

## LIMITED PERMEABILITY AND METABOLISM OF L-GLUTAMATE IN THE LOCUST GUT WALL

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### INTRODUCTION

The process of intestinal absorption in insects, despite its central importance in the physiology of these animals, has been subjected to only a few studies. Treherne (1957, 1958*a-c*, 1959) demonstrated that absorption of certain sugars, fats, and amino acids occurs primarily in the midgut caeca of *Periplaneta* and *Schistocerca*. The force driving absorption of amino acids appears to be a diffusion gradient generated by the rapid removal of water from the gut luminal fluid. Additional support for a diffusional mechanism was provided by Shyamala & Bhat (1966), who observed that dinitrophenol and cyanide did not reduce the rate of transport of amino acids across the wall of the silkworm midgut. Their results indicated, however, that some amino acids pass across the gut wall faster than others. This may reflect the involvement of facilitated diffusion, hindered diffusion, or metabolism of certain amino acids during absorption.

We were particularly interested in discovering what happens to L-glutamate during absorption. L-Glutamate is the most abundant amino acid in many common animal and plant proteins (Needham, 1965). Insects are believed to degrade dietary protein to its constituent amino acids during digestion. There would therefore regularly arise significant amounts of the free amino acid in the insect gut lumen and gut wall resulting from the hydrolysis of food protein. Absorption of dietary L-glutamate intact would cause its blood levels to rise. This could have important physiological consequences, which now will be considered.

L-Glutamate may be an excitatory transmitter substance at insect neuromuscular junctions (cf. Usherwood, 1971). One category of evidence for this includes observations that L-glutamate added to the saline bathing an *in vitro* muscle preparation causes the muscle to contract. For example, the threshold concentration for the metathoracic unguis retractor muscle of the locust is  $c. 7 \times 10^{-6}$  M (Usherwood & Machili, 1968). The authors noted that even when the bath concentration of L-glutamate is well below threshold ( $> 7 \times 10^{-12}$  but  $< 7 \times 10^{-7}$  M) the responses of the muscle to indirect stimulation are potentiated. Such findings led them to assert that structural or enzymic barriers between the blood and the neuromuscular synapses cannot be invoked. Furthermore, on the basis of blood analyses and studies of the pharmacological activity of L-glutamate added to blood, they suggested that there is very little 'free' L-glutamate in the blood of orthopteroid insects.

If these views are valid, then it would be expected that blood levels of 'free' L-glutamate would be kept low. The fact that mosquito larvae survive on a diet of glutamate or of casein (Wigglesworth, 1942), which contains 20% glutamate (Hipp, Basch

& Gordon, 1961), speaks for some sort of mechanism controlling L-glutamate in the blood. It appeared possible that the gut wall might play an important role in regulating the entry of dietary L-glutamate into the blood from the gut lumen. We therefore undertook to discover the relationship between gut-luminal and blood concentrations of L-glutamate in locusts. The present study examines the passage of L-glutamate through the intestinal wall of the locust *in vivo*. We sought a method that would enable the passage of amino acids through the gut wall to be studied under as natural conditions as possible. The techniques of gut infusion of radio-labelled precursors developed by Treherne (1958*b*) enables the disappearance of radioactivity from the gut lumen to be followed quantitatively, but it tells little about the nature of absorbed products. We found it possible to follow passage through the gut wall by combining gut infusion of the  $^{14}\text{C}$ -precursors with simultaneous perfusion of the haemocoel. Analysis of the perfusate gives information about the quantity and molecular nature of absorbed products.

To provide a better perspective for understanding the absorption/metabolism of L-glutamate, L-alanine and glycine were also studied. L-Alanine was chosen because, after deamination, its carbon skeleton can feed into the citric acid cycle and can be utilized for energy production, as can that of L-glutamate. A subsequent paper (Murdock & Koidl, 1972) will consider the nature of blood metabolites after gut infusion of the amino acids.

#### MATERIALS AND METHODS

##### *Animals*

*Schistocerca gregaria* and *Locusta migratoria* were bred in our laboratory from stock provided by the Anti-Locust Centre. Breeding conditions were as recommended by Hunter-Jones (1966). Locusts were not allowed food for 24–36 h prior to the experiment.

##### *Perfusion*

During emplacement of the hindgut cannula the animal was held to a glass rod by means of tape strips binding the thorax and abdomen (Fig. 1). Under the mouth-parts several turns of absorbent paper were attached to the rod; this served to absorb crop fluid that was sometimes regurgitated, preventing it from dripping into the perfusate-collecting tube. The wings of the animal were bent forward halfway along their length, and fastened with tape. This exposed the posterior abdominal segments and made the insertion of the perfusion needle easier.

The hindgut was cannulated with a Hamilton 100  $\mu\text{l}$  syringe having a permanently attached needle. To prevent injury during cannulation, the needle tip was fitted with a 2–3 mm collar of fine plastic catheter tubing. The needle was inserted 5–7 mm into the rectum. This was facilitated by lubricating the catheter tip with glycerol. After the cannula had been inserted, it was ligatured firmly in place with wetted cotton thread. In some experiments it was sealed in place with a beeswax–resin mixture.

With the cannula in place the syringe barrel was firmly bound to the glass rod with strips of tape; the extended plunger was also fixed to the glass rod with tape so that it could not slip and cause premature injection. The glass rod with the affixed insect and syringe was then clamped in a vertical position, with the insect's head some 10 cm above the table top.

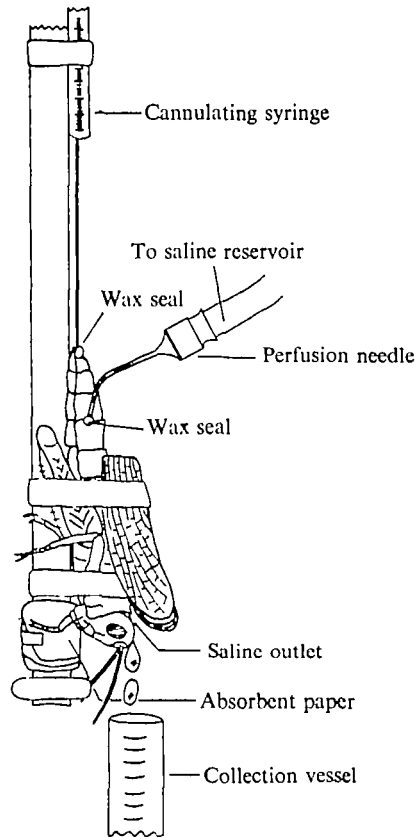


Fig. 1. Schematic representation of the perfused-locust preparation. 100  $\mu$ l of saline containing the  $^{14}\text{C}$ -labelled precursor were injected into the alimentary tract. Simultaneously, the haemocoel was perfused by means of a perfusion needle inserted into the abdominal haemocoel. The perfusate was collected in calibrated tubes and subjected to quantitative analysis.

To perfuse the haemocoel, a No. 27 B-D hypodermic syringe needle was mounted on the barrel of a disposable plastic syringe. The syringe barrel was connected to a saline reservoir by a length of plastic tubing. The reservoir was held 30–50 cm above the needle tip; this produced a hydrostatic head sufficient in most cases for a satisfactory perfusion rate. The barrel of the perfusion syringe was clamped in a micro-manipulator, which enabled the needle to be inserted into the animal with a minimum of injury. The perfusion needle was bent into a curve and was made to pierce the intersegmental membrane of one of the terminal abdominal segments in such a way that the needle tip entered and ran parallel to the long axis of the insect's body. The tip entered *c.* 5 mm. The junction of needle and intersegmental membrane was sealed over with beeswax-resin mixture; this was found to prevent back-leakage from the wound, and served also to minimize further damage when the animal made struggling movements.

To facilitate perfusion it was found useful to bend the insect's head forward and seal it in place with droplets of wax placed at the lateral junction of the head capsule and thoracic shield. A small cut was then made in the dorsal cervical membrane, taking care to cut as shallowly as possible. Usually blood began welling from the

wound. At this time the screw clamp on the tubing leading to the saline reservoir was loosened. After a few drops of saline had passed through the animal, the flow rate was increased to a value *c.* one-third faster than that finally desired, and the haemocoel was allowed to wash out for 30 min. With an initial value of 30 ml/hr the flow rate usually stabilized later at 15–25 ml/h. If a rate of at least 15 ml/h could not be attained, the experiment was discontinued.

During the final 3 min of pre-perfusion, a sample of perfusate was collected to serve as a background control in the radioactivity determinations. The plunger of the cannulating syringe was then depressed, over a period of *c.* 30 sec, expelling its contents into the alimentary tract. Beginning at the moment of injection, a calibrated 15 ml centrifuge tube was placed under the animal's head, and the saline dripping into it was collected during the following 3 min.

The volume was noted, and the next collecting tube was put into place. Samples were collected at intervals of 3 min over the following 30 min, and at 42–45 and 57–60 min after gut infusion of the radioactivity. When L-glutamate was being studied, the collections were continued at 3 min intervals for 45 min and then at 15 min intervals for an additional 75 min.

#### *Scintillation counting*

To determine the quantity of radioactivity absorbed, a 50  $\mu$ l sample of perfusate was taken immediately after the end of each 3 min collecting period. The sample was expelled under the surface of 1 ml of a 1:2 mixture of ethanolamine and ethylene glycol monomethyl ether (EA:EGME) in a scintillation vial. The EA:EGME solution was required to trap any  $^{14}\text{CO}_2$  present in the perfusate. When all samples had been collected, 10 ml of scintillation fluid (Bray, 1960) was added to each vial and the radioactivity was measured in a Packard Tri-Carb scintillation counter. Quenching was routinely monitored using the external standard accessory, but was found to vary insignificantly. By means of internal standards the counting efficiency was found to be 56%. The radioactive yield of each 3 min sample was calculated by multiplying the dpm/50  $\mu$ l aliquot by the total sample vol ( $\mu$ l). The percentage total yield in a 3 min sample was calculated by dividing the dpm/sample by the total dpm injected into the gut and multiplying by 100.

#### *Heat-volatile and acid-volatile metabolites*

To determine the amount of volatile radioactivity in samples from the L-glutamate experiments, a 50  $\mu$ l aliquot of perfusate was added to a glass scintillation vial. 100  $\mu$ l of 2 N-HCl was then added, the contents of the vial were mixed well, and brought to dryness at 105 °C. After allowing the vial to cool, 50  $\mu$ l of distilled water were added to redissolve the residue, followed by 1 ml of the EA:EGME solution and scintillation fluid as above. The difference between the total radioactivity determined by direct counting of a 50  $\mu$ l perfusate aliquot and that found after heat/acid treatment of another 50  $\mu$ l aliquot represents the portion of radioactivity in the perfusate due to  $^{14}\text{CO}_2$  and other volatile metabolites. Tests with precursor L-glutamate-1- $^{14}\text{C}$  carried through the heat/acid procedure showed that no radioactivity was lost from it.

*Assay of  $^{14}\text{CO}_2$* 

The  $^{14}\text{CO}_2$  determinations were made essentially as described by Tait (1970). A sample of perfusate was put into a scintillation vial, which was immediately closed with a rubber stopper. A metal hook attached to the lower end of the stopper held a  $1.5 \times 1$  cm square of glass-fibre paper that had been wetted with  $20\ \mu\text{l}$  of 10% KOH. When all samples from a given experiment had been collected, each vial was opened,  $100\ \mu\text{l}$  of 2 N-HCl were added and the vial was immediately recapped. Absorption was allowed to proceed for 30 min, after which the glass-fibre was removed, dried in a stream of warm air, and added to a scintillation vial containing 5 ml of scintillation fluid. Studies carried out with standard  $^{14}\text{C}$ -bicarbonate revealed a combined trapping and counting efficiency of 53%.

*Infusion and perfusion fluids*

The composition of the saline used was: NaCl, 200 mM; KCl, 10;  $\text{CaCl}_2$ , 2;  $\text{Na}_2\text{HPO}_4$ , 6;  $\text{NaH}_2\text{PO}_4$ , 4. Solutions infused into the gut contained also 50 mM of the amino acid to be studied, plus  $10\ \mu\text{Ci/ml}$   $^{14}\text{C}$  in the form of the amino acid. When L-glutamate was infused, the solution was made up in saline containing 150 mM-NaCl and 50 mM sodium L-glutamate. This was necessary because of the limited solubility of L-glutamic acid. The pH of the gut-infusion solution and the perfusion saline was 6.7. The initial experiments employed a high L-glutamate concentration because we sought to assess the capacity of the alimentary tract to protect against the entry of this amino acid. The concentrations of the other amino acids were also set at 50 mM to enable direct comparisons of their fate with that of L-glutamate.

*Radioisotopes*

L-Glutamate- $1\text{-}^{14}\text{C}$  was supplied by Calatomic (Los Angeles, Calif.); all other  $^{14}\text{C}$ -amino acids were purchased from NEN GmbH (Frankfurt/Main). Radioactive sodium bicarbonate- $^{14}\text{C}$  was supplied by NEN at a concentration of  $1\ \mu\text{Ci/ml}$  (sp. act.  $10\ \mu\text{Ci}/\mu\text{mole}$ ). Experiments in which counting efficiency was determined with internal standards made use of  $^{14}\text{C}$ -tridecane (Radiochemical Centre, Amersham).

## RESULTS

*Observations on the method of whole-animal perfusion*

We found that injection of  $100\ \mu\text{l}$  of fluid into the locust gut is a satisfactory method for establishing an approximately known concentration of a substance in the lumen of the alimentary tract. Preliminary experiments in which indicator dye solutions were injected confirmed that the fluid reaches the proventriculus-midgut junction, and that the gastric caeca in particular show pronounced increases in volume. We also found that gastric caeca in starved animals are often very much reduced in size. These observations indicate that the caeca are readily expansible reservoirs whose volume reflects the feeding state of the animal.

Pulsatory movements occur in the caeca. These pulsations appear to be centred at the several lines dividing the caeca along their length. They arise at irregular intervals at a spot on the longitudinal division-line and move some distance along it. It appears

that they involve an opening and shutting of a space between the longitudinal division of the caecum. They can best be observed in a freshly opened animal. Usually these movements, which are normally of small amplitude, cease after the caecum has been exposed to saline for a few minutes, and they have not been observed to start up again. Presumably they have something to do with the absorptive functions of the caeca.

During perfusion it often happened that the animals became stunned. Whether this is due to the unnaturally high pressures required to obtain a satisfactory flow rate, or has another cause, is not clear. Many of the animals that became stunned recovered within an hour or two after halting perfusion and removing the cannula, and some of these were observed to live for several days thereafter; they were seen to copulate, eat and defaecate, apparently normally. In other experiments, the locusts remained active and responsive throughout the experiment, and could walk and jump upon being freed from the perfusion assembly.

When the solution containing the radioisotope was first injected into the gut there was usually a marked decrease in perfusate flow rate. Probably this stems from the increased flow resistance due to filling of the haemocoel with the expanded gut. Small variations in flow rate were often seen. This is presumably caused by spontaneous position changes of the gut, or by small shifts in body position which change the flow resistance of the haemocoel. In some but not all experiments there was a slow downward drift in flow rate during the course of the experiment. For these reasons it was necessary to monitor the flow rate continuously.

Experiments were also made in which animals that had fed at different times before the experiment were cannulated and perfused. In general, animals that have fed shortly before the experiment are unsatisfactory because it is usually impossible to obtain the required perfusion rate. Apparently this is due to the full crop and gastric caeca which effectively plug the haemocoel in the crop region. Animals that had last fed 24–36 h prior to the experiment were found to be satisfactory.

To establish that a flow rate of  $\frac{1}{3}$  ml/min ensured a rapid washout of the radioactivity entering the haemocoel, L-glutamate- $U$ - $^{14}C$  was injected into the haemocoel of animals undergoing perfusion. These animals had been cannulated and gut-infused with 100  $\mu$ l of saline containing 50 mM 'cold' L-glutamate. Fig. 2 shows that nearly all of the haemocoel-injected radioactivity appeared in the collected perfusate in the first 3 min, and that in the next three time intervals the recovery was quantitative. This suggests that any radioactivity entering the haemocoel from the alimentary tract should be washed out of the animal rapidly, and will be quantitatively recovered.

#### *Absorption of radioactivity from L-glutamate- $I$ - $^{14}C$*

Fig. 3 shows the time-course of appearance of radioactivity in the perfusate after L-glutamate- $I$ - $^{14}C$  is injected into the alimentary tract of *Schistocerca*. Some  $30.9 \pm 5.0$  (S.E.) percent of the  $^{14}C$  appeared in the perfusate during the following hour. Peak release occurred between 15 and 40 min in the different experiments. These observations establish that a significant fraction of the infused  $^{14}C$  does reach the saline perfusing the haemocoel.

Attempts to determine the molecular nature of the radioactivity in samples of the perfusate by chromatography revealed that most of the  $^{14}C$  was easily volatilized in the presence of acid. The possibility that the acid-volatile radioactivity represented a form

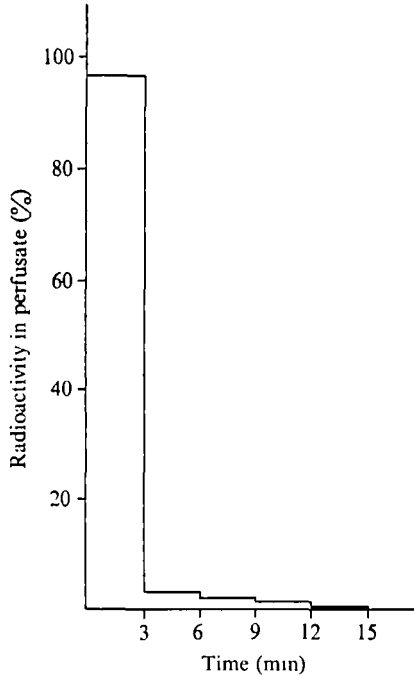


Fig. 2. Washout of L-glutamate injected into the saline-perfused haemocoel of *Schistocerca gregaria*. In two experiments overall recovery averaged 102.7%. Most of the radioactivity appeared in the first 3 min.

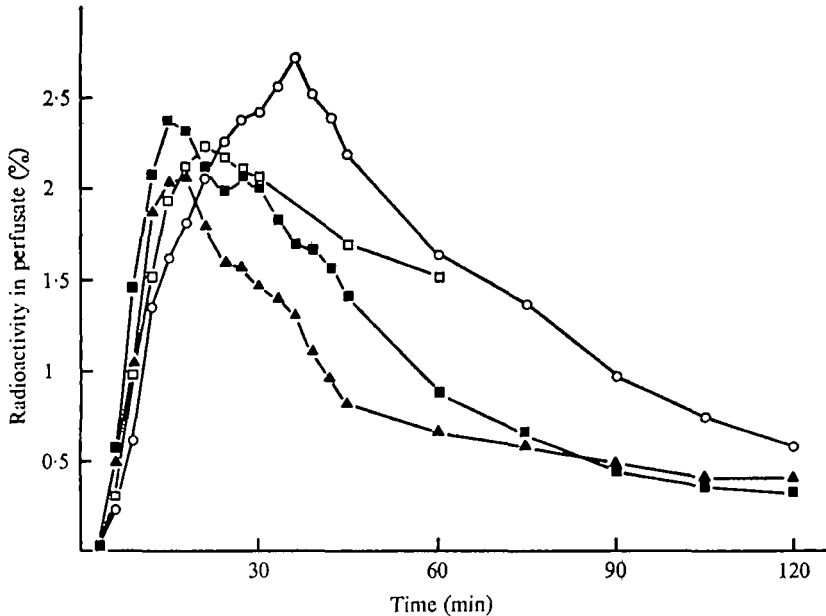


Fig. 3. Release of radioactivity into the haemocoel perfusate after injection of L-glutamate-1- $^{14}\text{C}$  into the alimentary tract of *Schistocerca*. Each point represents the percentage of the total injected dose that appeared in the perfusate in the preceding 3 min. The average 1 h yield for the four experiments shown as  $30.9 \pm 5.0$  (s.d.)%. Values for the period 45–57 min were extrapolated from the curves. The radioactivity released was predominantly  $^{14}\text{CO}_2$ /bicarbonate (see Table 1).

Table 1. *Percentage of haemocoel-perfusate radioactivity releasable as  $^{14}\text{CO}_2$  after gut infusion of L-glutamate-1- $^{14}\text{C}$* 

Experiment	Time*	$^{14}\text{CO}_2$ (%)†	
		Direct assay	Calculated from residue
1	6-9	75	67
	18-21	77	77
	30-33	91	84
	36-39	81	90
	72-75	91	80
2†	6-9	48	67
	18-21	71	68
	42-45	81	77
3	42-45	85	—
4	12-15	82	—
	18-21	62	—
	24-27	82	—
	30-33	80	—
	36-39	81	—
	72-75	90	—
5	12-15	79	—
	24-27	82	—
	42-45	96	—

\* Minutes after gut infusion of the labelled precursor.

† *Locusta migratoria*; all others are *Schistocerca*.

† As percentage of total radioactivity recovered during the 3 min period of the sample.

of  $^{14}\text{CO}_2$  was investigated. As can be seen from Table 1, an average of 80% of the perfusate radioactivity could be released by acid and trapped on KOH paper. Approximately the same quantity of volatile radioactivity could be calculated from determinations of the non-volatile residue and a knowledge of the total radioactivity in the sample. These observations suggest that the major share of radioactivity in the perfusate is not unchanged L-glutamate-1- $^{14}\text{C}$ , but rather that it is derived from a form of  $^{14}\text{CO}_2$  arising from metabolism of the gut-infused L-glutamate. Inspection of Table 1 indicates that the content of volatile radioactivity tends to be somewhat lower in initial fractions, perhaps rising slowly as perfusion continues. On the whole, however, the ratio  $^{14}\text{CO}_2$ :residual- $^{14}\text{C}$  is surprisingly constant at *c.* 4:1.

The metabolism producing the  $^{14}\text{CO}_2$  very likely takes place in the tissues of the alimentary tract wall. Gastric caeca, which appear to be the main sites of amino acid absorption (Treherne, 1959), do actively metabolize L-glutamate (Murdock & Koidl, 1972). The rapid washout of radioactivity entering the haemocoel (Fig. 2) would seem to preclude uptake and metabolism in other tissues.

The form of the  $^{14}\text{CO}_2$  in the perfusate may be either (i) the dissolved gas, or (ii) carbonic acid and bicarbonate derived from the reactions:  $\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3$  and  $\text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^-$ . To indicate the uncertainty of the molecular nature of this radioactivity, it will be referred to as  $^{14}\text{CO}_2$ /bicarbonate in the subsequent discussion.



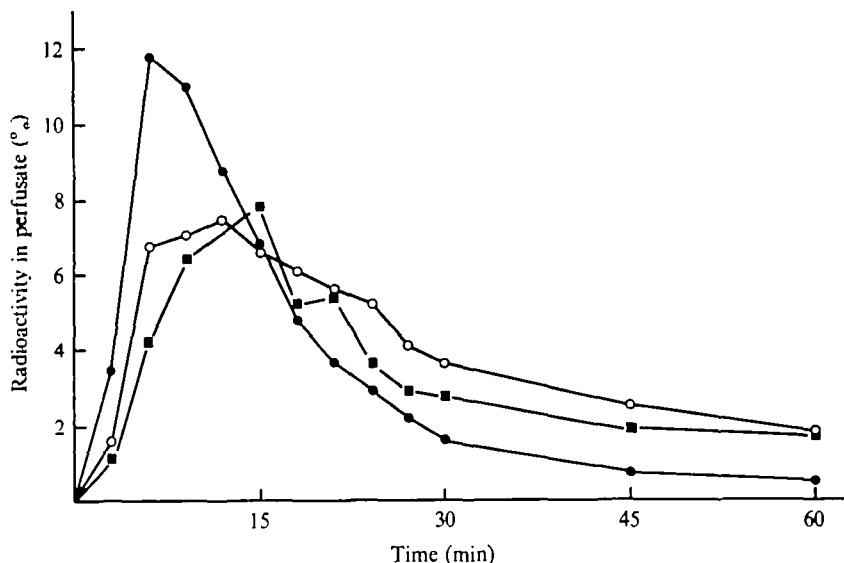


Fig. 4. Absorption of  $\text{NaH}^{14}\text{CO}_3$  infused into the alimentary tract of *Schistocerca gregaria* after infusion into the gut lumen. Each point represents the percentage of the total injected dose that appeared in the perfusate during the preceding 3 min. The total yields at 1 h were: ●, 70%; ■, 66%; ○, 79%. Values for the periods 30–42 min and 45–57 min were extrapolated from the curves.

#### Absorption of $^{14}\text{C}$ -bicarbonate

To learn more about the absorption of bicarbonate itself, a series of experiments were made in which  $^{14}\text{C}$ -bicarbonate was injected into the alimentary tract of perfused animals. The solution injected was of the same ionic composition as the perfusion saline, but contained in addition  $0.1 \mu\text{Ci NaH}^{14}\text{CO}_3$ . Fig. 4 shows some typical release curves. It is clear that bicarbonate enters the haemocoel perfusate very rapidly. Peak release occurred between 6 and 12 min. The total 1 h yield varied from 66 to 79%, most of which appeared in the initial 30 min.

In the L-glutamate- $1\text{-}^{14}\text{C}$  experiments a fraction (c. 20%) of the perfusate radioactivity was in a form stable to heat and drying in the presence of acid. Considering the high yield of  $^{14}\text{CO}_2$ /bicarbonate, it could have happened that some of this underwent carbon dioxide fixation reactions, forming products stable to heat and acid, thus contributing to the residual radioactivity. To evaluate this, samples of the perfusate from the bicarbonate-infusion experiments were subjected to heat/drying in the presence of acid. Residual radioactivity was sometimes found, but this was always small; c. 1.0%, at most, of the total radioactivity originally present in the perfusate sample. The residual radioactivity therefore represents mainly unchanged L-glutamate or its metabolic products other than  $^{14}\text{CO}_2$ /bicarbonate.

#### Absorption of L-alanine and glycine

An average of  $41.3 \pm 9.3$  (S.E.)% of the radioactivity injected into the alimentary tract of *Locusta* as L-alanine- $1\text{-}^{14}\text{C}$  appeared in the perfusate during the hour following injection (Fig. 5). Approximately 30% of this radioactivity was present as  $^{14}\text{CO}_2$ /bicarbonate. The metabolism of luminal L-alanine thus appears to be considerable, but much less than for L-glutamate. The time-course of release of radioactivity from

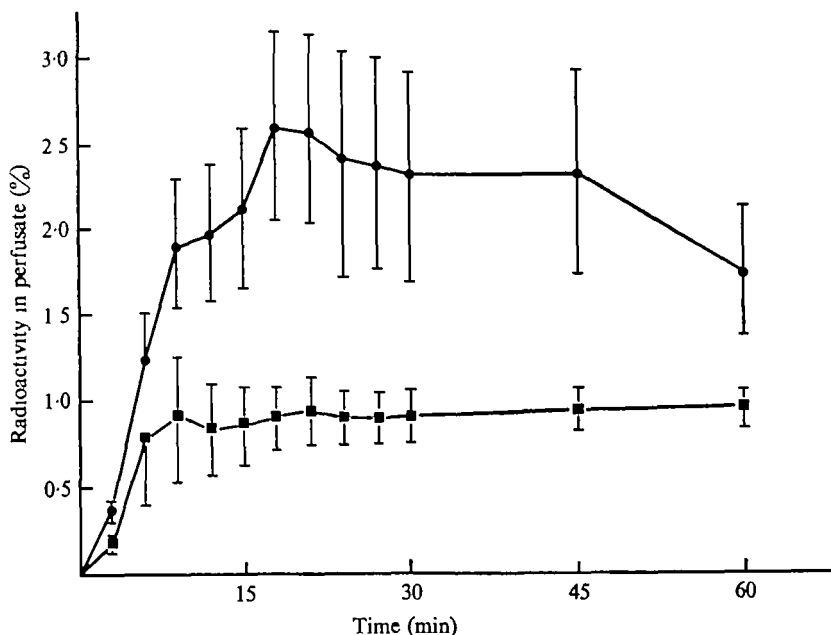


Fig. 5. Absorption of radioactivity after gut infusion of L-alanine-1-<sup>14</sup>C (●—●) and glycine-1-<sup>14</sup>C (■—■) into *Locusta*. Each point represents the average percentage of the total injected dose that appeared in the perfusate in the preceding 3 min. Total 1 h yields were: alanine— $41.3 \pm 9.3$  (S.E.) % and glycine— $17.6 \pm 3.3$  (S.E.) %, based on extrapolated values for the period 30–42 and 45–57 min.

L-alanine-1-<sup>14</sup>C was similar to that of L-glutamate in that peak release occurred between 15 and 30 min after gut infusion.

The time-course of release of radioactivity into the perfusate when glycine-1-<sup>14</sup>C was infused, however, differed from that seen with L-glutamate and L-alanine (Fig. 5). In the hour following injection of the precursor,  $17.6 \pm 3.3$  (S.E.) % of the gut-infused radioactivity appeared in the perfusate. Only 2.5 % of the perfusate radioactivity could be trapped on KOH paper, which is considered insignificant; the glycine-1-<sup>14</sup>C precursor contained 1–2 % apparent <sup>14</sup>CO<sub>2</sub>. Thus only a small fraction of the 1-<sup>14</sup>C of glycine, if any, becomes oxidized to <sup>14</sup>CO<sub>2</sub>. The release rate rose quickly to a peak of c. 9 min after gut infusion and remained at this level during the following hour.

#### DISCUSSION

The gut infusion/haemocoel perfusion technique presents a method by which certain aspects of the intestinal absorption of nutrients in locusts may be studied quantitatively *in vivo*. It may be valuable to consider the limitations of the method. First, the site of absorption is unknown; the appearance of radioactivity in the perfusate after infusion of the labelled precursor into the gut lumen merely shows that radioactivity has passed through the gut wall. In the case of locusts, however, it seems established that absorption occurs primarily in the gastric caeca (Treherne, 1957, 1958a–c, 1959), so it is not unreasonable to assume that much of the perfusate radioactivity found in our experiments entered the haemocoel at this site. Secondly, when a substance is studied which may undergo extensive metabolism in the gut-wall tissue

The appearance of radioactivity in the perfusate is no indication of absorption of the unchanged precursor. In such cases the time-course of release of radioactivity represents the sum of radioactivity released as  $^{14}\text{CO}_2$ /bicarbonate, other metabolites, and unchanged precursor. Additional methods must be employed to ascertain the nature of the perfusate radioactivity. Thirdly, the method as presently used is capable only of indicating one-way movement of radioactivity; it can therefore say little about the mechanisms driving absorption.

The perfusion experiments with L-glutamate-1- $^{14}\text{C}$  indicate that there is extensive metabolism of the substance in the tissues of the gut wall. The residual radioactivity of the perfusate (c. 20% of the total) could represent unchanged L-glutamate, its metabolites, or a mixture of the two. If we make the assumption that this residual radioactivity represents unchanged L-glutamate, it can be calculated that within 1 h approx. 6% of the radioactivity originally infused into the gut lumen appeared in the perfusate as unchanged L-glutamate. With glycine, which is not metabolized to any significant extent, the 1 h yield was c. 18%. It follows that the maximum influx of L-glutamate is only about one-third that of glycine. When L-alanine-1- $^{14}\text{C}$  was injected, c. 30% of the overall 41% 1 h yield was  $^{14}\text{CO}_2$ /bicarbonate, leaving 29% in a residual form.

Of this residual radioactivity, we estimate from chromatographic analyses that at least 80% is unchanged L-alanine, so in 1 h there is a net influx of alanine comprising c. 23% of the dose originally infused into the gut. Compared with L-glutamate, then, L-alanine crosses the gut wall some four times faster. It would thus appear that intact L-glutamate crosses the gut wall considerably more slowly than two other common amino acids.

Actually, a large proportion of the residual radioactivity in the L-glutamate infusion experiments is probably in the form of glutamine, for this amino acid is the main gastric caecal metabolite of L-glutamate which would contain the original 1-carbon of L-glutamate (Murdock & Koidl, 1972). If this is so, then the retardation of L-glutamate influx is far more striking than the comparison above indicates. Slow penetration of L-glutamate through the gut wall is not limited to locusts, for Shyamala & Bhat (1966) reported that passage of this amino acid across the intestinal wall of the silk-worm was slow compared to other amino acids.

When L-glutamate-1- $^{14}\text{C}$  is injected into the locust gut lumen, there is a rise and fall in the rate of appearance of radioactivity in the perfusate. There is a relatively constant (perhaps slowly rising) ratio  $^{14}\text{CO}_2$ /bicarbonate:residual- $^{14}\text{C}$  through the experimental period. This can be interpreted as indirect evidence for the release of a metabolic product instead of the unchanged precursor. For example, if L-glutamate were impermeant, but glutamine produced from it could cross the gut wall, then the release of glutamine would ultimately depend upon the rate at which it were synthesized. In the presence of adequate L-glutamate this rate would depend upon the rate at which co-substrate  $\text{NH}_4^+$  becomes available, that is, it would depend upon the rate at which the tissue was metabolizing, which in turn would be reflected in the rate at which  $^{14}\text{CO}_2$ /bicarbonate was being produced and released. There would thereby result an association of residual radioactivity and  $^{14}\text{CO}_2$ /bicarbonate radioactivity which would be reflected in the relatively constant ratio of the two types of radioactivity.

Although the apparent coupling of  $^{14}\text{CO}_2$ /bicarbonate and residual radioactivity

release could be explained in this way, the question of the ultimate cause of the rise and fall of overall metabolism has not been answered. Exhaustion of substrate can be ruled out, for *c.* 70% of the radioactivity remains in the lumen after 1 h. It may be that the stretching of the gut caused by the injection of a large volume of solution triggers compensatory metabolism, such as might be required for ionic pumps which would bring the composition of the luminal fluid to a new equilibrium position; as that equilibrium is approached the metabolism shuts down. If L-glutamate could serve as a metabolic substrate for such pumps, then its metabolism would be expected to rise and fall.

An alternative explanation is based on two assumptions: (1) the metabolism observed is largely a reflection of the L-glutamate concentration in the gut lumen; (2) a metabolite retaining the 1-carbon is produced from L-glutamate and comes to replace it in the gut lumen. This metabolite is itself subject to only slow metabolism. The fall off in metabolism would be a consequence of the fall off in the concentration of L-glutamate in the gut lumen. The postulated retention of the 1-carbon of L-glutamate in the metabolite is needed to account for the large amount of radioactivity remaining in the gut after the burst of metabolism is past. This metabolite could be produced in the gut wall and released back into the lumen of the gut. Metabolism of L-glutamate in the gut lumen itself can probably be ruled out, for we have been unable to find any evidence for metabolism when L-glutamate- $U-^{14}C$  is incubated with gut contents. Substantial metabolism by gut-lumen symbionts seems therefore unlikely, as does secretion into the lumen by the gut wall of enzymes metabolizing L-glutamate. Such a rise and fall in radioactivity release possibly occurs with L-alanine, but it may be less marked. Presumably this reflects the smaller metabolic capacity of the gut wall for L-alanine.

The large burst of metabolism and radioactivity release following injection of L-glutamate- $1-^{14}C$  into the gut may thus be a consequence of a sudden change in the composition of the luminal fluid, and therefore may be regarded as artifactual. However, the high percentage of  $^{14}CO_2$ /bicarbonate that appears in the perfusate continues long after peak release. We interpret this to mean that, even under more or less 'steady-state' conditions, metabolism is the predominant fate of luminal L-glutamate. The burst of metabolism seen with L-glutamate and not with glycine apparently reflects the particularly high capacity of the gut to oxidize L-glutamate compared to glycine.

Only glycine showed an absorption that was uncomplicated by metabolism. The absorption curve indicates a rapid rise to maximal entry rate, which continued for the remainder of the experimental period. Such an absorption curve can be rationalized by assuming that glycine passes through the gut wall at a limited number of sites on the surface of the alimentary tract, and that the concentration was sufficient to cause saturation. Since only 18% of the glycine was absorbed during the first hour, the concentration never fell below the saturation level, hence a constant rate of absorption.

When it is recalled that the gut-luminal concentration of L-glutamate used in these experiments was 50 mM, then it can be appreciated how effective the mechanisms are that prevent its entry into the haemocoel space. Probably in the normal animals such high luminal concentrations would never be attained. It may be that little, if any, unchanged L-glutamate is absorbed in locusts.

The limited passage of luminal L-glutamate across the gut wall and the extensive gut-associated metabolism may prevent, or at least suppress, entry of the intact amino acid into the blood. This being so, the gut plays an important part in regulating the blood concentration of L-glutamate. If large increases in blood L-glutamate should be able to cause non-adaptive excitation of somatic or other muscles, and the gut plays a part in preventing these rises from coming about, then the gut may be regarded as at least one link in the chain that guards against unnatural excitation of muscles by this amino acid. That there are other, complementary systems for controlling blood L-glutamate is probable (cf. Murdock & Koidl, 1972). It is possible to imagine a more subtle effect of rising and falling levels of blood L-glutamate. If low levels of the amino acid should be able to potentiate the response of muscles to neural excitation (Usherwood & Machili, 1968), then the response of muscles would vary to some extent with the level of L-glutamate in the blood. In light of the often occasional nature of feeding and the variation in the L-glutamate content of foods, considerable variations in blood L-glutamate might well occur if there were no systems to regulate its blood concentration. This comprises an additional argument for control of blood L-glutamate, in which we suggest the gut tissues play a significant part.

## SUMMARY

1. A method is described which enables the passage of nutrients through the gut wall of locusts to be studied quantitatively *in vivo*. The method consists of infusing the radio-labelled precursor into the gut lumen while the haemocoel is perfused with saline. The perfusate is collected and analysed.

2. Compared to L-alanine and glycine, intact L-glutamate crosses the locust gut wall slowly, if at all.

3. Metabolism of L-glutamate in the gut wall is extensive, that of alanine markedly less, while there is little metabolism of glycine.

4. It is suggested that limited permeability and extensive metabolism in the gut wall combined to severely restrict the entry of intact dietary L-glutamate into the blood.

5. The gut wall may be an important site of regulation of blood L-glutamate, preventing or hindering influx of this abundant dietary amino acid. The system could be a contributing factor in protecting neuromuscular synapses from non-adaptive excitation by helping to prevent the concentration of blood L-glutamate from rising.

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