The Development of Amine Oxidase Activity by Human Tissues after Birth

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Gale & Watchorn (unpublished results) found that conditions suitable for bacterial production of toxic amines may exist in the gut of the rat. Gale (1941) has shown that addition of tyramine to the diet produces a fatal diarrhoea in infant rats but that susceptibility to this amine disappears with age. Diets containing 2.5–20% free tyrosine are also toxic (Sullivan, Hess & Sebrell, 1932; Lillie, 1932; Martin, 1942) and it has been suggested (Martin, 1942) that the toxicity may be due to tyramine formation in the kidney of the rat. The adult animal is protected against toxic amines by the action of amine oxidase and it is consequently important to ascertain if the tissues of the newborn animal, whether rat or man, are deficient in this protective mechanism.

Ewins & Laidlaw (1910) found that tyramine is destroyed when it is perfused through pig's liver, the final product of the breakdown being *p*-hydroxyphenylacetic acid. Hare (1928) extracted an enzyme from liver and showed that in the presence of tyramine the O_2 uptake was increased, NH_3 and H_2O_2 being formed during the reaction; she later confirmed (Bernheim, 1931) that *p*-hydroxyphenylacetic acid was the final reaction product. Pugh & Quastel (1937), Richter (1937) and Kohn (1937) have produced evidence that the first stage in the oxidation is deamination of the amine to *p*-hydroxyphenylacetaldehyde and the name amine oxidase is now restricted to the enzyme responsible for this primary reaction. Blaschko, Richter & Schlossmann (1937) and Bhagvat, Blaschko & Richter (1939) have shown that amine oxidase is specific for monoamines in general, although the rate of attack varies widely for the different substrates. Of the amines tested, tyramine, *iso*amylamine and dihydroxyphenylethylamine are the most rapidly attacked; in all cases, 1 atom of oxygen is taken up per mol. substrate with the liberation of 1 mol. each of NH_3 and the appropriate aldehyde.

Amine oxidase is widely distributed in mammalian tissues and has been found in the liver, pancreas, heart, gut, spleen, thyroid, kidney, brain, testicle, uterus, adrenals, etc. of the cat, pig, sheep, ox, dog, rabbit, rat and guinea-pig; it is absent from the heart and brain of the ox and from the intestinal mucous membrane of the dog (Blaschko *et al.* 1937;

Bhagvat *et al.* 1939; Holtz, Heise & Spreyer, 1938; Pugh & Quastel, 1937; Werle & Mennicken, 1938). In man the enzyme is recorded for brain (Birkhäuser, 1940), liver (Holtz *et al.* 1938) and kidney (Holtz *et al.* 1938; Zeller, Stern & Wenk, 1940; Blaschko, 1942) and, by a study of the blood amine level after intravenous injection of *iso*amylamine, Richter, Lee & Hill (1941) find that adult man can deal adequately with amounts of this amine well beyond normal physiological occurrence.

Birkhäuser (1940) gives the brain amine oxidase activities of 4 'small children' compared with those of 20 adults, the latter classified as being over or under 60 years of age: the activities of the thalamus, nucleus and cortex were less for the children than for the adult groups, which were not significantly different from one another. Zeller *et al.* (1940), investigating kidney activities for adult and newborn man, found no variation in the medulla; the cortex of the infant was without amine oxidase activity while that of the adult was active; the number of observations made is not stated. Zeller, Birkhäuser, Mislin & Wenk (1939) have also studied the variation with age of histaminase activity in man; they found that the kidney medulla activity did not vary, while the activity of kidney cortex, liver, brain and adrenals all increased with age; the activity of newborn cortex and brain was negligible.

In the investigation reported below the tissues chosen were the liver, kidney (cortex and medulla) and mucosa from the duodenum, ileum and colon. An attempt has been made to determine whether there is a statistical difference between the activity of newborn and older tissues in man and, if there is such a difference, to find at what age activity is in general fully developed. The work was carried out on fresh post-mortem material at the Birmingham Children's Hospital.

METHODS

Extraction of enzyme. Philpot (1937) alternately froze and thawed rabbit liver, ground it in ice-cold Ringer's solution and strained the resulting mash through muslin; she found that the greater part of the amine oxidase activity lay in the sediment. Richter (1937) ground liver with sand and phosphate at pH 7.2, centrifuged the preparation and used the supernatant as a source of amine oxidase.

To find the best method of determining the total activity of a tissue, cat liver was ground in a mortar, suspended in $M/15$ -phosphate pH 7.5 and part of the mash centrifuged; the sediment was suspended in its original volume of buffer, and the activity of supernatant, sediment and whole mash tested. Both supernatant and sediment contained amine oxidase activity (Table 1), the latter being the more active,

Table 1. *Distribution of amine oxidase in liver (dog)*

Enzyme preparation	Activity (μ l. $O_2/10$ min.)
Supernatant after centrifuging	13
Sediment, resuspended	22
Whole mash	33

and later experiments were carried out with the whole mash after it had been rendered homogeneous by straining through muslin. In the estimation of kidney activities the cortex and medulla were separated by dissection and each then treated in the same way as the liver. In all experiments, measured portions of the preparations were dried to constant weight in a steam oven and activities expressed as $Q_{O_2} = \mu$ l. O_2 taken up/hr./g. dry weight tissue, the O_2 uptake being calculated from the initial rate (see below). All the 'Activity' values quoted are corrected for any 'blank' O_2 uptake.

As a general rule, the whole duodenum was used as a source of enzyme and the portions of the ileum and colon taken were those adjacent to the appendix. Richter (1937) opened the gut, left it to stand in a disinfecting solution for 10 min. and then ground it with sand. For this work, sand would have interfered with dry weight estimations, so the method of Zeller *et al.* (1939) was adopted, in which the mucous membrane is carefully scraped off and suspended in phosphate buffer. This treatment does not allow for enzymic activity in the gut wall but, if similar adjacent portions of the gut are treated in the two ways (Table 2), the greater part of the amine oxidase activity is found in the mucosa and the following results refer only to this

Table 2. *Disinfection and treatment of gut*

Source of gut	Enzyme preparation	Activity (μ l. $O_2/10$ min.)
Dog	Whole gut (1.8 g.)	12
	Mucosa layer from 1.8 g. gut	19
	Muscle layer from 1.8 g. gut	8
		Q_{O_2} of mucosa
Man, case 42.70	Gut washed without anti-septic; activity estimated in presence of semicarbazide	2780
	Gut disinfected with hydroxyquinoline; activity estimated in presence of semicarbazide	2870
Man, case 42.29	Gut disinfected with hydroxyquinoline; activity estimated in presence of semicarbazide	990
	Gut washed without anti-septic; activity estimated in presence of $M/300$ cyanide	970

mucosal activity. As a means of sterilization, Richter (1937) washed gut preparations in a 1:10,000 solution of 8-hydroxyquinoline, but experiments have shown that coliform organisms can still be isolated from gut treated in this way. $M/300$ -cyanide inhibits the oxidation of tyramine by bacteria (Gale, 1942), whereas amine oxidase is not affected by this concentration of cyanide (Philpot, 1937; Pugh & Quastel, 1937). Table 2 also shows gut activity (a) with and without treatment with 8-hydroxyquinoline and (b) with and without the treatment with $M/300$ -cyanide; there is no significant difference between the pairs of values obtained. Moreover, Gale (1942) found that bacterial suspensions oxidize tyramine only after a lag period proportional to the concentration of the substrate; under our conditions this lag period would greatly exceed the duration of the experiment. Hence it was decided not to attempt sterilization of the gut, and the preparations were merely washed thoroughly before separation of the gut mucosa.

Cadavers were brought to the mortuary within 3 hr. of death and kept in an ice-chest until autopsy; this was carried out each morning on children who died before 6 p.m. on the previous day. Tissues were taken at the time of autopsy and either used at once or, when several sets of material were available, the surplus was returned to the ice-chest until it was wanted. To discover the extent of deterioration between death and autopsy, it was possible on one occasion to obtain a piece of liver within 3 hr. of the child's death and there was no significant difference between the activity of this piece and that of the rest of the liver, removed 21 hr. later. As a further check, the activity of the whole range of tissues was estimated directly after autopsy and again after being kept in the ice-chest for 24 hr.; there was no significant difference between corresponding activities determined at the beginning and end of this period (Table 3). Full records have been kept of

Table 3. *Effect on amine oxidase activity of keeping human tissues in ice-chest*

Case no.	Tissue	Activity as Q_{O_2}	
		At autopsy	24 hr. later
42.23	Ileum	3500	4100
	Colon	3420	3030
	Liver	1210	1300
	Kidney medulla	1020	940
42.69	Ileum	1310	1280
	Colon	1290	1140
	Kidney cortex	850	900
	Kidney medulla	665	715

post-mortem findings but, except in two cases of nephritis for which activities are given later, there appears to be no correlation between the pathological condition of a tissue and its amine oxidase activity; uniformly low values were given by the tissues of two still-born children whose foetal hearts had last been heard 48 hr. and 7 days, respectively, before delivery.

After the above preparations had been carried out, estimations were made in Barcroft manometers at 37°. One manometer was used for both blank and experiment and the capacities of each pair of cups differed by less than 1%. $M/12$ semicarbazide was present to trap the aldehyde primarily formed. Previous workers have used $M/44$ tyramine,

but Hare (1928) records that concentrations above $m/100$ diminish the activity. With cat and dog liver $m/300$ was found to be the optimum concentration of substrate.

Manometer cups were therefore prepared with 2.5 ml. of enzyme preparation in $m/15$ phosphate pH 7.5, 0.2 ml. $m/2$ semicarbazide, 0.3 ml. $m/30$ tyramine solution in a Keilin 'dangler' cup (water in blank), and 0.4 ml. 10% NaOH in the centre cup. After equilibration, the contents of the Keilin cup were mixed with the main reaction fluid and readings were taken every 5, 10 or 15 min. All Q_{O_2} values were calculated from the initial rate (first 10 min.) as the rate decreases with time.

RESULTS

The activities of tissues from 58 children not more than 8½ years old were estimated; 27 were less than 3 months old and 53 less than 3 years. Since the activities for any one tissue show the expected wide degree of scatter, the cases were divided into age

the first 3 months of life than during the rest of the time covered by the experiment, and the mean activity steadily increases during those 3 months; (3) the mean activity of the duodenum is significantly less for children under 3 months than for older ones (the duodenum will be discussed more fully later since it is the only example in which the amine oxidase was absent in many cases); (4) the average activity of the liver during the first few weeks of life is less than it is afterwards; from the age of 1 month it remains substantially constant; (5) the mean activity of the colon is slightly less during the first 3 months than later, but no significant gradation can be calculated.

Table 5 gives the steady mean activity reached by each tissue with its standard deviation and range. The scatter is obviously very high for all tissues, being greatest for the ileum and least for the kidney

Table 4. *Variation of mean amine oxidase activities of tissues with age group*

For each tissue, italicized figures are significantly lower than roman figures.

Age group ...	Stillborn	0-2 weeks	0-1 month	0-3 months	1-3 months	3-6 months	6-12 months	1-9 years
No. of cases ...	5	15	19	27	8	6	8	16
Amine oxidase activity (Q_{O_2})								
Tissue								
Duodenum*	<i>452</i>	<i>277</i>	<i>323</i>	—	<i>828</i>	1279	1194	1011
Ileum	1738	1624	1558	1731	—	1442	2505	2260
Colon	<i>1122</i>	<i>1184</i>	<i>1228</i>	<i>1170</i>	—	1598	1694	1430
Kidney cortex	<i>253</i>	<i>423</i>	<i>430</i>	<i>522</i>	—	879	911	957
Kidney medulla	<i>242</i>	<i>323</i>	<i>334</i>	<i>458</i>	—	710	862	953
			2 weeks-1 month (4)		1-6 months (14)			
Liver	320	592	778		759		846	794

* See text for analysis of figures for duodenum.

groups and the mean activity within each group was calculated. A statistical determination was then made to discover in which age groups the mean activity was significantly below the steady mean value found to be reached by that tissue as its age increased: this is shown in Table 4, in which italicized figures are significantly lower than the others for the same tissue. From this table it appears that: (1) the mean activity of the ileum shows no significant variation with age; (2) the mean activity of the kidney cortex and medulla is lower during

cortex. Table 6 shows that an abnormally high activity in one tissue is not necessarily accompanied by high activities in other tissues from the same individual, and vice versa: in fact, it often happens that high activities in one or two tissues are associated with low values in others from the same case. Table 7 shows the abnormally low activities found for two stillborn children in whom the foetal hearts had last been heard 7 days and 24 hr., respectively, before delivery; it also shows the results obtained from two children with acute nephritis.

Table 5. *Mean oxidase activities of tissues at full development*

Tissue	Period	No. of cases	Amine oxidase activity (Q_{O_2})		
			Mean	Standard deviation	Range
Duodenum*	0-9 years	38	1151	471	255-2550
Ileum	0-9 years	56	1952	1017	380-5140
Colon	3 months-9 years	29	1530	710	710-3390
Liver	2 weeks-9 years	40	780	270	425-1670
Kidney cortex	3 months-9 years	27	928	176	650-1380
Kidney medulla	3 months-9 years	27	890	209	540-1300

* Zero activities have been omitted from this analysis.

Table 6. *Distribution of activities (Q_{O_2}) in sample cases*

Italicized figures represent activities greater than (mean + standard deviation), those in brackets less than (mean - standard deviation).

those in brackets less than (mean - standard deviation).						Kidney	
Case no.	Age (months)	Duodenum	Ileum	Colon Amine oxidase activity (Q_{O_2})	Liver	Cortex	Medulla
42.14	8	970	2500	1730	1670	900	1120
42.26	48	2550	1170	1060	670	1000	1040
42.36	16	(0)	5140	3390	930	(670)	770
42.53	22	(0)	1890	(710)	1280	1060	1300
42.72	6	(230)	1050	1360	(485)	900	(600)
42.84	7	1900	3100	2340	655	1380	985
Less than 3 months							
42.29	1 month	1270	990	1340	620	565	(240)
42.37	2½ months	1520	2970	1180	1120	1100	1260
42.49	8 days	(0)	(850)	920	325	620	740
42.50	8 days	1300	1770	(0)	640	485	340

Table 7. *Pathological conditions associated with low activities*

Activities as Q_{O_2} ; italicized figures omitted from statistical analyses.

Activities as Q_{O_2} ; italicized figures omitted from statistical analyses.						Kidney	
Case no.	Condition	Duodenum	Ileum	Colon	Liver	Cortex	Medulla
S2	Macerated stillbirth	<i>0</i>	<i>0</i>	<i>0</i>	<i>120</i>	<i>0</i>	<i>0</i>
L2	Macerated stillbirth	<i>0</i>	<i>500</i>	<i>0</i>	<i>150</i>	<i>0</i>	<i>0</i>
42.79	Nephritis 1 year	680	2370	1570	500	310	210
42.87	Nephritis 5 years	1970	2520	1480	600	220	150

DISCUSSION

Fig. 1 shows the scatter of activities with age for the kidney cortex. The correlation constant ($r = +0.64$) for the regression line calculated for ages 0–3 months gives $P < 0.01$. For older children there was no

significant increase in activity with age, and the line representing the mean value intersects the regression line sufficiently near to the 3-month abscissa to suggest that, for the kidney cortex, the amine oxidase activity does not increase after the third month of life.

Fig. 1. Variation of amine oxidase activity of kidney cortex with age. (Age scale telescoped after 3 months and figures over 2 years omitted.) Equation for correlation line $y = 7.03x + 377$. $N = 25$. $r = +0.64 \pm 0.116$. $P < 0.01$. Standard error = 193. 3 months–9 years: mean activity = 928. Standard deviation = 176.

Fig. 2. Variation of amine oxidase activity of kidney medulla with age. (Age scale telescoped after 3 months and figures over 2 years omitted.) Equation for correlation line $y = 8.1x + 272$. $N = 25$. $r = +0.628 \pm 0.124$. $P < 0.01$. Standard error = 150. 3 months–9 years: mean activity = 890. Standard deviation = 209.

Fig. 2 shows the corresponding analysis for the kidney medulla; again, the mean activity is fully developed by the end of the third month. The slopes

Fig. 3. Variation in amine oxidase activity of duodenum with age. (Age scale telescoped after 3 months and figures after 2 years omitted.) Tissues with amine oxidase activity: birth-9 years: mean activity=1151. Standard deviation=471.

Fig. 4. Variation of amine oxidase activity of liver with age. (Age scale telescoped after 3 months and figures after 2 years omitted.) 0-2 weeks, $N=10$, mean=592. 2 weeks-9 years: mean=780. Standard deviation=270.

of the regression lines calculated for the kidney cortex and medulla for the first 3 months are not significantly different.

Fig. 3 contains values found for the duodenum;

this tissue is remarkable in that 10 of the 15 children under 1 month old showed no amine oxidase activity. While an analysis of the figures suggests that the mean value calculated for children up to 3 months is significantly less than the steady level reached above this age, the apparent difference can be shown to be due to the large number of zero activities recorded for the lower age group; where the tissue has amine oxidase activity, the mean value and degree of scatter appear to be constant over the whole range included in this survey. There would appear to be some factor other than age involved in the development of amine oxidase activity by the duodenum.

Fig. 4 shows the activities obtained for the liver; the mean activity is doubled during the first fortnight of life but no satisfactory regression line can be drawn to cover the period of increase. Figures are not given for ileum and colon since, in spite of a wide degree of scatter, there appears to be no variation in mean activity with age for these tissues.

SUMMARY

1. Methods are described for the determination of the amine oxidase activity of human post-mortem material.

2. Investigation of the variation of amine oxidase with age in human tissues shows that (a) all tissues exhibit a wide degree of scatter in activity at each age; (b) the mean activity of the mucosa of the ileum shows no variation with age; (c) the kidney cortex and medulla of the newborn child are deficient in amine oxidase, in comparison with adult tissue; the activity increases until the child is 3 months old, when the mean activity appears to remain constant; (d) a large number of children less than 1 month old show no activity in the duodenal mucosa; in those in whom amine oxidase activity is found, the mean activity does not vary with age; and (e) although the mean activity of the colon is significantly less during the first 3 months of life than during the rest of the observed period, there does not appear to be a significant gradation of activity with age.

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REFERENCES

- Bernheim, L. C. H. (1931). *J. biol. Chem.* **93**, 299.
 Bhagvat, K., Blaschko, H. & Richter, D. (1939). *Biochem. J.* **33**, 1338.
 Birkhäuser, H. (1940). *Helv. chim. Acta*, **23**, 1071.
 Blaschko, H. (1942). *J. Physiol.* **101**, 337.
 Blaschko, H., Richter, D. & Schlossmann, H. (1937). *Biochem. J.* **31**, 218.
 Ewins, A. J. & Laidlaw, P. P. (1910). *J. Physiol.* **41**, 78.
 Gale, E. F. (1941). *Chem. Ind.* **19**, 721.
 Gale, E. F. (1942). *Biochem. J.* **36**, 64.

- Hare, L. C. H. (1928). *Biochem. J.* **22**, 968.
 Holtz, P., Heise, R. & Spreyer, W. (1938). *Arch. exp. Path. Pharmac.* **136**, 684.
 Kohn, H. I. (1937). *Biochem. J.* **31**, 1693.
 Lillie, R. D. (1932). *Publ. Hlth Rep., Wash.*, **47**, 372.
 Martin, G. J. (1942). *Arch. Biochem.* **1**, 397.
 Philpot, F. J. (1937). *Biochem. J.* **31**, 856.
 Pugh, C. E. M. & Quastel, J. H. (1937). *Biochem. J.* **25**, 2306.
 Richter, D. (1937). *Biochem. J.* **31**, 2022.
 Richter, D., Lee, M. H. & Hill, D. (1941). *Biochem. J.* **35**, 1225.
 Sullivan, M. X., Hess, W. C. & Sebrell, W. H. (1932). *Publ. Hlth Rep., Wash.*, **47**, 83.
 Werle, E. & Mennicken, G. (1938). *Biochem. Z.* **296**, 99.
 Zeller, E. A., Birkhäuser, H., Mislin, H. & Wenk, M. (1939). *Helv. chim. Acta*, **22**, 1381.
 Zeller, E. A., Stern, R. & Wenk, M. (1940). *Helv. chim. Acta*, **23**, 3.

Studies on Bacterial Amino-acid Decarboxylases

4. *l*(-)-HISTIDINE DECARBOXYLASE FROM *CL. WELCHII* TYPE A

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Gale (1941) has shown that strains of *Cl. welchii*, when grown under acid conditions or in the presence of fermentable carbohydrate, decarboxylate *l*(-)-histidine and *l*(+)-glutamic acid to histamine and γ -aminobutyric acid respectively. In this paper the extraction, purification and properties of *l*(-)-histidine decarboxylase from a strain of this organism are described.

ORGANISM AND METHODS

Organism and substrates used. The organism used as a source of *l*(-)-histidine decarboxylase was *Cl. welchii* (Type A) BW 21, National Collection of Type Cultures (N.C.T.C.) no. 6784. Histidine solutions were prepared from *l*(-)-histidine monochloride monohydrate (The British Drug Houses Ltd.), except for the quantitative experiments, in which *l*- and *dl*-compounds as supplied by Roche Products Ltd. were used.

Methods of estimating and expressing enzyme activity. As in the earlier papers of this series (Gale & Epps, 1944a; Epps, 1944) the activity of enzyme preparations was estimated in Warburg manometers. Unless otherwise stated, the reaction temperature was 30°, the gas phase air, the buffer 2 ml. *m*/5 acetate pH 4.5 and the substrate (in side-bulb) 0.5 ml. *m*/30 *l*(-)-histidine solution at pH 4.5.

At 30° the reaction curve is not linear (see below), so the manometers were read 2 min. after the substrate had been tipped into the main reaction vessel and then at 5 min. intervals, the rate of CO₂ evolution being calculated from the first of these 5 min. periods (under the conditions adopted the temperature of the manometer flask had regained 30° within 2 min. of mixing).

Enzyme units. The unit quantity of enzyme liberates 100 μ l. CO₂/5 min.; rates of enzymic activity are expressed as $Q_{CO_2}^0 = \mu$ l. CO₂ evolved/mg. C/hr. C was estimated by the wet combustion micro-method of Van Slyke & Folch (1940).

1. PRELIMINARY WORK ON THE PURIFICATION OF ENZYME PREPARATIONS

Growth of cultures and preparation of acetone powders. For this preliminary work cultures were grown for 17 hr. at 37° in 2% glucose tryptic-casein broth to which a small amount of heart muscle had been added. Anaerobic conditions were ensured by addition after autoclaving of 1 drop thiolacetic acid/l. broth, thiolacetic acid in this concentration having no harmful effect on growth or activity. After growth the bacteria were harvested, washed once, made into a thick suspension with distilled water and stirred into 5 vol. ice-cold acetone. The precipitate was left to flocculate, then filtered off on a small Buchner funnel and washed successively with acetone, a mixture of acetone and ether in equal parts and, finally, with ether. The dry powder was active towards *l*(-)-histidine and also, to a lesser extent, towards *l*(+)-glutamic acid; by altering the conditions of growth and acetone treatment it was possible to reduce this residual glutamic acid decarboxylase activity to zero.

Destruction of *l*(+)-glutamic acid decarboxylase activity during preparation of the acetone powder. The test conditions for activity towards glutamate were the same as for histidine, except for the substrate used.

(1) **Variation in growth time.** Glutamic acid decarboxylase shows no variation with growth times from 12 to 27 hr. at 37°; histidine decarboxylase is maximal when the culture is harvested between 15 and 21 hr.

(2) **Variation in growth temperature.** Powders prepared from cultures grown for 17 hr. at 27 and 37° respectively have the same activity towards glutamic acid; the activity towards histidine is five times greater in the powder from the culture grown at the higher temperature.

(3) **Temperature of acetone treatment.** The histidine decarboxylase activity of the powder is the same whether the suspension is treated with acetone at room temperature or at 0°; the glutamic acid decarboxylase activity is halved by the use of acetone at room temperature.