THE ABSORPTION AND METABOLISM OF SOME SUGARS IN THE LOCUST, *SCHISTOCERCA GREGARIA* (FORSK.)

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INTRODUCTION

The absorption of labelled glucose from the mid-gut of *Schistocerca gregaria* (Forsk.) has been shown to be related to the rate of its conversion to trehalose, which accumulates in the haemolymph (Treherne, 1958*a*). The rate of disappearance of glucose from the isolated alimentary canal suspended in a relatively large volume of circulating poisoned saline was similar to the absorption obtained in the intact animal when there was a rapid conversion to trehalose. On the basis of these observations it was suggested that the mechanism of glucose absorption might involve a diffusion across the gut wall, this process being facilitated by the rapid conversion to trehalose which would tend to maintain a steep concentration gradient. The similarity of this hypothesis to the early, and now outmoded, theories of Höber (1899) and Verzár (1931) for the absorption of hexoses from the mammalian intestine was noted (Treherne, 1958*a*, *b*).

In the present investigation experimental observations have been extended to mannose and fructose in an attempt to throw some further light on the nature of the absorptive processes involved. A detailed analysis has also been made of the absorption of labelled glucose at low concentration, for the uptake at these levels is of considerable theoretical interest in interpreting the extent of the facilitated diffusion and the role of any other absorptive processes in the absorption of sugars by this insect.

METHODS

Many of the techniques employed in this investigation were similar to those used in previous studies (Treherne, 1957, 1958*a*) and for this reason detailed descriptions have been limited to techniques incorporating novel features.

The experiments were performed on adult female *S. gregaria* (Forsk.) which were reared and maintained at 28·0 ± 1·0° C. and fed on fresh green wheat grown in pots. To empty the alimentary canal individuals were isolated in glass jars and deprived of food for 24 hr. preceding the experiment.

In order to fill the alimentary canal with the experimental solution a fine nylon hypodermic needle was thrust into the rectum of an insect anaesthetized with CO_2, and 0·15 ml. of fluid forced into the lumen of the gut. By this method the whole of the hind-gut and mid-gut was filled with the experimental solution.

The amount of ^{14}C-labelled monosaccharide absorbed from each part of the gut was determined using a dye, Amaranth (Azo-Rubin S), as a marker. This dye was not absorbed from the lumen of the gut and the percentage absorption of the labelled sugars was calculated from the glucose/dye ratio in the various parts of the alimentary canal. Amaranth has also been found suitable for use as a blood volume indicator by Yeager & Munson (1950). The dye concentration used in this investigation was 0·05 M/l. The experimental solution used was based on the saline described by Hoyle (1953). When the concentration of the sugars was altered, in the range 0·00134–0·30 M/l., the total osmolarity of the solution was maintained by appropriate adjustment of the NaCl concentration.

The ^{14}C-labelled sugars used in this investigation were glucose, fructose and mannose obtained from the Radiochemical Centre, Amersham. In each case the radio-active molecules were generally labelled with ^{14}C. The radio-activity was assayed in solution as previously described (Treherne, 1957) or counted on lens paper (Reid, 1947), using a thin-windowed G.M. tube (G.E.C. CV 2139).

Initially the experimental solution was collected from the gut lumen by removing the gut and dropping the washed ligatured portions into known volumes of a solution buffered to pH 10·0. In some later experiments it was found necessary to obtain samples of the contents of the mid-gut caeca in which there was no trace of contamination by the haemolymph. For this purpose 0·5 mm. diameter nylon tube was drawn out over a low flame and a 7·0 cm. length was fixed to an ordinary hypodermic needle-holder mounted on to a 1·0 ml. syringe. The dead space of the syringe and nylon tube was filled with liquid paraffin. The locust was anaesthetized with CO_2 and quickly opened to expose the alimentary canal. The nylon tube was then thrust into the rectum and pushed gently forward until the tip was in the region of the caeca. A small volume of fluid was then withdrawn into the tube by suction and the tube removed from the gut.

The separation and identification of carbohydrates from the haemolymph was accomplished by descending paper chromatography using Whatman No. 1 filter-paper. The following solvent systems were employed: ethyl acetate/acetic acid/water (Jermyn & Isherwood, 1949), *n*-butanol/ethanol/water (Hirst & Jones, 1949) and *n*-propanol/ethyl acetate/water (Baar & Bull, 1953). Reducing substances on the chromatograms were detected using the silver-nitrate method of Trevelyan, Proctor & Harrison (1950). The presence of non-reducing substances was revealed by spraying with 0·5% sodium-*meta*periodate (Evans & Dethier, 1957) before treatment with silver nitrate. Control experiments showed that single spots of haemolymph applied to the base line of the chromatograms did not interfere with any of the solvent systems used. When more than one spot was applied to the same portion of the paper then some interference resulted. It was therefore not possible to concentrate the haemolymph at the base line by successive applications of haemolymph to one portion of the paper. It was found, however, that if the haemolymph was coagulated by heating to 100° C. and then centrifuged the resulting supernatant did not interfere and the carbohydrate could be safely concentrated on the chromatogram by successive applications of this fluid.

To determine the concentration of glucose and trehalose in the haemolymph these substances were eluted from the chromatograms and determined by the anthrone method described by Dimler, Schaefer, Wise & Rist (1952). Glucose was found to be present in relatively small amounts and it was found necessary to apply at least 25 vol. of a 3·0 μl. pipette in a row along the base line of the chromatogram.

Blood volume determinations were carried out by the dye method of Yeager & Munson (1950).

RESULTS

The uptake of labelled mannose and fructose from the various parts of the alimentary canal during an experimental period of 15·0 min. was studied at concentrations of 0·002, 0·02 and 0·20 M/l. At all three concentrations the uptake was found to be confined to the caeca and ventriculus (Figs. 1, 2). The disappearance of mannose from the caeca was found to proceed more rapidly at a concentration of 0·002 M/l. than at 0·02 M/l., a difference which was statistically significant ($P < 0{\cdot}01$). There was a smaller difference between the percentage absorption at 0·02 and 0·20 M/l., which was nevertheless significant ($P \simeq 0{\cdot}02$). The disappearance of labelled fructose (Fig. 2) was similar at all three concentrations, except for the barely significant difference ($P \simeq 0{\cdot}05$) between the absorption from the ventriculus at 0·02 and 0·20 M/l. The results for the absorption of mannose and fructose from the caeca are summarized and compared with those for glucose (Treherne, 1958*a*) in Table 1. It will be seen that the percentage absorption of glucose was relatively high at 0·002 and 0·02 M/l., but was significantly less at 0·20 M/l. Mannose showed a progressive decline from a rate which was similar to that of glucose at 0·002 M/l., while fructose showed a relatively slow absorption at all three concentrations.

To follow the disappearance of mannose and fructose from the gut lumen *in vitro* the alimentary canal was removed from an anaesthetized insect and filled via the rectum with 0·15 ml. of 0·02 M/l. sugar solution. The alimentary canal was then ligatured at the crop and the rectum and suspended in 1·0 l. of saline which was kept circulating around the gut by a stream of air bubbles. The circulating saline contained KCN and iodoacetic acid each to a concentration of 2·0 mM/l. With both mannose and fructose it was found that the absorption from the caeca in the isolated poisoned alimentary canal was more rapid than in the intact insect (Fig. 3). These differences were found to be statistically significant ($P < 0{\cdot}01$ in both cases). The uptake of these sugars *in vitro* was similar to that for glucose where the percentage absorption from the caeca under identical experimental conditions was $54{\cdot}5 \pm 6{\cdot}0\%$ (Treherne, 1958*a*).

Experiments were carried out which were designed to investigate the relation between the monosaccharides in the gut lumen and the carbohydrates in the haemolymph. To do this solutions containing radio-active sugars were injected into the gut lumen and the ^{14}C-labelled compounds found in the haemolymph were examined by paper chromatography. Fig. 4 illustrates the distribution of radio-activity in the haemolymph for the three sugars. In this experiment the initial concentration of the sugars in the gut lumen was 0·02 M/l., the experimental period being 15 min.

It will be seen that in all three cases a peak of radio-activity was found which coincided with that for trehalose. Under these conditions all of the labelled glucose was converted to trehalose, but with both the other sugars substantial amounts of mannose and fructose remained in the haemolymph. The relation between the sugar

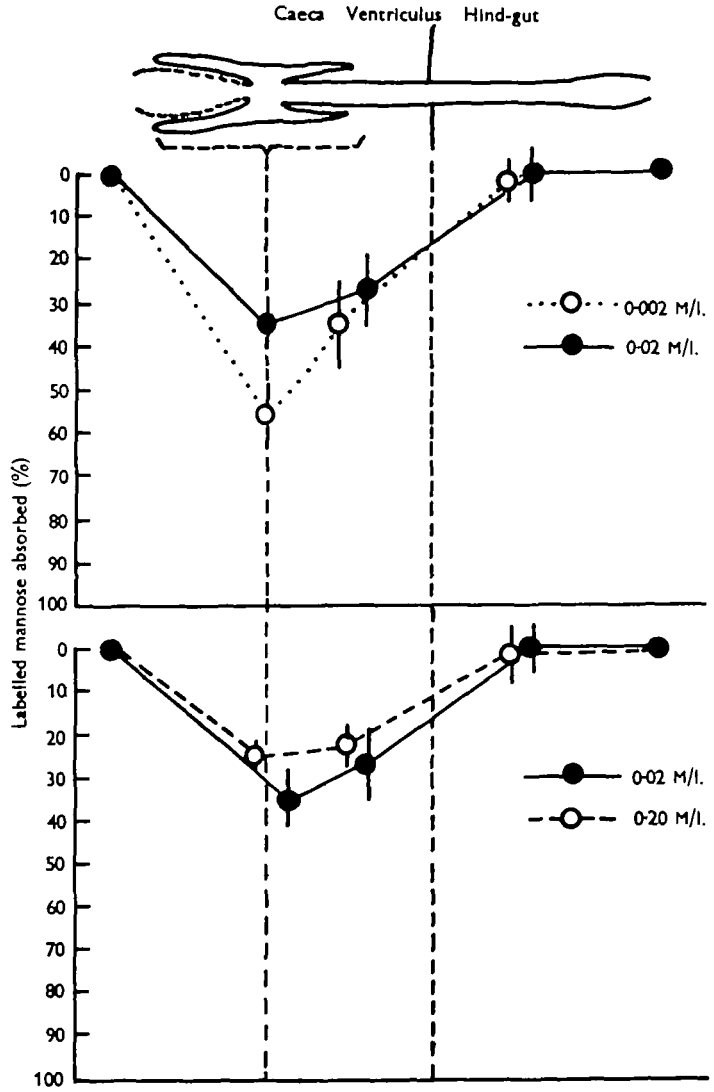

Fig. 1. The uptake of ^{14}C-labelled mannose from the alimentary canal at three concentrations. Each symbol represents the mean and extent of the standard deviation for five determinations.

concentration in the gut lumen and the amount of trehalose formed in the haemolymph is illustrated in Fig. 5. With glucose there was a complete conversion to trehalose until a concentration of just over 0·02 M/l. was reached in the lumen, after which there was a rapid fall in the percentage of trehalose formed in the haemolymph.

With mannose there was a complete conversion at low concentration, but the amount of trehalose formed fell off much more rapidly with increasing concentration than with glucose. With fructose there was only a relatively slow conversion to trehalose at all the concentrations studied.

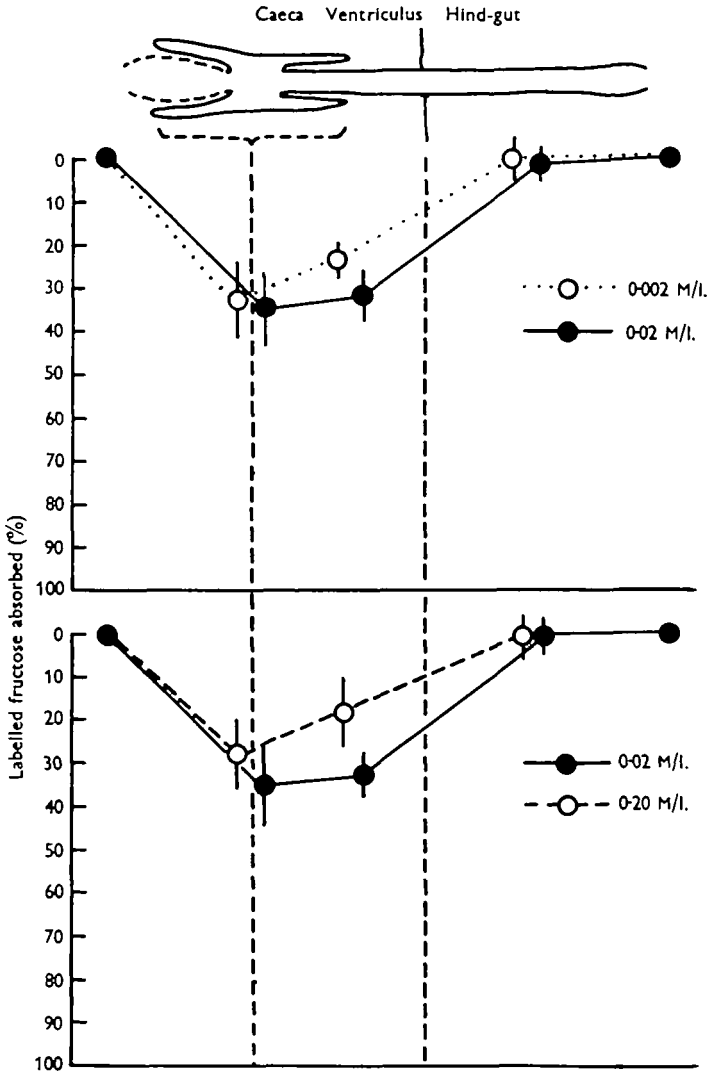

Fig. 2. The uptake of ^{14}C-labelled fructose from the alimentary canal at three concentrations. Each symbol represents the mean and extent of the standard deviation for five determinations.

In some experiments the extent of the conversion of labelled sugars absorbed by the mid-gut was compared with that obtained on direct injection of sugars into the haemolymph. With glucose, for example, it was estimated that with a concentration in the gut lumen of 0·02 M/l. a total of 0·138 mg. of labelled glucose was absorbed

Table 1. *The percentage uptake of* ^{14}C*-labelled sugars from the mid-gut caeca at three concentrations during an experimental period of* 15 *min.*

Sugar	Labelled sugar absorbed (%)		
	0·002 M/l.	0·02 M/l.	0·20 M/l.
Glucose	54·1 ± 6·7	55·7 ± 7·9	39·1 ± 6·1
Mannose	55·2 ± 7·2	35·5 ± 6·8	26·3 ± 1·5
Fructose	32·3 ± 9·5	34·8 ± 8·9	27·5 ± 8·1

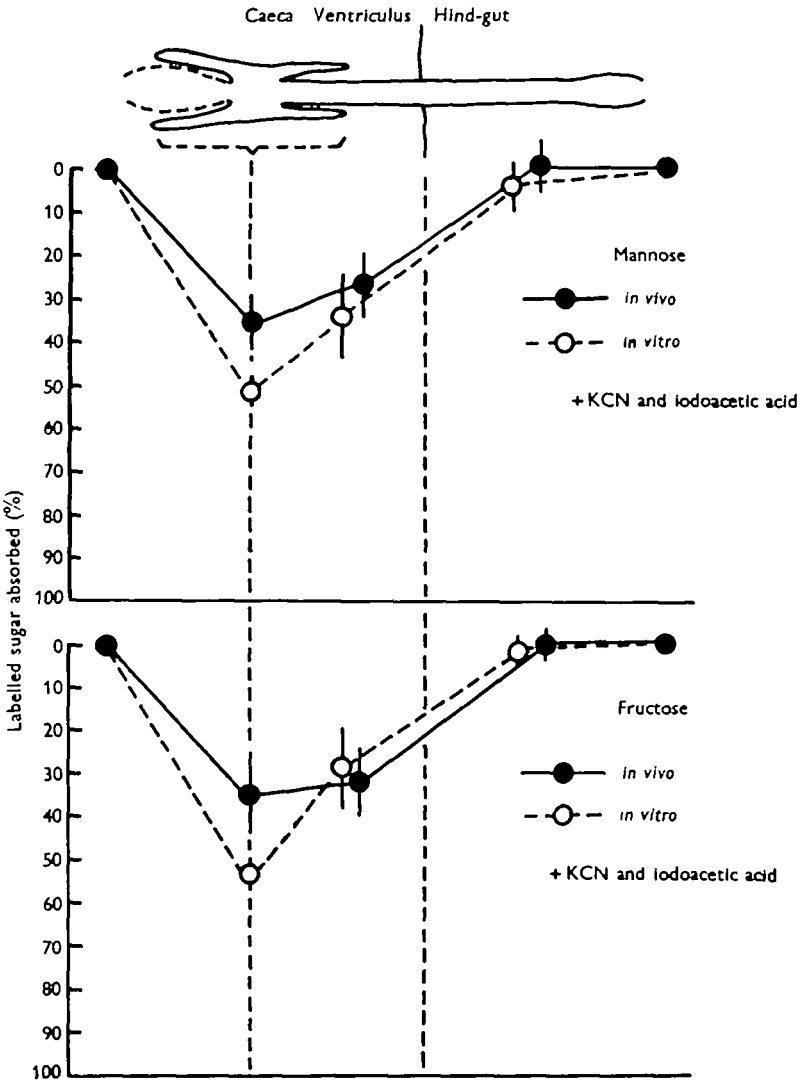

Fig. 3. The percentage absorption of labelled mannose and fructose from a poisoned gut isolated in circulating saline compared with that from the intact animal, during an experimental period of 15·0 min. The various symbols represent the mean and extent of the standard deviation.

from the caeca and the ventriculus during the experimental period of 15·0 min. Therefore in this experiment 0·138 mg. of ^{14}C-labelled glucose was injected into the haemolymph in 0·03 ml. saline using an Agla syringe. The results of these

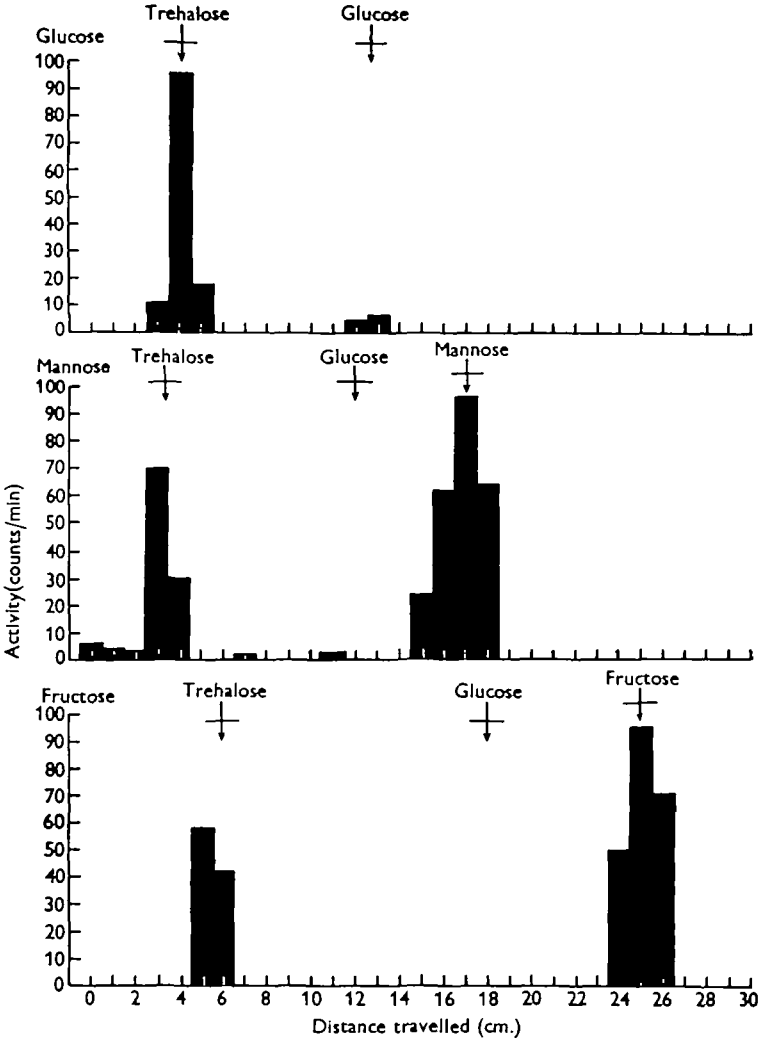

Fig. 4. The radio-activity on paper chromatograms of haemolymph taken 15·0 min. after the introduction of ^{14}C-labelled sugars into the alimentary canal. The symbols above the chromatograms represent the position and extent of the control spots, which were run adjacent to the experimental ones. The solvent system used in these experiments was ethyl acetate/acetic acid/water.

experiments are summarized in Table 2. It seems fairly clear that the percentage conversion to trehalose was approximately the same whether the sugars were absorbed via the gut or injected directly into the haemolymph.

The concentration of glucose and trehalose in the haemolymph was measured by

eluting these sugars from the paper chromatograms and determining their concentration by the anthrone method (Dimler *et al.* 1952). The results are given in Table 3. It will be seen that trehalose was present in fairly massive amounts. There was a considerable degree of variation in the results for glucose, but it is clear that

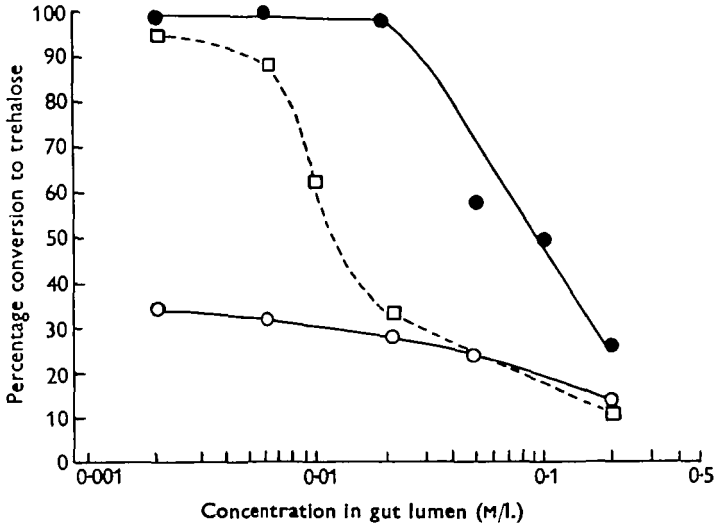

Fig. 5. The effect of concentration of ^{14}C-labelled sugars in the gut lumen on the amount of trehalose found in the haemolymph. —●—, glucose; - - □ - -, mannose; —○—, fructose.

Table 2. *A comparison of the rates of conversion to trehalose of* ^{14}C*-labelled sugars when absorbed via the mid-gut or injected directly into the haemolymph*

Sugar	Concentration in gut lumen	% incorporated as trehalose in the haemolymph	
		Absorbed via the mid-gut	Injected into the haemolymph
Glucose	0·002 M/l.	98·6 ± 2·3	97·9 ± 1·8
	0·02	98·6 ± 6·5	91·9 ± 7·5
Fructose	0·02 M/l.	28·3 ± 3·9	34·3 ± 4·5

this sugar formed only a small proportion of the total (about 3·4%). These findings in general are similar to those published by Howden & Kilby (1956) for this species, although the figures for glucose are somewhat lower than the values of 50–100 mg. % quoted by these authors.

The results in Table 3 indicate that at the lowest concentrations used in this investigation the concentration of glucose in the gut lumen approached that in the haemolymph. At these concentrations in the lumen the uptake of labelled glucose could be due either to an active transport or to passive exchange with the glucose in equilibrium with the trehalose in the haemolymph. In order to differentiate between these two possibilities it is essential to be able to measure the net glucose

absorption at very low concentrations in the lumen of the mid-gut. If much of the labelled glucose was being actively absorbed then it should be possible to demonstrate a substantial net glucose absorption; if the labelled glucose was merely exchanging with that in the haemolymph then there would be no demonstrable net glucose absorption. Unfortunately, it was not possible to measure directly the net glucose uptake at these concentrations because of the difficulty of making accurate determinations by chemical analysis of such small quantities of sugar. An attempt was made, therefore, to estimate the net absorption at low concentration by investigating the uptake of labelled glucose from the mid-gut caeca when the initial specific activity of the glucose in the haemolymph approximated to that in the gut lumen. This was done by injecting sufficient radio-active glucose solution into the haemolymph to give the same specific activity on both sides of the gut wall. Under these conditions any decrease in the labelled glucose in the lumen will be a measure

Table 3. *The concentration of glucose and trehalose in the haemolymph of adult female locusts*

Serial	Glucose concentration mg./100 ml.	Trehalose concentration mg./100 ml.
1	32·2	795·9
2	12·3	801·6
3	23·5	545·7
4	34·7	622·3
5	9·8	674·2
6	6·3	599·5
7	40·1	847·6
8	34·2	669·0
Mean ± S.D.	24·1 ± 12·9	694·5 ± 99·3

of the net glucose absorption. These results were compared with those in which the labelled glucose was absorbed, as in previous experiments, into the haemolymph containing no ^{14}C. Because of the rapidity with which the glucose exchanges and becomes incorporated as trehalose in the haemolymph, sufficient radio-active glucose was injected so that the specific activity of the glucose and the trehalose equalled that of the glucose in the gut lumen. To take an actual example, the blood volume of this insect was found to be 0·248 ± 0·038 ml., so that from the results in Table 3 it can be estimated that the total amount of glucose and trehalose (expressed as glucose units) was approximately 3·50 mg. Now the specific activity of a 0·00134 M/l. glucose solution in the gut lumen was 0·655 μc./mg., therefore it was necessary to inject 2·29 μc. of ^{14}C-labelled glucose into the haemolymph to bring the glucose in the haemolymph to the same specific activity as the glucose in the gut contents. The labelled glucose was allowed to come into equilibrium with trehalose for a period of 30·0 min. before the gut lumen was filled with the experimental solution. It was assumed that after this period the small amount of glucose in equilibrium with the trehalose in the haemolymph was of a similar specific activity to that in the gut lumen. The labelled glucose was injected into the haemolymph in a small

volume of saline with the aid of an Agla syringe. To minimize the effects of contamination of the gut contents by the haemolymph the fluid in the caeca was collected by means of the nylon tubing previously described. The results obtained

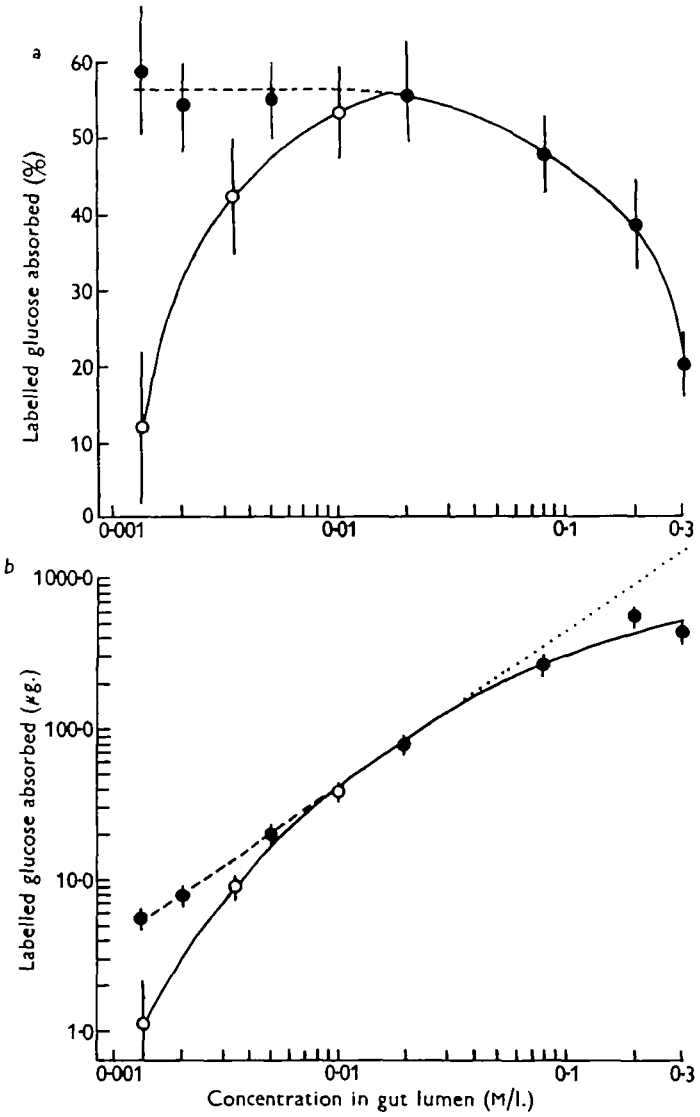

Fig. 6. (*a*) The effect of concentration on the movements of ^{14}C-labelled glucose between the lumen of the caeca and the haemolymph. The open circles represent the percentage absorption of labelled glucose when the initial radio-activity of the glucose in the haemolymph, in equilibrium with the trehalose, approximated to that in the gut lumen. The closed circles represent the uptake when there was no initial radio-activity in the haemolymph. The vertical lines illustrate the extent of the standard deviation. (*b*) A logarithmic plot illustrating the uptake of labelled glucose, in absolute units, calculated from the data illustrated in (*a*). The continuous line represents the net glucose absorption, while the broken one indicates the net absorption plus the apparent absorption due to exchange with unlabelled molecules.

using this technique are illustrated in Fig. 6*a*. It will be seen that at a concentration of 0·00134 M/l. the percentage absorption of labelled glucose from the caeca was much reduced in the experiments in which the specific activity in the haemolymph was similar to that in the gut lumen. This difference became smaller as the glucose concentration of the gut contents was increased, so that at 0·01 M/l. the percentage absorption of the labelled glucose was similar in both cases. In Fig. 6*b* the absolute amount of labelled glucose (in μg.) absorbed from the lumen of the caeca during the experimental period has been plotted on a logarithmic scale with respect to concentration. These figures were calculated from the data given in Fig. 6*a* and with the knowledge that the initial volume of the caeca contents was 0·039 $\pm$ 0·009 ml. In both graphs the continuous line can be regarded as an estimate of the net glucose absorption effected during the experimental period.

It seemed possible that the apparent reduction in the percentage absorption of labelled glucose in the caeca at low concentrations, when the specific activity of the haemolymph equalled that in the gut lumen, might be due, in part at least, to an entry of radio-active material into the lumen of the gut before the introduction of the experimental solution. The further possibility could not be eliminated that, despite careful precautions, there might be some contamination of the caeca contents by the highly radio-active haemolymph due to slight damage to the gut wall during collection of the caecal fluid. These possibilities were tested in the following way. 2·92 μc. ^{14}C-labelled glucose were injected into the haemolymph. The experimental fluid from the caeca was then immediately collected and the amount of radio-active material present determined in relation to the recovered dye. If any extraneous radio-active material had entered the lumen of the caeca during the equilibration period this would have been reflected in an increase in the amount of radio-activity relative to the recovered dye. If no radio-active material had entered the lumen then the glucose/dye ratio would not have altered and the apparent percentage absorption would have been zero. The apparent absorption in these experiments was $-0{\cdot}80 \pm 3{\cdot}80\%$, indicating that the entry of any extraneous material into the gut lumen during the equilibration period was relatively small.

DISCUSSION

The absorption of the labelled sugars was found to be largely confined to the midgut, a finding which is in accord with some previous investigations on glucose absorption in the cockroach (Treherne, 1957) and the locust (Treherne, 1958*a*). The proportion of the labelled sugars absorbed from the lumen of the caeca, however, showed some marked differences at different concentrations. The uptake of labelled glucose was relatively rapid at 0·002 and 0·02 M/l., but fell to a rate which was similar to that from the ventriculus at 0·20 M/l. With mannose the percentage absorption was similar to that for glucose at 0·002 M/l. but then declined much more rapidly, again to a rate similar to that from the ventriculus. Fructose showed a rather slow rate of absorption at all three concentrations.

The absorbed sugars were converted, in varying degrees, to trehalose which

accumulated in the haemolymph. This non-reducing disaccharide was first detected in insects by Wyatt & Kalf (1956) and its presence in *S. gregaria* was confirmed by Howden & Kilby (1956). It was recently shown that absorbed glucose was incorporated as trehalose in this insect (Treherne, 1958*a*). The conversion to trehalose of the three sugars used in this investigation showed considerable differences and variations when the concentration in the gut lumen was altered. In these experiments glucose showed a very rapid conversion to trehalose, until the concentration in the gut lumen exceeded 0·02 M/l. when excess glucose accumulated in the haemolymph. Mannose was rapidly converted to trehalose at a concentration of 0·002 M/l. in the gut lumen, but the rate of conversion fell off steeply with increasing concentration. With fructose the conversion proceeded relatively slowly at all concentrations studied. It seems clear that these rates of conversion to trehalose parallel the percentage absorption of the labelled sugars from the lumen of the caeca. These facts do not conflict with the hypothesis advanced in a previous publication (Treherne, 1958*a*) that glucose absorption is effected by diffusion across the gut wall and that the process is facilitated by the conversion to trehalose which would tend to maintain a steep concentration gradient across the gut wall. Evans & Dethier (1957) have reported changes in the blood sugar concentration in the blowfly which suggest a fairly rapid conversion of mannose and fructose to trehalose and it is possible that in this species sugar absorption may be similar to that in the locust.

When the alimentary canal was isolated in circulating poisoned saline the three sugars disappeared from the gut lumen at similar rates. The passage of glucose through the gut wall under these circumstances was at the same rate as in the intact animal when there was a complete conversion to trehalose. With mannose and fructose this disappearance from the gut lumen *in vitro* was more rapid than in the intact animal, where at 0·02 M/l. the conversion to trehalose was relatively slow. The behaviour of the sugars in this model system lends support to the hypothesis of absorption by facilitated diffusion, for in these circumstances it would be expected that the sugars would diffuse through the gut wall at similar rates. The slower absorption of mannose and fructose from the intact gut, as compared with that *in vitro*, can be attributed to the reduced concentration gradient across the gut wall due to the demonstrated accumulation of unconverted monosaccharides in the haemolymph at this concentration.

It was found that the conversion to trehalose occurred at closely similar rates whether the sugars were absorbed via the mid-gut or injected directly into the haemolymph. This suggests that the postulated absorption mechanism could operate by converting the absorbed sugars in the haemolymph, thus maintaining a concentration gradient across the whole width of the gut wall. These results do not, however, eliminate the possibility of conversion occurring within the mucosal cells.

Chemical analysis of the haemolymph showed that there was a small amount of glucose in equilibrium with the trehalose, the values obtained for glucose being somewhat smaller than those quoted by Howden & Kilby (1956). Thus at very low

concentrations the glucose in the gut lumen was at a similar level to that in the haemolymph (i.e. 0·00134 M/l.). The uptake of labelled glucose from the caeca at low concentrations was shown to be substantially reduced when the initial specific activity of the glucose in the haemolymph was adjusted to approximate to that in the gut lumen. This effect became negligibly small when a concentration of about 0·01 M/l. was attained in the gut lumen. Under these experimental conditions the absorption of the labelled glucose can be regarded as an estimate of the *net* glucose

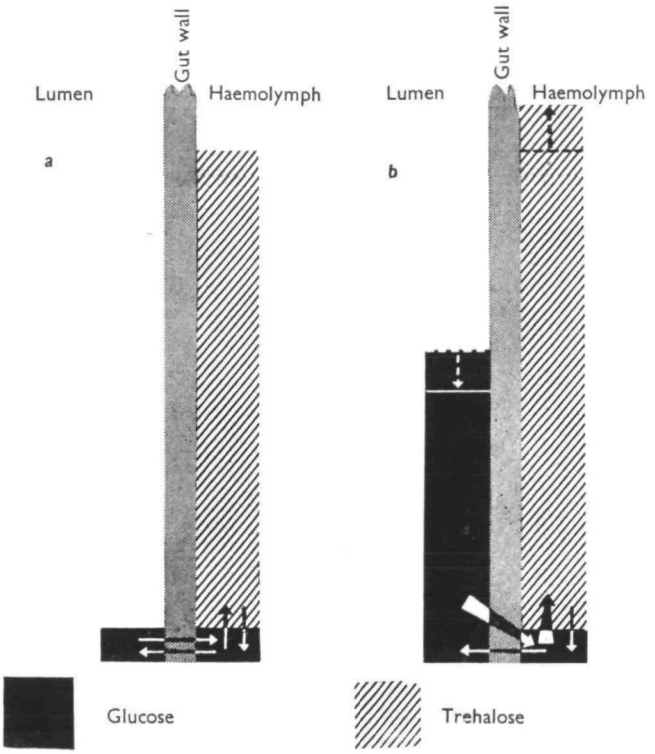

Fig. 7. A diagrammatic representation of the movements of labelled glucose between the lumen of the caeca and in the haemolymph: (*a*) At low concentration, when the glucose level in the lumen is similar to that in the haemolymph. (*b*) At a relatively high concentration when the glucose in the lumen exceeds that in the haemolymph. (Not drawn to scale.)

absorption effected from the mid-gut caeca. It seems fairly clear that at very low concentrations, close to that in the haemolymph, most of the absorption of labelled glucose from the caeca was due merely to exchange with that in the haemolymph. With increasing concentration in the lumen the proportion exchanging with that in the haemolymph became progressively smaller and the absorption of the labelled glucose molecules approximated to the net glucose absorption. The exchange and absorption of glucose between the gut lumen and in the haemolymph are illustrated diagrammatically in Fig. 7. There was no evidence for any appreciable amounts of mannose or fructose in the haemolymph; therefore in experiments with these sugars

the uptake of labelled molecules will represent fairly closely the net absorption effected by the mid-gut.

It will be appreciated that the conversion to trehalose could only produce a net glucose uptake when the concentration in the gut lumen exceeded that in the haemolymph. At very low concentrations most of the glucose movements would be an exchange with that in the haemolymph (Fig. 7*a*). Therefore, it is clear that at low concentrations, such as might exist towards the end of a meal, some additional mechanism must be postulated to explain any net glucose absorption observed. The experiments showed that the net uptake probably proceeded very slowly at low concentration, and it follows that any auxiliary transport mechanism would be relatively ineffective. It seems more likely, and would probably be an economical arrangement, that the uptake of the last traces of glucose might be linked to water movements. In this case any net water uptake would tend to concentrate the glucose in the gut lumen, which might then eventually diffuse passively into the haemolymph.

SUMMARY

1. The uptake of ^{14}C-labelled glucose, mannose and fructose from the alimentary canal of *Schistocerca gregaria* (Forsk.) has been studied using the dye Amaranth as a reference substance.

2. Absorption was confined to the mid-gut, the proportion absorbed by the caeca depending on the type of sugar and its concentration in the gut lumen.

3. The absorbed sugars were converted, in varying degrees, to trehalose which accumulated in the haemolymph. The extent of the conversion appeared to parallel the rate of absorption of the sugars at the various concentrations.

4. The sugars passed through the gut wall at similar rates in experiments in which the isolated alimentary canal was suspended in a large volume of circulating poisoned saline. The passage through the gut wall under these conditions was equivalent to the rapid absorption obtained *in vivo* when there was a rapid conversion to trehalose.

5. It is suggested that these observations support the hypothesis that the sugars are absorbed by diffusion across the gut wall and that the process is facilitated by the rapid conversion to trehalose in the haemolymph, which tends to maintain a steep concentration gradient.

6. At very low concentrations in the gut lumen, glucose was at a similar level to the relatively small amount of glucose in equilibrium with the trehalose in the haemolymph. When the specific activity of injected ^{14}C in the haemolymph approximated to that in the gut lumen the absorption of the labelled glucose from the mid-gut caeca was reduced at very low concentrations. Thus at these levels it is suggested that most of the absorption of labelled glucose can be attributed to an exchange with that in the haemolymph. At a concentration of 0·01 M/l. the proportion exchanging became negligibly small and the absorption of the labelled glucose molecules approximated to the net glucose absorption.

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