Studies of Hepatic, Brain Monoamine Oxidase and Brain Serotonin in Rats

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The effects of 7-ethyl-8-methylflavin (7-Et) and 7-methyl-8-ethyl-flavin (8-Et) on rat hepatic monoamine oxidase (MAO), brain MAO activity and 5-hydroxytryptamine (5-HT or serotonin) in rat brain were investigated. In the study of hepatic MAO activity, kynurenine a nonphysiological substrate for both A and B type MAO, was used for a spectrophotometric method, and [14C]-labeled amines were also used for a radiometric procedure for camparison with MAO activity determined by the spectrophotometric method. The rate of change in MAO activity of hepatic mitochondria from rats receiving Rb-def and 7-Et and 8-Et flavin showed the activity was severely reduced during 8 weeks. Rapid reduction of enzyme activity (50% in def-group, 35% in 7-Et group and 8% 8-Et flavin group) was observed at the end of 2 weeks. The enzyme activity lasted with slow decrement of enzyme level from 4 weeks to the end of 8 weeks as low as 16% in def, 18% in 7-Et and 3% in 8-Et flavin group. The trend of decrement of MAO activity when kynurenine was used as a substrate appears to be similar with the small variation of MAO activity when [14C]-labelled tyramine, dopamine, serotonin and tryptamine respectively were used as substrate. The rate of decay of brain mitochondrial MAO activity in rats receiving each respective flavin was not rapid and severely depressed as the MAO activity we have found in liver mitochondrial MAO of rats during the 8 week experimental time, but a similar tendency of decay of MAO in each group was observed. The potent inhibitory effect of 8-Et on brain MAO was confirmed by the study of the simultaneous measurement of MAO activity in each experimental group. When the reduction of brain MAO activity in rats receiving 8-Et after 6 weeks was approximately 80% of normal and in the same rats the concentration of brain 5-HT showed a 60% increment of that of the normal rats. During the experimental period there is no absolute parallelism between the MAO inhibition and 5-HT increase. However when the reduction of MAO activity reached 80% of normal value, the concentration of 5-HT increased dramatically as much as 60% of normal value. The results so far suggest clearly that 8-Et produces a much more potent inhibitory effect on the hepatic MAO as well as brain MAO in rats. Therefore our present and previous results suggest that 7-Et and 8-Et flavin should bind itself to hepatic, brain

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MAO apoenzyme in the condition of total absence of riboflavin in these animals, and the holoenzyme is catalytically inactive.

**Key Words:** Hepatic MAO, Brain MAO, Brain Serotonin, flavin coenzyme

Among the mammalian mitochondrial enzymes which are known to be covalently bound to flavin adenine dinucleotide (FAD), succinic dehydrogenase (SDH) (EC1.3.99.1), monoamine oxidase (MAO) (EC1.4.3.4) and sarcosine dehydrogenase (EC 1.5.3.1) are the known enzymes (Lambooy and Shaffner, 1977). In all of these enzymes, FAD is covalently attached to the enzyme protein through the 8-α methyl group of riboflavin (Walker et al., 1971). Monoamine oxidase is a mitochondrial outer mem-

![Fig. 1. Structure of the flavin peptide from mono-

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![Fig. 2. Basic flavin structure: Flavin R7 R8 Trivial Name
A CH3 - CH3 - Riboflavin
B C2H5 - CH3 - 7-Ethyl-8-methylflavin or 7-Et
C CH3- C2H5 - 7-Methyl-8-ethylflavin or 8-Et

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these two flavins could replace riboflavin as the coenzyme in liver SDH but also in all flavoprotein enzymes which are required for metabolism in these rats, and illustrated that the two homologs must be covalently linked to the enzymes, forming holoenzyme analogs.

The bond formation of 7-Et flavin may produce the normal structure of the covalent bond at the 8α-carbon as with FAD-containing SDH and 8-Et produces an abnormal structure at the pseudo-FAD junction in which the covalent bond at 8α-carbon position would need to be formed by using the α carbon of an 8 ethyl group (Fig. 3). A similar covalent bond is likely to be formed to produce a functional MAO. If the S of a cysteine residue replaces the N-3 of histidine residue, the two homologs are to function as coenzymes for MAO. This study was undertaken hoping to find how these flavin homologs influence the rat hepatic MAO activity (study I) as well as the brain MAO activity (study II) and whether the brain MAO activity influences the brain serotonin level (study III) during the period when these homologues were replacing FAD as the coenzyme. In the study of hepatic MAO activity (study I), kynuramine, a nonphysiological substrate for both A and B type MAO, was used for a spectrophotometric method, (Weissbach et al., 1960) and [14C]-labeled amines were also used for a radiometric procedure (Jain, et al., 1973) for comparison of MAO activity determined by the former spectrophotometric method.

In the study of brain MAO activity (study II), enzyme activities were determined using kynuramine only as a substrate for a spectrophotometric method. In study III, the 5-HT level of the brain was measured by the fluorometric method and discussed whether this criterion was related to the loss of brain MAO activity resulting from the replacement of the riboflavin component of the enzyme by either of the two homologs.

**MATERIALS AND METHODS**

**Chemicals and Instruments**

**Study I and II:** Kynuramine dihydrobromide, 1-[14C]-tyramine, 1-[14C]-dopamine, 2-[14C]-serotonin, 2-[14C]-tryptamine, O-phthaldialdehyde(Opt), 5-hydroxytryptamine (serotonin) creatine sulfate, L-cysteine and L-dopa were purchased from the Sigma Chem. Co., and riboflavin from Eastman Org. Chem. Co. U.S.A. 7-Et and 8-Et were coordinately provided by Dr. J.P. Lambooy (Dept. of Biochem., Univ. of Maryland, Baltimore, Maryland 21201, U.S.A.). Liquid scintillation counter (Packard Tri-Carb 300) was used for the Radio-metric assay of hepatic MAO activity and the Gilford spectro-
photometer, Model 240 (Recorder 242) was used for the spectrophotometric assay of hepatic MAO and brain MAO activity. A Turner fluorometer Model III was used for the assay of brain 5-HT. Mitochondrial protein was determined by the Lowry method using the Shimadzu recording spectrophotometer (UV-240).

Animals and Diets

Study I: For the measurement of the hepatic MAO activity, one hundred and seven male rats weighing between 60 and 70 gm were used and they were divided into 4 groups which consisted of a riboflavin group (group Rb), a riboflavin deficiency group (group Rb-def), a 7-ethyl-8-methylflavin group (group 7-Et) and a 7-methyl-8-ethylflavin group (group 8-Et). The group Rb-def was fed, ad libitum, a basic diet described by Lambooy, Smith and Kim (1971). The group Rb, group 7-Et and group 8-Et, each was fed a basic diet supplemented by 20mg/Kg of riboflavin, 20.8mg/Kg of 7-Et and 20.8mg/Kg of 8-Et respectively. The rats were fed their respective diet for 8 weeks. The composition of the basic diet is shown in Table 1.

| Table 1. The composition of the basic diet |
|-------------------------------|---------|
| Vitamin free sucrose          | 680 gm  |
| Vitamin free casein            | 180 gm  |
| Corn oil                       | 100 gm  |
| U.S.P. Salt mixture            | 40 gm   |
| **Total**                      | **1 Kg**|

The basic diet, 1Kg, contains vitamin mixture 2ml cod liver oil 20gm, choline chloride 1.5 gm, pteroyl glutamic acid 1.8mg, biotin 0.6mg, thiamine-HCl 20mg, pyridoxine-HCl 20mg, menadione 50mg, calcium pantothenate 60mg, myoinositol 100mg, and cyanocobalamin 0.04mg.

At two week intervals, 3 rats from each group were sacrificed for the measurement of hepatic MAO activity. Equal portions of the liver of the three rats from each group were pooled by group and triplicate assays performed on each such pooled sample. On days 57, 59 and 60, four additional rats from each group were sacrificed for MAO assays using 1-[14C]-tyramine, 1-[14C]-dopamine, 2-[14C]-serotonin and 2-[14C]-tryptamine, respectively, as substrates.

Study II and III: One hundred and twenty eight male rats weighing between 60 and 70gm were used for study II and III. Grouping, feeding and diet composition for the animals were essentially the same as in the study I. The group of rats were fed their corresponding respective diet for 6 weeks. At two week intervals, eight rats from each group were sacrificed for assay of brain MAO activity and 5-HT level. Brains of four rats from each group were pooled for the determination of brain MAO and triplicate assays performed on each such pooled sample. The brains of the remaining four rats were pooled for the assay of 5-HT and triplicate assays on each pooled sample were made.

Preparation of Rat Liver Mitochondria (study I)

The rats lightly anesthetized with ether were decapitated and the livers were promptly removed and placed in cold 0.25M sucrose. Approximately 0.7g portions was taken from the right median lobe of each of the three rats from each group and they were pooled and 10% homogenate in cold 0.25M sucrose was prepared. The homogenate was filtered through four layers of No. 80 gauge cheese cloth. The filtrate was centrifuged at 600g for 10 min, the supernatant suspension was saved and the pellet resuspended in 18 ml of 0.25M sucrose. This pellet suspension was centrifuged again as above and the combined supernatant suspensions centrifuged at 9000g for 10 min. After discarding the supernatant, the pellet was suspended in 20ml of cold 0.25M sucrose and the suspension centrifuged at 9000g for 10 min. The pellet obtained from the last centrifugation was resuspended in 10 ml of 0.25M sucrose. This mitochondrial
preparation was used undiluted for the MAO assay.

**Preparation of Rat Brain Mitochondria (Study II)**

The rats, lightly anesthetized with ether were killed by decapitation and the brain removed rapidly and washed with ice-cold Medium A (Med A) (0.4M sucrose containing 1x10^-3 M EDTA adjusted to pH 7.4 with Tris 0.1M). The four brains were pooled and homogenized in four volumes of this medium per gm of tissue. Med A was added so that the final homogenate was 1:2 (three times dilution). The suspension was subjected to differential centrifugation by using Ficoll according to Achee et al. (Achee et al., 1974).

**Assay of hepatic MAO (Study I)**

The undiluted liver mitochondrial preparation was used for MAO assay. MAO activities were determined using either kynuramine as a substrate for a spectrophotometric assay (Weissbach, et al., 1960) or [14C]-labeled amines for a radiometric procedure (Jain et al., 1973). Three determinations were made in all cases. MAO activities determined by using kynuramine were expressed as n moles substrate oxidized/min/mg protein. Enzyme activities using [14C]-labeled amines as substrates were expressed as mU/mg, where one mU equals 1 n mole/min. Proteins of the mitochondrial preparation were measured by the procedure of Lowry et al., (Lowry et al., 1951) using bovine serum albumin as a standard.

**Assay of Brain MAO Activity (Study II)**

For brain MAO assay, the washed mitochondrial fraction was dissolved in 1.5-2.0 ml of Med A.

MAO activities were measured using kynuramine as a substrate in the same way as described in study I. All assays were performed three times. Protein concentrations of the mitochondrial preparations were determined as described in study I (Lowry et al., 1951).

**Extraction and Assay of Brain 5-HT (Study III)**

The procedure of extraction and assay of brain 5-HT was based on the method of Curzon and Green (Curzon and Green, 1971).

The whole brain of a rat was homogenized in 10 volumes of cold acidified butanol (0.85ml of concentrated HCl, was added to 1 liter of n-butanol). After centrifugation at 3000 rpm for 5 min, 10 ml of the supernatant with 20 ml n-heptane and 1.6 ml 0.1 N-HCl containing 0.1% cysteine was shaken vigorously for 20 min in a 50 ml glass stoppered tube. The phases were separated by Sovrall centrifugation at 3000 rpm for 5 min and the organic phase was aspirated off. 0.5ml of aqueous phase was mixed well with 3ml of 0.004% o-phthalaldehyde (OPT) in 10 N-HCl and heated in a boiling water bath for 15 min. After cooling in ice water, the fluorescence was measured on a Turner fluorometer Model III using an activation filter of 365 nm and an emission wavelength of 485 nm. 5-HT level was expressed as ug per gm brain tissue in wet weight. A standard of 5-HT was prepared as a 60 ug/ml solution in deionized water, diluted for use as the concentration of 0.3 ug/ml, 0.6 ug/ml, 1.2 ug/ml with 0.1 N HCl containing 0.1% cysteine. The standard for 5-HT was carried out in parallel with the brain samples.

The fluorescence and the amount of the standard 5-HT was linear over the range we used and similar curves were obtained in each assay. The reagent blank was prepared by mixing 3 ml of OPT solution with 0.5ml 0.1 N HCl containing 0.1% cysteine only. The brain tissue blanks were prepared by mixing 0.5 ml of the aqueous phase with 3 ml 10 N-HCl only.

**RESULTS**

The graph in Fig. 4 shows the rate of change
of the hepatic mitochondrial MAO activity (study I) which was plotted from the data of kynuramine use only as a substrate shown in Table II. The rate of change in MAO activity of the hepatic mitochondria from rats receiving Rb, Rb-def and 7-Et and 8-Et flavin showed that the activity was severely reduced during the 8 weeks.

Rapid reduction of enzyme activity (50% in the def-group, 35% in 7-Et group and 8% in 8-Et flavin group) was observed at the end of 2 weeks. The enzyme activity showed slow decrement of enzyme level from 4 weeks to the end of 8 weeks as low as 16% in the def group, 18% in 7-Et and 3% 8-Et flavin group. The values for MAO activity are expressed as percentage of the activity found at the same time interval for the animals receiving a Rb-containing diet.

The results shown in Table II include the results of hepatic MAO activities using [14C]-labeled amines as substrates. Enzyme activity of MAO when kynuramine which is known as a nonphysiological substrate for both type of MAO A and B, is used as the substrate, was compared with the enzyme activity using a physiological substrate, serotonin, dopamine, for MAO A and tyramine and tryptamine, for both A and B type MAO. Radiometric assay of MAO activity was performed. As indicated in Table II, the decrease of MAO activity when kynuramine was used as a substrate appears to be similar with little variation, to the MAO activity when tryamine, dopamine, serotonin and tryptamine, were used as substrate, respectively.

On the basis of these results brain MAO
### Table 2. Hepatic monoamine oxidase activity from rats receiving riboflavin (Rb), no riboflavin (def), 7-ethyl-8-methylflavin (7-Et) or 7-methyl-8-ethylflavin (8-Et). (study 1).

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<th>28</th>
<th>42</th>
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<tr>
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<td>100c</td>
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</table>

a. All assay values determined as n moles substrate oxidized/min/mg protein. The normal values for the enzyme activities in group Rb were: Time "0" 4.2±0.12; day 14, 4.4±0.14; day 28, 4.0±0.11; day 42, 4.2±0.20, day 56, 4.2±0.20. All values in the table are expressed as percentage of the value for Group Rb done in parallel with the experimental Groups.

b. The assay values for the enzyme activity for group def were: Time "0" 4.2±0.12; day 14, 1.7±0.12; day 28, 1.1±0.02; day 42, 0.67±0.02; day 56, 0.66±0.04. The "P" value for the difference between group Rb and group Def for each time interval is less than 0.0001.

c. The assay values for the enzyme activity in Group Rb was 8.8±0.36 mU/mg, where one mU=1 nmole/min. The other assays by the radiometric procedure were of comparable precision.

Activity was determined using only kynuramine as a substrate. The rate of change in MAO activity of brain mitochondria from rats receiving Rb, def, 7-Et and 8-Et flavin when kynuramine is used as a substrate was shown in Table III and Fig. 5. The rate of decay of brain mitochondrial MAO activity in rats receiving each respective flavin was not rapid and severely depressed as the MAO activity we have found in liver mitochondrial MAO of rats during the 8 weeks experimental time, but a similar tendency of decay of MAO in each group was observed. The change of 5-HT level in the rat brain in each group was shown in Fig. 6. The 5-HT level was expressed as a percentage of the value for Rb group done at the same time interval as the experimental group. As shown in Fig. 6, a significant increase of the 5-HT level in rat brain in 8-Et and 7-Et flavin group was observed as high as 60% in 8-Et and 25% in 7-Et flavin.
Table 3. Brain monoamine oxidase activity from rats receiving riboflavin (Rb), no riboflavin (def), 7-ethyl-8-methylflavin (7-Et) or 7-methyl-8-ethylflavin (8-Et). (Study II)

<table>
<thead>
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<th>Group</th>
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<th>14</th>
<th>28</th>
<th>42</th>
</tr>
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<tbody>
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<td>Riboflavin (Rb)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Riboflavin deficient (def)</td>
<td>100</td>
<td>87</td>
<td>75</td>
<td>65</td>
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<tr>
<td>7-ethyl-8-methylflavin (7-Et)</td>
<td>100</td>
<td>105</td>
<td>69</td>
<td>70</td>
</tr>
<tr>
<td>7-methyl-8-ethylflavin (8-Et)</td>
<td>100</td>
<td>57</td>
<td>47</td>
<td>20</td>
</tr>
</tbody>
</table>

a. All assay values determined as nmoles substrate oxidized/min/mg protein. Kynuramine was the substrate. The values for the enzyme activities in Group Rb were: time "0" 6.20±0.41; day 14, 6.10±0.33; day 28, 6.80±0.29; day 42, 5.92±0.53. All value for Rb done in parallel with the experimental groups.

b. The assay values for the enzyme activity for Group def were: Time "0", 6.50±0.51; day 14, 5.30±0.30; day 28, 5.1±0.41; day 42, 3.85±0.32. The "P" value for the difference between Group Rb and Group def for day 28 and 42 is less than 0.05.

Fig. 6. The change in brain 5-HT level in rats receiving riboflavin (Rb), no riboflavin (def), 7-ethyl-8-methylflavin (7-Et) or 7-methyl-8-ethylflavin (8-Et). Four rats from each group were pooled by group and triplicate assays were performed on each such pooled sample. 5-HT concentrations are expressed as percent change of 5-HT level for group Rb found at the same time interval as the experimental groups. Each point represents mean±S.D.

Fig. 7. Time course and effects of 7-methyl-8-ethylflavin (8-Et) and 7-ethyl-8-methylflavin (7-Et) on the change in rat brain MAO activity and 5-HT concentration. MAO activity (○—○), 5-HT concentration (●—●). Each point represents mean±S.E.

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a result of decreased MAO activity. Fig. 7 illustrates the evidence that the accumulation of 5-HT is dependent on MAO activity in brain cells and indicates that the MAO activity in brain cells is one of the unique factors which induces the accumulation of 5-HT.

However, the accumulation of 5-HT in the brain cells of the deficient group was not observed in spite of decreased MAO activity in the brain, indicating that synthesis of 5-HT might be reduced in the brain of rats in the def group.

DISCUSSION

In study I, it is shown that the rate of change in hepatic MAO activity of rats receiving 7-Et or 8-Et produced a very different pattern from that of hepatic SDH activity in the same experimental conditions reported by earlier workers (Kim and Lambooy 1967, Dombrowski and Lambooy, 1973). Early workers reported that when 7-Et or 8-Et was the only metabolically active flavin utilized in the rat, the reduction of hepatic SDH activity was about 90% and 50% of control rats respectively. It is difficult to understand the mechanism involved in such inhibitory effects of these homolog compounds on hepatic SDH activity. However, it was reported that 7-Et possesses 8α methyl group as in riboflavin, therefore the bond between 8α methyl of 7-Et and the N-3 of the imidazole ring of a histidine residue of SDH apoenzyme may be a normal covalent structure as in SDH-FAD holoenzyme. Whereas the homolog 8-Et which possesses 8 ethyl groups would use the 8α carbon position of an ethyl group to form the covalent bond with the N-3 of a histidine residue which must be considered abnormal in relation to the normal FAD bond (Dombrowski and Lambooy, 1973, Lambooy and Shaf-

Our results on the change in the activity of hepatic MAO which is a similar kind of flavo-protein as hepatic SDH, showed that rats receiving 8-Et produced a much lower activity than rats receiving 7-Et throughout the experimental period and after 8 weeks, almost no activity was detected in this group (Fig. 4 and Table II). The potent inhibitory effect of 8-Et on hepatic MAO activity in comparison with 7-Et was much more dramatic when kynuramine or tryptamine (or tryamine), a common substrate for both types of MAO A and B (Houslay and Tipton, 1974) was used than when dopamine or serotonin was used as the substrate. It is known that in the mammalian brain dopamine (Waldmeier, et-al., 1976) and serotonin (Johnston, 1968, Goridis and Neff, 1971a and Neff and Goridis, 1972) are important biogenic amine neurotransmitters which are metabolized specifically by MAO A. There is some evidence of species variation for dopamine: dopamine has been reported to be a substrate for type A MAO in rats (Waldmeier, et al., 1976) and for type B MAO in man (Glover, et al., 1971). Therefore this result gives some indirect evidence to suggest that there may exist some differences in the characteristic of hepatic MAO and brain MAO in rats.

However continuation of our study on the effects of these homologs on the activity of brain MAO produced a similar result that rats receiving 8-Et as the only available flavin source showed the least enzyme activity in comparison with other groups. After only six weeks (Fig. 5 and Table III) the activity of the brain MAO in the 8-Et group was reduced to 80% of normal. Recently Lambooy reported that the 80% reduction of brain MAO activity in rats receiving 8-Et homolog was already present in four weeks of treatment (Dix and Lambooy, 1981). Other workers reported that the half life of a rat brain
MAO is 10 to 13 days (Goridis and Neff, 1971b, Planz et al., 1972, Luine and McEwen, 1977, Nelson et al., 1979). Considering the results of these reports, the experimental period of 6 weeks in our study appears to be a sufficiently long time for replacing riboflavin by 8-Et during the period of the biosynthesis of MAO holoenzyme in a rat brain. At the present time we can not explain the exact nature of replacement, but as described earlier, the homolog, 8-Et would form an abnormal covalent bond structure at pseudo-FAD junction whereas 7-Et produces a normal covalent bond structure as SDH-holoenzyme does.

It is also very difficult to understand how these homologs produce such contrasting inhibitory effects on MAO. The only known structural differences between SDH and MAO holoenzyme reside in the fact that SDH is covalently bound to FAD from N-3 of the imidazole ring of a histidine residue to the 8α methyl group of riboflavin (Singer and Kenney, 1974) and MAO-FAD involves a bond between the-S-atom of a cysteine residue in the enzyme and the 8α methyl group of riboflavin (Walker, et al., 1971).

The potent inhibitory effect of 8-Et on brain MAO was confirmed by the study of the simultaneous measurement of the concentration of brain 5-HT with the measurement of MAO activity (Fig. 6) in each experimental group. When the reduction of brain MAO activity in rats receiving 8-Et after 6 weeks was approximately 80% of normal and in the same rats the concentration of brain 5-HT shows a 60% increment of that of the normal rats. During the experimental period there is no absolute parallelism between the MAO inhibition and 5-HT increase, however when the reduction of MAO activity reached 80% of normal value, the concentration of 5-HT increased dramatically to as much as 60% of normal value. This result is comparable with the report of other workers that the enzyme has to be inhibited at least to 85% before the 5-HT content of the brain rises when the hydrazine series compound, iproniazid, was treated in rats as an inhibitor of MAO (Pletcher et al., 1965 and Pletscher, 1966). The increase of 5-HT in rats receiving 8-Et as the only flavin source is considered to result from the inhibition of MAO A in the brain while the rat brain has both MAO A and B (Edwards and Malsbury, 1978 and Tipton et al., 1976). This seems logical because it is well known that serotonin and norepinephrine in mammalian brain can be metabolized only by MAO A (Johnston, 1968, Goridis and Neff, 1971a). It is also well reported that the rats in which the activity of MAO B was completely blocked by administration of MAO B specific inhibitor showed no change in the concentration of 5-HT or norepinephrine in the brain (Neff and Yang, 1973). The results so far suggest strongly that 8-Et produces a much more potent inhibitory effect on the hepatic MAO as well as the brain MAO in rats. It is very difficult to explain about the exact mechanism involved in the greater inhibitory effect of 8-Et on the hepatic MAO as well as brain MAO in comparison with that of 7-Et. However the possible answer for this question may be that either 8-Et fails to bind itself to MAO as a coenzyme or if it binds, the holoenzyme becomes catalytically inactive. It is suggested that the latter would be more provable because of the following reasons. Reports by early workers describe that when the rat was totally deprived of riboflavin for 8 weeks and thus no detection of riboflavin was seen in the tissue of the rat, these two homologs of riboflavin reduced the activity of SDH in the liver, kidney and heart although the degree of reduction was different. However these animals showed no differences with the riboflavin fed rats with respect to the efficiency of food
utilization, physical appearance and growth response. (Lambooy, 1961, Kim and Lambooy, 1967, Lambooy, et al., 1971, Dombrowski and Lambooy, 1973). This experiment was repeated in our study too. This observation was interpreted in such a way that these homologs function as a coenzyme of not only SDH but of all other flavoprotein enzymes in these rats. Recently, Lambooy and Kim (Lambooy and Kim, 1983) reported that $[^{14}C]$-(2)-7-ethyl-8-methyl-10-(1-D-ribityl) isalloxazine bonds to the SDH and MAO apoenzymes by the use of $[^{14}C]$-(2)-riboflavin and $[^{14}C]$-(2)-7-ethyl-8-methyl-10-(1'-D-ribityl) isalloxazine. Therefore it is believed that 7-Et and 8-Et flavin should bind itself to MAO in the condition of total absence of riboflavin in these animals.

REFERENCES


Houslay MD, Tipron KF: A kinetic evaluation of monoamine oxidase activity in rat liver mitochondrial outer membranes. Biochem J 139:645, 1974


Lambooy JP: Growth promoting properties of 6-ethyl-7-methyl-9-(1'-D-ribityl) isalloxazine and 6-methyl-7-ethyl-9-(1'-D-ribityl) isalloxazine. J Nutr 75:116, 1961


Neff NH, Goridis C: Neuronal monoamine oxidase:
specific enzyme types and their rates of formation.


Planz G, Quring K, Palm D: Turnover rates of monoamine oxidase: Recovery of the irreversibly inhibited enzyme activity and the influence of isoprenaline. Life Sci 11:147, 1972


Waldmeier PC, Delini-Stula A, Maitre L: Preferential deamination of dopamine by an A type monoamine oxidase in Rat brain. Naunyn-Schmiedeberg's Arch Pharmac 292:9, 1976