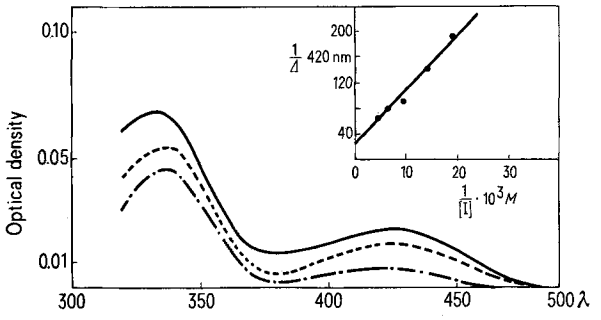

Fig. 2. Spectra in phosphate buffer pH 6.8: ——, enzyme; – – –, enzyme plus $7 \cdot 10^{-5}$ M seryl-trihydroxy-benzyl hydrazine; –·–, enzyme plus $21 \cdot 10^{-5}$ M seryl-trihydroxy-benzyl-hydrazine. The spectra are corrected for the absorption of the compound added. In the inset: absorbance at 420 nm of the enzyme plus seryl-trihydroxy-benzyl-hydrazine as a function of its varying concentrations presented as double reciprocal plot.

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be interesting to check the binding sites specificity and their functional properties.

The other possibility might involve only one substrate binding site and a second intermediate, in addition to the Michaelis complex. The relative magnitudes of rate constants of 2 intermediates could be quite different, depending on the substrates used. As suggested by Krupka and Laidler[9], an apparent non-competitive inhibition may be at play in the sense that the inhibitor becomes attached to the second intermediate and not to the Michaelis complex. It must be pointed out in this connection that, for Dopa decarboxylase, some intermediate substrate-enzyme complexes have already been characterized and their properties have been connected with the structural variations of substrate[10].

Besides these hypotheses, the results show that the trihydroxybenzylhydrazide seryl derivative is not a powerful inhibitor of the aromatic amino acid decarboxylase in vitro. Owing to a large substituent in 1 position of the aromatic ring and the lack of a free hydrazinic group, the requirements for a good interaction at the active site are probably not met. The powerful inhibition observed in vivo therefore probably reflects the interaction between the peripheral decarboxylase and the compound formed by hydrolysis of the seryl hydrazine linkage.

Enzymic basis for the nutritional requirement of arginine in insects[1]

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Summary. The fat body of the cockroach, Blaberus cranifera, and the silkmoth, Hyalophora gloveri, has been tested for some enzymes of the urea cycle. While ornithine transcarbamoylase and argininosuccinase activities could not be detected, arginase is present in the fat body of these insects. These results explain the essentiality of arginine in the diet of insects.

The arginine biosynthetic pathway, believed to be present in whole or in part in all organisms, has been successfully exploited for ammonia detoxification during the evolution of ureotelism associated with the invasion of the terrestrial habitat by animals[4]. Terrestrial insects, which are uricotelic, seem to have lost the ability to synthesize arginine, and thus the potential for forming urea as an excretory product. This is indicated by the nutritional requirement for arginine by many insects[5]. However, in some species of insects, arginine in the diet can be replaced by citrulline but not by ornithine[6,7]. These findings suggest that insects, like birds[8,9], can form arginine from citrulline, but are unable to synthesize citrulline from CO_2, NH_3 and ornithine. To find out the biochemical basis for these nutritional results, we have studied the distribution of some of the urea cycle enzymes in the fat body of the cockroach, Blaberus cranifera, and the silkmoth, Hyalophora gloveri. These results are reported here.

Material and methods. The procurement and maintenance of the insects, Blaberus cranifera and Hyalophora gloveri, and the methods of arginase assay (L-arginine amidinohydrolase, E.C. 3.5.3.1) and protein determination have been described earlier[10]. Ornithine transcarbamoylase (Carbamoylphosphate: L-ornithine carbamoyltransferase, E.C. 2.1.3.3) and argininosuccinase (L-argininosuccinate arginine-lyase, E.C. 4.3.2.1) assays were performed in 10% (w/v) fat body extracts essentially by the methods described by Campbell and Speeg[11].

Results and discussion. The distribution of the 3 urea cycle enzymes in the fat body of the cockroach and silkmoth is shown in the table. Ornithine transcarbamoylase (OTCase) and argininosuccinase (ASase) activities could not be detected in the fat body of these insects. The recovery of citrulline added to the OTCase assay system was 104%. There is no inhibitor of OTCase in fat body tissue of Blaberus cranifera, as determined by experiments in which a purified bacterial OTCase was mixed with fat body extracts of this insect and assayed. Further, the methods used for the assay of these enzymes are

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Distribution of some urea cycle enzymes in the insect fat body

Species	Stage in development	OTCase	ASase	Arginase* μmoles urea formed per h per g tissue	Arginase* μmoles urea formed per h per mg protein
B. cranifera	2nd instar nymphs	B. L. D.	–	–	–
	6th instar nymphs	B. L. D.	–	63	1.8
				47	1.2
	9th instar nymphs	B. L. D.	B. L. D.	66	0.9
				75	1.5
	11th instar nymphs	B. L. D.	–	88 ± 22 (3)	2.0 ± 0.4 (3)
	Adults	B. L. D.	B. L. D.	126 ± 27 (10)	2.3 ± 0.6 (10)
H. gloveri	Diapause pupae	B. L. D.	B. L. D.	112 ± 34 (9)	1.0 ± 0.1 (8)
	Adult moths	B. L. D.	B. L. D.	2453 ± 907 (9)	24.5 ± 5.1 (9)

* Wherever 3 or more assays were done, the mean values and the standard deviations are given with the number of assays in the parentheses. Otherwise individual observations are given. B. L. D.: Below the level of detection.

quite sensitive and have been successfully employed to demonstrate these activities in the tissues of vertrebrates, as well as invertebrates like snails, flatworms and annelids[11,12].

These results are consistent with those of Porembska and Mochnacka[13], who failed to detect the synthesis of citrulline and arginine in the fat body and muscle extracts of Celerio euphorbiae, and Kameyama and Miura[14], who could not detect the NH_4-dependent carbamoylphosphate synthetase and OTCase in Aldrichina grahami. Thus it would appear that the insects studied by us, as well as Celerio euphorbiae[13], are not only incapable of synthesizing citrulline from NH_3, CO_2 and ornithine, but also cannot affect the conversion of citrulline to arginine. However, the nutritional studies of Hinton[6] on Drosophila, and Davis[7] on Oryzaephilus surinamensis, as well as the isotopic studies of Inokuchi, Horie and Ito[15] on Bombyx mori, suggest that some insects at least are capable of converting citrulline to arginine. The possibility remains that some insects have lost the entire arginine biosynthetic pathway making arginine irreplaceable in their diet, whereas others have retained a part of the pathway enabling them to substitute dietary citrulline for arginine.

Arginase is the only enzyme of the urea cycle present in the fat body of the cockroach and silkmoth (table). The arginase activity increases 20- to 30fold during the metamorphosis of silkmoth pupae into adults (table), and this increased arginase activity has been shown to play a role in the conversion of exogenous arginine to proline, which is necessary in the flight muscle metabolism of the adult moth[16]. There is no such dramatic increase in the fat body arginase activity during development of the cockroach (table) consistent with the limited capacity of this insect to fly.

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Enterohepatic cycling of O-(β-hydroxyethyl) rutosides and their biliary metabolites in the rat

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Summary. O-(β-Hydroxyethyl)rutosides are shown to undergo reabsorption from the intestine following their secretion in bile.

Although biliary excretion has been established as a major route of excretion of O-(β-hydroxyethyl) rutosides following absorption from the gut or parenteral administration to the rat[3,4] the possibility of re-absorption of biliary metabolites of these compounds from the lumen of the intestine and subsequent enterohepatic cycling does not appear previously to have been examined, although evidence has been presented that these glycosides are ultimately excreted largely as their aglycones in faeces[3,4]. Use of an intercannulated rat preparation has now permitted some assessment to be made of the extent of reabsorption of each of the hydroxyethylrutosides and/or their conjugates following their secretion in bile.

Materials and methods. 3′,4′,5,7-Tetra-O-(β-hydroxyethyl)rutoside, (tetra-HR) 3′,4′,7-tri-O(β-hydroxyethyl)-rutoside (tri-HR) (constituents of the therapeutic agent Paroven, Zyma S.A., Nyon, Switzerland) and 7-mono-O-(β-hydroxyethyl)rutoside (7-mono-HR) (the therapeutic value of which is currently under investigation) were

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