Mean L-gulonolactone oxidase activity (as μmoles ascorbate g^{-1} h^{-1}) in liver and kidney homogenates for the 6 species of mammals in which L-gulonolactone oxidase has been detected in kidney

Species		Sex and N	Liver	Kidney X-Section	Cortex	Medulla
Mammalia: Prototheria						
Echidna	*Tachyglossus aculeatus*	1♂, 2♀♀	Nil	18.8	24.4	1.9
Platypus	*Ornithorhynchus anatinus*	1♂, 2♀♀	Nil	10.1	11.0	4.8
Mammalia: Marsupialia						
Long-nosed bandicoot	*Perameles nasuta*	1♂	1.7	2.8	3.8	0.6
Brindled bandicoot	*Isoodon macrourus*	3♂♂	4.4	5.3	6.1	1.7
Red-necked wallaby	*Macropus rufogriseus*	1♂, 1♀	5.6	0.6	1.1	0.2
Red-necked wallaby	*Macropus rufogriseus*	3♂♂, 1♀, 1?[a]	4.2	Nil	Nil	Nil
Red-necked pademelon	*Thylogale thetis*	2♀♀[b]	5.2	0.4	n.d.[c]	n.d.
Red-necked pademelon	*Thylogale thetis*	1♂, 1♀	3.0	Nil	n.d.	n.d.

All animals were adults except as noted. [a] Includes one unsexed pouch young; [b] both individuals were pouch young; [c] not determined.

manner of reptiles. 2 species of bandicoots (Marsupialia; Peramelina) have relatively high levels of L-gulonolactone oxidase activity in both liver and kidney. In the kangaroos and relatives (Marsupiala; Diprotodonta) the primary locus of ascorbate synthesis is the liver, but a few individuals of at least 2 species have L-gulonolactone oxidase at low levels in the cortex of the kidney. Thus, mammals, like birds[9], include 4 groups of species: those that synthesize ascorbate only in kidney, the primitive, reptilian condition; those that synthesize both in kidney and liver, presumably an intermediate evolutionary stage; those that synthesize only in liver, presumably a derived condition; and those that are incapable of ascorbate biosynthesis, which presumably is adaptive despite the fact that such species are subject to diseases associated with low ascorbate levels. Most mammals synthesize ascorbate in liver, whereas in birds most synthesize in kidney. In both groups, however, it is members of the more recently derived lineages that have L-gulonolactone oxidase in liver.

Within the kidney of both prototherians and marsupials, most synthesis takes place in the cortex (table). The low levels of activity observed in medullary tissue may have resulted from a low level of L-gulonolactone oxidase in medullary cells or it could have resulted from contamination of the medullary preparations with some cells from the cortex. Cortex clearly appears to be the primary region of ascorbate biosynthesis within the kidney in the few mammals capable of synthesis there.

Levels of enzyme activity were consistently higher in the echidna than in the platypus. We suspect the ascorbate economy of the 2 differs in ways presently not understood. Bandicoots have roughly equal activity levels of enzyme in liver and kidney. Nevertheless, because the liver is larger than the kidneys more ascorbate biosynthesis presumably takes place in liver, assuming that all other aspects of biosynthesis in the 2 organs are equal. Some macropods (Macropodidae) apparently have limited biosynthetic capability in kidney, but most synthesis in the 2 species reported clearly takes place in liver.

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A thin-layer chromatographic assay for measuring pineal hydroxyindole-O-methyltransferase activity

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Summary. A thin-layer chromatographic procedure for measuring pineal HIOMT activity is described, based on the methylation of NAS to melatonin. The method enables simple and accurate determination in small aliquots from a single pineal organ homogenate.

Hydroxyindole-O-methyltransferase (HIOMT), the enzyme responsible for the conversion of N-acetylserotonin (NAS) to melatonin, has been identified in the pineal and Harderian glands and in the retina of several species of mammals and birds. HIOMT, which modulates the synthesis of the pineal hormone melatonin, has been most

thoroughly studied in the pineal where its activity is influenced by different environmental stimuli, especially light[1] and heat[2]. An assay for measuring HIOMT activity, involving methylation of NAS with labelled S-adenosylmethionine and extraction of enzymically formed radioactive melatonin into chloroform, was developed by Axelrod et al[3].

We are reporting here a rapid and sensitive procedure based on the same principle, which permits microdetermination of HIOMT activity in the rat pineal. The radioactivity formed by melatonin can be separated quantitatively by simple TLC using very small aliquots of a single organ homogenate.

Materials and methods. ^{14}C-Methyl-S-adenosylmethionin (56 mCi/mmole) was purchased from New England Nuclear Corporation. All indoleamines used were obtained from Regis Chemical Co., Chicago, USA.

Precoated plastic sheets 20× 10 cm (Polygram Sil G/UV$_{254}$) were acquired from Macherey Nagel and Co., Düren, FRG. Male rats of the Hebrew University's 'Sabra' strain weighing 180–200 g each were kept under diurnal lighting (lights on from 06.00 h to 18.00 h) for at least 5 days before decapitation between 10.00 h and 11.00 h.

For HIOMT assay 1 pineal was homogenized in 60 µl 0.05 M phosphate buffer pH 7.9. Aliquots of 20 µl homogenate were transferred into the bottoms of small serological test tubes each containing 5 µg NAS in 5 µl of the phosphate buffer. After adding 5 nCi ^{14}C-S-adenosylmethionine in 5 µl phosphate buffer the tubes were sealed with parafilm and incubated for 1 h at 37 °C. All samples were made in duplicate. The reaction was stopped by adding 30 µl absolute ethanol containing approximately 2 µg melatonin, or a standard carrier mixture of 2 µg melatonin, 5-methoxytryptophol (5-MTOH), 5-methoxyindoleacetic acid (5-MIAA) and 5-methoxytryptamine. From the final reaction mixture 40 µl were applied to precoated plastic 20× 10 cm sheets under a continuous gentle stream of nitrogen to form a 10–12 mm spot. To each sheet 3 spots were applied. Chromatographs were developed in darkness in a solvent system composed of chloroform/methanol/glacial acetic acid 90:10:1, or a system consisting of chloroform/methanol 90:10 which was found similarly satisfactory for separating the radioactive melatonin from other possible indole compounds formed, the labelled S-adenosylmethionine, or from any adherent radioactive impurities. The separated spots of melatonin were cut out from the sheet and each placed in a counting vial to which 10 ml scintillation fluid were added. The samples were then counted by a standard scintillation technique.

Results and discussion. Under our experimental conditions the rate of accumulation of melatonin was linear for up to 1.5 h incubation (figure). There was also a linear relationship between the methylation of NAS and the quantity of pineal homogenate taken. We further tested that the radioactivity of the melatonin spot obtained in our 1-dimensional chromatographic system was not polluted by overlapping radioactive compounds with similar R_f values – i.e. 5-MTOH, 5-MIAA – that could be produced in the system. 2 aliquots from the same homogenate were applied to the precoated plastic sheets. After separating the melatonin and 5-MTOH/5-MIAA spots by the solvent system used by us, 1 set of spots was taken for radioactivity counting and another set was further purified by a 2 dimension system consisting of ethylacetate, known to separate all other methoxylated indole compounds from melatonin[4]. No difference was found between the counts of the melatonin and 5-MTOH/5-MIAA spots obtained in both systems, thus indicating that under the conditions of our experiments the melatonin is completely separated and all radioactivity obtained in its 1-dimensional chromatography spot is of melatonin only (table 1). In order to test the results obtained by our procedure (method 2) against those of Axelrod et al. (method 1), levels of pineal HIOMT were measured by both methods in rats exposed to extreme conditions of ambient light. Adult male rats were kept in continuous light (40 W 'daylight' overhead tubes) or darkness for 7 days, and their pineal glands were then examined for HIOMT activity. The results obtained by the 2 procedures were the same (table 2). Our method gives accurate and reproducible results, is speedy and less tedious than any described in the literature.

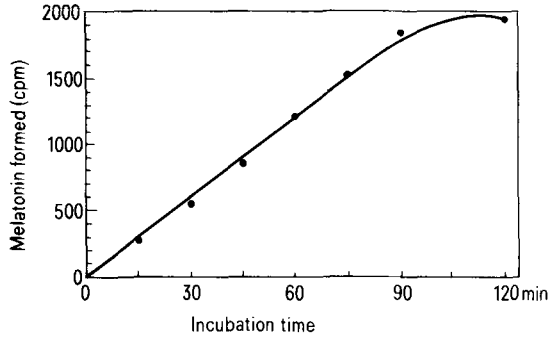
Table 1. Melatonin, 5-methoxytroptophol (5-MTOH), 5-methoxyindoleacetic acid (5-MIAA) produced (in cpm) in the system and estimated by 1- and 2-dimensional separations

Samples	Aliquots A separated by the 1-dimensional system		Aliquots B separated by the 2-dimensional system	
	Melatonin	5-MTOH and 5-MIAA	Melatonin	5-MTOH and 5-MIAA
1	1908	92	2040	67
2	1663	108	1608	81
3	2537	147	2467	71
Blank	108	136	85	80

Table 2. Comparison of results of pineal HIOMT activity obtained in rats exposed to continuous light or darkness

Samples	Light		Darkness	
	Method 1	Method 2	Method 1	Method 2
1	4214	3866	6010	5976
2	4467	4028	6394	6070
3	2710	2718	5658	5418
4	2531	2475	4672	4505
5	1802	1993	4659	4329

Values given in cpm of melatonin produced.



Time course of methylation of N-acetylserotonin. Relation between melatonin formed and incubation time.

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