lesser, effect to that of butanol in the systems they studied the effect of ethanol on the invertase system was reexamined. In the concentration range of 2–10% ethanol, inhibition was of the purely non-competitive type; a K_m of 33 mM and 44 mM for cell wall and soluble invertase, respectively, being deduced from the common points of intersection on the horizontal axis in LINEWEAVER–BURK plots. The lower K_m for cell-wall preparations may reflect some hindrance imposed by the site of the bound enzyme. It was unlikely to be due to a local variation of effective pH at the site of the bound enzyme for it was shown that the K_m of the bound invertase did not increase until the pH of the medium had been lowered to between pH 3 and 4 and in this region there was also an increase in the K_m of the soluble enzyme.

As pointed out by FOLK *et al.*[4] these results may be interpreted either on the basis of modification of k_0 (rate constant for dissociation of enzyme–substrate complex to products and free enzyme) or of modification of the free enzyme. Such a large number of kinetic studies of invertase action are available that it appears likely that it may prove a useful system for further investigation of this effect in spite of possible complication by the demonstrable transferring activity of invertase.

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Urea biosynthesis in invertebrates: [^{14}C]Urea formation in the land snail and earthworm

The failure to demonstrate urea synthesis by Helix hepatopancreas tissue in early studies of invertebrate nitrogen metabolism[1,2] and the controversial findings with Tetrahymena[3,4] have led to the general conclusion that urea synthesis does not take place in invertebrate animals by the Krebs–Henseleit ornithine cycle[5,6]. Observations on the earthworm[7,8] and more recent studies of the land snail, *Otala lactea*[9], have, however, shown that the question of urea synthesis and metabolism in certain of these animals should remain an open one. Otala hepatopancreas was shown to possess ornithine transcarbamylase (EC 2.1.3.3), argininosuccinate lyase (EC 4.3.2.1), and possibly argininosuccinate synthetase (EC 6.3.4.5) activity in addition to high levels of arginase (EC 3.5.3.1)[9]. Ornithine transcarbamylase activity has also been shown

to be widely distributed among flatworms[10,11] and this enzyme activity is now known to occur in *Lumbricus terrestris* (approx. 1500 μmoles citrulline formed per g gut tissue per h at 28° and pH 7.8) as well as in other species of earthworms[12]. In spite of these findings of the wide distribution of ornithine transcarbamylase activity in invertebrates, it has not been possible to demonstrate the synthesis of carbamyl phosphate by tissue preparations of several of these animals using *in vitro* assay systems for carbamyl phosphate synthetase activity. Because of this, the present work was directed toward studying the incorporation of $^{14}CO_2$ into urea and citrulline by intact tissue preparations of Lumbricus and Otala. Such an incorporation into urea has previously been shown with the flatworm, Hymenolepis[10]. An incorporation of $^{14}CO_2$ into both the ureido carbon of citrulline and urea would be highly suggestive that a urea cycle or very similar series of reactions is operative in these invertebrates[13].

The saline used for Otala contained, in mM: NaCl, 120; KCl, 4; $CaCl_2$, 1; $MgCl_2$, 2.5; $NaHCO_3$, 1; Na_2SO_4, 1.4; NaH_2PO_4, 3.5; $(NH_4)_2SO_4$, 1; L-ornithine, 2; and sodium succinate, 5. The final pH was adjusted to 8.0 with NaOH. The composition for Lumbricus was: NaCl, 125; KCl, 2.7; $CaCl_2$, 1.8; $MgCl_2$, 0.4; $NaHCO_3$, 1; Na_2SO_4, 0.4; NaH_2PO_4, 3.5; $(NH_4)_2SO_4$, 1; L-ornithine, 2; and sodium succinate, 5. The final pH was adjusted to 7.5. These solutions were sterilized by filtration and 0.7 mg penicillin G and 0.5 mg dihydrostreptomycin sulfate were added per ml. Incubations were carried out at 30° with constant shaking in cotton-stoppered flasks which had previously been sterilized. 1 g Otala hepatopancreas and 0.5 g Lumbricus gut tissue were incubated in 5 ml of the appropriate saline for the times indicated in Table I following the addition of 990 000 counts/min $NaH^{14}CO_3$ to each flask. The reaction was terminated by the addition of 1 N H_2SO_4 and the tissue debris and precipitated protein were removed by centrifuging at 20 000 × *g*. The supernatant and wash fluids from this centrifugation were combined and gassed extensively with CO_2 and then neutralized to pH 7.0. The solution was then transferred to a closed vessel and treated with jackbean urease. Following the urease treatment, the solution was acidified with lactic acid and the $^{14}CO_2$ formed due to the action of the urease was aerated into a separate vessel containing CO_2-free NaOH. Carrier $NaHCO_3$ was added and the CO_2 was precipitated as $BaCO_3$ by standard methods. Determination of the radioactivity in the $BaCO_3$ was with a low-background (1–2 counts/min) gas-flow counter and corrections were made for background radiation and self-absorption.

Following the removal of the $^{14}CO_2$, 25 μmoles L-citrulline were added to each of the reaction mixtures and any remaining protein was precipitated with ethanol and removed by centrifuging. The ethanolic supernatant fiuid was extracted with chloroform and the aqueous layer was collected[14] and passed through a column of Dowex-50 (H^+). The column effluent and wash were discarded and the amino acids were eluted with 2 N ammonia solution. An average of 94% of the original citrulline was recovered in this procedure. Approx. 14 μmoles of the recovered citrulline from each reaction mixture were arsenolyzed[15] with purified rat liver ornithine transcarbamylase[16] in a closed Warburg flask. The $^{14}CO_2$ liberated was trapped in NaOH in the center well and was precipitated in the presence of carrier $NaHCO_3$ as $BaCO_3$ and counted as described above.

Alanine, aspartic acid and glutamic acid are known to become labelled during incubations of Otala hepatopancreas with $^{14}CO_2$ due to CO_2-fixation[17]. Because of this, non-radioactive citrulline was arsenolyzed in the presence of from 1000–2000

counts/min each of uniformly labelled L-[^{14}C]alanine, L-[^{14}C]aspartic acid, and L-[^{14}C]glutamic acid. In the presence of these amino acids, either alone or in combination, no liberation of $^{14}CO_2$ was detected during the arsenolysis reaction. With the experimental material, the amount of $^{14}CO_2$ liberated was also found to correspond to the amount of citrulline broken down. The $^{14}CO_2$ liberated in the experiment was thus assumed to originate in the ureido carbon of the citrulline or in a compound very much like citrulline.

TABLE I

Flask contents and treatment	*Counts/min $Ba^{14}CO_3$*			
	Urease treatment		*Citrulline arsenolysis*	
	Otala	*Lumbricus*	*Otala*	*Lumbricus*
Controls:				
1 Zero-time incubation	4.3	1.8	2.5	0
2 Heat-inactivated tissue, incubated 3 h	1.0	3.9	0	0
3 Supernatant saline solution from tissue preincubation: incubated 3 h*	2.0	4.9	0	0
Experimentals:				
4 Resuspended tissue from Control No. 3: incubated 3 h*	262.8	414.4	142.0	34.3
5 Tissue incubated 1 h	89.9	125.5	111.7	18.0
6 Tissue incubated 2 h	104.9	169.1	62.9	16.3
7 Tissue incubated 3 h	70.0	190.0	75.0	13.2

* See text for explanation.

As shown in Table I, a significant incorporation of $^{14}CO_2$ into both the ureido carbon of citrulline and the urea carbon took place in the presence of Otala hepatopancreas and Lumbricus gut tissue. This incorporating ability was heat labile and present in the tissues as shown by Controls No. 2 and 3. Control No. 3 was prepared by preincubating each tissue for 1 h in 5 ml saline. At the end of this time, the tissue was separated from the saline solution by low-speed centrifugation (800 $\times$ g for 10 min). The supernatant saline was then incubated with $H^{14}CO_3^-$ as the control. The sedimented tissue was resuspended in 5 ml saline and also incubated with $H^{14}CO_3^-$ as indicated in the table (No. 4, Experimentals). In all cases, preincubation of the tissues caused an increased level of isotope incorporation.

In conjunction with previous observations on the earthworm[7,8,12] and snail[9], the data presented here strongly suggest that at least a low level of urea synthesis may be achieved in these two invertebrates by the urea cycle or by a very similar series of reactions. The incorporation of $^{14}CO_2$ into the ureido carbon of citrulline is, at present, most explicable in terms of an intermediate formation of carbamyl phosphate although the synthesis *in vitro* of this compound has not been demonstrated in invertebrate animals. The importance of the time and place of appearance of enzyme systems for the synthesis and metabolism of carbamyl phosphate during biochemical evolution has previously been discussed[18].

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The ornithine pathway in the yeast *Candida utilis*

The pathway of the ornithine synthesis in the yeast *Candida utilis* ((Henneberg) Lodder et Kreger-Van Rij (syn. *Torulopsis utilis* (Henneberg) Lodder) was partly elucidated by ABELSON AND VOGEL[1]. Experiments with tracer techniques revealed that L-ornithine was synthesized in *C. utilis* from L-glutamic acid, most probably *via* L-glutamic γ-semialdehyde[1].

Since cell-free extracts of *C. utilis* showed only minor activities of L-ornithine δ-transaminase (L-ornithine: 2-oxoacid amino transferase, EC 2.6.1.13), the enzyme that converts L-glutamic γ-semialdehyde to L-ornithine, a major role for a pathway involving non-acetylated intermediates is not likely. For this reason the role of acetylated intermediates in the synthesis of L-ornithine from L-glutamate by *C. utilis* was checked in the present investigation.

The production of L-ornithine *via* acetylated intermediates was demonstrated by VOGEL[2] in *Escherichia coli* and by UDAKA AND KINOSHITA[3] in *Micrococcus glutamicus*. In both organisms N-α-acetyl-L-ornithine is formed from L-glutamate *via* N-acetyl-L-glutamate, N-acetyl-L-glutamic γ-phosphate, and N-acetyl-L-glutamic γ-semialdehyde. In *E. coli* the N-α-acetyl-L-ornithine is split by the enzyme acetylornithinase to L-ornithine and acetate. In *M. glutamicus*, however, a transacetyla-