Biochemical Pharmacology, Vol. 29, pp. 1221–1230.
Pergamon Press Ltd. 1980. Printed in Great Britain.

DEVELOPMENT OF BENZYLAMINE OXIDASE AND MONOAMINE OXIDASE A AND B IN MAN

RACHEL LEWINSOHN, VIVETTE GLOVER and M. SANDLER
Bernhard Baron Memorial Research Laboratories, and Institute of Obstetrics and Gynaecology, Queen Charlotte's Maternity Hospital, London W6 0XG, U.K.

(*Received* 27 *September* 1979; *accepted* 6 *December* 1979)

Abstract—We describe the widespread distribution of the enzymes benzylamine oxidase (BzAO) and monoamine oxidase A and B (MAO A, MAO B) in human tissues at three stages of development: fetal, neonatal and adult. Relatively low activity of each is present in fetal tissues, but the specific activities of BzAO and MAO B in fetal liver are similar to those in the adult, suggesting that these enzyme systems are functionally mature in the liver at 20 weeks gestation. Specific activity of BzAO in the lung, twice as high in the adult as in the fetus, reaches adult value at birth. In fetal brain, lung, aorta and digestive tract, MAO A emerges before MAO B. Estimation of total activity developed by tissue or organ sheds a new light on the importance of each enzyme in body economy and shows skeletal muscle to be by far the most active whole body source of MAO B, whilst the liver has the highest total and specific activity of MAO A. A similar approach clearly demonstrates that BzAO is essentially a tissue rather than a plasma enzyme, which tends to predominate in blood vessel walls.

In an earlier paper [1], we described the widespread distribution of benzylamine oxidase (BzAO) and monoamine oxidase (MAO) B in human and rat tissues. This study was undertaken in an attempt to elucidate the significance of the finding by one of us [2] of low serum BzAO activity in patients with severe burns or cancer. It then seemed reasonable to investigate the activity of BzAO in rapidly-growing tissues. In this paper, we describe the distribution of BzAO in human tissues and compare activities in fetus, neonate and adult. The development of MAO A and MAO B activity was investigated in parallel.

Despite considerable research effort in recent years [3–9], the physiological role of the amine oxidases in the degradation of a multiplicity of endogenous and exogenous substrates remains poorly understood. Benzylamine (Bz) is the best substrate for both MAO B and BzAO [1] and, indeed, is the only known substrate for human BzAO. This compound, together with the MAO A substrate, 5-hydroxytryptamine (5-HT, serotonin) [7] and the putative MAO B substrate, phenylethylamine (PEA) [10], has been used to chart the development of the amine oxidases in a wide variety of tissues.

MATERIALS AND METHODS

Chemicals

Benzylamine hydrochloride methylene [^{14}C], sp. act. 56 mCi/mmole, radiochemical purity 98%, and 5-hydroxy[*side-chain*-2-^{14}C] tryptamine sulphate, sp. act. 58 mCi/mmole, radiochemical purity 98%, were purchased from Radiochemical Centre Ltd., Amersham, U.K. Phenylethylamine hydrochloride, beta-[ethyl-1-^{14}C], sp. act. 50.98 mCi/mmole, radiochemical purity 97%, was purchased from New England Nuclear, Boston, MA, U.S.A. (-)-Deprenyl was generously donated by Professor J. Knoll, Budapest, clorgyline by May & Baker Ltd., Dagenham, U.K., and phenelzine by William R. Warner & Co. Ltd., Eastleigh, Hants, U.K. All other reagents were obtained from commercial sources or as described earlier [1].

Tissues

(a) *Fetal.* Six human fetuses (gestational age 19–21 weeks) were obtained from the fetal tissue bank of the Royal Marsden Hospital, London, through the kind offices of Dr. Sylvia D. Lawler; one fetus was obtained from Chelsea Hospital for Women, London (Mr. John Shepherd). Abortion in all cases had been induced by intra-amniotic injection of 80 g urea in 200 ml Hartmann's solution, followed by 5 mg prostaglandin E_2 [11]. Six fetuses were male and one female; the mean crown–rump length was 16.3 cm (range 15–17.7 cm) and the mean weight 343 g (range 259–450 g). Two fetuses were moderately macerated, with the abdominal cavity full of blood clot, but the livers were intact. In one of them, one lung was grossly haemorrhagic, and tissue was taken from the other lung, which was spottily haemorrhagic. Skeletal muscles used were pectoral and thigh, assayed separately; activities shown are means of pooled values. Tissues of the digestive tract were split open and washed thoroughly in running water before treatment as described below. The fetuses were kept at 2–4° up to the time of dissection, which was carried out by one of us (R.L.) within 12 hr of extraction. Except for lung, liver, skin, scalp and muscle, the organs were homogenized in their entirety. The entire brain was coarsely mixed and an aliquot taken for homogenization and assay.

(b) *Neonatal.* In this group were two stillbirths (one male, one female) and one male neonate, a

premature of 34 weeks gestational age who died at 7 days. In the interval between death and dissection (post-mortem lag, 50–64 hr), the bodies were kept at 2–4°. Birth weights were 2845, 4160 and 2060 g and crown–rump lengths 34, 38 and 32.3 cm, respectively. The brain region assayed was the frontal cortex; heart tissue was a section of the left ventricle. Renal cortex and some medulla were used. The data for skeletal muscle are means of pooled values of the pectoral and thigh muscles, assayed individually. Tissues of the digestive tract were split open and their entire thickness used, after washing as described above. Subsequent treatment was as described under "Freezing and homogenizing procedures".

(c) *Adult.* Tissues from adults were obtained at autopsy from three Coroner's cases, two male and one female, aged 64, 55 and 68 yr, respectively. Liver and aorta were used from three and one additional autopsies, respectively. All died from coronary heart disease; no evidence of neoplastic or infectious disease was found in any of them. Post-mortem lag was 12–36 hr; in the interval the bodies were kept at 2–4°. As with the neonatal tissues, frontal cortex and left ventricular region represented brain and heart; renal cortex, wedge-shaped sections of the adrenals and the sharp edge of liver and lung were used. Atherosclerotic lesions were seen in all aortae; these were avoided and tissue specimens taken from apparently normal regions. Abdominal skin was freed from fat and subcutaneous tissue at dissection. Tissues of the digestive tract were whole wall thickness of all but the colon, where taenia coli were avoided. These tissues were scraped on the mucosal side and thoroughly washed in running cold water before treatment as described under "Freezing and homogenizing procedures".

It is well known that autolysis sets in immediately after death and sometimes earlier. It would not, of course, occur uniformly throughout the digestive tract and this may, indeed, provide a partial explanation for the variability in activity of the parts of the digestive tract examined. Nevertheless, in order to get some approximate idea of activity, it was decided to proceed with the assay as though breakdown of tissues occurred uniformly. Any effect of the intestinal flora must also be taken into account. However, since all the tissues were treated similarly, emptied of contents and the mucosal surfaces scraped and thoroughly washed, we hoped that any enzyme activity deriving from bacteria would be reduced to a minimum.

Pectoral and psoas muscles were examined individually and the values presented are means of the two.

Freezing and homogenizing procedures

All tissues were treated in identical fashion. At dissection, they were freed from blood by rinsing in cold 0.9% saline, dried between layers of filter paper, coarsely minced with scissors or scalpel blade, and quick-frozen in solid CO_2 (−80°). Within a few days they were pulverized as described earlier [1] and homogenized at 0° in an Ultra-Turrax homogenizer with a 10-N shaft (Sartorius Instruments, Surrey, U.K.) for 5–10 sec at top speed. A 10% (w/v) homogenate was prepared with 0.1 M potassium phosphate buffer (K_2HPO_4/KH_2PO_4, pH 7.2), divided into aliquots and stored at −20° until required.

Assay procedures

The basic procedure employed was the radiometric microassay (extraction method) described earlier [1]. With Bz as substrate, the assay tube contained, in a total volume of 240 μl: 0.1 M Tris buffer (pH 9.0), 100 μl; inhibitor solution or water, 100 μl; tissue homogenate, 20 μl; and substrate, 20 μl. With PEA or 5-HT as substrates, the assay tube contained, in a total volume of 140 μl: 0.1 M potassium phosphate buffer (pH 7.2), 100 μl; tissue homogenate, 20 μl; and substrate, 20 μl. The final concentration of substrate in the assay tube was 42 μM for Bz, 150 μM for PEA and 371 μM for 5-HT. Inhibitors used were (-)-deprenyl (final concentration 4×10^{-7}M) or phenelzine (final concentration 2.2×10^{-6}M). Water blanks were prepared by substituting water for tissue homogenate. Where inhibitors were used, the mixture was preincubated at room temperature for 20 min before labelled substrate was added. Incubation was carried out for 30 min in a shaking water bath at 37°, and the reaction stopped by adding 2 M citric acid (100 μl) to the solution, which was immediately mixed on a Vortex mixer at top speed. Three millilitres of toluene (Bz, PEA) or ethyl acetate/toluene 1:1 (5-HT) was used for extraction. The remaining steps were as described previously [1]. Blanks gave radioactivity counts representing the following percentages of total substrate activity: Bz ≤ 0.5 per cent, PEA ≤ 3 per cent, 5-HT ≤ 0.01 per cent. Extraction efficiency with the three substrates, tested in 4–6 different human tissues, gave closely similar values for all tissues with each substrate. The mean extraction efficiency was 95, 96.2 and 91.2 per cent, respectively, for Bz, PEA and 5-HT, and has been corrected for in the specific enzyme activities quoted in this paper.

Both Bz and PEA were used to determine MAO B activity in each tissue tested. Phenelzine, used in all experiments as an inhibitor of BzAO, was potent, but relatively non-selective, with poor replication of results. Whilst it served to confirm the presence of BzAO at the concentration employed, it was not able to separate the different enzyme forms as successfully as deprenyl or clorgyline [12].

All assays were carried out at least in duplicate; results express the means of these replicates. All assays on every series of tissues from a particular subject were carried out on the same day. The Lowry method [13] was used to determine total tissue protein, with bovine serum albumin as standard.

Estimation of total tissue or organ activity

This was based on total protein content of the tissue, multiplied by specific activity of the particular enzyme. The values for organ or tissue percentages of total body weight used in these calculations (Table 5) were taken from various sources [14–19]. Where such data were unobtainable (e.g. for the digestive tract), they were supplemented by our own measurements and those kindly supplied for some fetal tissues by the fetal tissue bank of the Royal Marsden Hospital (Dr. L. Wong).

Total body weights used in these calculations were as follows: fetus, 300 g; neonate, 3000 g; adult, 70 kg.

Variability of method

Replicate determinations on aliquots of 2 neonatal tissues (lung = 11; liver = 10) provided additional controls of substrate and inhibitor efficacy. One previously unthawed aliquot of each of these tissue homogenates was assayed together with a series of tissues. Mean specific activity of lung was 22.7 (±6%), 2.5 (±6%), 5.3 (±5%) and 25.8 (±8%) nmoles/mg protein/30 min for deprenyl-resistant Bz-oxidizing activity, D*Bz (deprenyl-sensitive Bz-oxidizing activity), PEA and 5-HT, respectively. The corresponding values for liver were 4.1 (±5%), 77.4 (±5%), 22.9 (±5%) and 56.3 (±6%), respectively. Percentages between brackets represent the standard error of the mean.

RESULTS

We have interpreted the enzyme activities observed with Bz, PEA and 5-HT as follows:

(a) Bz—activity insensitive to 4×10^{-7}M deprenyl = BzAO;
(b) Bz—activity sensitive to 4×10^{-7}M deprenyl = MAO B;
(c) PEA—activity in all human tissues except vessels, placenta and (partly) lung = MAO B;
(d) 5-HT—activity in all human tissues = MAO A.

Bz is a substrate for both BzAO and MAO B [1], but is a very poor substrate for MAO A [12]. Both PEA and 5-HT can be substrates for MAO A and B, and at higher substrate concentrations each becomes less specific [20]. The use of a particular substrate at a single concentration may therefore give only an approximate indication of the activity of a particular enzyme form.

Concerning (a) above, it is true that 10^{-4}M deprenyl inhibits both MAO A and B activity, whereas 10^{-7}M will inhibit the latter but not the former. We have chosen the lower concentration, however, because fetal tissues seem to be more sensitive to the inhibitor than neonatal or adult tissues: in several experiments on fetal tissues with 10^{-4}M deprenyl, Bz-oxidizing activity was inhibited in all tissues to a similar degree. Moreover, in the adult human lung, rich in MAO A, there was no difference in Bz activity at 10^{-7} and 10^{-4}M deprenyl [12]. The question arises, however, whether the deprenyl-insensitive Bz activity we detect could be partly catalyzed by MAO A. We think it very unlikely that MAO A contributes more than a trace of activity because we have shown that Bz is a very poor substrate for human MAO A, e.g. in placenta (K_m = 400 μM; V_{max} = 0.7 per cent of that for 5-HT) [12]. Moreover, we have found no relation between the distribution patterns of MAO A and deprenyl-insensitive Bz activity; in fetal brain and adult cerebral vessels, both totally insensitive to deprenyl, the ratio of Bz to 5-HT-oxidizing activity was 0.03 and 5.8, respectively (see also Discussion).

As for (c) above, both our work and that of others [12, 21–23] has produced evidence for major or total deamination of PEA by MAO A in a few tissues only. In the vast majority of tissues examined by us, the bulk of activity elicited with PEA as substrate

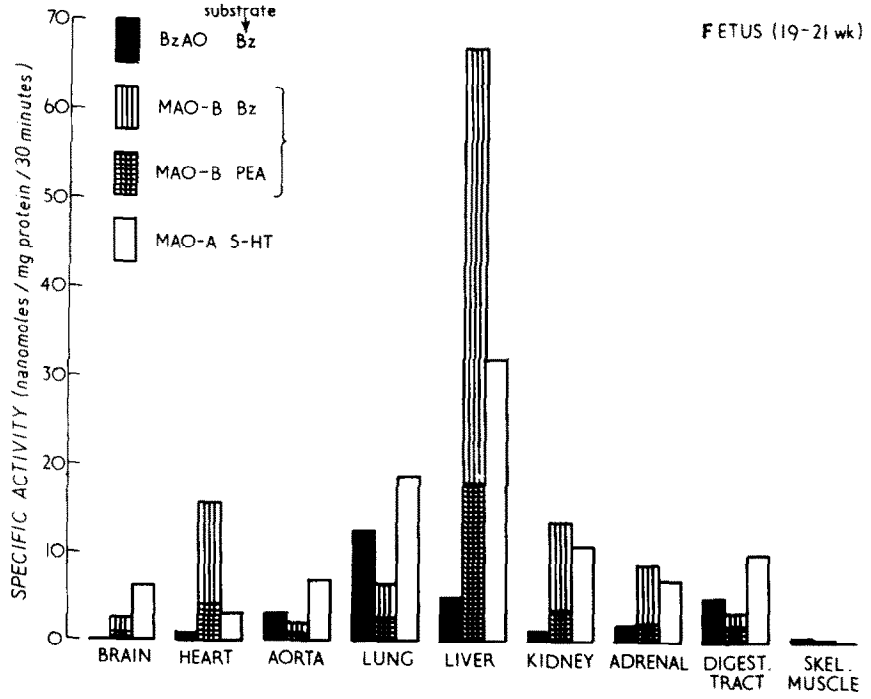

Fig. 1. Distribution of BzAO, MAO B and MAO A in the human fetus (19–21 weeks). For assay conditions, see Materials and Methods. Middle column shows MAO B activity assayed with Bz and PEA; total height of column = D*Bz, deprenyl-sensitive moiety of benzylamine-oxidizing activity; cross-hatched portion = activity registered with PEA as substrate. For interpretation of PEA activity, see text.

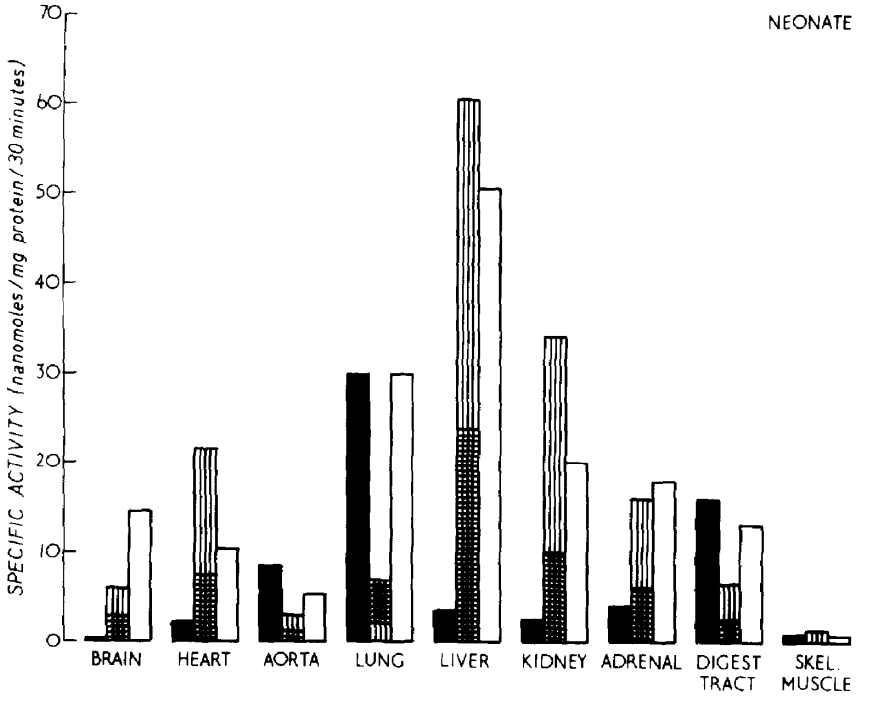

Fig. 2. Distribution of BzAO, MAO B and MAO A in the human neonate. Legends and observation as in Fig. 1.

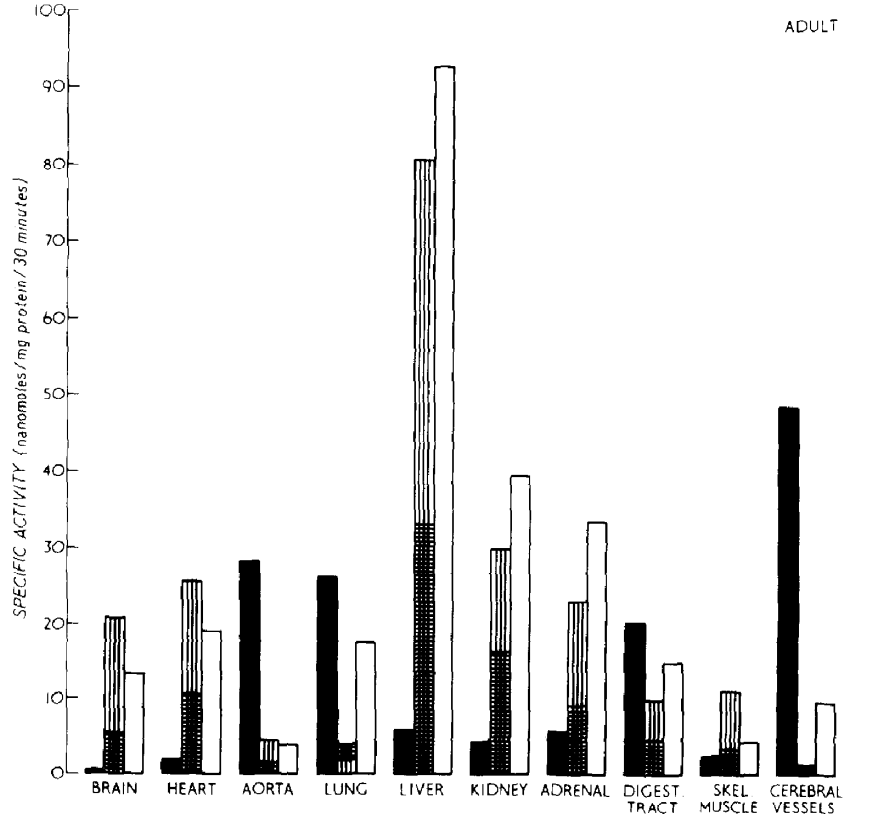

Fig. 3. Distribution of BzAO, MAO B and MAO A in the human adult. Legends and observation as in Fig. 1.

was in all likelihood predominantly due to MAO B. Though variable in the various tissues, the D*Bz/PEA ratio [mean 2.74 ± 0.24 (S.E. of mean), 2.66 ± 0.31 and 2.48 ± 0.21 for fetal, neonatal and adult tissues, respectively] was significantly different only in umbilical vessels (0.4), placenta (0.26), lung (0.4) and one or two other tissues (see ref.12). A low specific activity was found in cerebral vessels with PEA as substrate, but D*Bz gave no detectable activity. On the other hand, no activity could be registered with PEA as substrate in several tissues with low D*Bz values, such as fetal tongue, fetal and neonatal muscle and skin, adult skin and a few others. Oxidation of PEA is undoubtedly less sensitive or reliable than D*Bz as a measure of MAO B activity; nevertheless we feel justified in using PEA-deamination in most human tissues as a rough indication of the activity of MAO B.

The specificity of 5-HT as a substrate for MAO A, on the other hand, is well established, notwithstanding several reports on the deamination of the substrate by MAO B. Edwards and Chang [20] considered 5-HT a poor substrate for MAO B in the human platelet, since its K_m was much higher than that observed for either rabbit platelets or rabbit brain regions. Other authors have shown 5-HT oxidation by MAO B in beef heart [24], certain circumventricular regions of the rat [25] and rat area postrema [26]. From all these reports it seems obvious, however, that the contribution of MAO B to the deamination of 5-HT is slight, unless high concentrations of 5-HT are employed. At the substrate concentration used in our experiments, it is likely that 5-HT was deaminated almost exclusively by MAO A.

Enzyme distribution

The distribution of BzAO, MAO A and MAO B in human tissues at three stages of development is shown in Tables 1–3. All three enzyme forms were detectable in every tissue studied, apart from MAO B in cerebral vessels. It is clear from these tables and Figs. 1–3 that each has its distinctive distribution and developmental pattern. In almost all tissues, the specific activity is lowest in the fetus, intermediate in the neonate and highest in the adult. In certain instances, such as BzAO in aorta or MAO B in brain and skeletal muscle, there is a 10-fold or greater increase in specific activity between fetus and adult. In no case (except for MAO B in lung) is there any substantial decrease with age. The developmental pattern varies from tissue to tissue, however, and there is relatively little change, for example, in the specific activity of the three enzyme forms between fetus and adult in the heart, and even less between the specific activity of BzAO and MAO B in fetal and adult liver.

Figures 1–3 show activity obtained with PEA, compared with deprenyl-sensitive activity using Bz as substrate (D*Bz) as a measure of MAO B. D*Bz gives a higher specific activity than PEA throughout (see above).

BzAO (Table 1). The findings in the present study confirm and extend our earlier data [1]. BzAO is most active in adult cerebral vessels (not done in fetus or neonate), by far the highest value for this enzyme in any human tissue. Activity is also high in aorta, lung and digestive tract. BzAO activity in blood vessels, which we commented upon earlier [1], is the subject of a separate study (in preparation). The extremely low activity in brain, the lowest of any adult tissue studied, may derive from traces of vascular tissue. Specific activity is also low in liver, heart, kidney and skeletal muscle, as well as fetal skin. Values for fetal scalp (not done in neonate or adult) are similar to those for other skin areas.

Table 1. Specific activity of BzAO in human tissues (nmoles/mg protein/30 min). Residual activity with Bz as substrate (42 μM) after inhibition with deprenyl (4×10^{-7}M)

Tissue	Fetal		Neonatal		Adult	
	Mean	Range	Mean	Range	Mean	Range
Brain	0.2	0.1–0.4	0.4	0.36–0.44	0.5	0.4–0.6
Cerebral vessels					47.8	33.2–55.3
Heart	1.0	0.3–1.5	2.1	1.6–2.8	1.9	1.5–2.8
Aorta	2.9	0.6–5.7	8.0	4.0–11.0	27.9	20.0–41.1
Lung	12.5	7.4–19.2	29.7	22.9–38.1	25.4	19.8–31.0
Liver	5.2	3.4–6.8	3.4	2.2–5.0	6.0	3.4–13.8
Kidney	0.8	0.1–1.3	2.4	1.4–3.2	4.6	3.5–5.3
Adrenal	2.1	0.5–3.9	3.9	2.1–6.8	5.2	2.5–7.9
Spleen	0.5	0.3–0.8	1.0	0.8–1.3	2.8	2.3–3.2
Tongue	0.6	0.1–1.6	1.3	0.6–2.1	4.1	3.8–4.4
Oesophagus	9.7	6.8–15.6	11.3	7.0–16.8	23.0	22.1–24.0
Stomach	5.3	0.7–12.3	13.5	7.1–21.5	19.7	16.8–24.1
Ileum	2.7	1.7–4.2	17.8	8.7–24.2	15.6	9.6–20.6
Colon	2.5	0.5–4.7	20.1	14.0–29.5	19.2	11.4–26.9
Mesentery and mesocolon*	5.0	1.9–8.2	6.5	2.8–16.6	23.5	22.6–24.3
Pancreas	0.8	0.4–1.3	2.8	1.2–4.0	4.5	N=1
Skeletal muscle*	0.6	0.4–0.7	0.9	0.5–1.4	2.0	1.4–2.9
Diaphragm	0.7	0.1–1.4	2.3	1.8–2.8	3.3	1.5–4.1
Skin	0.5	0.02–1.3	2.7	1.3–4.6	1.7	1.1–2.4

* Pooled values from individual assays (see Materials and Methods, Tissues). For assay procedure, see Materials and Methods.

Table 2. Specific activity of MAO B in human tissues (nmoles/mg protein/30 min)*

Tissue	Fetal Mean	Fetal Range	Neonatal Mean	Neonatal Range	Adult Mean	Adult Range
Brain	2.7	1.6–4.1	6.1	5.4–7.0	21.1	13.8–29.4
Cerebral vessels					‡	‡
Heart	15.4	7.1–19.8	21.4	20.5–22.6	25.2	23.5–28.3
Aorta	2.2	0.8–3.9	3.1	2.0–4.2	4.4	1.8–7.0
Lung	6.6	4.2–9.1	2.4	0–4.9	1.8	0–3.5
Liver	66.4	50.4–99.2	59.8	51.4–66.4	80.0	49.4–123.6
Kidney	14.1	6.0–24.8	33.5	22.6–49.2	29.5	24.1–32.8
Adrenal	7.8	4.9–10.1	16.6	10.1–20.2	22.6	22.4–22.8
Spleen	1.3	0.1–2.8	0.5	0.5–0.6	3.4	2.6–4.3
Tongue	0.6	0.3–0.9	3.5	2.0–4.7	8.2	7.2–8.7
Oesophagus	1.8	0.5–2.6	6.6	4.7–8.9	8.5	6.7–10.0
Stomach	3.0	1.1–5.9	3.4	1.1–6.2	8.4	5.9–12.7
Ileum	2.8	2.7–3.0	10.1	4.6–17.5	18.1	12.0–27.3
Colon	3.1	1.2–6.1	3.4	3.1–3.9	11.8	9.7–13.9
Mesentery and mesocolon†	8.1	3.4–10.8	3.9	2.3–6.0	12.1	11.1–13.1
Pancreas	4.4	2.4–5.7	3.4	2.3–4.7	3.0	N = 1
Skeletal muscle†	0.4	0.2–0.7	1.6	0.9–2.2	11.2	10.9–11.8
Diaphragm	0.8	0.1–1.7	9.3	7.5–11.2	10.9	8.4–13.3
Skin	0.5	0.2–0.9	2.7	1.9–3.2	1.7	1.4–1.9

* Activity expresses moiety sensitive to 4×10^{-7}M deprenyl (D*Bz), with Bz as substrate (42 μM). For assay procedure, see Materials and Methods.

† See footnote, Table 1.

‡ No detectable activity.

MAO B (Table 2). Liver, at all stages of development, showed the highest specific activity for MAO B of any human tissue studied. While there is virtually no change in specific activity of cardiac MAO B from fetus to adult, activity in kidney and adrenal increases sharply. In brain, MAO A predominates at the fetal and neonatal stages, but MAO B becomes dominant in the adult. Adult kidney, heart, adrenal and brain are relatively rich in MAO B; considerable activity is also found in adult digestive tract and skeletal muscle.

MAO A (Table 3). In fetal brain, MAO A emerges before MAO B. Specific activity of hepatic MAO A rises steadily from fetal to adult stage. Excepting only the term human placenta [12], adult liver is the human tissue showing by far the highest activity of MAO A. Although all fetal MAO A specific activity is relatively low, compared with the adult, fetal liver, lung, kidney, adrenal, aorta and digestive tract show relatively high values. Peak MAO A activity in brain, lung and colon is seen in the neonate.

Ratio MAO A/B (Table 4). The relative tissue pro-

Table 3. Specific activity of MAO A in human tissues (nmoles/mg protein/30 min)*

Tissue	Fetal Mean	Fetal Range	Neonatal Mean	Neonatal Range	Adult Mean	Adult Range
Brain	6.7	3.6–13.5	14.5	11.5–18.9	12.9	8.7–16.9
Cerebral vessels					8.3	6.4–11.3
Heart	2.9	2.1–4.0	10.2	8.7–10.9	19.0	15.7–23.8
Aorta	6.8	2.4–15.8	5.1	4.0–6.0	4.1	3.2–5.6
Lung	18.2	14.2–21.8	29.9	25.8–36.9	17.4	9.4–25.8
Liver	31.4	14.6–55.0	50.2	27.4–74.8	92.0	29.8–215.4
Kidney	11.0	7.5–14.3	19.6	9.3–25.7	38.8	37.1–41.9
Adrenal	7.1	4.8–8.0	17.5	5.1–28.3	33.4	30.3–36.5
Spleen	3.8	2.9–5.1	0.8	0.3–1.2	1.8	1.2–2.4
Tongue	1.8	0.7–2.5	3.9	1.6–6.2	12.9	12.4–13.1
Oesophagus	15.6	10.3–24.0	7.8	4.4–12.5	18.6	10.2–21.6
Stomach	11.2	8.9–13.4	9.1	8.0–11.1	8.2	7.1–9.7
Ileum	4.3	3.8–5.2	13.5	11.6–16.4	15.8	12.7–17.7
Colon	6.8	2.5–10.9	19.8	15.5–25.2	13.5	13.1–13.8
Mesentery and mesocolon†	11.7	6.7–19.4	4.9	3.5–5.7	28.0	21.9–34.0
Pancreas	17.9	11.2–20.3	12.4	7.3–19.6	26.3	N = 1
Skeletal muscle†	0.3	0.2–0.7	0.6	0.2–1.3	4.3	1.8–6.4
Diaphragm	1.1	0.6–3.1	3.6	1.5–5.7	6.8	5.6–7.9
Skin	2.0	0.3–3.0	4.5	3.8–5.9	0.7	0.7–0.8

* Substrate, 5-HT (371 μM). No inhibitor. For assay procedure, see Materials and Methods.

† See footnote, Table 1.

Table 4. Ratio MAO A/B (5-HT/D*Bz)*

Tissue	Fetal	Neonatal	Adult
Brain	2.43	2.39	0.61
Cerebral vessels			‡
Heart	0.19	0.47	0.75
Aorta	3.15	1.65	0.93
Lung	2.77	12.27	9.78
Liver	0.47	0.84	1.15
Kidney	0.78	0.59	1.31
Adrenal	0.91	1.06	1.48
Spleen	2.98	1.44	0.52
Tongue	2.88	1.12	1.58
Oesophagus	8.46	1.17	2.56
Stomach	3.78	2.65	1.0
Ileum	1.53	1.35	0.87
Colon	2.22	5.77	1.14
Mesentery and mesocolon†	1.45	1.26	2.31
Pancreas	4.08	3.62	8.89
Skeletal muscle†	0.75	0.41	0.39
Diaphragm	1.31	0.39	0.62
Skin	4.39	1.70	0.44

* Substrates as in Tables 2 and 3.
† See footnote, Table 1.
‡ No detectable MAO B activity.

portions of MAO A and B, as determined by the 5-HT/D*Bz ratio, are shown in Table 4. In general, the A/B ratio is higher in fetus than adult. This phenomenon is particularly noticeable in brain and oesophagus, where the A/B ratio in the fetus is 4 times greater than that of the adult; the ratio in skin is ten times greater in fetus than adult. In the adult, with the exception of placenta (see ref. 12), the lung has the highest A/B ratio.

Total activity in tissue or organ (Table 5). Although specific enzyme activity in a given tissue may be low, the activity of the enzyme in the total tissue or organ mass—and, therefore, in the total body economy—may be considerable, as shown in Table 5. Obviously, estimates of total activity such as those shown can, at best, be only a rough approximation and should be approached as indicating orders of magnitude rather than precise values.

DISCUSSION

It is evident from the data presented here that the distribution and development of the copper enzyme [4] BzAO, and the flavin enzyme forms [4] MAO A and B differ in every tissue.

By demonstrating a series of ontogenetic differences not only between BzAO and monoamine oxidase, but also between MAO A and MAO B, support may be provided for the view that MAO A and MAO B are different functional enzymic entities. We have also produced evidence to show that Bz (D*Bz) is a far more sensitive and reliable substrate for MAO B than PEA (Figs. 1–3). In general, D*Bz measurement gives higher absolute values for MAO B specific activity than PEA. An exception is the lung in neonate and adult (Figs. 2 and 3). As we have shown [12], PEA in adult lung preparations is metabolized partly by MAO A and partly by MAO B. This experiment was not carried

Table 5. Total enzyme activity of organ or tissue (μ moles)*

Tissue		Per cent of body weight	Increase in total protein (F = 1)	BzAO	MAO B (D*Bz)	MAO B (PEA)	MAO A
Skeletal muscle	F	25		4	3	0	2
	N	25	40	264	438	0	180
	A	43	730	10,602	59,370	16,857	23,006
Liver	F	4		10	133	36	63
	N	4.2	14	94	1634	651	1386
	A	2.1	150	1764	23,520	9643	27,048
Digestive tract	F	3.8		3	3	1	6
	N	3.5	9	145	69	38	142
	A	4.2	300	5027	4259	1728	4136
Lungs	F	2		6	3	1	9
	N	2	16	235	19	55	237
	A	1.2	255	3293	231	530	2258
Brain	F	13.4		0.6	7	2	18
	N	11.7	19	20	308	149	734
	A	2.3	85	119	4667	1216	2850
Kidneys	F	0.7		0.2	3	0.8	2
	N	0.8	17	9	121	35	71
	A	0.4	250	242	1552	859	2040
Heart	F	0.6		0.2	3	1	0.6
	N	0.7	13	6	59	20	28
	A	0.5	260	109	1416	582	1069
Skin	F	13		2	2	0	7
	N	15	10	108	105	0	179
	A	7	120	757	739	0	326
Aorta	A	0.14		251	39	13	37
Plasma	A	4.2		37	—	—	—

* For assay procedure and calculations, see text. F = fetus; N = neonate; A = adult.

out with neonatal lung, although a similar situation seems likely to prevail (Fig. 2). As for adult cerebral vessels (Fig. 3), no D*Bz activity was registered, and the deamination of PEA is undoubtedly catalyzed by MAO A, as in the case of placenta [12].

The D*Bz/PEA ratio varies in the tissues studied (Figs. 1–3). This may be explained in part by MAO B sensitivity towards deprenyl with Bz as substrate, which in most tissues differs with the developmental stage. The main reason, however, may well be that MAO A contributes to a variable extent to PEA deamination in tissues other than the lung and vessels.

The argument that Bz might be oxidized to some extent by MAO A is mainly based on the work of Lyles and Callingham [27–29]. These authors found that rat heart (but not the human heart, ref. 30) has a Bz-oxidizing activity which gives a biphasic curve when clorgyline is used as inhibitor, suggesting the presence of mitochondrial MAO in all the fractions studied. Similar results were obtained in our experiments with human placenta and lung [12]. Since all these tissues are very rich in MAO A, it is not impossible that this enzyme contributed to the deamination of Bz in these experiments. Another explanation, however, is equally plausible: clorgyline may be less selective for MAO A than has been postulated, and in certain tissues it may inhibit the activity of BzAO. Lyles and Callingham [28] do not entirely dismiss this possibility, although they point out that the clorgyline-sensitive moiety of Bz-oxidizing activity, whilst sensitive to semicarbazide, was insensitive to cyanide, a selective inhibitor of plasma amine oxidase. A similar sensitivity pattern, with tyramine as substrate, has been shown by Coquil *et al.* [31] in a component of rat artery amine oxidase, suggesting that rat tissues in general may be insensitive to cyanide.

Support for our interpretation of inhibition of BzAO by clorgyline is afforded by Houslay and Tipton's experiments with a purified preparation of beef plasma and Bz as substrate, in which clorgyline was shown to be a reversible inhibitor of BzAO [32]. These authors stress the need for care in avoiding blood-contamination of the tissues studied. As pointed out in our previous paper, however [1], contamination with blood is unlikely to be a cause of substantial error in the interpretation of results, since the specific activity of plasma BzAO is negligible, compared with that of most tissues. If there is contamination, it is far more likely to be due to the presence of vascular fragments, particularly in crude homogenates, a possibility pointed out by Lyles and Callingham [28].

BzAO. Fragments of vascular tissue may account for the relatively high BzAO value in fetal liver, where specific activities of BzAO and MAO B (with Bz but not PEA as substrates—see Fig. 1) are similar to those found in the adult (Tables 1 and 2, Fig. 3), suggesting that at 19–21 weeks gestation, these enzyme systems have reached a comparable level of maturity. Nor is this surprising, in view of the intense metabolic activity of the fetal liver. Vascularization is well advanced at this stage, when the need for blood vessels must be similar to, if not greater than, that of the adult organ [33, 34]. In the fetus, indeed, total BzAO activity in the liver equals that of all the other main tissues together (Table 5).

Tongue, diaphragm and skeletal muscle show values sufficiently similar at all three stages of development to indicate that BzAO activity in striated muscle does not differ, whatever the origin of the muscle. Conversely, the wide scatter of BzAO values noted in the different parts of the fetal digestive tract narrows with development and, in the adult, specific activities appear to belong to a homogeneous system.

Except for lung, liver, aorta and digestive tract, specific activity of BzAO is very low in the fetus. Evidence continues to mount suggesting an association of this enzyme with blood vessels. The localization of BzAO in blood vessel walls of human placenta and rat liver has recently been demonstrated histochemically in these laboratories: intense staining was present in the media, whilst the endothelium appeared unstained [35]. One possible explanation for the increase in specific activity of BzAO from fetal to adult stage might be the increase in vascularity of the tissues examined. This interpretation may be particularly relevant to the gastric musculature of the fetus, which is somewhat deficient, and the fetal intestine with its relatively greater thickness of mucosa compared to muscle [36].

The virtual absence of BzAO in brain parenchyma is of particular interest, for another copper enzyme of parallel function, diamine oxidase, has also not been demonstrated in this tissue in rabbit, guinea-pig and rat [45].

MAO A and B. Like BzAO, MAO A and B are widely distributed in the fetus. Although MAO B activity in fetal brain is barely demonstrable, specific activity of MAO A at 20 weeks' gestation is already about one-half that of the adult. The emergence of MAO A before MAO B in the fetal brain has also been demonstrated in rat [37] and mouse [38], and seems to cut across the view [39] that MAO B is a pure protein bound in a lipid environment and capable of being converted into MAO A with an additional lipid complement. If this hypothesis were correct, MAO B might have been expected to precede MAO A ontogenetically.

Our results agree with those of Suzuki and Yagi [40], who found MAO A values similar to those shown by us in human fetal and adult brain. However, their values for MAO B using PEA as substrate are higher than ours, perhaps reflecting the higher substrate concentration they employed, which might have resulted in some deamination by MAO A.

Studies of human fetal liver with a variety of substrates [41] have revealed some fluctuation of activity between the 16th and 32nd week of gestation; from then on there was a steady increase until birth. Since Bz, 5-HT and tyramine were among the substrates employed, the enzymes involved were, presumably, MAO B and A.

Our study shows that, in human tissues, the development of the activity of the three enzymes investigated is related to functional rather than morphological maturity. Differing rates of functional and morphological ontogenesis of enzyme systems are well described in man [36, 42] and rat [33, 43, 44] and seem likely to represent a widespread phenomenon.

Specific activity of MAO A in fetal liver is one third that of the adult, whilst that of pulmonary MAO A, similar in fetus and adult, shows a rise in the neonate. Perhaps the fetal lung and digestive tract contribute to the deamination of 5-HT to a relatively greater extent than in the adult, with the lung playing a particularly prominent role.

The widespread distribution of MAO B is perhaps more puzzling. Because of the absence of diamine oxidase in brain [45], mentioned above, one of its major functions may be related to the metabolism of *tele-N*-methylhistamine, the major histamine metabolite in this tissue. Recent research has shown that *tele*-methylhistamine is a specific substrate for MAO B in both rat [46, 47] and man (J. D. Elsworth, V. Glover and M. Sandler, in preparation). It would be of interest to determine the extent to which peripheral MAO B, for example in skeletal muscle, is present to inactivate local *tele*-methylhistamine produced from the decarboxylation of muscle *tele*-methylhistidine [48, 49].

Total enzyme activity. The estimated total activity of the three enzymes (Table 5), most striking in skeletal muscle, deserves comment. The very large increase in activity between fetus and adult seems to shed new light on the importance of these enzymes, and, in particular, of skeletal muscle amine oxidase, in the total body economy. No doubt the increase in mass from accretional growth accounts in part for the increase from total fetal to adult activity seen e.g. in skeletal muscle and digestive tract.† Increase in vascular tissue probably also contributes substantially to the increase both in specific and total activity of BzAO between fetus and adult. The total activity of MAO B (D*Bz) in adult skeletal muscle is about twice the total activity of this enzyme in all the other major organs. Even with PEA as substrate, the total value for MAO B in skeletal muscle exceeds the activity of all the other tissues together (Table 5). Estimated total activity of MAO A in skeletal muscle is second only to that in the liver, in which the specific activity of MAO A is so high that the 1.5 kg mass of the adult produces a greater total activity than the 20-fold larger mass of skeletal muscle. On the other hand, the entire digestive tract develops only about 15 per cent of the MAO A activity of the adult liver.

Despite their semiquantitative nature, these figures may provide a more realistic insight into the relative importance of enzyme activity in the round, compared with the more limited perspective provided by specific activity alone.

Far from simplifying the issue, these observations seem to add a further dimension of complexity to our knowledge of the amine oxidases in the human body. One conclusion, however, is implicit in the relatively low specific activity of all three enzymes in fetal tissues: neither BzAO nor MAO A or B can be related to rapid growth in tissues *per se*.

Acknowledgements—The generous help of Dr. P. M. Sutton (University College Hospital Medical School, London), Dr. J. Pryse-Davics and Dr. Gillian S. Gau (Institute of Obstetrics and Gynaecology, Queen Charlotte's Maternity Hospital, London), is gratefully acknowledged. We thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brasília, Brazil (Grant No. 1112.2356/76) and The Wellcome Trust, London, U.K. (Grant No. 7085/I.5) for defraying the salary of R.L., and the Parkinson's Disease Society, London, for that of V.G.

† Including oesophagus, stomach, intestine and mesentery.

REFERENCES

1. R. Lewinsohn, K.-H. Böhm, V. Glover and M. Sandler, *Biochem. Pharmac.* **27**, 1857 (1978).
2. R. Lewinsohn, *Clin. chim. Acta* **81**, 217 (1977).
3. E. Costa and M. Sandler (Eds.), *Monoamine Oxidases—New Vistas. Advances in Biochemical Psychopharmacology*, Vol. 5. Raven Press, New York (1972).
4. H. Blaschko, *Rev. Physiol. Biochem. Pharmac.* **70**, 83 (1974).
5. D. L. Murphy and C. H. Donnelly, in *Neuropsychopharmacology of Monoamines and Their Regulatory Enzymes. Advances in Biochemical Psychopharmacology* (Ed. E. Usdin), Vol. 12, p. 71. Raven Press, New York (1974).
6. G. E. W. Wolstenholme and J. Knight (Eds.), *Monoamine Oxidase and its Inhibition. Ciba Foundation Symposium* No. 39. Elsevier-Excerpta Medica-North-Holland, Amsterdam (1976).
7. M. D. Houslay, K. F. Tipton and M. B. H. Youdim, *Life Sci.* **19**, 467 (1976).
8. C. J. Fowler, B. A. Callingham, T. J. Mantle and K. F. Tipton, *Biochem. Pharmac.* **27**, 97 (1978).
9. T. P. Singer, D. L. Murphy and R. W. von Korff (Eds.), *Monoamine Oxidase: Structure, Function and Altered Functions*. Academic Press, New York, in press.
10. H.-Y. T. Yang and N. H. Neff, *J. Pharmac. exp. Ther.* **187**, 365 (1973).
11. P. Bowen-Simpkins, *J. Obstet. Gynaec. Br. Commonw.* **80**, 824 (1973).
12. R. Lewinsohn, V. Glover and M. Sandler, *Biochem. Pharmac.* **29**, 777 (1980).
13. D. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
14. K. Diem and C. Lentner (Eds.), *Scientific Tables (Documenta Geigy)*, 7th Edn., pp. 518 and 710. Geigy Pharmaceuticals, Macclesfield, U.K. (1975).
15. E. L. Potter and J. M. Craig, in *Pathology of the Fetus and Infant*, 3rd Edn., pp. 18 and 21. Year Book Med. Publ., Lloyd-Luke, London (1976).
16. P. Gruenwald, *Adv. reprod. Physiol.* **2**, 279 (1967).
17. W. S. Spector, (Ed.), *Handbook of Biological Data*, p. 70. W. B. Saunders, Philadelphia (1956).
18. J. W. T. Dickerson and E. M. Widdowson, *Biochem. J.* **74**, 247 (1960).
19. E. M. Widdowson and J. W. T. Dickerson, *Biochem. J.* **77**, 30 (1960).
20. D. J. Edwards and S.-S. Chang, *Life Sci.* **17**, 1127 (1975).
21. T. Egashira, *Jap. J. Pharmac.* **26**, 493 (1976).
22. E. D. Dial and D. E. Clarke, *Biochem. Pharmac.* **27**, 2374 (1978).
23. O. Suzuki, Y. Katsumata, M. Oya and T. Matsumoto, *Biochem. Pharmac.* **28**, 953 (1979).
24. T. J. Mantle, M. D. Houslay, N. J. Garrett and K. F. Tipton, *J. Pharm. Pharmac.* **28**, 667 (1976).
25. J. S. Kim, M. Hirano, H. Uchimura, M. Saito, T. Matsumoto and T. Nakahara, *J. Neurochem.* **32**, 253 (1979).
26. K.-H. Böhm, V. Glover, M. Sandler, M. Petty and J. L. Reid, *J. Neurochem.* **33**, 607 (1979).
27. G. A. Lyles and B. A. Callingham, *J. Pharm. Pharmac.* **26**, 921 (1974).

28. G. A. Lyles and B. A. Callingham, *J. Pharm. Pharmac.* **27**, 682 (1975).
29. G. A. Lyles and B. A. Callingham, *J. Pharm. Pharmac.* **31**, 755 (1979).
30. D. Parkinson and B. A. Callingham, *Biochem. Pharmac.* **27**, 2374 (1978).
31. J. F. Coquil, C. Goridis, G. Mack and N. H. Neff, *Br. J. Pharmac.* **48**, 590 (1973).
32. M. D. Houslay and K. F. Tipton, *Biochem. Pharmac.* **24**, 429 (1975).
33. J. T. Coyle and D. Henry, *J. Neurochem.* **21**, 61 (1973).
34. F. Elias, in *Development and Structure of the Cardiovascular System* (Ed. A. A. Luisada), p. 175. McGraw-Hill, New York (1961).
35. T. A. Ryder, M. L. McKenzie, J. Pryse-Davies, V. Glover, R. Lewinsohn and M. Sandler, *Histochemistry* **62**, 93 (1979).
36. C. A. Smith, *The Physiology of the Newborn Infant*, 3rd Edn., p. 230. Blackwell, Oxford (1959).
37. T. J. Mantle, N. J. Garrett and K. F. Tipton, *Fedn. Eur. biochem. Soc. Lett.* **64**, 227 (1976).
38. J. A. Diez and J. L. Maderdrut, *Brain Res.* **128**, 187 (1977).
39. K. F. Tipton, M. D. Houslay and T. J. Mantle, in *Monoamine Oxidase and its Inhibition* (Eds. G. E. W. Wolstenholme and J. Knight). *Ciba Foundation Symposium* No. 39, p. 5. Elsevier–Excerpta Medica–North-Holland, Amsterdam (1976).
40. D. Suzuki and K. Yagi, in *Maturation of Neurotransmission* (Eds. A. Vernadakis, E. Giacobini and G. Filogamo), p. 100. Karger, Basel (1978).
41. T. G. Nachushkina, *Zh. Evol. Biokhim. Fiziol.* **9**, 91 (1973).
42. R. Warwick and P. L. Williams (Eds.), *Gray's Anatomy*, 35th Edn. Longman, Edinburgh (1973).
43. R. Balazs, *Br. Med. Bull.* **30**, 126 (1974).
44. J. T. Coyle, in *The Neurosciences*, 3rd Study Program (Eds. F. O. Schmitt and F. G. Worden), p. 877. M.I.T. Press, Cambridge (1974).
45. R. Kapeller-Adler, *Amine Oxidases and Methods for their Study*, p. 104. Wiley-Interscience, New York (1970).
46. P. C. Waldmeier, J. J. Feldtrauer and L. Maître, *J. Neurochem.* **29**, 785 (1977).
47. L. B. Hough and E. F. Domino, *J. Pharmac. exp. Ther.* **208**, 422 (1979).
48. J.-C. Schwartz, C. Rose and H. Caillens, *J. Pharmac. exp. Ther.* **184**, 766 (1973).
49. I. B. Holbrook, E. Gross and M. H. Irving, *Br. J. Nutr.* **41**, 15 (1979).