sists of cleomeprenols esterified with these fatty acids. Comparison of the fatty acid composition of cleomeprenol esters of the feces with that of the esters present in the leaves of *C. spinosa* showed that the concentrations of stearic and oleic acids in the former were about 3 times those of the acids in the latter, while linolenic acid was one-third. Other fatty acids in both the esters were similar in composition.

In pig liver, more than half of the dolichols are present as the fatty acid esters[8]. Formation of the dolichol esters is observed during the incubation of dolichols with rat liver microsomes[9]. However, no accumulation of cleomeprenol esters in the larval body of *P. rapae crucivora* was observed. Thus, it was found that the larvae transform the cleomeprenols of *C. spinosa* to long chain fatty acid esters to excrete them in this form in the feces.

1 Y. Higashi, J.L. Strominger and C.C. Sweeley, Proc. natl Acad. Sci. USA *57*, 1878 (1967).
2 J.F. Caccam, J.J. Jackson and E.H. Eylar, Biochem. biophys. Res. Commun. *35*, 505 (1969).
3 R.M. Baar and F.W. Hemming, Biochem. J. *126*, 1193 (1972).
4 F.W. Hemming, in: Natural Substances Formed Biologically from Mevalonic Acid, p. 105. Ed. T.W. Goodwin. Academic Press, London 1970.
5 T. Suga, T. Shishibori, S. Kosera, T. Tanaka and M. Itoh, Chem. Lett. *1975*, 771.
6 T. Shirōzu, in: Butterflies of the Japan in Colour, p. 159. Hokuryu-kan, Tokyo 1965.
7 P.J. Dunphy, J.D. Keer, J.F. Pennock and K.J. Whittle, Chem. Ind. *1966*, 1549.
8 P.H. Butterworth and F.W. Hemming, Archs Biochem. Biophys. *128*, 503 (1968).
9 R.W. Keen and M.E. Kruczek, Biochemistry *15*, 1586 (1976).

Triglyceride metabolism in *Ephestia cautella* pupae exposed to carbon dioxide[1]

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Summary. The triglyceride content of *Ephestia cautella* pupae exposed to increased carbon dioxide atmospheres at low relative humidity was not markedly affected. There was a significant increase in weight loss of pupae exposed to low relative humidity. Results indicate that for *E. cautella*, metabolic water formation by fat utilization can hardly regulate water exigencies for the pupae and cannot fully compensate for water losses in high carbon dioxide atmospheres.

Recently the effect of atmospheres containing low oxygen or high carbon dioxide concentrations was studied in an attempt to apply the controlled-atmosphere method for controlling insects in large bulks of dry grain[3]. To clarify the effect of carbon dioxide on stored product pests, we investigated the basic biochemistry of its influence on *Ephestia cautella* (Walker) pupae[4]. It is well known that stored-product insects can survive at very low humidities. Therefore, the means of conserving water in these insects is an important aspect of their structure and physiology.

Although carbon dioxide plays an important role in keeping insect spiracles open, thus exposing the organism to desiccation[5], it is now generally accepted that the effect of carbon dioxide is complex and not restricted to this physiological effect only[6].

As a process of dehydrogenation, the oxidation of organic substrates takes place with the consequent formation of metabolic water[7]. The present investigation was carried out to clarify the effect of carbon dioxide on triglyceride metabolism, and to ascertain whether metabolic water, formed in fat metabolism, may be a regulating phenomenon that replaces water lost through increased desiccation rate induced by carbon dioxide under conditions of low relative humidity.

E. cautella pupae were reared according to Navarro and Gonen[8]. They were examined after exposure to various gas compositions and relative humidities at 26 °C as described by Navarro and Donahaye[9]. Weight loss was determined by weighing each sample before and after treatment, and was calculated as percent of the starting weight. Triglycerides were extracted by the method of Bligh and Dyer[10], which is essentially as follows: The sample (0.5 g, about 60 insects) were homogenized in the extraction fluid (chloroform-methanol 1:2 v/v, 5 cm^3). The homogenate was filtered and the filtrate treated as described in the original paper[10]. After removal of the aqueous phase, an aliquot of the chloroform phase was diluted 100-fold and used for the determination of triglycerides by the method of Wahlfeld[11], with the following modifications: The aliquot was evaporated in a small test tube and heated subsequently to 100 °C

Effects of different atmospheric compositions at 2 relative humidities on triglyceride levels and weight loss of *E. cautella* pupae (in relation to time)

Atmospheric composition (%)			Relative humidity	Exposure time	Triglycerides	Weight loss
O_2	N_2	CO_2	(%)	(h)	(% ± SE)	(% ± SE)
20	80	0	100	24	12.04 ± 0.630	0.83 ± 0.177
20	80	0	100	48	13.00 ± 0.772	1.62 ± 0.524
20	80	0	100	144	9.87 ± 0.445	1.62 ± 0.630
20	80	0	20	48	13.45 ± 0.697	4.46 ± 0.793
20	80	0	20	144	9.46 ± 0.766	12.17 ± 0.650
20	0	80	100	24	15.64 ± 1.020	3.02 ± 0.281
20	0	80	100	48	12.91 ± 1.186	5.06 ± 0.294
20	0	80	100	144	10.06 ± 0.835	7.53 ± 1.513
20	0	80	20	48	12.60 ± 0.514	25.91 ± 1.414
20	0	80	20	144	9.16 ± 1.496	58.77 ± 1.173

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