53. BENZEDRINE (β -PHENYLISOPROPYLAMINE) AND BRAIN METABOLISM

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It has been shown by Quastel & Wheatley [1933] that the addition of a variety of amines, including tyramine and *iso*amylamine, to brain tissue respiring in a glucose-phosphate medium results in a fall in the rate of O_2 uptake by the tissue. The oxidations of glucose, lactic acid, pyruvic acid and glutamic acid by brain were found to be markedly inhibited by exposure of the tissue to such amines. Succinic acid oxidation is not impaired, however, except in the case of brain tissue exposed to tyramine. In this case a large fall in the rate of succinic acid oxidation takes place.

A study of the mechanism by which several aliphatic amines which retard brain respiration undergo breakdown in isolated tissue resulted in the finding [Pugh & Quastel, 1937, 1] that brain contains an enzyme capable of oxidizing aliphatic amines with the liberation of NH_3 . Typical instances are butylamine, *iso*amylamine and heptylamine. The oxidase in question was found to be quite distinct from amino-acid oxidase.

Subsequent work carried out independently by Pugh & Quastel [1937, 2] and by Blaschko *et al.* [1937, 1] showed that the aliphatic amine oxidase was identical with tyramine oxidase and the enzyme is now known as amine oxidase. The former authors proved by analysis of the reaction products formed when *iso*amylamine and butylamine are oxidized by liver extracts that the following transformations take place:

Richter [1937; see also Kohn, 1937] showed that the enzyme oxidizes phenylethylamine, tyramine and arterenol to form NH_3 ; adrenaline, epinine and sympatol to produce methylamine; and alkamine and hordenine to give ethylamine and dimethylamine respectively. The corresponding aldehydes are also formed; these were isolated as the dinitrophenylhydrazones. The general reaction was represented as follows:

where R^1 = hydrogen or an alkyl group. Blaschko *et al.* examined a large number of amines and showed that only compounds containing an amino group at the end of a hydrocarbon chain are oxidized. When the α carbon atom was substituted as in the cases of *isopropylamine* and *ephedrine* no attack by the enzyme took place. It was suggested that the free hydrocarbon chain was an essential part of the substrate since molecules such as *cadaverine* and *histamine* are not attacked.

When intact tissue slices (brain, liver, kidney) are examined under suitable physiological conditions it is found that the addition of tyramine or β -indol-ethylamine to the medium in which the slices are respiring does not result in

an increased O_2 uptake, although there is no doubt that the amine is undergoing oxidation. This is shown by the liberation of NH_3 and of the corresponding aldehyde [Pugh & Quastel, 1937, 2]. The rate of O_2 uptake of the tissue slices falls, the rate of fall increasing with time; this effect is much more marked with brain cortex slices than with liver or kidney cortex slices. To show an increased O_2 consumption of the intact tissues in presence of amines it is necessary to inhibit the normal respiration of the tissues by the addition of cyanide or of arsenite.¹ Amine oxidase is unaffected by cyanide or arsenite and in the presence of the latter molecules it is possible to show that tyramine oxidation by intact tissues results in the absorption of one atom of O_2 and the formation of one molecule of NH_3 for each molecule of amine oxidized. The same applies to the oxidation of indolethylamine, although here there is evidence that in the presence of arsenite more than one atom of O_2 is consumed for each molecule of amine decomposed. It is obvious that for an investigation of the properties of amine oxidase it is necessary to use tissue extracts or preparations where other oxidizing systems have been eliminated as far as possible.

Similar conclusions to these were arrived at by Blaschko *et al.* [1937, 2] in their studies of adrenaline inactivation by tissues.

Competition for amine oxidase takes place between various amines. Thus it was found by Pugh & Quastel [1937, 2] that competition between the following amines occurs: tyramine, *iso*amylamine, β -indolethylamine and β -phenylethylamine. Blaschko *et al.* [1937, 1] showed that competition occurs between tyramine, adrenaline, *iso*amylamine and β -indolethylamine. The latter authors also showed that *l*-ephedrine, triethylamine, tri*iso*amylamine, hordenine—amines which are either slowly or not attacked by amine oxidase—have an affinity for the enzyme and inhibit the oxidation of amines which are attacked by the oxidase. Kohn [1937] arrived at similar results.

Benzedrine (β -phenylisopropylamine) is either very feebly or not at all attacked by amine oxidase [Pugh & Quastel, Blaschko *et al.*]. It has nevertheless a high affinity for this enzyme and is a powerful inhibitor of amine oxidation.

It is the purpose of this communication to show that the presence of benzedrine at low concentration markedly stimulates the respiration of brain *in vitro* whether this is examined in the form of intact tissue slices or as a mince so long as the respiration takes place in the presence of a variety of amines, and that this stimulation is directly related to the affinity of benzedrine for amine oxidase.

TECHNIQUE

O_2 uptakes were determined with the Warburg respiratory apparatus. The methods for the preparation and examination of tissue slices are described in an earlier paper [Pugh & Quastel, 1937, 2] and will not be further commented upon here.

EFFECTS OF BENZEDRINE ON THE RESPIRATION OF BRAIN TISSUE IN PRESENCE OF TYRAMINE, INDOLETHYLAMINE AND *ISO*AMYLAMINE

Brain cortex slices

The presence of tyramine, indolethylamine, *iso*amylamine or certain other amines results in a progressive fall in the respiration of brain cortex slices respiring in a glucose-phosphate-Locke medium at 37° [Quastel & Wheatley, 1933; Pugh & Quastel, 1937, 1, 2]. Benzedrine at relatively high concentrations also reduces

¹ Or of a high phosphate concentration [Philpot, 1937].

the respiration of brain cortex slices examined in a glucose medium, but it has little or no effect at small concentrations (e.g. $10^{-4}M$).

When benzedrine¹ at small concentrations is added to a glucose medium containing tyramine, indolethylamine or *iso*amylamine the fall in the rate of O_2 uptake takes place much less rapidly than in the absence of the benzedrine. Typical results relating to rat brain cortex slices are shown in Table 1. In the case of tyramine at a concentration of $7.7 \times 10^{-3}M$ the respiration falls from a value of $Q_{O_2}=9.3$ during the 1st hour of the experiment, to 4.8, during the 2nd hour, and to 2.6 during the 3rd hour. When benzedrine at a concentration of $4.3 \times 10^{-4}M$ which has little or no inhibitory action on the respiration of brain cortex slices is added to the glucose medium containing tyramine $7.7 \times 10^{-3}M$ the fall in respiration is given by $Q_{O_2}=9.7$ during the 1st hour, 8.4 during the 2nd hour and 6.0 during the 3rd hour. The benzedrine effects a partial neutralization of the inhibition of respiration of brain cortex slices brought about by the presence of tyramine. The effect may be observed similarly with the inhibitions produced by *iso*amylamine and indolethylamine. In the latter case the inhibitory action of $0.33 \times 10^{-3}M$ indolethylamine on rat brain cortex respiration was almost completely neutralized by the presence of $0.43 \times 10^{-3}M$ benzedrine.

It is evident that the presence of benzedrine has the effect of stimulating the O_2 uptake of brain cortex slices when these respire in media containing amines such as tyramine, indolethylamine or *iso*amylamine. The stimulant action of benzedrine, so long as it is not used at such high concentrations (e.g. $4 \times 10^{-3}M$) that it itself exercises inhibitory effects, is inversely proportional to the concentration of tyramine or indolethylamine.

Table 1

Effects of benzedrine ($4.3 \times 10^{-4}M$) on brain cortex respiration in presence of amines.
Glucose-phosphate-Locke. O_2 . 37°.

Amine	<i>M</i> conc.	Q_{O_2}					
		1st hour		2nd hour		3rd hour	
		Benze- drine absent	Benze- drine present	Benze- drine absent	Benze- drine present	Benze- drine absent	Benze- drine present
Nil	—	12.7	11.2	10.9	10.1	9.7	9.7
Tyramine	7.7×10^{-3}	9.3	9.7	4.8	8.4	2.6	6.0
	1.9×10^{-3}	9.7	9.7	5.2	8.6	3.0	6.0
β -Indolethyl- amine	0.67×10^{-3}	10.4	12.1	6.8	10.6	3.0	6.8
	0.33×10^{-3}	10.2	11.2	8.3	10.3	5.2	8.4
<i>iso</i> Amylamine	1.67×10^{-3}	10.5	10.7	7.6	9.2	4.9	7.6

Table 2

Effects of benzedrine on brain cortex respiration in presence of tyramine.
Glucose-phosphate-Locke. O_2 . 37°.

Tyramine <i>M</i> conc.	Benzedrine <i>M</i> conc.	Q_{O_2}		
		1st hour	2nd hour	3rd hour
Nil	Nil	11.4	10.1	9.0
1.9×10^{-3}	Nil	9.6	5.3	3.1
1.9×10^{-3}	1.4×10^{-4}	10.1	8.5	6.3
5.7×10^{-3}	Nil	8.8	4.9	2.7
5.7×10^{-3}	1.4×10^{-4}	11.0	6.3	2.9

This is seen in the results quoted in Tables 1 and 2.

¹ As the sulphate.

Minced tissue

The effect of benzedrine in decreasing the fall of respiration of brain tissue when this is exposed to tyramine, etc., may also be seen very clearly with minced brain tissue. Typical results obtained with a suspension of minced rat brain tissue in saline (1.3 g. brain in 7 ml. saline) are shown in Table 3; respiration was allowed to occur in a glucose-phosphate-saline medium in the presence of air.

Table 3

O₂ uptakes (μ l.) of 1 ml. minced rat brain tissue at 37° in glucose (0.01 *M*)-phosphate (0.033 *M*; pH 7.4)-saline in the presence of tyramine and various concentrations of benzedrine. Tyramine conc. = 1.9×10^{-3} *M*.

Time	No amine present	Tyramine alone present	Tyramine + benzedrine Benzedrine concentrations			
			7.1×10^{-5} <i>M</i>	1.4×10^{-4} <i>M</i>	2.8×10^{-4} <i>M</i>	7.1×10^{-4} <i>M</i>
During 1st hour	304.3	240.9	234.7	236.8	233.1	238.2
During 2nd hour	249.7	145.2	156.1	173.7	186.2	192.8
During 3rd hour	205.8	74.9	109.9	128.3	162.7	180.2

It will be seen that the neutralizing action of benzedrine increases with its concentration.

Moreover, it will be noted that using a concentration of tyramine of 1.9×10^{-3} *M* the presence of so low a concentration of benzedrine as 7.1×10^{-5} *M* effects a partial neutralization of the inhibitory action of the tyramine.

The latter concentration corresponds to a concentration of β -phenylisopropylamine of about 1 mg. per 100 ml. Doubtless smaller concentrations of benzedrine would be equally effective in partially neutralizing the inhibitory effects of amines such as tyramine when these are present in very low concentrations.

Another method of observing the action of benzedrine on amine inhibition of brain respiration is to expose the minced brain tissue to mixtures of benzedrine and toxic amines for an arbitrary period after which glucose is added to the system. O₂ uptakes are now recorded. The experiments can be carried out conveniently in the Warburg apparatus, glucose being placed in the side tube of the manometer flask. Minced brain tissue is placed in a phosphate-Locke medium containing tyramine or mixtures of tyramine and benzedrine and allowed to incubate at 37° in air, the manometric apparatus being allowed to shake in the usual way for 2 hr. The levels of the fluid in the manometer are read after opening and reclosing the taps of the flask and the glucose in the side tube is tipped into the body of the flask. The rates of O₂ uptake are now read for 1 hr. Typical results obtained with minced guinea-pig brain suspension (3 g. brain in 15 ml. Locke solution) are shown in Table 4. It will be seen that when benzedrine is present in the medium the addition of tyramine brings about

Table 4

O₂ uptakes (μ l.) in 1 hr. at 37° by 2 ml. minced guinea-pig brain tissue in a glucose (0.01 *M*)-phosphate (0.033 *M*; pH 7.4)-Locke medium after exposure for 2 hr. in the absence of glucose to tyramine and to a mixture of tyramine and benzedrine.

Amine present	μ l. O ₂
Nil	238.7
Benzedrine (1.4×10^{-3} <i>M</i>)	177.7
Tyramine (1.9×10^{-3} <i>M</i>)	90.1
Benzedrine (1.4×10^{-3} <i>M</i>)	170.8
+ tyramine (1.9×10^{-3} <i>M</i>)	

little or no fall in the brain respiration. In another experiment carried out in a similar manner an O_2 uptake of $71.1 \mu\text{l.}$ found after exposure to tyramine ($1.9 \times 10^{-3} M$) alone was increased to $196.3 \mu\text{l.}$ by the addition to the medium of benzedrine ($0.7 \times 10^{-4} M$).

Thus the effect of adding benzedrine to brain tissue consuming O_2 in the presence of glucose and of tyramine is greatly to stimulate the O_2 uptake.

SUCCINIC ACID OXIDATION BY BRAIN IN PRESENCE OF TYRAMINE AND OF MIXTURES OF TYRAMINE AND BENZEDRINE

It was noticed by Quastel & Wheatley [1933] that the presence of tyramine greatly inhibited succinate oxidation by brain tissue and that tyramine was unique, among the amines investigated, in this respect.

It may be shown that benzedrine neutralizes this inhibitory action of tyramine. This fact is most conveniently demonstrated by exposing brain tissue aerobically to tyramine and to a mixture of tyramine and benzedrine for 2 hr. at 37° in the absence of other substrates, adding sodium succinate solution and measuring the subsequent rate of O_2 uptake. Typical results are shown in Table 5, in which values of O_2 uptake obtained with a minced rat brain suspension (1.4 g. brain suspended in 7 ml. saline) are recorded.

Table 5

O_2 uptakes ($\mu\text{l.}$) in 1 hr. by 1 ml. minced rat brain suspension in a succinate ($0.013 M$)-phosphate ($0.033 M$; pH 7.4)-saline medium after exposure for 2 hr. in the absence of succinate to tyramine and to a mixture of tyramine and benzedrine. Air. 37° .

Amine present	$\mu\text{l. } O_2$
Nil	215.1
Tyramine ($1.9 \times 10^{-3} M$)	112.2
Tyramine ($1.9 \times 10^{-3} M$) + benzedrine ($4.3 \times 10^{-4} M$)	174.1

MECHANISM OF ACTION OF BENZEDRINE

If amines such as tyramine, β -indolethylamine or *iso*amylamine exert their inhibitory effects on glucose oxidation by brain by combination with some essential respiratory catalyst, it follows that benzedrine at concentrations which have no inhibitory effects on glucose oxidation can have no affinity for the respiratory catalyst in question. Hence it cannot displace the inhibitory amines from this catalyst or neutralize their inhibitory actions. Since, however, the evidence already submitted shows that the inhibitory amines and benzedrine do compete in such a way that the presence of the latter molecule neutralizes the development of an inhibition of respiration, it follows that the free amines themselves at the concentrations in question are not wholly responsible for the inhibitions. It is more likely that inhibitory agents are developed as a result of metabolism of the amines and that the benzedrine exerts its effect by retarding the development of such agents. Evidence in favour of this view is given by the fact that the inhibitions exerted by tyramine, etc., increase markedly with time (see Table 1, and Pugh & Quastel, 1937, 2) pointing to the gradual development of an inhibitory agent. That this agent is the aldehyde¹ corresponding to the inhibitory amine is made likely by the following evidence:

1. Brain contains an amine oxidase capable of oxidizing tyramine, β -indolethylamine and *iso*amylamine to the corresponding aldehydes.

¹ Or a substance formed as a result of further metabolism of the aldehyde.

2. Those amines which are not attacked by amine oxidase have little or no inhibitory effects on the respiration of brain in presence of glucose. Such amines, for instance, are cadaverine, putrescine and histamine [Quastel & Wheatley, 1933], which suffer no oxidation by amine oxidase [Blaschko *et al.* 1937, 1; Pugh & Quastel, 1937, 2]. Again benzedrine which is not oxidized by this enzyme has no inhibitory action on brain respiration at concentrations which will show effective neutralizing powers.

3. Aldehyde formation can be shown to take place gradually when brain slices or suspensions respire in presence of the inhibitory amines. The development of the aldehyde, as indicated by the dinitrophenylhydrazine-alkali test [Pugh & Quastel, 1937, 2], is much decreased by the presence of those concentrations of benzedrine which are effective in neutralizing the inhibitory action of the amines.

4. Aldehydes corresponding to, or of a similar structure to, those formed by *isoamylamine* and *tyramine*, exert considerable inhibitory effects on the respiration of brain in presence of glucose and these effects are not neutralized by the addition of benzedrine to the system.

Such aldehydes exert their inhibitory effects immediately in contrast to the corresponding amines whose effects develop relatively slowly with time.

The inhibitory effects of *p*-hydroxybenzaldehyde and of *isovaleric* aldehyde on rat brain cortex respiration in a glucose-phosphate-Locke medium and the lack of neutralizing action of benzedrine will be seen in the results given in Table 6.

Table 6

Effects of aldehydes on respiration of rat brain cortex slices in presence and absence of benzedrine. Glucose-phosphate-Locke. O_2 . Benzedrine = $4.3 \times 10^{-4} M$.

Aldehyde	<i>M</i> conc.	Q_{O_2}					
		1st hour		2nd hour		3rd hour	
		Benze- drine absent	Benze- drine present	Benze- drine absent	Benze- drine present	Benze- drine absent	Benze- drine present
<i>p</i> -Hydroxy- benzaldehyde	2.7×10^{-3}	9.6	8.8	7.3	7.0	4.9	5.2
<i>iso</i> Valeric aldehyde	4.7×10^{-3}	6.8	7.4	4.6	5.2	2.6	3.4
Nil	—	12.7	11.2	10.9	10.1	9.7	9.7

*iso*Valeric aldehyde and *p*-hydroxybenzaldehyde inhibit the respiration of minced brain tissue in a glucose-phosphate medium their toxicities increasing with their concentrations. Typical results are shown in Tables 7 and 8. In these experiments rat brain suspensions (1.5 g. brain suspended in 7 ml. saline) was employed. The lack of neutralizing effect of benzedrine is shown in Table 9. The toxicity of *isovaleric* aldehyde is perhaps lower than would have been anticipated from that of *isoamylamine*, but the question of the relative rates of penetration into the cell has to be borne in mind, as well as the fact that the amine *per se* may exert some inhibitory influence at the concentrations used.

The conclusion¹ that benzedrine exerts its stimulating action on the O_2 uptake of brain when this respire in presence of certain amines by diminishing the rate of formation of toxic aldehydes or of toxic products formed from such aldehydes is supported by the following evidence which shows that succinate

¹ A somewhat analogous case in which the presence of uric acid may accelerate oxygen uptake in the xanthine oxidase system has been noted by Harrison [1932].

Table 7

O₂ uptakes (μ l.) by 1 ml. rat brain suspension in glucose (0.01 *M*)-phosphate (0.033 *M*; pH 7.4)-saline in the presence of various quantities of *isovaleric* aldehyde. Air. 37°.

<i>isovaleric</i> aldehyde conc.	O ₂		
	1st hour	2nd hour	3rd hour
Nil	277.6	219.2	186.4
10 ⁻³ <i>M</i>	244.2	191.9	169.3
3 × 10 ⁻³ <i>M</i>	198.5	156.1	140.8
6 × 10 ⁻³ <i>M</i>	128.3	94.8	79.5
10 ⁻² <i>M</i>	82.9	47.8	29.0

Table 8

O₂ uptakes (μ l.) by 1 ml. minced rat brain suspension in glucose (0.01 *M*)-phosphate (0.033 *M*; pH 7.4)-saline in presence of various quantities of *p*-hydroxybenzaldehyde. Air. 37°.

<i>p</i> -Hydroxybenzaldehyde conc.	μ l. O ₂ uptake	
	1st hour	2nd hour
Nil	253.5	211.9
2.67 × 10 ⁻³ <i>M</i>	223.5	181.9
5.33 × 10 ⁻³ <i>M</i>	193.7	138.6

Table 9

As in Table 7 but with the addition of various concentrations of benzedrine.

Conc. <i>isovaleric</i> aldehyde	Conc. benzedrine	μ l. O ₂ uptake	
		1st hour	2nd hour
6 × 10 ⁻³ <i>M</i>	Nil	125.4	102.2
6 × 10 ⁻³ <i>M</i>	1.4 × 10 ⁻⁴ <i>M</i>	120.3	109.3
6 × 10 ⁻³ <i>M</i>	5.6 × 10 ⁻⁴ <i>M</i>	99.5	86.7
Nil	Nil	253.5	211.9

oxidation by brain is inhibited by the aldehyde corresponding to tyramine (i.e. *p*-hydroxyphenylacetaldehyde).

It is of importance to mention at this point that benzedrine, in common with a number of other amines, e.g. mezcaine, will bring about inhibitions of brain respiration when used at relatively high concentrations. Since there is no evidence that benzedrine suffers oxidation by the brain at these concentrations it is clear that the inhibitions in question cannot be caused by oxidation products of benzedrine. It seems more likely that these inhibitions may be related to the presence of the non-polar phenylisopropyl group acting in a manner similar to such groups in the narcotics. A discussion of this type of inhibition is, however, not relevant to the subject of this paper, for only concentrations of benzedrine are being considered which do not themselves markedly affect the respiration of brain *in vitro*.

EFFECT OF SEMICARBAZIDE ON THE OXIDATION OF SUCCINATE BY BRAIN IN PRESENCE OF TYRAMINE

The addition of semicarbazide to brain tissue suspended in a phosphate-saline medium containing tyramine results in a complete neutralization of the latter's inhibitory effects on succinate oxidation. Typical findings are shown in Table 10. In the experiment whose results are recorded in this table minced rat brain (1.3 g. suspended in 7 ml. saline) was exposed to tyramine and to mixtures of tyramine and semicarbazide for 2 hr. at 37° before the addition of succinate. Recordings of O₂ uptake were made immediately after this addition.

Table 10

As in Table 5 with the exception that semicarbazide is substituted for benzedrine.

Substrates present	μ l. O ₂ uptake
Succinate (0.033 <i>M</i>)	332.9
Succinate (0.033 <i>M</i>) + tyramine (0.0019 <i>M</i>)	126.9
Succinate (0.033 <i>M</i>) + tyramine (0.0019 <i>M</i>) + semicarbazide (0.013 <i>M</i>)	309.3
Succinate (0.033 <i>M</i>) + tyramine (0.0019 <i>M</i>) + semicarbazide (0.033 <i>M</i>)	335.3
Succinate (0.033 <i>M</i>) + semicarbazide (0.033 <i>M</i>)	339.3

This result is convincing evidence that an aldehyde, or a product of further change of an aldehyde, derived from tyramine is responsible for the inhibition of succinate oxidation by this amine in presence of brain tissue.

Tyramine and succinate oxidation by tissues

Further evidence in support of the above conclusion may be obtained from the following observations.

When succinate is oxidized by brain tissue anaerobically by ferricyanide the presence of tyramine has no inhibiting effect whatever on the rate of oxidation. On the other hand, if brain tissue is incubated aerobically for 2 hr. in the presence of tyramine and, after this period, succinate is added to the system, the subsequent rate of anaerobic oxidation of succinate by ferricyanide is greatly decreased from the normal. These results are shown in Table 11. The technique of measuring the rate of anaerobic oxidation of succinate manometrically has been explained in detail by Quastel & Wheatley [1938] and this technique was followed in the experiments recorded in Table 11. The rate of CO₂ formation is a measure

Table 11

Rat brain (1.3 g.) was minced and suspended in Locke solution. This was centrifuged and the deposit made up to 8 ml. with Locke solution. 1 ml. suspension was placed in 0.025 *M* bicarbonate in presence and absence of 0.0019 *M* tyramine. This was exposed either (a) to 95% O₂ + 5% CO₂ or (b) to 95% N₂ + 5% CO₂ for 2 hr. at 37°. The gas in the former case was then changed to 95% N₂ + 5% CO₂. Succinate (0.033 *M*) was added, followed by 0.2 ml. ferricyanide solution.

	μ CO ₂ formed
Exp. (a) 2 hr. previous exposure to 95% O ₂ + 5% CO ₂	
No tyramine added	770.9 (1 hr.)
Tyramine present	449.5 (1 hr.)
Exp. (b) 2 hr. previous exposure to 95% N ₂ + 5% CO ₂	
No tyramine added	518.5 (45 min.)
Tyramine present	601.3 (45 min.)

of the rate of oxidation of succinate. It is evident from these experiments that tyramine *per se* is ineffective in inhibiting succinate oxidation by brain and that a product of oxidation of tyramine is the responsible agent. This is clearly an aldehyde as shown by the results given in Table 10 and is most probably *p*-hydroxyphenylacetaldehyde since this aldehyde has been isolated as a product of oxidation of tyramine by amine oxidase [Richter, 1937].

EFFECTS OF TYRAMINE ON SUCCINATE OXIDATION IN PRESENCE OF MIXTURES OF MUSCLE SUCCINIC DEHYDROGENASE AND LIVER AMINE OXIDASE

It is evident that if the conclusions which have been recorded are correct, an inhibition of succinate oxidation after the addition of tyramine should take place in the presence of a mixture of liver amine oxidase and muscle succinic dehydrogenase, similar to that which occurs in the presence of brain suspensions. The following experiments indicate that this phenomenon does in fact occur.

Liver amine oxidase was prepared by grinding guinea-pig or rat liver with sand for 20 min. and mixing with an equal volume of 0.066 *M* phosphate buffer pH 7.4. This was squeezed through muslin and dialysed overnight against 0.066 *M* phosphate buffer pH 7.4. The dialysed solution was centrifuged and the centrifugate made up to twice the initial volume. This preparation contained an active amine oxidase and possessed also a little succinic dehydrogenase activity.

Muscle succinic dehydrogenase was prepared by mincing guinea-pig or rat muscle (using the Latapie mincer) and washing three or four times with ten times its volume of water, squeezing the preparation through muslin after each wash. The washed finely divided muscle was finally made up to twice its initial volume with 0.02 *M* phosphate buffer pH 7.4. This preparation of succinic dehydrogenase is practically free from amine oxidase.

When sodium succinate solution is added to a mixture of amine oxidase and succinic dehydrogenase to which tyramine has been previously added and aerobic incubation for 15–30 min. at 37° carried out, it is found that the velocity of O₂ uptake due to succinate oxidation is much smaller than if tyramine had not been added to the system. A typical result is shown in Table 12 where

Table 12

O₂ uptakes (μl.) at 37° by a mixture of liver amine oxidase and muscle succinic dehydrogenase during the period of 30 min. following the addition of succinate (0.033 *M*) to the system containing tyramine. The systems were allowed to incubate aerobically at 37° for 30 min. before addition of the succinate.

Mixed enzymes No substrate	Mixed enzymes + tyramine (0.0019 <i>M</i>)	Mixed enzymes + succinate (0.033 <i>M</i>)	Mixed enzymes + tyramine (0.0019 <i>M</i>) + succinate (0.033 <i>M</i>) added after 30 min. incubation
7.5	26.9	308.7	75.9

a mixture of 0.7 ml. succinic dehydrogenase preparation and 0.5 ml. amine oxidase preparation in phosphate buffer (0.033 *M*; pH 7.4) was used. In the absence of amine oxidase succinate oxidation by muscle succinic dehydrogenase is but little impeded by the addition of tyramine. Thus in one experiment the O₂ uptake by a succinic dehydrogenase preparation in presence of sodium succinate (0.033 *M*) in 1 hr. was 144.5 μl. and this was reduced by previous aerobic exposure to tyramine (0.019 *M*) for 1 hr. to 115.5 μl. There is evidently a small reduction in velocity of succinate oxidation due to the tyramine but whether this is due to the presence of a small amount of amine oxidase in the muscle preparation or to the presence of the amine itself has not yet been made clear. It is sure however that this reduction is small compared with that effected by the addition of liver amine oxidase to the system.

THE EFFECT OF ADDING BENZEDRINE TO A MIXTURE OF AMINE
OXIDASE AND SUCCINIC DEHYDROGENASE

When benzedrine is added to the system amine oxidase, succinic dehydrogenase, tyramine and succinate, the fall in the rate of succinate oxidation is impeded and may be completely prevented. This is seen in the results recorded in Table 13, where a mixture of 0.5 ml. succinic dehydrogenase preparation

Table 13

O₂ uptakes (μl.) at 37° by a mixture of amine oxidase and succinic dehydrogenase during the period of 2 hr. following the addition of succinate to the systems indicated below. The systems were allowed to incubate aerobically at 37° before the addition of the succinate.

	Mixed enzymes Tyramine (0.0019 <i>M</i>) + succinate (0.033 <i>M</i>)	Mixed enzymes Benzedrine (0.0014 <i>M</i>) + succinate (0.033 <i>M</i>)	Mixed enzymes Tyramine (0.0019 <i>M</i>) + benzedrine (0.0014 <i>M</i>) + succinate (0.033 <i>M</i>)
Mixed enzymes Succinate (0.033 <i>M</i>)	286.3	86.9	262.8
			262.0

and 0.1 ml. amine oxidase preparation in *M*/30 phosphate buffer pH 7.4 was employed. The succinate was not added to the systems until the mixed enzyme preparation had been allowed to incubate in presence of tyramine or a mixture of tyramine and benzedrine for 1 hr. at 37°.

Thus the effect of benzedrine in neutralizing the inhibition of succinate oxidation effected by tyramine in presence of brain tissue can be seen equally well when a mixture of liver amine oxidase and muscle succinic dehydrogenase is substituted for the brain suspension.

PROTECTION OF SUCCINIC DEHYDROGENASE BY SUCCINATE FROM
THE EFFECT OF *p*-HYDROXYPHENYLACETALDEHYDE

The addition of a mixture of succinate and tyramine to a minced brain suspension in phosphate buffer solution (pH 7.4) at 37° leads to a velocity of O₂ uptake which differs not appreciably from that obtained when no tyramine is added. It is only when the tissue has been in contact aerobically with tyramine for a period of 15 min. or over that the diminished velocity of succinate oxidation is observed. A typical result is shown in Table 14.

Table 14

O₂ uptakes (μl.) at 37° in 2 hr. by minced rat brain tissue in presence of succinate or of a mixture of succinate and tyramine. In one part of the experiment succinate was added at the same time as the tyramine; in the other succinate was added 1 hr. after the brain tissue had been in contact with the tyramine.

Succinate added at the same time as the tyramine		Succinate added 1 hr. after incuba- tion with tyramine had commenced	
Succinate (0.033 <i>M</i>) alone	Tyramine (0.0014 <i>M</i>) + succinate (0.033 <i>M</i>)	Succinate (0.033 <i>M</i>) alone	Tyramine (0.0019 <i>M</i>) + succinate (0.033 <i>M</i>)
455.1	474.9	405.5	300.9

This phenomenon is also seen when a mixture of liver amine oxidase and muscle succinic dehydrogenase is used instead of a brain suspension. Results are quoted in Table 15. These show that the addition of succinate at the same

Table 15

O₂ uptakes (μ l.) in 15 min. at 37° by a mixture of amine oxidase (0.5 ml.) and succinic dehydrogenase (0.7 ml.) in presence of succinate and of a mixture of tyramine and succinate. The succinate was added either at the commencement of the experiment (i.e. with the tyramine) or at intervals of 15 min. and 30 min. after aerobic incubation with the tyramine had commenced.

Mixed enzymes Tyramine (0.0019 <i>M</i>) Succinate (0.033 <i>M</i>) added at commencement of the experiment	Mixed enzymes Tyramine (0.0019 <i>M</i>) + succinate (0.033 <i>M</i>) added at commencement of the experiment	Mixed enzymes Tyramine (0.0019 <i>M</i>) + succinate (0.033 <i>M</i>) added 15 min. after commence- ment of the experiment	Mixed enzymes Tyramine (0.0019 <i>M</i>) + succinate (0.033 <i>M</i>) added 30 min. after commence- ment of the experiment
166.8	171.9	113.1	41.0

time as tyramine to a mixture of the two enzymes leads to no change in the velocity of oxidation of the succinate. Previous aerobic incubation of the enzymes with tyramine leads to a greatly decreased rate of oxidation of succinate when this is subsequently added. The longer the period of previous incubation the smaller is the subsequent rate of succinate oxidation.

Since tyramine oxidation by the mixture of enzymes takes place in presence of succinate as shown by the development of the aldehyde (dinitrophenylhydrazine-alkali test) it is evident that succinate protects succinic dehydrogenase from the product of tyramine oxidation.

This protection of succinic dehydrogenase by succinate from the effect of a toxic agent is not a new observation. It was shown by Hopkins *et al.* [1938] that succinate protects its dehydrogenase from the inactivating influence of oxidized glutathione. These phenomena can indeed be regarded as special cases of a general rule relating to the protection of enzymes by their substrates (and molecules of allied molecular structure) from toxic agents (cf. Quastel [1931; 1932] on the protection of fumarase and urease by their substrates from the influence of toxic dyestuffs, or Dixon & Keilin [1936] on the protection of xanthine oxidase by its substrate from the action of cyanide). The results of Hopkins *et al.* would indicate that a thiol group in the succinic enzyme is partly responsible for the attachment of its substrate. It is not unreasonable to suggest that the toxic action of *p*-hydroxyphenylacetaldehyde might also be due to a condensation with a thiol group of the enzyme. It is evident however that if this is the case there is not the same reactivity with all aldehydes, for as has already been stated the inhibition of succinate oxidation in presence of brain takes place only with tyramine among the amines investigated.

COMPETITION OF AMINES FOR AMINE OXIDASE

The evidence submitted above shows the great importance of amine oxidase in brain in accounting for the stimulation of O₂ uptake by brain tissue effected by benzedrine when respiration takes place in presence of tyramine, etc. A knowledge of the nature of the competition between benzedrine and other amines for amine oxidase will be of value in assessing the relative importance of various amines as inhibitors of amine oxidase and in giving some conception of the concentrations at which various amines may be likely to stimulate brain respiration.

The fact that amines combine with amine oxidase in a strictly reversible manner may be shown by work which has been carried out on the competition between benzedrine and tyramine for amine oxidase (rat liver extracts).

Let us assume that the following reversible reactions take place:

where E = amine oxidase, T = tyramine, B = benzedrine or benzedrine derivative. Let x and y be the molar concentrations of tyramine and benzedrine respectively, and K_m and K_y the dissociation constants of ET and EB respectively. Let v = velocity of O_2 uptake effected by the amine oxidase in presence of tyramine alone and v' the velocity of O_2 uptake by the same amount of enzyme in presence of a mixture of tyramine and benzedrine.

Then, according to the mass action law,

$$\frac{v'}{v} = \frac{1}{1 + \frac{K_y y}{K_m x + K_m}}.$$

If I = fractional inhibition of the activity of the enzyme effected by the benzedrine, then

$$I = \frac{v - v'}{v}$$

and

$$\frac{1 - I}{I} = \frac{1}{\frac{v}{v'} - 1} = \frac{K_y (x + K_m)}{K_m y}. \quad \dots(1)$$

That this expression is followed may be seen from Table 16 where velocities of O_2 uptake due to tyramine and to mixtures of tyramine and benzedrine in presence of amine oxidase (rat liver extract) are noted. Some of these results (Exp. 1, 2) were obtained by Pugh & Quastel (unpublished) using different quantities of extracts. The velocities of O_2 uptake with given quantities of tyramine, or tyramine and benzedrine, are constant over the initial periods of the experiments and the velocities recorded refer only to such initial periods. The results indicate that benzedrine competes with tyramine for amine oxidase in a strictly reversible manner. Taking $K_m = 0.00178$ [Blaschko *et al.* 1937, 1] and K_y = dissociation constant of benzedrine-amine oxidase complex = 0.0004 approximately, the calculated inhibitions of O_2 uptake due to the presence of benzedrine are approximately equal to the observed inhibitions (see Table 16).

Table 16

Inhibitions effected by benzedrine on O_2 uptake by amine oxidase preparations of rat and guinea-pig liver in presence of tyramine.

I (calc.) is given by equation (1). $K_m = 0.00178$; $K_y = 0.0004$.

Exp.	M conc. tyramine	M conc. benzedrine	Rate of O_2 uptake in absence of benzedrine $\mu l. O_2$ per 30 min.	Rate of O_2 uptake in presence of benzedrine $\mu l. O_2$ per 30 min.	I fractional inhibition of O_2 up- take due to benzedrine	I calc.
1 (rat)	0.0066	0.0033	180	57	0.68	0.65
2 (rat)	0.0066	0.0066	114	26	0.77	0.78
3* (guinea-pig)	0.01	0.00125	160.6	105.9	0.34	0.32
	0.01	0.0025	160.6	79.6	0.50	0.49
	0.01	0.0075	160.6	45.9	0.71	0.74

* The O_2 uptakes recorded in this experiment refer to a period of 45 min.

This indicates that the affinity of benzedrine for amine oxidase is over four times that of tyramine for the enzyme.¹

l-Ephedrine and tyramine

It may be shown in a similar manner that *l*-ephedrine combines reversibly with amine oxidase. There is fair agreement between calculated and observed inhibitions of the O₂ uptakes when the dissociation constant of the ephedrine-amine oxidase complex is taken as 0.0057. On substituting this value together with $K_m=0.00178$ in equation (1), the results given in Table 17 are obtained.

Table 17

Inhibitions effected by *l*-ephedrine on O₂ uptakes by guinea-pig liver amine oxidase in presence of tyramine.

I (calc.) is given by equation (1). $K_m=0.00178$; $K_y=0.0057$.

M conc. tyramine	M conc. ephedrine	Rate of O ₂ uptake in absence of ephedrine. μ l. O ₂ in 30 min.	Rate of O ₂ uptake in presence of ephedrine. μ l. O ₂ in 30 min.	I fractional inhibition of O ₂ uptake due to ephedrine	I calc.
0.01	0.025	75.2	44.7	0.40	0.40
0.01	0.05	75.2	31.0	0.59	0.57
0.01	0.075	75.2	25.7	0.66	0.66
0.01	0.1	75.2	22.9	0.70	0.73
0.01	0.125	75.2	16.3	0.78	0.77

3:4-Methylenedioxyphenylisopropylamine and tyramine

3:4-Methylenedioxyphenylisopropylamine is a powerful inhibitor of amine oxidase, its effect being at least equal to that of benzedrine in this respect. Results illustrating this fact are shown in Table 18, where the dissociation constant for the 3:4-methylenedioxybenzedrine-amine oxidase complex is taken as 0.0004, that for tyramine-amine oxidase being 0.00178.

Table 18

M conc. tyramine	M conc. 3:4-methylenedioxy- phenylisopropylamine	I (obs.) fractional inhibition of O ₂ uptake (guinea-pig liver amine oxidase)	I (calc.)
0.01	0.00125	0.32	0.32
0.01	0.0025	0.48	0.49
0.01	0.005	0.61	0.65

3-Methoxy-4-hydroxyphenylisopropylamine and tyramine

The benzedrine derivative, 3-methoxy-4-hydroxyphenylisopropylamine, also combines reversibly with amine oxidase. Taking the dissociation constant of this amine with amine oxidase to be 0.005 there is good agreement between the observed and calculated inhibitions of O₂ uptake due to rat liver amine oxidase in presence of tyramine. Results are shown in Table 19.

It will be observed that the affinity of 3-methoxy-4-hydroxybenzedrine for amine oxidase is a little more than that of *l*-ephedrine and is much less than that of benzedrine.

¹ The assumption is made in these calculations that the velocity of O₂ uptake during the initial period of the experiment is due mainly to the oxidation of tyramine to the corresponding aldehyde. Investigations are now being carried out to determine how far the apparent equilibria constants of amines with amine oxidase are affected by secondary oxidations in the presence of the enzyme.

Table 19

 $K_m = 0.00178$; $K_y = 0.0050$.

<i>M</i> conc. tyramine	<i>M</i> conc. 3-methoxy:4- hydroxybenzadrine	<i>I</i> (obs.) fractional inhibition of amine oxidase	<i>I</i> (calc.)
0.01	0.0135	0.29	0.29
0.01	0.0217	0.38	0.39
0.01	0.0352	0.51	0.51

The following¹ is a table of the approximate dissociation constants of amine oxidase complexes of *l*-ephedrine, benzedrine and of benzedrine derivatives, taking that of the tyramine complex to be 0.00178:

Benzadrine	...	...	...	0.0004
3:4-Methylenedioxybenzadrine	...	...	...	0.0004
Tyramine	...	...	...	0.00178
3-Methoxy-4-hydroxybenzadrine	...	...	...	0.0050
<i>l</i> -Ephedrine	...	...	...	0.0057

The structures of these molecules are given below:

Benzedrine

3:4-Methylenedioxyphenylisopropylamine

3-Methoxy-4-hydroxyphenylisopropylamine

Ephedrine

Tyramine

¹ The magnitudes of these constants are subject to revision in view of the present uncertainty of the K_m of the tyramine complex. This is given, for example, as 0.0005 by Kohn [1937]. The relative values, however, will remain approximately those shown in the table.

It would be expected from these results that *l*-ephedrine and 3-methoxy-4-hydroxybenzadrine would be less effective than benzedrine in neutralizing the inhibitory effects of tyramine oxidation on brain respiration in presence of glucose or succinate and that 3:4-methylenedioxyphenylisopropylamine would be at least equal to benzedrine in this respect. These predictions may be verified as shown below.

EFFECTS OF EPHEDRINE AND 3:4-METHYLENEDIOXYISOPROPYLAMINE
ON TYRAMINE INHIBITIONS OF BRAIN OXIDATIONS

As shown in Table 20 *l*-ephedrine is much inferior to benzedrine in retarding the inhibition of succinic acid oxidation due to the exposure of brain tissue to tyramine. The stimulant action of ephedrine on such an oxidation after exposure of brain tissue to tyramine (0.0019 *M*) may be quite plainly observed with

Table 20

O₂ uptakes (μ l.) in 1 hr. at 37° by minced rat brain in presence of succinate, tyramine etc., as shown below. Succinate was added 2 hr. after the brain tissue had been in contact at 37° with the tyramine and with the mixtures of tyramine and ephedrine, and tyramine and benzedrine. Succinate = 0.013 *M*.

Succinate	Succinate + tyramine (0.0019 <i>M</i>)	Succinate + ephedrine (0.00083 <i>M</i>) + tyramine (0.0019 <i>M</i>)	Succinate + benzedrine (0.0004 <i>M</i>) + tyramine (0.0019 <i>M</i>)
215.1	112.2	124.9	174.1

relatively high concentrations of *l*-ephedrine, e.g. 0.004 *M*. For example under these conditions an O₂ uptake by brain tissue in presence of succinate (0.013 *M*) and tyramine of 123 μ l. was increased to 191.6 μ l. by the addition of *l*-ephedrine (0.0041 *M*). 3:4-Methylenedioxyphenylisopropylamine was equal in activity to benzedrine in neutralizing the inhibiting influence of tyramine oxidation on succinate breakdown. Its powerful activity can also be seen in its retarding action on the fall of respiration of brain tissue in presence of glucose when tyramine is present in the system. A typical result is given in Table 21.

Table 21

Conditions as in Table 3. Glucose = 0.01 *M*. Tyramine = 0.0019 *M*. Air. 37°.

	μ l. O ₂ uptake		
	1st hour	2nd hour	3rd hour
Glucose	276.1	209.3	182.3
Glucose + tyramine	209.9	98.4	37.1
Glucose + tyramine + 3:4-methylenedioxy-phenylisopropylamine (7.7×10^{-5} <i>M</i>)	215.7	112.9	50.7
Glucose + tyramine + 3:4-methylenedioxy-phenylisopropylamine (1.5×10^{-4} <i>M</i>)	211.9	122.7	68.7
Glucose + tyramine + 3:4-methylenedioxy-phenylisopropylamine (7.7×10^{-4} <i>M</i>)	178.5	117.5	93.5

A comparison of the effects of benzedrine, *l*-ephedrine and 3-methoxy-4-hydroxybenzadrine in retarding the inhibitory action of tyramine on the respiration of brain cortex slices in presence of glucose is given in Table 22. It will be noted that both *l*-ephedrine and 3-methoxy-4-hydroxybenzadrine are less effective than benzedrine in effecting an increased rate of O₂ uptake.

Table 22

Rates of O_2 uptake by rat brain cortex slices in glucose (0.01 *M*)-phosphate (0.03 *M*; pH 7.4)-Locke media in the presence of tyramine and mixtures of tyramine and benzedrine, *l*-ephedrine or 3-methoxy-4-hydroxybenzedrine.

Amines present	Q_{O_2}		
	1st hour	2nd hour	3rd hour
Tyramine (1.9×10^{-3} <i>M</i>)	12.3	7.7	4.6
Tyramine (1.9×10^{-3} <i>M</i>) + benzedrine (4.3×10^{-4} <i>M</i>)	12.5	11.0	9.9
Tyramine (1.9×10^{-3} <i>M</i>) + ephedrine (1.5×10^{-3} <i>M</i>)	12.0	9.6	7.0
Tyramine (1.9×10^{-3} <i>M</i>) + 3-methoxy-4-hydroxybenzedrine (1.4×10^{-3} <i>M</i>)	12.1	10.4	8.3

THE ACTION OF BENZEDRINE ON THE INHIBITION OF BRAIN RESPIRATION CAUSED BY LUMINAL, CHLORETONE AND BULBOCAPNINE

Benzedrine has apparently no neutralizing action, at the concentrations investigated, on the inhibiting influence of the narcotics luminal (phenylethyl-barbituric acid) and chloretone, and of bulboCAPNINE on the respiration of brain tissue in presence of glucose. This lack of activity on the part of benzedrine would be expected if its influence is mainly related to its affinity for amine oxidase. Illustrative results are shown in Table 23.

Table 23

O_2 uptakes by minced rat brain tissue in glucose (0.01 *M*)-phosphate-saline at 37° in the presence of luminal, chloretone and bulboCAPNINE with and without benzedrine.

Substrates		$\mu l. O_2$		
		1st hour	2nd hour	3rd hour
Exp. 1	Glucose	307.9	248.5	209.6
	Glucose + luminal (0.08 %)	155.7	104.9	83.3
	Glucose + luminal + benzedrine (7.1×10^{-4} <i>M</i>)	132.4	90.8	78.2
Exp. 2	Glucose	279.1	223.5	187.5
	Glucose + chloretone (0.033 %)	163.8	131.6	111.1
	Glucose + chloretone + benzedrine (5.7×10^{-4} <i>M</i>)	144.4	107.8	97.9
Exp. 3	Glucose	288.1	239.5	217.1
	Glucose + bulboCAPNINE (0.01 %)	159.3	146.6	139.3
	Glucose + bulboCAPNINE + benzedrine (4.3×10^{-4} <i>M</i>)	171.5	154.1	143.0

POSSIBLE CLINICAL SIGNIFICANCE OF THE ACTION OF BENZEDRINE ON BRAIN METABOLISM

It is now well known that benzedrine administration has very considerable value in cases of narcolepsy and in conditions where a stimulation of the central nervous system is required. It was indeed the clinical value of benzedrine administration which led us to the investigation which has just been described.

The effects of benzedrine *in vitro* in partially neutralizing the inhibition of glucose oxidation by brain due to the presence of tyramine and other amines take place at concentrations which are not markedly greater than those which have pharmacological effects *in vivo*. Moreover, since it has been shown that competition between benzedrine and other amines takes place at the amine

oxidase according to the laws of mass action it follows that the influence of benzedrine may be exerted in the body at much lower concentrations than those which we have investigated. It seems not unreasonable to suggest that the action of benzedrine *in vivo* is linked with its ability to compete with amines giving rise in the body to toxic substances; the lower the concentration of such amines the lower the quantity of benzedrine which will be required to compete successfully with them.

Ephedrine is known clinically to be less powerful than benzedrine as a stimulant of the central nervous system and it has been shown that such a difference between their effects on brain respiration occurs also *in vitro*. According to Gunn *et al.* [1939] 3:4-methylenedioxyphenylisopropylamine is a somewhat more powerful stimulant of the central nervous system than benzedrine, and it has just been seen that the former amine has at least the same activity as benzedrine in neutralizing the fall of brain respiration in a glucose medium due to tyramine.

Again, according to Gunn *et al.* [1939], 3-methoxy-4-hydroxyphenylisopropylamine is a less powerful stimulant of the nervous system than benzedrine. This result is consistent with the relatively poor activity of the former amine in neutralizing the inhibitory action of tyramine on brain oxidations. Such results are suggestive of a possible connexion between amine metabolism in the brain and the development of the clinical condition (fatigue) known to be relieved by benzedrine administration.

It will be of much interest to investigate cases of narcolepsy with a view to observing whether aldehydes, or similar toxic bodies, are elaborated in this disorder, and if so, whether their rates of formation are retarded by benzedrine administration.

SUMMARY

1. When brain respiration in a glucose medium takes place in the presence of tyramine, β -indolethylamine or isoamylamine a fall in respiration occurs. This fall in respiration is neutralized by the presence in the system of benzedrine (β -phenylisopropylamine sulphate). This phenomenon applies to the respiration of intact brain cortex slices or of minced brain tissue.

2. The same phenomenon obtains in the case of tyramine when glucose is replaced by sodium succinate.

3. The fall in brain respiration due to the presence of tyramine, etc., is not due wholly to the amine itself but to a product of oxidation of the amine, i.e. the corresponding aldehyde, or a product formed by further metabolism of the aldehyde.

4. Succinate protects succinic dehydrogenase from the toxic influence of the oxidation product of tyramine (probably *p*-hydroxyphenylacetaldehyde).

5. Benzedrine owes its stimulating influence to its ability to compete reversibly with amines for the amine oxidase of brain and of other organs, thereby reducing the rate of formation of inhibitory aldehyde.

6. *l*-Ephedrine, 3:4-methylenedioxyphenylisopropylamine and 3-methoxy-4-hydroxyphenylisopropylamine also compete reversibly with tyramine for amine oxidase and they all show effects similar to benzedrine in their retarding actions on the fall of respiration of brain tissue in glucose or succinate media due to the presence of tyramine. Ephedrine and 3-methoxy-4-hydroxyphenylisopropylamine are much less active than benzedrine, and 3:4-methylenedioxyphenylisopropylamine has about the same activity as benzedrine. The equilibrium constants of these amines with amine oxidase have been determined.

7. Aldehydes such as *isovaleric* aldehyde, and *p*-hydroxybenzaldehyde are inhibitory to the respiratory processes of brain and their inhibitory effects are not neutralized by the addition of benzedrine.

8. Benzedrine has no retarding action on the fall of respiration of brain tissue in a glucose medium due to the presence of luminal, chloretone or bulbo-capsine.

9. The possible clinical significance of these results is discussed. It is pointed out that the relative stimulating actions of ephedrine, benzedrine and benzedrine derivatives on brain respiration in the presence of tyramine bear a parallelism to the relative stimulating actions of these substances on the central nervous system as observed *in vivo*.

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