to remove all traces of solvent which might interfere with lipolysis. After cooling, the reagent solution, containing the lipase-esterase mixture, was added and the test tube was incubated at 30 °C for 90 min, after which the enzymatic-spectroscopic determination was carried out as described by Wahlfeld[11]. Each test was replicated 4 times.

The results (table) indicate that weight loss after 144 h is greatest in the presence of carbon dioxide at low relative humidity. It has been shown that a concentration of 4.3% carbon dioxide increased the water loss of *E. cautella* pupae to a detrimental level when exposed to 20% relative humidity[12]. The critical water loss of 30%, above which complete mortality of pupae was obtained, can be reached with a combination of high carbon dioxide and low relative humidity[12]. The results of the present study indicate that weight losses above this critical level were obtained when insects were exposed to 80% carbon dioxide and 20% relative humidity. Since the oxidation of 1 g of a fat of average saturation would theoretically yield about 1.1 g of water[7], and there is only a slight change in triglyceride content, the rate of metabolic water production in relation to the situation of water balance should be viewed simply as an incidental consequence of insect metabolism.

It has been demonstrated that to produce a given unit of body weight, more food is eaten by *E. kuehniella* larvae when they are exposed to low humidities[13]. This is because part of the food is utilized as water. However, for *E. cautella* in the pupal stage, with the existing organic substrates, there are significant changes in triglyceride content between 24 and 144 h of exposure. However, exposure to different atmospheric compositions did not result in pronounced differences in triglyceride levels.

Since the change in triglyceride content for *E. cautella* pupae is slight, and since we have shown previously[4] that carbohydrate content of the insect is less than 1% of its fresh body weight (and cannot therefore contribute significantly to metabolic water), the formation of metabolic water in pupal tissue would only slightly regulate the transpiratory and excretory losses. However, this process would not regulate the full losses for water caused under conditions of high carbon dioxide and low humidity.

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3 S.W. Bailey and H.J. Banks, Proc. 1st int. Work Conf. Stored Prod. Ent., p. 375. Savannah, Georgia 1974.
4 A. Friedlander and S. Navarro, Insect Biochem. *9*, 79 (1979).
5 B. Burkett and H. Schneiderman, Science *156*, 1604 (1967).
6 E.J. Bond and C.T. Buckland, J. econ. Ent. *71*, 307 (1978).
7 E. Bursell, in: The Physiology of Insecta, vol. 2, p. 65. Ed. M. Rockstein, Academic Press, New York 1974.
8 S. Navarro and M. Gonen, J. stored Prod. Res. *6*, 187 (1970).
9 S. Navarro and E. Donahaye, J. stored Prod. Res. *8*, 223 (1972).
10 E.G. Bligh and D.J. Dyer, Can. J. Biochem. Physiol. *37*, 911 (1969).
11 A.W. Wahlfeld, in: Methods of Enzymatic Analysis. Ed. H.U. Bergmeyer. Academic Press, New York 1974.
12 S. Navarro and M. Calderon, Israel J. Ent. *8*, 143 (1973).
13 G. Fraenkel and M. Blewett, Bull. ent. Res. *35*, 127 (1944).

Ascorbic acid biosynthesis in the mammalian kidney[1]

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Summary. The egg-laying mammals (Prototheria) synthesize L-ascorbic acid only in kidney, as is characteristic of reptiles. Bandicoots (Marsupialia) synthesize it in both kidney and liver. 2 other species of marsupials (kangaroos) synthesize it primarily in liver, but some individuals also synthesize in kidney.

The biosynthetic pathway by which ascorbic acid is synthesized in those vertebrates capable of synthesizing it was extrapolated from work with rat liver preparations[4]. All species known to require dietary ascorbate lack the enzyme L-gulonolactone oxidase[5,6], which catalyzes the oxidation of L-gulonolactone to L-ascorbic acid. In amphibians, reptiles, and some birds, L-gulonolactone oxidase is located in the kidney[7-9]. All species of placental mammals (Eutheria) reported to be capable of synthesizing ascorbate have L-gulonolactone oxidase solely in the liver[5,10]. The only species of marsupial previously investigated, the Virginia opossum *(Didelphis virginiana)*, likewise was reported to have the enzyme in liver[11]. Because the egg-laying mammals (Prototheria; Monotremata) exhibit a number of reptilian characteristics[12], we have investigated the locus of ascorbate biosynthesis in this group. Also, we have studied ascorbate biosynthesis in additional species of marsupials. Results of these studies demonstrate greater variability in the tissue locus of ascorbic acid biosynthesis in mammals than previously was known.

Material and methods. Assays of tissues for L-gulonolactone oxidase (EC 1.1.3.8) were performed by the method of Ayaz et al.[10]. This method involves: 1. homogenizing the tissue in a buffer containing sodium deoxycholate (DOC) to disperse the enzyme; 2. centrifuging to remove debris; 3. incubating the homogenate with L-gulonolactone; 4. stopping the reaction with a mixture of trichloracetic and metaphosphoric acids; 5. oxidizing ascorbic acid to dehydroascorbic acid and filtering; and 6. determining the dehydroascorbic acid in the filtrate by reaction with 2,4-dinitrophenylhydrazine and colorimetric analysis. Tissues were removed from animals quickly after killing and either analyzed immediately for L-gulonolactone oxidase or wrapped in parafilm or plastic wrap, quickly frozen in liquid nitrogen or a freezer, and kept at -20 °C or below until assayed. One *Tachyglossus aculeatus,* one pouch young *Thylogale thetis,* the *Perameles nasuta,* and all 3 *Isoodon macrourus* were from laboratory populations. Other animals were captured or shot from natural environments in northeastern New South Wales, Australia. All specimens were preserved as vouchers and are on deposit in the Collection of Mammals, Carnegie Museum of Natural History, Pittsburgh, Pennsylvania, USA.

Results and discussion. As shown in the table, we found that the egg-laying mammals (Prototheria; Monotremata) contain L-gulonolactone oxidase only in the kidney, in the

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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4 S. Dagley and D.E. Nicholson, An Introduction to Metabolic Pathways, p. 130. Blackwell, Oxford 1970.
5 I.B. Chatterjee, Science *182*, 1271 (1973).
6 E.C. Birney, R. Jenness and K.M. Ayaz, Nature, Lond. *260*, 626 (1976).
7 A.P. Grollman and A.L. Lehninger, Archs Biochem. Biophys. *69*, 458 (1957).
8 R.N. Roy and B.C. Guha, Nature, Lond. *182*, 319 (1958).
9 C.R. Chaudhuri and I.B. Chatterjee, Science *164*, 4 95 (1969).
10 K.M. Ayaz, R. Jenness and E.C. Birney, Analyt. Biochem. *72*, 161 (1976).
11 Y. Nakajima, T.R. Shantha and G.H. Bourne, Histochemie *18*, 293 (1969).
12 M. Griffiths, Echidnas, p. 216. Pergamon Press, Oxford 1968.

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