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ONTOGENESIS OF ENZYME SYSTEMS DEAMINATING DIFFERENT MONOAMINES

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1 A detailed investigation into the postnatal development of the activity of the enzyme monoamine oxidase (MAO) in the rat and domestic pig was carried out.

2 MAO activity was measured in littermate male rats aged between 3 and 122 days belonging to six breeding colonies. The tissues studied were three brain regions in which monoamines may play a role in neuronal transmission (septum, hypothalamus, corpus striatum) and, for comparison, in the cerebellum. Liver, heart and adrenal glands were the peripheral organs studied. The following substrates were used to measure MAO activity in each tissue homogenate: kynuramine, tyramine, dopamine, tryptamine and 5-hydroxytryptamine (5-HT).

3 MAO activity towards kynuramine, tyramine and dopamine increased after birth in all brain regions and also in the liver, to reach maximal values between days 40 and 80. In the heart and the adrenal glands enzyme activity remained low up to 30–40 days and then increased steeply. This was the case in all litters examined.

4 All tissues deaminated more tyramine than dopamine. In the liver, the ratio of the quantities of tyramine deaminated/dopamine deaminated was approx. 2 at all ages. In the homogenates of whole brains (including or excluding the hypothalamus and striatum) this ratio was also 2 at all ages. In contrast in the isolated striatum and hypothalamus it was first much higher and reached a value of 2 only at an age of about 20 days. This may indicate an independent development of a dopamine and a tyramine deaminating enzyme system in discrete brain regions. It was suggested, that the low ability to deaminate dopamine in discrete brain regions may be due to the local presence of an enzyme inhibitor which becomes too diluted to be active in homogenates of whole brain.

5 Deamination of tryptamine in the striatum decreased between day 5 and 20 in 3 out of 4 colonies tested. There was a large fall in the deamination of 5-HT in all tissues of one group of rats, but in another 4 groups the tissues of the 5 day old rats deaminated smaller amounts of 5-HT than those of the older rats.

6 Purified hypothalamic mitochondria from 40 day old rats deaminated more tyramine and dopamine but not tryptamine per mg protein than those from 5 day old rats.

7 In the domestic pig there was a significant rise in the values in hippocampal MAO activity towards dopamine and tyramine from the foetus (55 day gestation) to the 1 week old piglet. A further steady rise up to week 6 was indicated, but this rise was not statistically significant. The difference between rat and pig probably reflected the much higher degree of maturity of the latter at birth.

8 In the hippocampus of the pig the ratio between the amount of tyramine deaminated/dopamine deaminated decreased from > 10 (foetus) to 4.8 in the 6 week old pig and 2 in the adult.

Introduction

Changes in the activity of the enzyme monoamine oxidase (MAO; E.C. 1.4.3.4.) during the postnatal development of rats have been reported on several occasions. These studies led to the general conclusion that the enzyme's activity increases with age (see e.g. Novick, 1961; Karki, Kuntzman & Brodie, 1962; Bennett & Giarman, 1965; Vaccari, Maura, Marchi & Cugurra, 1972; Youdim, Holzbauer & Woods, 1974). In most of these previous studies only a single substrate has been used or only one tissue has been investigated.

In the present work a more detailed investigation has been conducted. The enzyme activity was measured in four discrete brain regions and in three peripheral organs, five different substrates being used for most samples. Litter-mate male rats from five

breeding colonies aged between 5 and 80 days were used. The work was designed particularly to investigate whether the postulated multiple forms of MAO develop at the same rate during maturation, and whether any differences exist in the development of deamination of different monoamines by various organs. Some of the results have been presented to the British Pharmacological Society (Blatchford, Holzbauer & Youdim, 1975).

Methods

Rats

The experiments were carried out on litter-mate male rats from different strains and breeding colonies. All rats were weaned at day 21 and fed on a diet of Oxoid 41B (water *ad lib*). Each experimental group consisted of the male rats of three to six litters and each age group (five to six rats) included at least one rat from each litter. The average body weights for the different groups at various ages are listed in Table 1.

The rats were killed by rapid decapitation. The tissues tested for MAO activity were three brain regions rich in monoamines (hypothalamus, corpus striatum and septum) and for comparison either the posterior telencephalon or the cerebellum. The peripheral organs examined were the heart, liver and adrenal glands. The tissues were dissected out as quickly as possible and stored at −18°C. To prevent desiccation a small quantity of buffer solution was added. On the day of the enzyme assay they were homogenized in ice cold 0.1 M sodium phosphate buffer (pH 7.4) with a Polytron homogenizer. The volume of buffer used was 1 ml for the septum, striatum, hypothalamus and adrenal glands; for the cerebellum, the atria of the heart and the piece of liver (dissected from the frontal lobe) 2–10 ml was used depending on the size of the tissue.

Domestic pigs

The hippocampus and adrenal glands from two litters of pigs (breed: Large White) became available and the MAO activity in these tissues was estimated with dopamine, tyramine and tryptamine as substrates. Piglets were killed by an intracardiac injection of sodium pentobarbitone on the day of birth and 1, 2, 4 and 6 weeks of age. Each age group consisted of males and females from each litter. The MAO activity in the left and right adrenal gland and in the two halves of the hippocampus of each animal was measured separately where possible.

Enzyme assay

The MAO activity in each individual homogenate of the rat tissues was measured towards the following substrates: kynuramine, tyramine, dopamine, tryptamine and 5-hydroxytryptamine (5-HT). Enzyme activity towards kynuramine was assessed by a fluorimetric method (Kraml, 1965) as described previously (Holzbauer & Youdim, 1973). For the other amines a radiochemical method similar to that of Robinson, Lovenberg, Keiser & Sjoerdsma (1968) was used (for details see, Davis, Holzbauer, Sharman & Youdim, 1975). The same methods were used for the tissues of the piglets. The protein content of the tissue homogenates was assayed by the method of Lowry, Rosebrough, Farr & Randall (1951). Enzyme activity is expressed as nmol amine deaminated per mg protein per minute.

Thermal inactivation of the enzyme was studied by incubating the tissue homogenates (in phosphate buffer pH 7.4, 0.05 M) at 48°C. Samples for enzyme assay were taken at intervals of 20 minutes.

To study the subcellular distribution of MAO activity in the hypothalami of male Porton rats, ten hypothalami of five day old rats were pooled to form sample 1 and four hypothalami of 40 day old rats were pooled to form sample 2. The tissues were homogenized in 0.32 M sucrose. A P_1 'nuclear fraction' was obtained after centrifugation at 900 *g* for 5 minutes. The supernatant was centrifuged for 30 min at 12,000 *g*. The 'crude mitochondrial fraction' thus obtained was layered over a continuous sucrose gradient (1.4–2.0 M) and centrifuged at 100,000 *g*, for 1 h to obtained a 'true mitochondrial fraction' (Holzbauer, Bull, Youdim, Wooding & Godden, 1973).

Results

In a preliminary experiment on a group of Wistar rats (Babraham colony) kynuramine was the only substrate against which MAO activity was measured. The rats were killed when 3, 5, 9, 20, 35 and 122 days old. The tissues studied were the liver, the apex of the heart and the following brain regions: septum, striatum, hypothalamus and the posterior portion of the telencephalon including part of the hippocampus and several nuclei of the amygdaloid complex. The results obtained on the hypothalamus, posterior telencephalon, heart and liver (expressed in percent of the values on day 3) are shown in Figure 1. The homogenates of the whole brain tissues showed a rise in MAO activity with age and maximal values were seen in the 80 day old rats. This was also the case for the septa and striata (not shown on the graph). The highest absolute values (enzyme activity per mg protein per min) in the brain were found in the hypothalamus. The activity in the liver on day 3 was 3.5 times higher than in the hypothalamus; on days 35 and 122 it was 1.4 times higher. In the liver the values reached a plateau in the beginning of the second month. The heart showed low enzyme activity up to

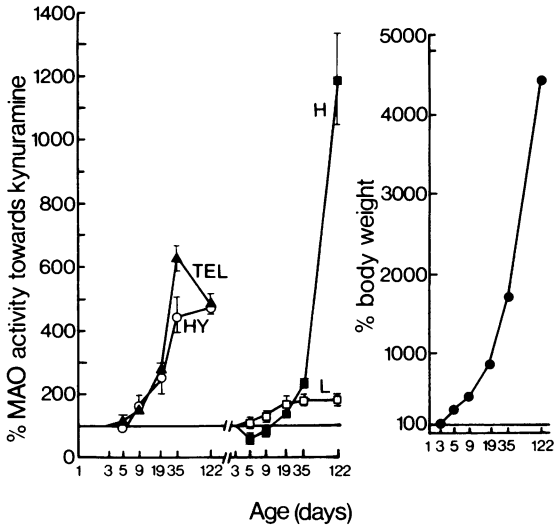

Figure 1 Relative increase in monoamine oxidase (MAO) activity (day 3 = 100%) towards kynuramine (calculated per mg protein min^{-1}) in the hypothalamus (HY, ○), posterior telencephalon (TEL, ▲), heart (H, ■) and liver (L, □) in litter-mate male rats aged 3–122 days (Babraham colony). Mean values are given; vertical lines show s.e. mean; $n = 5$. Mean body weights (●). (Preliminary experiment on a group of Wistar rats, Babraham colony).

day 35 when it amounted to only about 10% of that in the liver. Thereafter it rose steeply and in the 4 months old rat it was about 50% of that in the liver.

I. Substrate differences in the development of monoamine oxidase activity with age

Experiments were carried out on six groups of rats aged between 5 and 80 days. In each tissue homogenate MAO activity was tested against several substrates. The results are presented in Figures 2–6. In all figures the mean values obtained for each tissue at different ages are joined by straight lines irrespective of the length of the interval. These lines must not be interpreted as the exact time course at which MAO activity changes with age. The different breeding colonies are referred to as groups 1–6 (see Table 1).

(a) *Kynuramine*

Group 1. The rats used in this experiment were 5, 10,.20, 40 or 80 days old. The brain regions analysed were the hypothalamus, septum and striatum and for comparison the cerebellum. In addition the liver, the atria of the heart and the adrenal glands were studied. The results obtained with kynuramine as substrate are shown in Figure 2. Enzyme activity increased in all tissues with age. In the brain tissues the highest values were found in the 40 day old rats, in the liver in the 20 day old rats.

Group 2. MAO activity towards kynuramine was also measured in the 5 and 40 day old rats of group 2. The rise in activity with age was 80% in the whole brain homogenate, 145% in the adrenal glands and 325% in the heart. No significant rise was seen in the liver homogenate.

(b) *Tyramine.* The results obtained with tyramine as substrate in groups 1–6 are summarized in Figure 3. They are expressed as nmol tyramine deaminated mg^{-1} protein min^{-1}. The results obtained for the septum in the different groups of rats and at the various ages were very similar to those obtained for the striatum and have been omitted from the graph for the sake of clarity. The standard errors have similarly been omitted for the results obtained with the cerebellum of the rats of group 2.

Group 1. (Figure 3). In all brain regions examined and in the livers the highest enzyme activity towards

Table 1 Experimental animals

				Age (days): 5	8	10	20	30	40	80
Rat strain		Colony	Month of birth	Mean body weight (g)						
Wistar rats	Group 1	Babraham	March–May 1974	10.6	—	13.8	30.6	—	98.0	276
	Group 2	Babraham	May–June 1974	11.6	—	—	—	—	140.8	—
	Group 3	Tuck	October 1974	9.9	—	—	32.5	—	—	—
	Group 4	Tuck	March 1975	9.2	11.9	17.5	34.1	60.8	—	—
	Group 5	Porton (Babraham)	November 1974	9.6	—	—	61.0	—	—	—
Hooded rats	Group 6	Babraham	December 1974	10.4	—	—	—	76.6	—	—

Six litters of Wistar rats (Babraham colony) born in August 1973 were used in a preliminary experiment (see Figure 1). The group numbers are the same as those used in Figures 2–9. The rats belonging to the Tuck colonies were born in Babraham from mothers purchased already pregnant from Tuck.

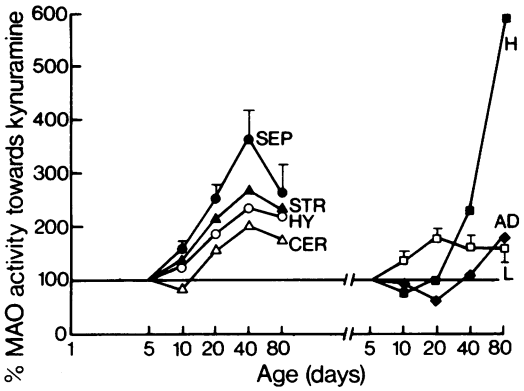

Figure 2 Relative increase in monoamine oxidase (MAO) activity (day 5 = 100%) towards kynuramine in four brain regions, heart, liver and adrenal glands. Group 1 (see Table 1), same tissue homogenates as used for the assays with tyramine, dopamine, tryptamine and 5-hydroxytryptamine in Figures 3–6, group 1. Septum (SEP, ●); striatum (STR, ▲); hypothalamus (HY, ○); cerebellum (CER, △); liver (L, □); heart (H, ■) and adrenal glands (AD, ◆) in littermate male rats aged 5–80 days; mean values are given. For the sake of clarity standard errors (vertical lines) are only given for septum and liver. $n = 4–5$. For most of the other estimates the coefficients of variation were smaller than 20%.

tyramine was seen in the 40 day old rats. In the heart and adrenal homogenates activity was low up to day 20. In both tissues there was still a considerable rise between days 40 and 80. Highest activity per mg protein in the brain was in the hypothalamus.

Group 2. (Figure 3). The rats of group 2 were killed either 5 or 40 days after birth and the whole brain, the atria of the heart, the liver and the adrenal glands were used. All tissue homogenates of the 40 day old rats deaminated more tyramine than those of the 5 day old rats. In the whole brain extracts the difference was 30%, in the liver 100%, in the heart 750% and in the adrenal glands 1000%.

Group 3. (Figure 3). The rats used in group 3 were either 5 or 20 day old. As in group 1, considerably more tyramine was metabolized by the hypothalami, striata and livers of the 20 day old rats than of the 5 day old ones. The cerebellum showed again the lowest activity of all brain regions tested.

Group 4. (Figure 3). The rats of this group were killed 3, 8, 10, 20 and 30 days after birth and only the striata were analysed. There was a threefold rise in tyramine deamination between days 5 and 20 and no further increase by day 30.

Group 5. (Figure 3). The rats of this group were killed 5 or 20 days after birth and the tissues examined were the hypothalami, the striata and the remainder of the brain including the cerebellum and medulla oblongata, and the liver. As in the other groups of rats there was a considerable rise with age in teh deamination of tyramine in all tissues studied.

Group 6. (Figure 3). This group consisted of hooded rats killed 5 or 30 days after birth. With tyramine as substrate only the hypothalami, the remainder of the brain as for group 5 and the livers were tested. Again all tissues showed an increase with age in their ability to deaminate tyramine.

Thus, in all groups there was a significant rise, with age, in the deamination of tyramine by all tissues studied. There were differences in the specific activity of the enzyme (nmol tyramine deaminated mg^{-1} protein min^{-1}). The MAO activity in the hypothalami of the 5 day old Porton rats (group 5) was nearly twice that of the 5 day old rats of group 1. The hypothalami of groups 3 and 5 on day 20 also exhibited a higher MAO activity per mg protein than those of group 1. The highest activity in the livers was seen in the 20 day old Porton rats.

(c) *Dopamine* The results obtained with dopamine as substrate are shown in Figure 4. All the homogenates studied deaminated less dopamine than tyramine (compare Figure 3). In many individual samples of homogenates of the four brain regions no dopamine deamination could be detected in rats aged 5 or 10 days. In the 20 day old rats of group 3 the homogenates of the striata showed no dopamine deamination, however the hypothalami had developed measurable activity. In contrast, the homogenates of whole brain of the 5 day old rats of group 2 and of group 6 (Hooded rats) deaminated considerable quantities of dopamine and there was no increase with age. No dopamine deamination was detected in the heart and adrenal homogenates of the 5, 10 and 20 day old rats. By day 40 (groups 1 and 2) and 80 (group 1) about half as much dopamine as tyramine was deaminated by the hearts. The livers deaminated dopamine about half as efficiently as tyramine at all ages. In the hypothalami of all groups tested the increase with age in dopamine metabolism was much steeper than the increase in tyramine metabolism. This was not the case for the livers.

(d) *Tryptamine.* With tryptamine as substrate, a fall with age in the MAO activity of the brain regions studied became apparent (Figure 5). Because a decrease in MAO activity with age was an unexpected finding, special care was taken to test the validity of the enzyme assay method. Enzyme kinetic studies (on homogenates of hypothalami of 5 and 40 day old rats) using tryptamine as substrate showed however that deamination of this amine followed first order kinetics.

There were differences between the groups of rats studied, in the rate at which this decrease with age occurred and also in the absolute quantities of

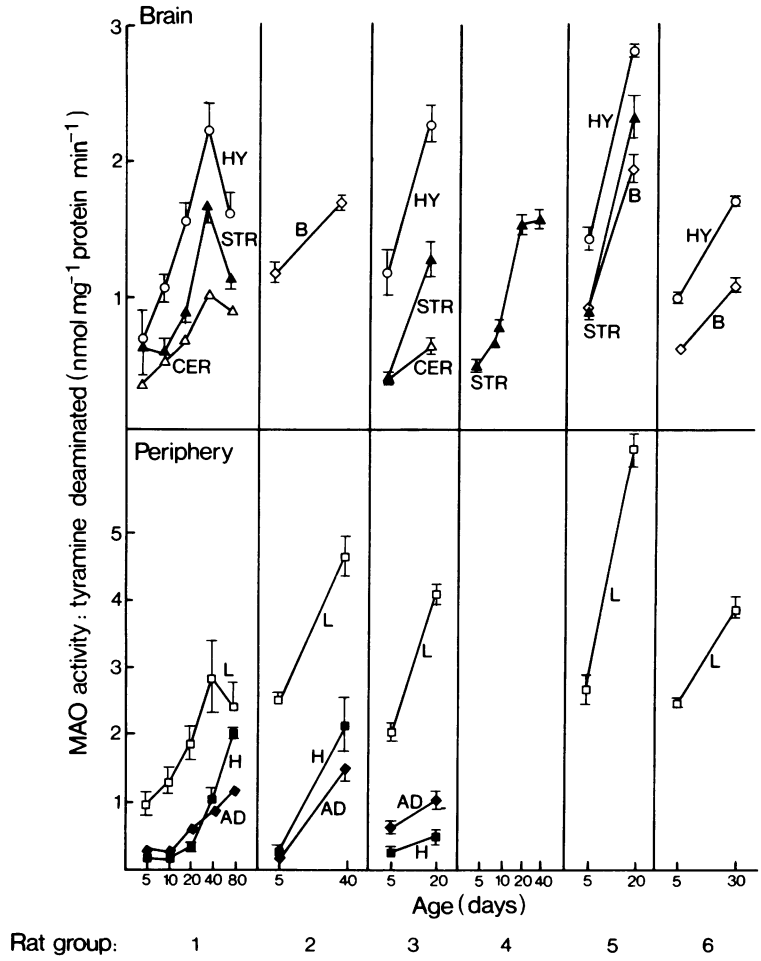

Figure 3 Postnatal development of monoamine oxidase (MAO) activity towards tyramine (nmol deaminated mg^{-1} protein min^{-1}) in litter-mate male rats aged between 5 and 80 days. Groups 1 and 2: Wistar rats bred in Babraham. Groups 3 and 4: Wistar rats bred at Tuck's. Group 5: Porton rats (bred at Babraham); Group 6: Hooded rats (bred at Babraham). (For definition of groups see also Table 1). Hypothalamus (HY, ○); striatum (STR, ▲); cerebellum (CER, △); whole brain homogenate (B, ◇); liver (L, □); heart (H, ■); adrenal glands (AD, ◆). Mean values are given; vertical lines show s.e. mean; $n=4-5$. (The same tissue homogenates of group 1 were also used for the assays with kynuramine in Figure 2).

tryptamine deaminated per mg protein. Highest activities were seen in group 3. In group 1 the values obtained for the cerebellum and septum were similar to those for the striatum and are therefore omitted from the figure. In group 6 the change in enzyme activity with age in the hypothalamus and striatum went in opposite directions. In the brain homogenates of groups 2 and 5 no significant difference in tryptamine deamination between days 5 and 40 or 5 and 20 could be detected. In contrast to the brain regions tryptamine deamination by the liver increased with age in all groups as seen for the other substrates. In all groups the quantity of tryptamine deaminated on day 5 was smaller than that of tyramine and the increase with age was less steep. Very little tryptamine was deaminated by the hearts and adrenal glands of the 5–20 day old rats. Deamination increased by day 40 and 80 but the rise was less steep than that for tyramine.

(e) *5-Hydroxytryptamine.* The ability of MAO to deaminate 5-HT at different ages varied between the groups of rats (Figure 6). In group 1 there was a steep fall between days 5 and 40 in all the tissues tested, the

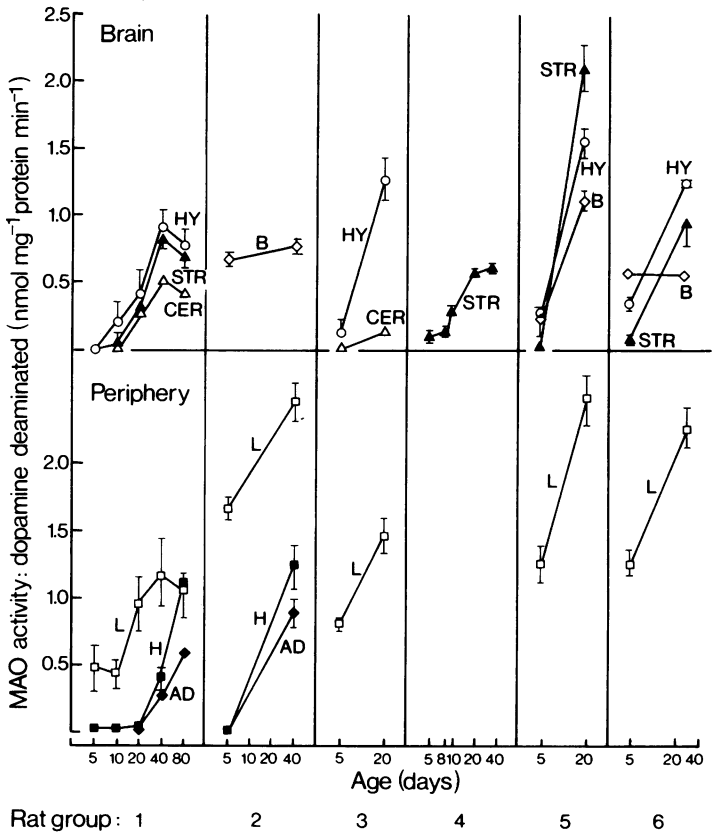

Figure 4 Postnatal development of monoamine oxidase (MAO) activity towards dopamine (nmol deaminated mg^{-1} protein min^{-1}). The same tissue homogenates were tested as in Figure 3. Symbols as in Figure 3.

other groups showed a rise with the exception of the whole brain homogenates of groups 2 and 6 in which no significant difference between day 5 and 40 occurred. The smallest quantities of 5-HT were deaminated by the hearts and adrenal glands of groups 2 and 3. More 5-HT than tyramine was deaminated by the hypothalami of the 5 day old rats in groups 1 and 3, less in groups 5 and 6.

II. Postnatal development of multiple forms of monoamine oxidase

1. *Differences in the development of a tyramine and a dopamine deaminating enzyme system in the rat brain.* Figure 7 illustrates the increase with age in the activity of MAO towards three different substrates in the hypothalami of the rats of group 1. The activities are expressed in percent of the highest activity observed in the 40 day old rats. MAO activity towards kynuramine and tyramine was doubled between days 10 and 40 whereas activity towards dopamine increased four-fold during the same period. This observation suggested the possibility that in the hypothalamus the enzyme system deaminating dopamine might develop with age independently from the tyramine deaminating system. Similar observations were made in other groups of rats. In the rats of group 3 the dopamine MAO activity in the hypothalamus on day 20 was about eight times higher than on day 5 (see Figure 4) whereas tyramine MAO activity only doubled during the same time (see Figure 3). In group 5 (Porton strain) dopamine deamination by the hypothalamic and striatal tissues on day 21 was seven to eight times that on day 5 but the increase in tyramine deamination was only two-fold.

In the hearts and adrenal glands a differential development of dopamine and tyramine deamination also became apparent. Deamination of tyramine by both tissues was slow but clearly demonstrable in 5, 10 and 20 day old rats. In the same homogenates no dopamine deamination could be detected. Between

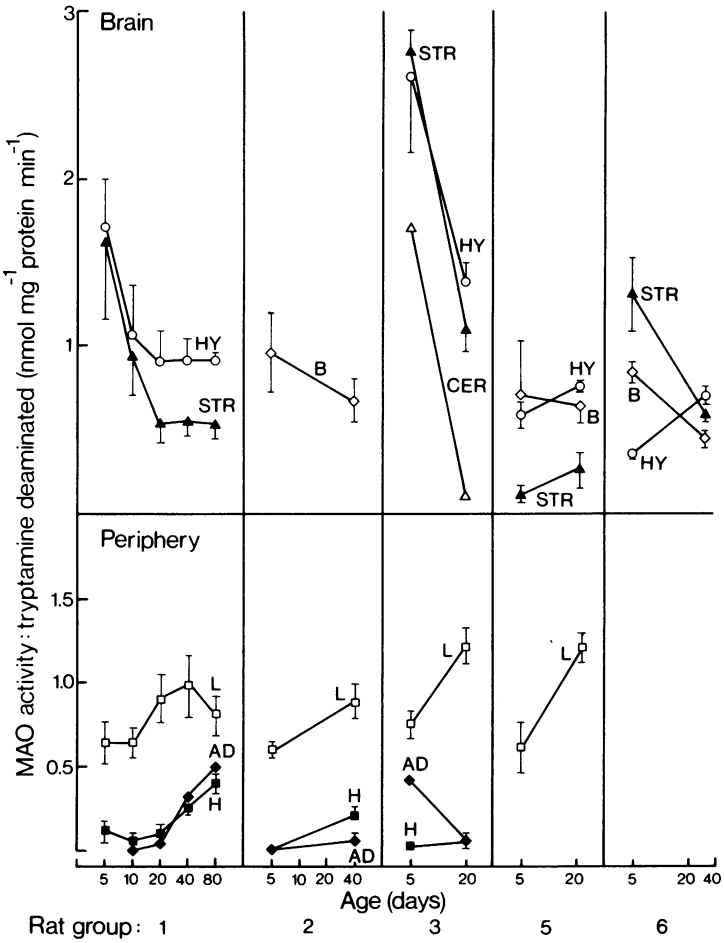

Figure 5 Postnatal development of monoamine oxidase (MAO) activity towards tryptamine (nmol deaminated mg^{-1} protein min^{-1}). The same tissue homogenates were tested as in Figures 3 and 4. Symbols as in Figure 3.

days 20 and 80 tyramine deamination in the heart increased six-fold, dopamine deamination however increased more than twenty-fold. In the adrenal gland there was a two-fold rise in tyramine deamination between days 20 and 80 in contrast to a more than twenty-fold rise in dopamine deamination (see Figures 3 and 4).

It was surprising to see that in group 2 and in group 6 (Hooded rats) the dopamine deamination in extracts of whole brain did not increase with age whereas it did so in the hypothalamic homogenates (Figure 4). In the same rats the rise in tyramine inactivation by the whole brain homogenate was similar to the rise in tyramine inactivation in the hypothalamus (Figure 3).

In the livers of all groups studied tyramine and dopamine deamination developed at a similar rate. Thus in groups 1, 3 and 5 both tyramine and dopamine deamination increased two-fold between days 5 and 20.

Assuming that tyramine and dopamine were deaminated by the same enzyme system one would expect the ratios of MAO activities expressed as nmol amine deaminated mg^{-1} protein min^{-1} towards these two substrates to remain constant during development in a given tissue. From Figure 8 it can be seen that this is in fact the case for the liver. In all groups of rats the ratio of tyramine to dopamine deaminated was about 2 at all ages tested. The whole brain homogenates of 5 day old rats also deaminated only about twice as much tyramine as dopamine, as did the livers. In contrast, in the hypothalamus and striatum of 5 day old rats this ratio was much larger (between 4 and >10) than in the same tissues of 20 to 80 day old rats when it was similar to that in the liver.

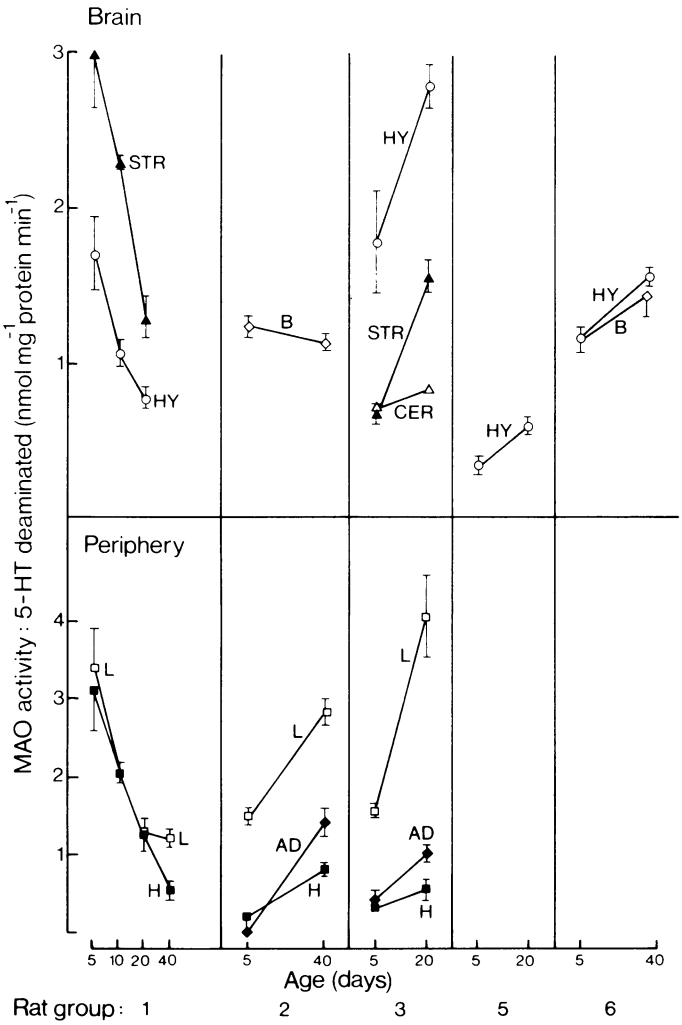

Figure 6 Postnatal development of monoamine oxidase (MAO) activity towards 5-hydroxytryptamine (5-HT) (nmol deaminated mg^{-1} protein min^{-1}). The same tissue homogenates were tested as in Figures 3, 4 and 5. Symbols as in Figure 3.

In the homogenates of the hearts and adrenal glands no dopamine metabolism could be detected in the 5 day old rats and on day 20 only on rare occasions. At these ages the ratios of tyramine deaminated to dopamine deaminated were well over 20.

2. *Independent development of a tryptamine deaminating enzyme system in the rat striatum and hypothalamus.* A comparison between the results in Figure 3 and Figure 5 leaves little doubt that the enzyme deaminating tryptamine develops in the young rat differently from the enzyme deaminating tyramine.

Figure 9 shows the ratios between tyramine deamination to tryptamine deamination in the hypothalami and livers of 3 groups of rats. Because of the rise in tyramine and the fall in tryptamine deamination with age these ratios increased with age. In group 1 the ratios obtained for the septum, the cerebellum and the striatum (not shown in the figure) were the same as those for the hypothalamus. The ratios also increased in the livers because the rise with age of tyramine deamination was faster than that of tryptamine deamination. Similar changes in the tyramine : tryptamine ratios were seen in the hearts and adrenal glands.

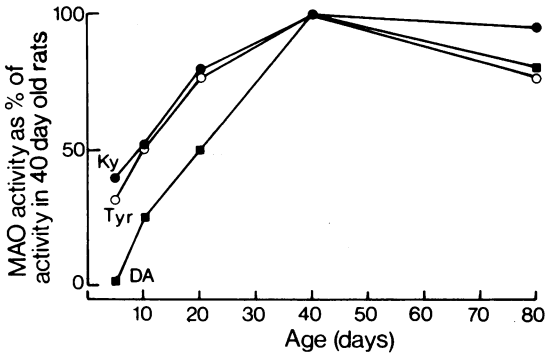

Figure 7 Monoamine oxidase (MAO) activity towards kynuramine (Ky), tyramine (Tyr) and dopamine (DA) in the hypothalamus of the rats of group 1 (see Figures 2, 3, 4 and Table 1) at different ages expressed as percent of the highest activity observed in the 40 day old rats. Kynuramine (Ky, ●); dopamine (DA, ■); tyramine (Tyr, ○).

III. Subcellular distribution of monoamine oxidase activity in the hypothalamus of 5 and 40 day old Porton rats

MAO activity was estimated (see methods section) in subcellular fractions of hypothalamic tissue of male rats belonging to the Porton strain which were killed when 5 or 40 days old. The results are shown in Figure 10. Per mg protein, the largest quantities of all the amines tested were deaminated by a mitochondrial fraction which sedimented at about 1.5 M on the sucrose gradient (P_2). This fraction showed in the 40 day old rats, per mg protein, about 1.7 times more MAO activity towards tyramine, 1.6 times more activity towards dopamine and 1.2 times more activity towards 5-HT than in the 5 day old rats. There was no difference with age in the tryptamine deamination. MAO activity was also found in the P_1 fraction (nuclear) and was higher in the 40 than in the 5 day old rats for all substrates. Activity in the combined supernatant and microsomal fraction was very low and did not increase with age.

IV. Comparison of monoamine oxidase activity in the liver with that in the hypothalamus and heart

Per mg protein the liver deaminated on average 1.5 times more kynuramine and tyramine than the hypothalamus. This was seen in most breeding colonies at all ages studied. For dopamine the figure was about 2 in rats aged 20 days or older. At an earlier age dopamine deamination in the hypothalamus was very low (see Figure 4). The hepatic deamination of the two indolalkylamines was equal to or somewhat lower than in the hypothalamus in rats older than 10 days.

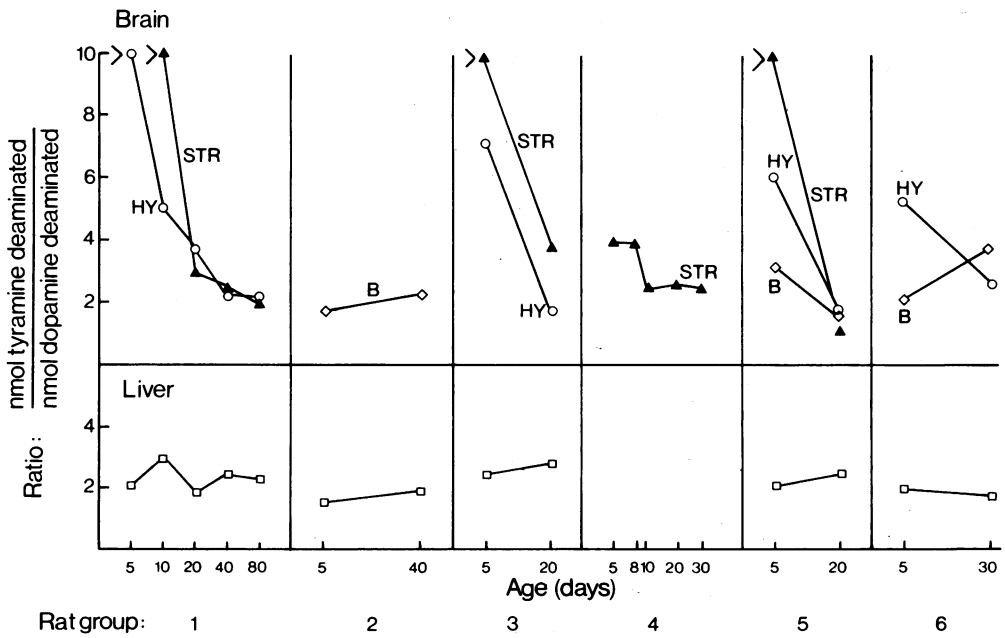

Figure 8 Variations with age in the ratio of monoamine oxidase (MAO) activity towards tyramine to MAO activity towards dopamine in the hypothalamus (○), striatum (▲), whole brain homogenates (◇) and livers (□) of all rats tested (compare values of Figures 3 and 4).

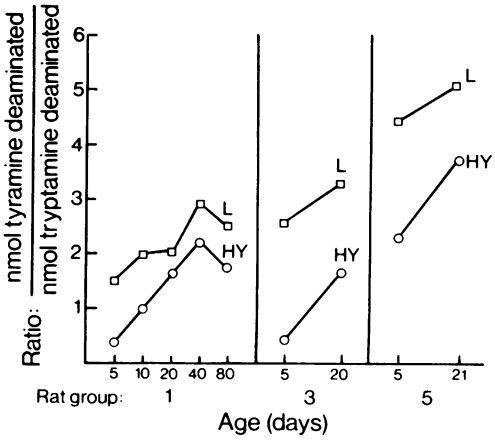

Figure 9 Variations with age in the ratio of monoamine oxidase (MAO) activity towards tyramine to MAO activity towards tryptamine in the hypothalamus (HY,O) and livers (L,□) of the rats of groups 1, 3 and 5.

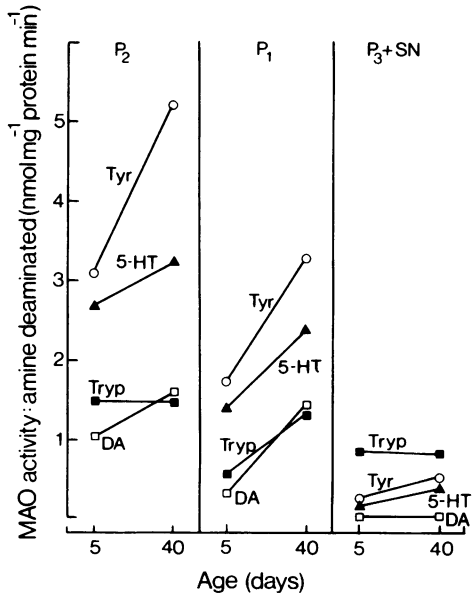

Figure 10 Monoamine oxidase (MAO) activity in subcellular fractions of the pooled hypothalami of either 5 ($n=10$) or 40 ($n=4$) day old Porton rats. P_2=purified mitochondrial fraction; P_1='nuclear' fraction; P_3='microsomal' fraction; SN: high speed supernate. MAO activity expressed as nmol substrate deaminated mg^{-1} protein min^{-1}; substrates: tyramine, (Tyr, O); 5-hydroxytryptamine, (5-HT, ▲); dopamine, (DA, □); tryptamine, (Tryp, ■).

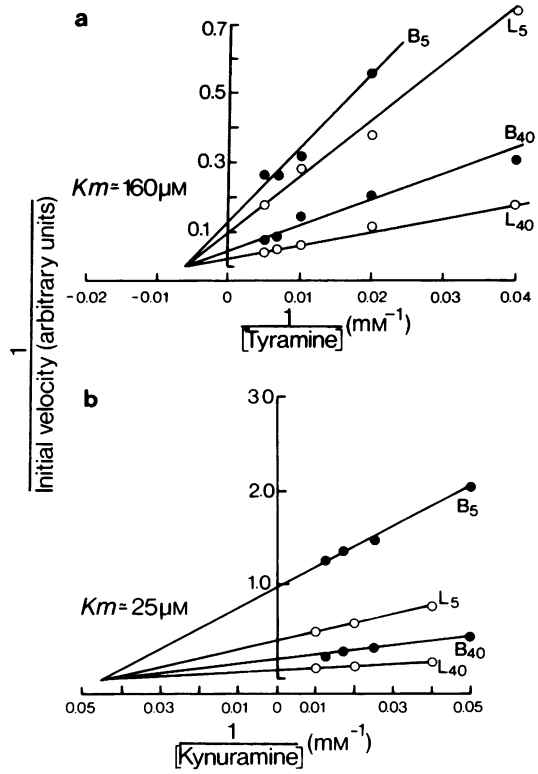

Figure 11 *Km* values for the deamination of (a) tyramine ($Km \simeq 160$ μM) and (b) kynuramine ($Km \simeq 25$ μM) by brain homogenates of 5 day (●, B5) and 40 day (●, B40) old rats and liver homogenates (O, L5; O, L40) of the same rats.

The time course of the development of MAO towards all substrates studied differed considerably between liver and heart. Up to 40 days MAO activity in the heart was relatively low and in groups 1, 2 and 3 about 10 times more kynuramine, tyramine, dopamine and tryptamine was deaminated per mg protein in the liver than in the heart. At 40 and 80 days this ratio was below 5.

V. Physico chemical properties of monoamine oxidase at different ages

The *Km* values for the deamination of kynuramine and tyramine by liver and whole brain homogenates of 5 and 40 day old rats are shown in Figure 11 (a) & (b). Neither tissue showed a difference in the *Km* values obtained at different ages. For kynuramine the value was 25 μM, and for tyramine 160 μM.

The heat inactivation (48°C) of MAO in various tissues of 5 and 40 day old rats was also tested using

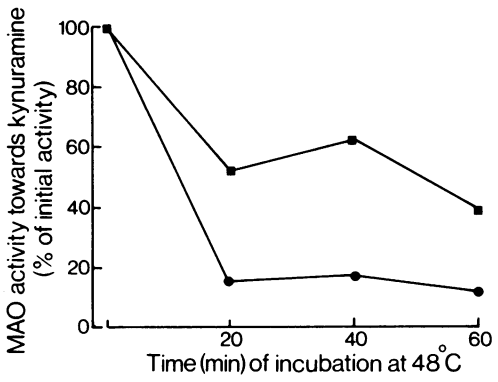

Figure 12 Heat inactivation of monoamine oxidase (MAO) activity towards kynuramine in adrenal homogenates (AD) of 5 (●) and 20 (■) day old litter-mate male rats (Wistar, Babraham bred). Incubation at 48°C in Na-phosphate buffer (0.05 M, pH 7.4).

different substrates. Figure 12 shows that the ability of adrenal homogenates of 5 day old rats to deaminate kynuramine decreased by more than 80% after 20 min whereas in the 40 day old adrenal glands it was decreased by only 50% after the same time. Figure 13 shows heat inactivation curves of MAO in liver and brain homogenates of 5 and 40 day old rats. The loss in enzyme activity was greater in the younger rats. This was especially pronounced when kynuramine was used as substrate. The decreased heat stability of the enzyme in the tissue of young rats may be caused by the absence of stabilizing compounds. In the brain it is known that the lipid content per g tissue increases more than ten-fold between day 12 and 48 (Crawford & Sinclair, 1972; Mead & Dhopeshwarkar, 1972).

VI. Monoamine oxidase activity during the development of the domestic pig

Results obtained on the hippocampus and the adrenal glands are listed in Table 2. In the hippocampus of the foetal pig, the MAO activity towards tyramine and tryptamine was only about one-third of that in the 1 week old piglet and no dopamine (<0.01 nmol mg^{-1} protein min^{-1}) was deaminated. The ability to deaminate dopamine increased gradually with age. The homogenates of the hippocampus of the 6 weeks old piglet deaminated per mg protein about twice as

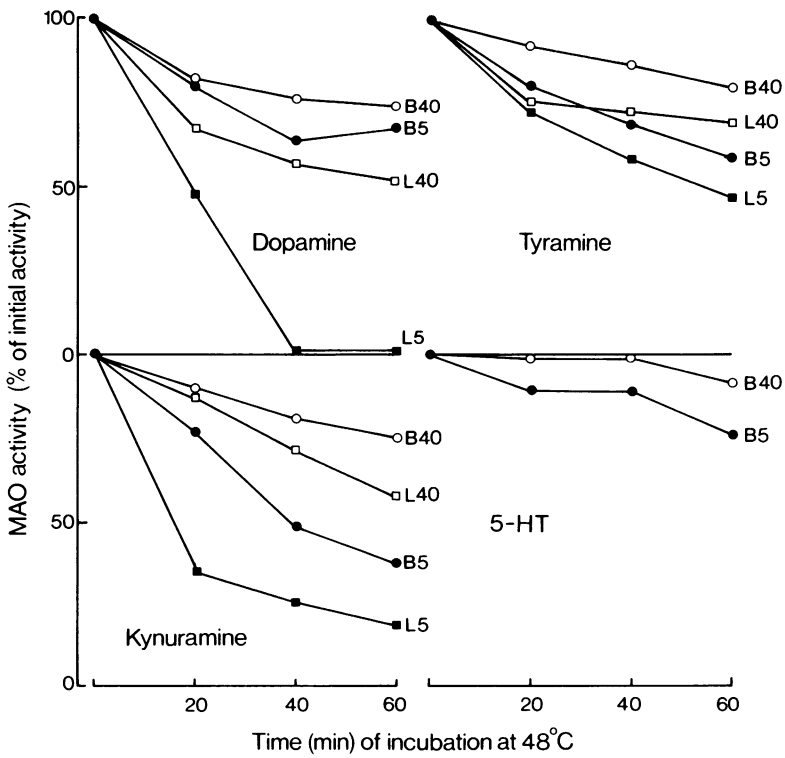

Figure 13 Heat inactivation of monoamine oxidase (MAO) activity in brain (B) and liver (L) homogenates of 5 and 40 day old litter-mate male rats (Wistar, Babraham bred) towards different substrates: (●) 5 day old brain; (○) 40 day old brain; (■) 5 day old liver; (□) 40 day old liver.

much dopamine as that of the 1 week old piglet. There was also a very small rise in MAO activity towards tyramine. Because of large individual variations and the small number of samples available, the changes were statistically not significant. It is of interest that the ratio between the amount of tyramine deaminated to the amount of dopamine deaminated by the hippocampus decreased with age as it did in discrete brain regions of the rat. It was >10 in the foetus, 7.7 in the 1 week old piglet, 7.3 at 2 weeks, 5.1 at 4 weeks and 4.8 in the 6 week old piglet. In two adult pigs (unpublished results) this ratio was found to be 2. No foetal adrenal tissue was available from this litter of pigs. The adrenal MAO activity towards all three substrates was lowest on day 1. Any further increases were not statistically significant.

Discussion

The results of this investigation have elucidated two major points. First, an independent development of at least three enzyme systems deaminating different monoamines has been observed in discrete brain regions, the heart and the adrenal glands, but not in the livers of the rat. Second, there were differences between breeding colonies which were especially pronounced when 5-HT was used as substrate. This finding emphasizes the danger of considering observations made on a single rat colony as representative for the whole species.

The independent development of enzyme systems deaminating one specific monoamine in discrete brain regions but not in the liver could be due to the different chemical environments prevailing in these tissues. Similarly the independent development of MAO activity deaminating tyramine or dopamine or tryptamine does not provide proof that different enzyme proteins are involved in the deamination of these substrates. Developmental changes in some components in the immediate environment of the enzyme protein (allotopic or allosteric effectors) which are required for conformational stability and for the most efficient deamination of a given monoamine could account for the differences observed when various substrates are used to test MAO activity in discrete brain regions.

This point could be carried even further to explain the general phenomenon of increasing enzyme activity with age as seen in most organs towards most substrates. This is not necessarily due to a change in the quantity of enzyme protein present per mg total protein but could result from developmental changes of allotopic or allosteric effectors and co-enzymes. This is exemplified by the postnatal increase in the tissue content of flavin (Kuzuya & Nagatsu, 1969) which is a co-factor of MAO (for review see Youdim, 1976). The activity of the enzyme effecting flavin synthesis is known to increase with age (Rivlin, Fazekas, Huang & Chaudhuri, 1976). It is also possible that an enzyme responsible for the covalent binding of FAD to the MAO apo-enzyme is not fully developed at birth. Phospholipids situated in close proximity to MAO in the outer mitochondrial membrane are also known to be of importance for certain properties of MAO. It was suggested that interaction between the enzyme protein and phospholipids was responsible for the multiple forms of MAO, for substrate-dependent differences in the rate of its heat inactivation and for inhibitor specificity (Tipton, Youdim & Spires, 1972; Houslay & Tipton, 1973; Tipton, Houslay & Garrett, 1973). Like the

Table 2 Developmental changes of monoamine oxidase (MAO) activity in the piglet*

		MAO activity: (nmol amine deaminated mg^{-1} protein min^{-1})		
Age	*Substrate:*	*Dopamine*	*Tyramine*	*Tryptamine*
	(1) Hippocampus			
Foetus (55 day) ($n=4$)		<0.01	0.24 ± 0.09	0.05 ± 0.03
1 week ($n=4$)		0.10 ± 0.04	0.77 ± 0.15	0.14 ± 0.07
2 weeks ($n=4$)		0.12 ± 0.07	0.88 ± 0.09	0.21 ± 0.09
4 weeks ($n=4$)		0.19 ± 0.05	0.97 ± 0.09	0.13 ± 0.05
6 weeks ($n=2$)		0.21 ± 0.04	1.00 ± 0.07	0.10 ± 0.05
	(2) Adrenal gland			
1 day ($n=4$)		0.19 ± 0.05	0.69 ± 0.11	0.07 ± 0.05
1 week ($n=8$)		0.28 ± 0.03	1.34 ± 0.11	0.20 ± 0.08
2 weeks ($n=6$)		0.32 ± 0.03	0.90 ± 0.15	0.13 ± 0.06
4 weeks ($n=8$)		0.22 ± 0.03	0.91 ± 0.09	0.12 ± 0.04
6 weeks ($n=2$)		0.25 ± 0.04	0.68 ± 0.07	0.11 ± 0.06

* Breed: Large White; n = number of samples.

flavins, the brain lipids are low at birth and increase gradually with age (see e.g. Mead & Dhopeshwarkar, 1972; Crawford & Sinclair, 1972).

MAO forms a constituent of the outer mitochondrial membrane (Schnaitman, Erwin & Greenawalt, 1967). Thus studies on the development and life span of the mitochondria in the organs investigated could be of importance for the understanding of the processes involved in the developmental changes in MAO activity as would be a knowledge of the turnover rate of the MAO protein at various ages. The difficulties in assessing the accurate life span of a mitochondrion are numerous. Most of the measurements are based on the turnover rates of mitochondrial constituents which are known to have different half lives thus indicating that synthesis of different proteins can take place independently in the intact organelle (for review see Munn, 1974). Gregson & Williams (1969) found that in the brain the number of mitochondria per g wet weight does not increase with age, however the activity (per g wet weight) of the two mitochondrial enzymes succinate dehydrogenase and cytochrome oxidase increased four and six-fold respectively. From this work we may infer that after birth the MAO activity (towards tyramine, dopamine and kynuramine) increases per mitochondrion. The interpretation of findings on brain MAO is further complicated by the simultaneous presence of neuronal and glial tissue in the homogenates. Furthermore, Maitre, Delini-Stula & Waldmeier (1976) obtained evidence that MAO may be present in the brain in at least two pools, a 'bulk' MAO and a small MAO pool. The latter is characterized by its relative resistance to inhibition, a rapid turnover and preference for dopamine deamination. It is possible that in discrete brain regions the population of mitochondria deaminating different monoamines develops independently. The existence in the adult rat brain of mitochondrial fractions which vary in their ability to deaminate dopamine or 5-HT has been described by Kroon & Veldstra (1972) and by Youdim (1974).

In contrast to most other organs, the cardiac MAO activity in the rat did not reach maximal values by the end of the 4th month. A study of the development of cardiac MAO activity towards 5-HT has been carried out by Horita (1967) in rats aged 2–16 weeks. The heart of the male rats deaminated per g tissue six times less 5-HT at four weeks than at 16 weeks, when a plateau was reached. The postnatal development of the enzymes succinic dehydrogenase and cytochrome oxidase did not follow the same time course as that of MAO. Callingham & Della Corte (1971) reported that the half life of cardiac MAO increased with age. In rats with a mean body weight of 155 g it was 6.7 days, in rats weighing about 230 g it was 10.2 days and in rats weighing 340 g 17.6 days. These authors found a similar increase with age in the half life of $NADH_2$-cytochrome C reductase. A difference in the postnatal development in the heart of two forms of MAO (type A and B) distinguished by their behaviour towards different inhibitors (Johnston, 1968) has recently been described by Callingham & Lyles (1975).

The observation made on the rats of group 1 that MAO activity mg^{-1} protein min^{-1} towards 5-HT decreases during the first 20 days after birth was in conflict with the results in all other groups studied and with previous reports in the literature (Karki *et al.*, 1962; Bennett & Giarman, 1965; Horita, 1967). We ascribe it to differences between breeding colonies.

The observation that dopamine was hardly metabolized by the homogenates of hypothalami, striata, heart and adrenal glands of 5 and 10 day old rats of all groups studied seems of special physiological interest. The fact that the whole brain homogenates from 5 day old rats metabolized considerable quantities of dopamine in contrast to striata and hypothalami draws attention to the importance of analysing discrete brain regions.

There was also a discrepancy in the findings on the development of dopamine deamination by homogenates of the hypothalamus and by purified hypothalamic mitochondria. MAO activity towards dopamine in the homogenates of hypothalami increased seven-fold between days 5 and 21. In contrast in the purified hypothalamic mitochondria of rats of the same colony there was only a 1.5-fold increase between days 5 and 40. Is it possible that in the homogenates of the hypothalami of 5 day old rats an enzyme inhibitor is present which is being removed during the purification of the mitochondria? A localized enzyme inhibitor in the brain of young rats may also be responsible for the observation that MAO activity per mg protein towards dopamine in the homogenates of whole brains of 5 day old rats was much higher than in the isolated hypothalami or striata and that there was no increase with age. In the whole brain homogenates a localized inhibitor of dopamine deamination might have become so diluted that it was no longer effective.

The development of MAO activity towards dopamine in the striata appears to follow the maturation of the neuronal system employing dopamine as a transmitter substance. In a detailed fluorescence histochemical study, Loizou (1972) observed that during the first week after birth the presumed dopamine containing varicosities were sparse whereas by the end of the third week the innervation was so dense that it was no longer possible to distinguish the individual varicosities.

Is it possible to draw from the experiments on tissue homogenates *in vitro* any conclusions about the *in vivo* function of MAO during development? This question has been investigated for dopamine deamination by measuring the MAO activity towards dopamine and simultaneously the two acidic metabolites of

dopamine, homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) in the striata of littermate rats aged 5–30 days. When expressed as percent of the values obtained on day 5 the rise with age in the striatal concentrations of HVA and DOPAC and the increase in the deamination of dopamine was similar (Davis *et al.*, 1975). Thus, at least for dopamine deamination it appears that the results of the *in vitro* experiments may reflect the *in vivo* situation. However, this may be a purely fortuitous occurrence as the development of dopamine deamination *in vitro* followed a similar time course with age in other brain regions (e.g. the cerebellum) in which no extensive dopamine containing systems have so far been described. This was also true for the adrenal glands and the heart, but not for the liver.

There are also differences between species in the increase of MAO activity during postnatal development. Thus, Karki *et al.* (1962) found that the ability of the brain of the rat to deaminate 5-HT was three times higher in the adult rat than in the newborn rat, whereas in the guinea-pig this difference between the adult and the newborn animal did not exist. Likewise, Horita (1968) was unable to find a rise with age in cardiac MAO activity towards 5-HT in the guinea-pig, rabbit, cat and mouse. In the adrenal gland and the hippocampus of the domestic pig the rise with age in MAO activity towards dopamine, tyramine and tryptamine was also much less pronounced than in the rat (see Table 2). However, the domestic pig has reached a much higher degree of maturation at birth than the rat and many developmental changes which occur in the rat during the first weeks of life will have taken place *in utero* in the pig. The decrease with age in the ratio of the quantity of dopamine to tyramine deaminated by the pig hippocampus could indicate that, as in the rat, the development of a dopamine deaminating system may take a different time course from a tyramine deaminating system. In certain regions of the pig brain, in which dopamine may play a role in neuronal transmission, the concentration of homovanillic acid increased during the first 6 weeks after birth (Fry, Sharman & Stephens, 1976) as has also been found in the rat (Davis *et al.*, 1975). Stanton, Cornejo, Mersmann, Brown & Mueller (1975) found higher MAO activity towards tyramine in the 'hind Brain' of pigs (crossbred: Hampshire X Yorkshire X Duroc), between birth and 70 days than at 150 days. Tyramine deamination by the adrenal glands of these pigs remained apparently constant during the first 5 months.

In order to explore the possibility that the differential deamination of specific monoamines by MAO is of physiological significance, analysis will have to be conducted on very small brain regions (individual nuclei) and simultaneous measurements made of the enzymes responsible for the synthesis of a given monoamine, the tissue concentration of that amine and those of its catabolic products and the activity of the enzymes responsible for the fromation of these catabolites.

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