 3·0 |
| | Indolethylamine 0·0066 *M* | 14·8 | 11·7 | 12·3 | 3·9 | 0·5 | 1·4 |
| | Phenylethylamine 0·0066 *M* | 15·7 | 14·5 | 12·5 | 12·2 | 0·9 | 0·7 |
| | Mescaline 0·0066 *M* | 17·0 | 14·2 | 15·2 | 13·1 | 0·4 | 0·7 |
| | Histamine 0·0066 *M* | 13·7 | 18·2 | 12·2 | 13·9 | 0·6 | 0·4 |
| | Benzedrine 0·0066 *M* | 16·3 | 5·3 | 12·6 | 2·0 | 1·0 | 0·3 |
| Rat liver | Tyramine 0·0052 *M* | 10·7 | 9·5 | 6·2 | 4·9 | 1·9 | 8·3 |
| | Indolethylamine 0·0066 *M* | 9·2 | 9·1 | 6·4 | 5·7 | 1·3 | 4·4 |
| | Phenylethylamine 0·0066 *M* | 8·1 | 9·7 | 1·3 | 2·2 | 1·5 | 3·2 |
| | Mescaline 0·0066 *M* | 11·9 | 11·6 | 7·8 | 7·5 | 1·1 | 1·0 |
| | Histamine 0·0066 *M* | 8·9 | 9·8 | 2·0 | 2·8 | 1·6 | 2·3 |
| | Benzedrine 0·0066 *M* | 10·3 | 13·3 | 7·6 | 8·6 | 1·4 | 1·0 |
| Guinea-pig kidney | Tyramine 0·0052 *M* | 25·8 | 21·9 | 23·1 | 17·9 | 0·9 | 6·5 |
| | Indolethylamine 0·0066 *M* | 19·3 | 18·3 | 17·6 | 7·8 | 0·6 | 4·8 |
| | Phenylethylamine 0·0066 *M* | 17·6 | 20·9 | 17·6 | 20·0 | 1·2 | 2·0 |
| | Mescaline 0·0066 *M* | 19·4 | 19·7 | 18·0 | 18·8 | 0·6 | 0·7 |
| | Histamine 0·0066 *M* | 16·4 | 17·1 | 16·9 | 16·0 | 0·9 | 0·9 |
| | Benzedrine 0·0066 *M* | 20·7 | 20·4 | 19·9 | 18·4 | 0·9 | 0·3 |

typical values of the respiration, $Q_{O_2}$, during the 1st and 2nd hr. of the experiment and of the ammonia production, $Q_{NH_3}$, calculated over the whole experimental period.

Tyramine exercises a progressively toxic action on brain respiration in presence of glucose, the $Q_{O_2}$ falling to a low value at the end of the experimental period of 2 hr. Tyramine, however, is oxidized by brain cortex, ammonia being liberated. The $Q_{NH_3}$ rises from 0·2 to 3·0 in a typical experiment. One of the products of oxidation is an aldehyde precipitated in acid solution by 2:4-dinitrophenylhydrazine; the hydrazone dissolves in alkali to form a deep red-brown solution.

Aldehyde formation may be conveniently shown by a positive dinitrophenylhydrazine-alkali test. The test is carried out by adding to 1 ml. of the solution containing the aldehyde 1 ml. of a saturated solution of 2:4-dinitrophenylhydrazine in *N* HCl, allowing the mixture to stand at room temperature for 30 min. and finally adding 2 ml. *N* NaOH. The formation of a deep brown or red-brown coloration, which remains stable for at least 1 hr., is indicative of the presence in the original solution of an aldehyde or ketone.

The formation of an aldehyde by the action of liver on tyramine has already been noted by Philpot [1937].

Tyramine, at the concentration used, does not diminish the respiration of liver and kidney cortex slices to the same extent as it does that of brain cortex, but, on the other hand, there is no evidence of increased respiration of these tissues in presence of the amine (see Fig. 1 and Table I). There is no doubt, however, as is known from previous work [Hare, 1928; Philpot, 1937] of the attack by liver tissue on tyramine. Results showing the increase of $Q_{NH_3}$ are shown in Table I.

It is evident that since tyramine inhibits normal respiratory processes in intact brain, liver and kidney slices, no accurate measure can be made of the oxygen consumed by the amine in presence of the normal intact tissue. To obtain a measure of the oxygen taken up by the amine, the normal respiratory

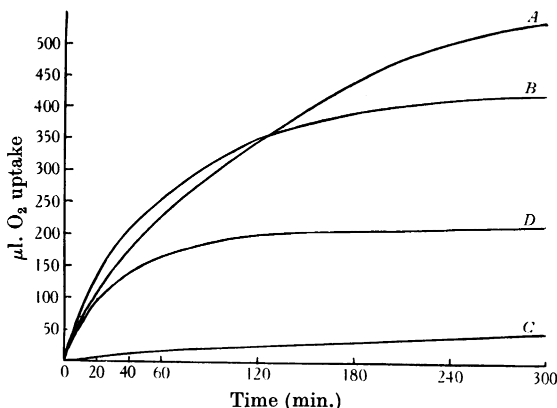



Fig. 1. Respiration of rat liver slices in presence and absence of tyramine and of cyanide. *A*, tissue alone (18·0 mg. dry weight). *B*, tissue (20 mg. dry weight) + tyramine (354 μl). *C*, tissue (19·3 mg. dry weight) + KCN 0·003 *M*. *D*, tissue (18·4 mg. dry weight) + KCN 0·003 *M* + tyramine 354 μl. Theoretical $O_2$ uptake for 1 atom $O_2$ = 177 μl.

processes of the tissue must be retarded by a poison which will not affect tyramine oxidation or resort must be made to the use of tissue extracts. Experiments on these lines will be described later.

Philpot [1937] has found a definitely increased respiration of liver slices in presence of tyramine. Our failure to do so must clearly depend on the different conditions under which the experiments were carried out. It is to be noted that Philpot used a high phosphate concentration *M*/15 in the medium in which the liver slices were immersed. Quastel & Wheatley [1933, 2] have pointed out that such a high phosphate concentration reduces liver slice respiration and renders conditions unphysiological probably by removal of calcium ions. With such a high phosphate concentration, for instance, fatty acids are but feebly attacked by liver slices. We have, therefore, used a phosphate-Locke medium in which the phosphate concentration is approximately *M*/50 to secure more favourable physiological conditions. It seems very probable that the increased respiration which Philpot obtained with liver slices in presence of tyramine is due to the suppression of normal respiratory processes in the liver by the high phosphate concentration. It will be shown later that cyanide has the same effect.

The activities of the tissues investigated in breaking down tyramine as shown by ammonia liberation follow the order:

Rat liver > guinea-pig kidney > rat brain.

It is an extraordinary fact that rat kidney slices have relatively little power to oxidize tyramine, there being little or no extra ammonia liberation in presence of the amine and the dinitrophenylhydrazine-alkali test being usually negative. The relative inertness of rat kidney to tyramine is paralleled by its inertness to the aliphatic amines [Pugh & Quastel, 1937, 1].

In our experiments with tyramine, there has always been slight pigment production, which could not be accounted for as methaemoglobin, as it was

obtained even with tissue extracts washed free from haemoglobin. Whether this is due to a slight impurity in the tyramine is a subject for further investigation.

*Indolethylamine.* The behaviour of this amine towards tissue slice respiration is very similar to that of tyramine. There is a large inhibitory effect which increases with time on the normal respiration of brain (*v.* Table I and Fig. 2) and

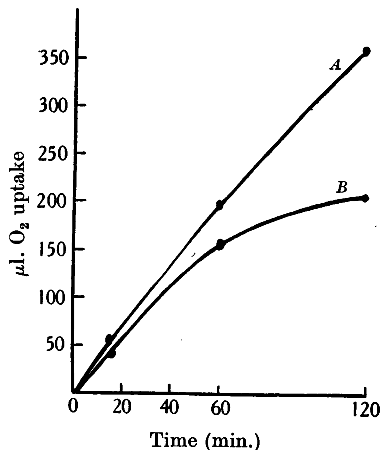



Fig. 2.

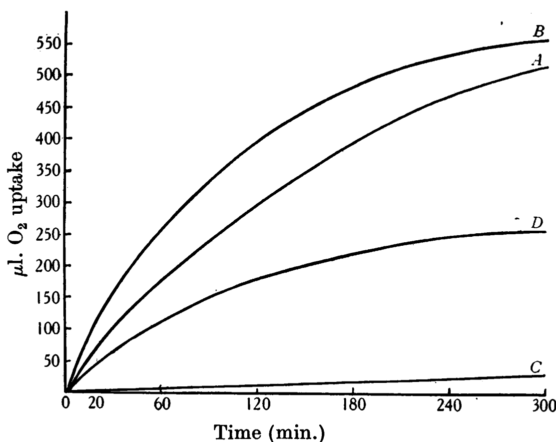



Fig. 3.

Fig. 2. Respiration of rat brain slices in presence and absence of indolethylamine. *A*, tissue alone (13·2 mg. dry weight). *B*, tissue (13·2 mg. dry weight) + indolethylamine (0·0066 *M*).

Fig. 3. Respiration of rat liver slices in presence and absence of indolethylamine and of cyanide. *A*, tissue alone (12·1 mg. dry weight). *B*, tissue (14·9 mg. dry weight) + indolethylamine (444 μl.). *C*, tissue (18·4 mg. dry weight) + KCN 0·003 *M*. *D*, tissue (13·2 mg. dry weight) + KCN 0·003 *M* + indolethylamine (444 μl.) Theoretical $O_2$ uptake for 1 atom O = 222 μl.

lesser effects on that of liver or kidney (Table I and Fig. 3). That oxidation of the amine by these tissues takes place is shown by ammonia formation and by aldehyde production as indicated by the positive dinitrophenylhydrazine-alkali test. The activities of the tissue slices investigated in oxidizing indolethylamine follow the order:

$$\text{Rat liver} > \text{guinea-pig kidney} > \text{rat brain}.$$

Rat kidney is relatively inert.

*Pigment formation from indolethylamine.* A constant feature of indolethylamine oxidation in presence of tissues is the formation of a melanin-like pigment. The pigment is brown or black and it is adsorbed firmly on the tissue slices. Brain cortex slices which have respired in presence of indolethylamine take on a brown colour. The pigment is not soluble in ether or in acid solution but appears gradually to dissolve in alkaline solution. It appears not to be reduced by sodium hydrosulphite solution. It is formed from indolethylamine by liver extracts from which haemoglobin has been removed by repeated washing with distilled water, so that there is little doubt that the pigment is not due to secondary oxidation of the haemoglobin in the tissues.

It is worth recalling, in connexion with pigment formation from indolethylamine, that Eppinger [1910] has advanced the view that tryptophan is the parent substance of the melanogen found in a case of melanotic sarcoma.

*Phenylethylamine.* The attack of tissue slices on phenylethylamine is very much less than on tyramine. Only rat liver shows any appreciable activity in

liberating ammonia from the amine (Table I). The dinitrophenylhydrazine-alkali test is usually only feebly positive or negative with the tissues investigated. Hare [1928] has shown that liver attacks phenylethylamine.

*Mescaline* (*3:4:5-trimethoxyphenylethylamine*). As has been previously noted [Quastel & Wheatley, 1933, 1], mescaline diminishes the normal respiration of brain cortex slices (*v.* Table I) and it has little or no action at the concentration tried on the respirations of liver and kidney slices. It is but feebly or not at all attacked under the conditions of these experiments.

*Histamine.* Histamine has no inhibitory effect on the respiration of brain, liver or kidney slices. There is, on the other hand, evidence of an increased respiration by brain in presence of the amine (Table I) but there is no increased ammonia formation. The dinitrophenylhydrazine-alkali test is usually negative, as in the cases of mescaline and benzedrine. Rat liver slices appear to have only a relatively small effect on the amine so far as ammonia production is concerned and guinea-pig kidney is without action.

*Benzedrine* (*phenylisopropylamine*). This amine has a large inhibitory action on the respiration of brain cortex slices (Table I) but none apparently on that of liver or kidney. No extra ammonia formation takes place when tissues respire in presence of this amine, but, on the contrary, there appears to be a lessened ammonia production.

*Respiration of brain cortex slices in presence of tyramine and indolethylamine*

The effects of tyramine and indolethylamine on the respiration of the brain cortex of rat, guinea-pig and sheep are shown in Table II. It will be noted that

Table II. *Respiration of brain cortex slices in presence of tyramine and indolethylamine*

| | | $Q_{O_2}$ (over 1st hr) | | $Q_{O_2}$ (over 2nd hr.) | | $Q_{NH_3}$ | |
|---|---|---|---|---|---|---|---|
| Amine | Animal | Amine absent | Amine present | Amine absent | Amine present | Amine absent | Amine present |
| Tyramine 0·0052 *M* | Rat | 15·5 | 9·1 | 12·2 | 4·6 | 1·2 | 3·9 |
| | Guinea-pig | 19·6 | 12·1 | 16·4 | 4·7 | 1·2 | 3·4 |
| | Sheep | 7·6 | 4·5 | 5·0 | 3·1 | 1·3 | 4·4 |
| Indolethylamine 0·0066 *M* | Rat | 14·8 | 11·7 | 12·3 | 3·9 | 0·5 | 1·4 |
| | Guinea-pig | 19·6 | 11·1 | 16·4 | 4·0 | 1·2 | 1·9 |
| | Sheep | 7·6 | 4·4 | 5·0 | 2·1 | 1·3 | 1·9 |

the behaviour towards guinea-pig and sheep is similar to that with rat. Increased ammonia formation takes place with the brain tissues of the three animals in presence of the amines, the ammonia formation being considerably greater in the case of tyramine. In all cases the dinitrophenylhydrazine-alkali test is positive.

*Effects of glucose*

In the absence of glucose the respiration of brain cortex slices falls rapidly. In presence of tyramine the rate of fall is not so great although the initial respiration is lower than in the absence of the amine. The $Q_{O_2}$ of brain in the presence of tyramine (0·0052 *M*) may be greater during the 2nd hour than in its absence, so long as glucose is not present. These results are seen in Table III. This increased $Q_{O_2}$ in presence of the amine is obviously due to oxidation of the amine. Ammonia formation from cortex slices is greater in the absence of glucose than in its presence, but there is no clear evidence that glucose affects appreciably the oxidation of tyramine by tissue slices as indicated by ammonia liberation.

Table III. *Rat brain cortex slices*

Glucose, 0·08 %; tyramine, 0·0052 *M*

| | $Q_{O_2}$ (over 1st hr.) | | $Q_{O_2}$ (over 2nd hr.) | | $Q_{NH_3}$ | |
|---|---|---|---|---|---|---|
| | Glucose absent | Glucose present | Glucose absent | Glucose present | Glucose absent | Glucose present |
| Exp. 1 | | | | | | |
| Tyramine present | 2·4 | 9·1 | 3·3 | 4·6 | 4·5 | 3·9 |
| Tyramine absent | 5·4 | 15·5 | 1·4 | 12·2 | 2·5 | 1·2 |
| Exp. 2 | | | | | | |
| Tyramine present | 5·5 | 12·5 | 4·0 | 5·0 | 6·3 | 4·7 |
| Tyramine absent | 7·0 | 13·5 | 1·7 | 10·5 | 2·2 | 1·6 |

*The action of cyanide*

The presence of cyanide whilst inhibiting the normal respiration of tissue slices does not prevent the activity of the amine oxidase. Results with indolethylamine, tyramine and *iso*amylamine in presence of rat liver slices are shown in Table IV where the $Q_{O_2}$ (estimated over the 1st hr. of the experiment) and the

Table IV. *Rat liver slices*

| | Cyanide absent | | | | Cyanide present *M*/300 | | | |
|---|---|---|---|---|---|---|---|---|
| | $Q_{O_2}$ (over 1st hr.) | | $Q_{NH_3}$ | | $Q_{O_2}$ (over 1st hr.) | | $Q_{NH_3}$ | |
| Amine | Amine absent | Amine present | Amine absent | Amine present | Amine absent | Amine present | Amine absent | Amine present |
| Indolethylamine 0·0066 *M* | 5·0 | 5·5 | 3·6 | 6·2 | 0·0 | 3·1 | 2·8 | 6·0 |
| Tyramine 0·0052 *M* | 8·4 | 12·7 | 1·8 | 9·7 | 0·0 | 8·0 | 2·8 | 7·6 |
| *iso*Amylamine 0·0066 *M* | 6·2 | 8·8 | 3·3 | 9·5 | 0·0 | 3·9 | 4·8 | 10·5 |

$Q_{NH_3}$ (over the entire experimental period) are given. It will be noted that there is a marked oxygen uptake when the amine is present with the tissue in presence of cyanide whilst the respiration in absence of amine is reduced nearly to zero. Ammonia formation is also markedly increased in the presence of the amine and cyanide, indicating an oxidation of the amine. The results lead to the conclusion that there is little or no inhibitory effect on the oxidation of amines by cyanide.

Philpot [1937] has observed that cyanide does not affect oxidation of tyramine by liver, and Bernheim [1931] has previously noted that tyramine takes up an atom of oxygen in presence of liver and cyanide.

Curves showing the effects of cyanide on tyramine and indolethylamine oxidation by liver slices are shown in Figs. 1 and 3.

*Total oxygen uptake by tyramine in presence of cyanide*

The total oxygen uptake by tyramine in presence of rat liver slices and in presence of cyanide amounts to approximately 1 atom of oxygen per mol. of

Table V. *Rat liver slices*

Cyanide concentration *M*/300

| Exp. | Amount of tyramine present µl. | Oxygen consumed in absence of amine µl. | Oxygen consumed in presence of amine µl. | $NH_3$ formed in absence of amine µl. | $NH_3$ formed in presence of amine µl. |
|---|---|---|---|---|---|
| 1 | 354 | 40 | 208 | 204 | 434 |
| 2 | 354 | 169 | 356 | 289 | 507 |

amine. The results of two experiments are shown in Table V. The experimental period was 4 hr., in order to allow oxidation of the amine to go as far as possible. The amount of tissue in Exp. 1 was 18·4 mg. dry weight and that in Exp. 2, 28·6 mg. dry weight. The amounts of tyramine and ammonia are expressed in $\mu$l. gas to make easier comparison with the volumes of oxygen consumed.

Assuming that the actual amount of oxygen taken up by the amine is the difference between the amounts consumed in the presence and in the absence of amine we see that

$$\frac{\text{Amine present}}{\text{Oxygen consumed}}=\frac{354}{168}=2{\cdot}1 \text{ (Exp. 1)} \quad \text{and} \quad \frac{354}{187}=1{\cdot}9 \text{ (Exp. 2)},$$

i.e. that 1 atom of oxygen is consumed per mol. of amine. Yet this conclusion does involve the assumption that, in presence of cyanide, tyramine does not interfere with the residual respiration of the liver slices, an assumption which seems justified by the fact that with different amounts of tissue the same ratio of amine oxidized to oxygen consumed is obtained. When the ammonia formation is considered a more difficult problem is involved, for there is as yet no evidence to indicate how far tyramine inhibits spontaneous ammonia formation from the tissue. If 1 mol. of ammonia is produced per mol. of amine then in Exps. 1 and 2 (Table V) 354 $\mu$l. $NH_3$ should have been formed whereas the actual amounts lie presumably between 230 and 434 $\mu$l. in the first experiment and between 218 and 507 $\mu$l. in the second. It is obvious that no commitment can be made as to the actual amount of ammonia produced from the amine, but it is true to say that the results are not inconsistent with the theoretical value of 354 $\mu$l., granting the production of 1 mol. of ammonia by oxidation of 1 mol. of tyramine. Philpot [1937] came to the same conclusion using rat liver slices in presence of a high phosphate concentration.

*Action of cyanide on indolethylamine oxidation by tissue slices*

Table VI. *Rat liver slices*

Cyanide concentrations $M/300$

| Exp. | Amount of indolethylamine present $\mu$l. | Oxygen consumed in absence of amine $\mu$l. | Oxygen consumed in presence of amine $\mu$l. | $NH_3$ produced in absence of amine $\mu$l. | $NH_3$ produced in presence of amine $\mu$l. |
|---|---|---|---|---|---|
| 1 | 444 | 30 | 263 | 252 | 419 |
| 2 | 444 | 93 | 317 | 352 | 524 |

It is clear from the results shown in Table VI that in presence of cyanide approximately 1 atom of oxygen is consumed per mol. of indolethylamine. The ratio

$$\frac{\text{Amine present}}{\text{Oxygen consumed}}=\frac{444}{233}=1{\cdot}9 \text{ (Exp. 1)} \quad \text{and} \quad =\frac{444}{224}=1{\cdot}9 \text{ (Exp. 2)},$$

again making the assumption that the amine does not interfere with the residual respiration of liver in presence of cyanide. We have observed in experiments lasting 6–7 hr. that there is a further very feeble oxidation taking place beyond the stage at which 1 atom of oxygen is taken up. The ammonia formation from the amine is again difficult to estimate for the reasons outlined in the case of tyramine but the results are not inconsistent with the value of 1 mol. of ammonia being produced from 1 mol. of indolethylamine.

One of the most noteworthy features of the effect of cyanide on indolethylamine oxidation by tissue slices is the fact that the pigment production is very

greatly reduced. It would seem therefore that two mechanisms exist in tissues which affect indolethylamine oxidation:

(1) A cyanide-resistant mechanism which results in the oxidation of the amino-group and liberation of ammonia.

(2) A cyanide-sensitive mechanism which results in the formation of a pigment either from indolethylamine directly or from its product of oxidation by the amine oxidase.

Conceivably the second process, i.e. pigment formation, is due to the introduction of oxygen into the indole nucleus and this may involve the participation of a cyanide-sensitive mechanism.

*The effects of arsenite*

Arsenite, whilst diminishing the normal respiratory processes of tissue slices, does not appreciably inhibit amine oxidation; nor, in the case of indolethylamine, does it markedly inhibit pigment formation.

Table VII. *Rat liver slices*

Sodium arsenite $M/1000$

| Amount of amine | Oxygen consumed in presence of amine μl. | $NH_3$ formed μl. |
|---|---|---|
| No amine added | 212 | 180 |
| Tyramine 354 μl. | 395 | 524 |
| Indolethylamine 444 μl. | 491 | 481 |

The experiments noted in Table VII were carried out over an experimental period of 5 hr., 23 mg. dry weight liver slices being present in each experiment. Sodium arsenite was added at a concentration of $M/1000$.

The results show that tyramine is oxidized in presence of arsenite in much the same way as in presence of cyanide, the oxygen uptake and ammonia formation corresponding approximately to 1 atom of oxygen and 1 mol. of ammonia

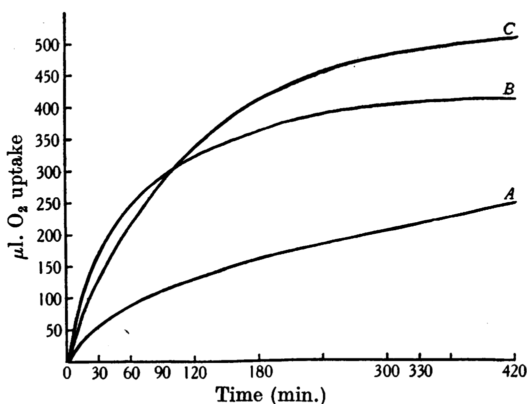



Fig. 4. Respiration of rat liver slices in presence of arsenite (0·001 $M$) and amines. $A$, tissue (22·9 mg. dry weight) + arsenite. $B$, tissue (22·6 mg. dry weight) + arsenite + tyramin e(354 μl.). $C$, tissue (23·7 mg. dry weight) + arsenite + indolethylamine (444 μl.).

per mol. of tyramine. With indolethylamine, however, it is evident that more oxygen is consumed than corresponds to 1 atom of oxygen and it seems likely

since pigment formation obviously takes place in the presence of arsenite, that the extra oxygen uptake is involved in the pigment production. The ammonia formation is not inconsistent with the conclusion, as in the case of cyanide, that 1 mol. of ammonia is produced from each mol. of indolethylamine.

Typical curves of the rates of oxygen uptake by tyramine and indolethylamine in presence of rat liver slices and sodium arsenite are shown in Fig. 4.

### *Effects of liver extracts on amine oxidation*

As shown in our earlier paper, extracts of rat brain and liver and guinea-pig kidney oxidize the higher aliphatic amines, and this applies also to indolethylamine and tyramine. It is evident, however, that in order to obtain satisfactory data which will indicate the course of breakdown of the amines active extracts must be used which have very little or no residual respiration and from which there is little spontaneous ammonia formation.

Such an extract may be obtained from rat liver by mincing and lightly grinding with sand fresh liver in an equal volume by weight of phosphate-Locke solution at *p*H 7·4. The tissue suspension is filtered through muslin and the filtrate centrifuged. The centrifuged deposit of tissue is separated from the clear centrifugate and from traces of sand, and it is well mixed with distilled water and again centrifuged. The deposit is mixed again with distilled water and again centrifuged and this process is repeated until the centrifugate is quite clear and shows no sign of haemoglobin spectroscopically. The residue is finally made up in phosphate-Locke solution, *p*H 7·4, or in distilled water.

With this extract, which shows very little residual respiration, active oxidation of butylamine, *iso*amylamine, tyramine and indolethylamine is observed.

### *Dialysis*

An experiment was carried out to see if dialysis of an active sheep liver extract, made similarly to that described above for rat liver, would result in loss of its ability to oxidize tyramine or *iso*amylamine. Dialysis, however, through a cellophane membrane against a phosphate-Locke solution for 20 hr. did not result in any appreciable loss of activity. For example:

$O_2$ uptake in 2 hr. by tyramine 0·0052 *M* with undialysed sheep liver extract = 300 $\mu$l.
$O_2$ uptake in 2 hr. by tyramine 0·0052 *M* with dialysed sheep liver extract = 301 $\mu$l.
$NH_3$ formation by tyramine 0·0052 *M* with undialysed sheep liver extract = 420 $\mu$l.
$NH_3$ formation by tyramine 0·0052 *M* with dialysed sheep liver extract = 401 $\mu$l.
$O_2$ uptake in 2 hr. by *iso*amylamine 0·0066 *M* with undialysed sheep liver extract = 174 $\mu$l.
$O_2$ uptake in 2 hr. by *iso*amylamine 0·0066 *M* with dialysed sheep liver extract = 161 $\mu$l.

The velocities of oxygen uptake in presence of the amines were the same with both dialysed and undialysed extracts. It appears, therefore, that the activity of the amine oxidase in the liver preparation is not dependent on an easily diffusible coenzyme.

### iso*Amylamine oxidation by rat liver extract*

To obtain a clear idea of the course of oxidation of amines it was decided to analyse as completely as possible the products of oxidation of a typical amine and to obtain a balance sheet. *iso*Amylamine was first chosen as it is one of the most readily attacked of the amines and its oxidation is uncomplicated by pigment formation.

As shown earlier [Pugh & Quastel, 1937, 1] *iso*amylamine is oxidized to a product which readily gives a dinitrophenylhydrazone. This was isolated and analysed in the following way.

*Isolation and identification of 2:4-dinitrophenylhydrazone of* iso*valeraldehyde*

45 mg. *iso*amylamine hydrochloride were dissolved in 10 ml. phosphate buffer solution *p*H 7·4. To this was added the total extract obtained from 50 g. fresh sheep liver and the volume made up with water to 80 ml. The suspension was gently shaken in an atmosphere of oxygen for 4 hr. in a water-bath at 40°. 50 ml. 10% trichloroacetic acid were added, the precipitate of protein filtered off and to the clear filtrate were added 100 ml. of a saturated solution of 2:4-dinitrophenylhydrazine in *N* HCl. There was immediate formation of a yellow precipitate and this was allowed to stand at room temperature overnight. The yellow crystalline precipitate was filtered, washed with a little *N* HCl, and then with a little water and finally dried. The weight of dinitrophenylhydrazone was 75 mg. This represented 79% of the theoretical yield. The substance was then analysed after recrystallization from alcohol, the following values being obtained: C, 49·7%; H, 5·1% (Weiler). The calculated results for the dinitrophenylhydrazone of *iso*valeraldehyde are C, 49·6%; H, 5·2%.

It is evident that the amine is oxidized to the corresponding aldehyde, thus:

$$R.CH_2NH_2 \rightarrow R.CHO + NH_3.$$

*Estimation of aldehyde, volatile acid and ammonia*

The aldehyde formed during the oxidation of the amine was estimated iodimetrically after bisulphite fixation. The procedure was as follows: to the solution containing the aldehyde was added 1 ml. 1% sodium bisulphite solution and the mixture was allowed to stand at room temperature for 30 min. A few drops of starch solution were added and the solution titrated with *N*/10 iodine solution from a microburette. Sodium bicarbonate was then added in excess and the bisulphite liberated from the aldehyde complex titrated with *N*/10 iodine solution.

Volatile acid was estimated by distillation into standard alkali from the solution containing the liver extract and products of oxidation of *iso*amylamine after rendering the latter solution acid with *N* sulphuric acid.

Ammonia was estimated, as stated earlier, by the method of Pugh & Quastel [1937, 2].

Control experiments were carried out in the absence of *iso*amylamine.

As stated in our earlier paper, oxidation of *iso*amylamine by tissues is accompanied by the production of a substance whose odour greatly resembles that of *iso*amyl alcohol. Since this is not formed when *iso*amylamine is placed in contact with tissues anaerobically, it seems likely that *iso*valeraldehyde produced from *iso*amylamine by oxidation undergoes a Cannizzaro reaction, in presence of liver aldehyde mutase [*v.* Dixon & Lutwak-Mann, 1937], into *iso*amyl alcohol and *iso*valeric acid.[1] It will be assumed that the amount of *iso*amyl alcohol formed is equivalent to the amount of *iso*valeric acid, estimated experimentally as volatile acid.

[1] Since not more than 1 atom of oxygen has been found to be consumed per mol. of amine during the oxidation of *iso*amylamine by a liver extract, it is evident that oxidation of the aldehyde by the Schardinger enzyme must be too small to affect these results.

### *Results with* iso*amylamine*

In a number of manometric vessels was placed *iso*amylamine $M/150$ in 3 ml. phosphate-Locke solution, containing an active liver extract. The same amount of liver extract was placed in each vessel. After the experimental period of 2 hr. the oxygen uptakes were determined and the solutions in the vessels were analysed, one vessel for ammonia estimation, one for volatile acid estimation and one for aldehyde estimation.

The following results were obtained, the values being given in terms of $\mu$l. gas:

| | | |
|---|---|---|
| *iso*Amylamine originally present | $= 444\ \mu$l. | |
| Oxygen uptake | $= 188\ \mu$l. | |
| $NH_3$ produced | $= 377{\cdot}1\ \mu$l. | |
| Aldehyde formed | $\equiv 0{\cdot}13$ ml. $M/10$ | $= 291\ \mu$l. |
| Volatile acid | $\equiv 0{\cdot}026$ ml. $N/10$ | $= 58\ \mu$l. |
| Alcohol (by hypothesis) | | $= 58\ \mu$l. |

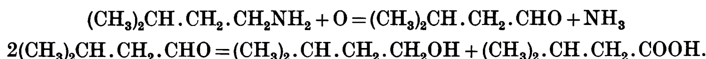
Total aldehyde formed
$= \text{aldehyde} + \text{volatile acid} + \text{alcohol} = 291 + 58 + 58\ \mu$l.
$= 407\ \mu$l.

$$\frac{\text{Total aldehyde formed}}{NH_3 \text{ produced}} = \frac{407}{377} = 1{\cdot}08.$$

$$\frac{\text{Ammonia formed}}{\text{Oxygen uptake}} = \frac{NH_3}{O} = \frac{377{\cdot}1}{188} = 2{\cdot}05.$$

It is also evident that not more than 1 atom of oxygen has been consumed per mol. of *iso*-amylamine.

These results are consistent with the following equations expressing the breakdown of *iso*amylamine by liver extract:



$$(CH_3)_2CH.CH_2.CH_2NH_2 + O = (CH_3)_2CH.CH_2.CHO + NH_3$$
$$2(CH_3)_2CH.CH_2.CHO = (CH_3)_2.CH.CH_2.CH_2OH + (CH_3)_2.CH.CH_2.COOH.$$

### *Results with butylamine*

When butylamine is oxidized in presence of intact liver slices acetoacetic acid is formed [Pugh & Quastel, 1937, 1]. With liver extracts, however, no evidence for acetoacetic acid production has been found. This is doubtless due to the fact that liver extracts, unlike the intact tissue, cannot oxidize the butyric acid which is formed by mutase action from the aldehyde.

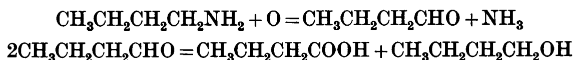
Experiments carried out, as described with *iso*amylamine, gave the following results:

| | |
|---|---|
| Butylamine originally present | $= 444\ \mu$l. |
| Oxygen uptake | $= 167$ ,, |
| $NH_3$ produced | $= 332$ ,, |
| Aldehyde formed | $= 168$ ,, |
| Volatile acid formed | $= 90$ ,, |
| Alcohol (by hypothesis) | $= 90$ ,, |
| Total aldehyde formed | $=$ aldehyde + volatile acid + alcohol |
| | $= 348\ \mu$l. |

$$\frac{NH_3}{O} = \frac{332}{167} = 1{\cdot}9.$$

$$\frac{\text{Total aldehyde formed}}{NH_3 \text{ formed}} = \frac{348}{332} = 1{\cdot}05.$$

The results are consistent with the equations:



$$CH_3CH_2CH_2CH_2NH_2 + O = CH_3CH_2CH_2CHO + NH_3$$
$$2CH_3CH_2CH_2CHO = CH_3CH_2CH_2COOH + CH_3CH_2CH_2CH_2OH$$

*Results with indolethylamine*

When indolethylamine is oxidized by a well washed liver extract, pigment formation takes place and the presence of the pigment makes it difficult to obtain a good end-point in the iodimetric estimation of aldehyde. The following are typical results:

| | |
|---|---|
| Indolethylamine originally present | $=444\ \mu$l. |
| Oxygen uptake | $=396\ \mu$l. |
| Aldehyde formed | $=123\ \mu$l. (approx.). |
| Volatile acid formed | =nil. |
| $NH_3$ produced | $=192\ \mu$l. |

These results show $\frac{NH_3 \text{ formed}}{\text{aldehyde formed}}=\frac{192}{123}=1{\cdot}5$. This high figure is probably due to the aldehyde estimation being too low owing to the difficulty in ascertaining the correct end-point in the iodine titration.[1] Another experiment gave a lower ratio, i.e. 1·2. The most significant finding, however, is that the ratio $\frac{NH_3}{O}=\frac{192}{396}=0{\cdot}48$ is much lower than was obtained with *iso*amylamine or butylamine. Another experiment gave a ratio $\frac{NH_3}{O}=0{\cdot}59$. Such a ratio is consistent with the view that between 3 and 4 atoms of oxygen are consumed for every molecule of ammonia formed.

It is clear that if between 3 and 4 atoms of oxygen are consumed for each mol. of indolethylamine, the oxidation of the base in the presence of tissue slices must cease appreciably before the indolethylamine is fully oxidized. For in experiments designed to obtain the total oxygen uptake by indolethylamine in presence of intact liver slices, values of total oxygen uptake not greater than 700 $\mu$l. have been obtained, the amount of indolethylamine present being initially 444 $\mu$l. This value includes the autoxidation of the tissue slices themselves. In experiments also with tissue extracts total values not greater than 400 $\mu$l. were obtained with the same initial quantity of indolethylamine. The conclusion seems to be that the products of oxidation of indolethylamine partially inhibit the oxidation of the amine itself. An experiment was carried out to see if indoleacetic acid would inhibit the oxidation of indolethylamine but the results showed no inhibitory effect whatever.

*Hydrogen peroxide formation*

Both Hare [1928] and Philpot [1937] have shown that during tyramine oxidation by liver extracts hydrogen peroxide is formed.

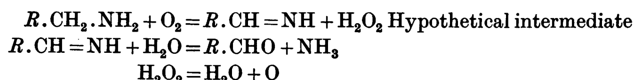
It is clear too, that during *iso*amylamine oxidation hydrogen peroxide is formed. This may be shown by the coupled oxidation of ethyl alcohol which ensues when the latter is added to *iso*amylamine in presence of rat liver extract. The oxygen uptake in presence of the amine is doubled on addition of alcohol (0·036 $M$). This fact is shown by curves $A$ and $B$, Fig. 5. Moreover, aldehyde formation as estimated by bisulphite addition and subsequent titration with iodine solution is also doubled.[2] For example in one experiment: aldehyde formation in 2 hr. by oxidation of *iso*amylamine in presence of 1 ml. rat liver extract = 224 $\mu$l.; aldehyde formed in 2 hr. by oxidation of *iso*amylamine plus ethyl alcohol by 1 ml. rat liver extract = 493 $\mu$l.

These facts indicate hydrogen peroxide formation by the amine [Keilin & Hartree, 1936]. Alcohol oxidation itself by rat liver extract is very feeble and insufficient to influence the values given above.

[1] It is also possible that some indoleacetic acid is formed. This point has not yet been investigated.

[2] The formation of acetaldehyde may be shown by the positive nitroprusside-piperidine test.

It is evident that the course of oxidation of *iso*amylamine may be represented as follows:



$$R.CH_2.NH_2 + O_2 = R.CH = NH + H_2O_2 \text{ Hypothetical intermediate}$$
$$R.CH = NH + H_2O = R.CHO + NH_3$$
$$H_2O_2 = H_2O + O$$

When ethyl alcohol is added to indolethylamine in presence of rat liver extract, the oxygen uptake of the amine is not doubled. Values obtained, in one experiment, are shown in curves $C$ and $D$, Fig. 5. A possible interpretation of this

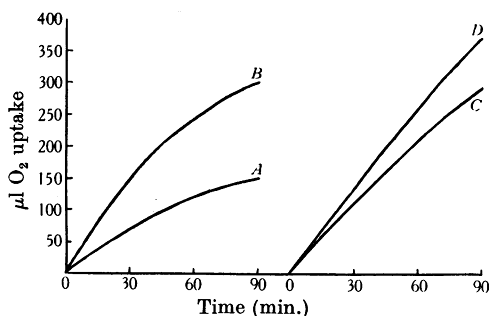



Fig. 5. $A$, 0·5 ml. liver extract + *iso*amylamine (0·0066 $M$). $B$, 0·5 ml. liver extract + *iso*amylamine (0·0066 $M$) + ethyl alcohol (0·036 $M$). $C$, 0·5 ml. liver extract + indolethylamine (0·0066 $M$). $D$, 0·5 ml. liver extract + indolethylamine (0·0066 $M$) + ethyl alcohol (0·036 $M$).

result is that the hydrogen peroxide formed during indolethylamine oxidation is responsible for further oxidation of the amine with subsequent pigment production. The presence of alcohol, in such circumstances, would not necessarily increase the oxygen uptake obtained with the amine alone. Further progress in this problem will depend on further knowledge being secured as to the chemical constitution of the pigment.

*Is amine oxidase a single enzyme?*

It seems very likely from the biological distribution of the amine oxidase, that the same enzyme is involved in the oxidation of the aliphatic amines, tyramine and indolethylamine.

A number of experiments were carried out to test this point.

Using rat liver extract it was found that the oxygen uptake of a mixture of butylamine and tyramine was not the same as the sum of the oxygen uptakes of the two amines taken separately but a value approximating to one of them.

For example:

Total $O_2$ uptake in 60 min. with butylamine 0·0066 $M$ = 42 μl.
Total $O_2$ uptake in 60 min. with tyramine 0·0052 $M$ = 229 μl.
Total $O_2$ uptake in 60 min. with butylamine 0·0066 $M$ + tyramine 0·0052 $M$ = 228 μl.

Similar results were obtained with mixtures of tyramine and *iso*amylamine and of tyramine + indolethylamine.

Ammonia formation from a mixture of two amines is also not the sum of the values obtained with the amines singly. For example, using rat liver extract,

(1) $NH_3$ in 2 hr. from butylamine 0·0066 $M$ = 100 μl.
$NH_3$ in 2 hr. from tyramine 0·0052 $M$ = 163 μl.
$NH_3$ in 2 hr. from butylamine 0·0066 $M$ + tyramine 0·0052 $M$ = 168 μl.

(2) $NH_3$ in 2 hr. from tyramine 0·0052 $M$ = 331 μl.
$NH_3$ in 2 hr. from *iso*amylamine 0·0066 $M$ = 176 μl.
$NH_3$ in 2 hr. from tyramine 0·0052 $M$ + *iso*amylamine 0·0066 $M$ = 210 μl.

Typical curves of the rates of oxygen uptakes of mixtures of amines are shown in Figs. 6, 7 and 8.

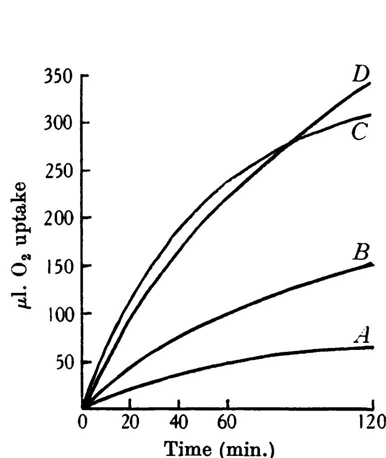



Fig. 6.

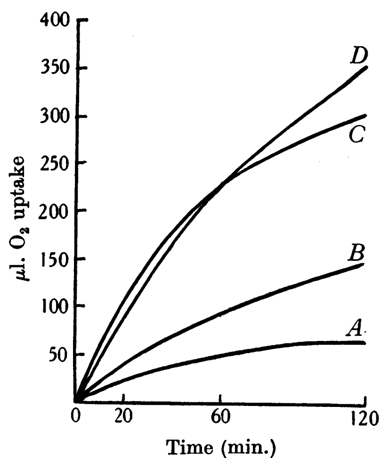



Fig. 7.

Fig. 6. *A*, 0·5 ml. liver extract alone. *B*, 0·5 ml. liver extract + *iso*amylamine (0·0066 *M*). *C*, 0·5 ml. liver extract + tyramine (0·0052 *M*). *D*, 0·5 ml. liver extract + tyramine (0·0052 *M*) + *iso*-amylamine (0·0066 *M*).

Fig. 7. *A*, 0·5 ml. liver extract alone. *B*, 0·5 ml. liver extract + butylamine (0·0066 *M*). *C*, 0·5 ml. liver extract + tyramine (0·0052 *M*). *D*, 0·5 ml. liver extract + butylamine (0·0066 *M*) + tyramine (0·0052 *M*).

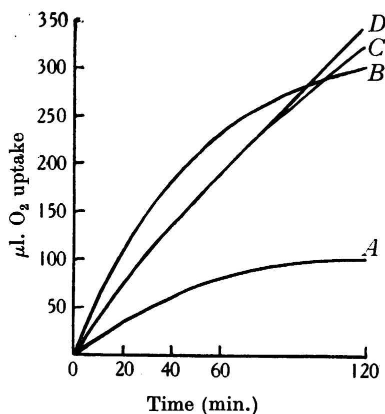



Fig. 8. *A*, 0·5 ml. liver extract alone. *B*, 0·5 ml. liver extract + tyramine (0·0052 *M*). *C*, 0·5 ml. liver extract + indolethylamine (0·0066 *M*). *D*, 0·5 ml. liver extract + tyramine (0·0052 *M*) + indolethylamine (0·0066 *M*).

These results lead to the conclusion that a common amine oxidase system is concerned with oxidation of the amines.

This is borne out also by the fact that an amine which is not vigorously oxidized by amine oxidase will inhibit the oxidation of another amine. For example, it was found that the presence of phenylethylamine will inhibit ammonia formation from tyramine. Thus in one experiment, using rat liver

slices in presence of phosphate-Locke-glucose medium, the following results were obtained:

| | No amine present | With tyramine 0·0052 $M$ | With $\beta$-phenylethylamine 0·0066 $M$ | With tyramine 0·0052 $M$ + $\beta$-phenylethylamine 0·0066 $M$ |
|---|---|---|---|---|
| $Q_{NH_3}$ (over 2 hr.) | 1·0 | 3·6 | 1·3 | 1·9 |

It is proposed to term the enzyme amine oxidase, this being identical apparently with tyramine oxidase.

## Summary

1. Indolethylamine and tyramine, which greatly inhibit the respiration of brain cortex slices in a glucose medium, are oxidized by an amine oxidase in brain with liberation of ammonia and an aldehyde. Mescaline, benzedrine (phenyl-*iso*propylamine) and $\beta$-phenylethylamine which also inhibit brain respiration are attacked feebly or not at all. There is no evidence for deamination of histamine by brain.

2. Rat liver slices and guinea-pig kidney slices oxidize and deaminate indolethylamine, tyramine and phenylethylamine and their effects on mescaline, histamine and benzedrine are small or negligible. The order of activity of the tissues on the amines is rat liver > guinea-pig kidney > rat brain. Rat kidney has relatively little activity.

3. The presence of cyanide does not affect the activity of the amine oxidase. In presence of cyanide, 1 atom of oxygen is taken up for each mol. of indolethylamine or tyramine consumed and 1 mol. of ammonia is liberated.

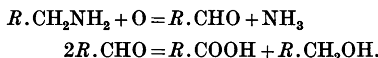
4. Oxidation of indolethylamine by tissues is always accompanied by the formation of a melanin-like pigment. In presence of cyanide, although oxidation of the amine and deamination take place, pigment formation is greatly reduced. It appears therefore that indolethylamine is attacked by a cyanide-resistant mechanism (amine oxidase) and a cyanide-sensitive system resulting in pigment formation.

5. Arsenite ($M$/1000) does not inhibit the activity of amine oxidase, nor does it inhibit appreciably pigment formation from indolethylamine.

6. Well washed liver extracts oxidize butylamine, *iso*amylamine, tyramine and indolethylamine. Dialysis of the extract has no apparent diminishing effect on its activity.

7. From the products of oxidation of *iso*amylamine by liver extract *iso*-valeraldehyde has been isolated as the dinitrophenylhydrazone (79% yield) and identified.

8. Analyses of the aldehyde, ammonia and volatile acid formed during the oxidation of *iso*amylamine and butylamine by liver extracts show that the following reactions take place during the oxidation of the amines:



$$R.CH_2NH_2 + O = R.CHO + NH_3$$
$$2R.CHO = R.COOH + R.CH_2OH.$$

9. Hydrogen peroxide is formed during amine oxidation by liver extracts as shown by coupled oxidation of ethyl alcohol in presence of the amine. Alcohol does not, however, double the oxygen uptake in presence of indolethylamine.

10. When indolethylamine is oxidized by a liver extract, there is an uptake of between 3 and 4 atoms of oxygen for each mol. of ammonia produced. It is concluded that the extra oxygen uptake, above that required for the aldehyde formation, is concerned with the pigment formation.

11. Experiments carried out with mixtures of amines show that a common amine oxidase system is concerned with the oxidation of the higher aliphatic amines, tyramine, $\beta$-phenylethylamine and indolethylamine. It is proposed to term the enzyme amine oxidase, this being identical with tyramine oxidase.

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