THE ABSORPTION OF GLUCOSE FROM THE ALIMENTARY CANAL OF THE LOCUST *SCHISTOCERCA GREGARIA* (FORSK.)

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INTRODUCTION

In a recent investigation (Treherne, 1957) it was demonstrated that glucose absorption in the cockroach was largely confined to the mid-gut caeca. It was also shown that the amount of glucose absorbed was related to crop emptying, so that the limiting process in absorption was not the transfer of glucose across the gut wall but the rate at which fluid was allowed to leave the crop. This made it impossible to study the process of glucose absorption in the caeca, which was effectively masked by the relatively slow emptying of the crop. In the present investigation, therefore, glucose absorption has been studied in the adult locust, in which it was found possible to fill the whole alimentary canal and thus to eliminate the effect of crop emptying on the total glucose absorption.

METHODS

All experiments were performed on adult female *Schistocerca gregaria* (Forsk.), reared and maintained at 28·0 ± 1·0° C., which had been isolated in glass jars and deprived of food for 24 hr.

Two methods were used to fill the alimentary canal with the experimental solutions used in this investigation. In the first, the insect was anaesthetized with CO_2 and was force-fed through a short length of 0·5 mm. bore nylon tube which was sealed into the mouth with wax. By this method the crop was filled with experimental solution and the absorption of glucose was followed as the fluid passed along the remainder of the alimentary canal. With the second method a fine nylon hypodermic needle was made by drawing out 0·5 mm. diameter nylon tube over a low flame and mounting this on an ordinary hypodermic needle holder. This nylon needle was inserted into the rectum of an anaesthetized insect and sealed into position with wax. When the insect had recovered from the anaesthetic the experimental solution was forced into the gut from an 'Agla' syringe and the needle withdrawn from the rectum. It was found that a volume of 0·15 ml. was sufficient to fill the whole of the hind- and mid-gut. The wax used was a mixture of bee's wax and resin given by Krogh & Weis Fogh (1951).

The experimental solution used in this investigation was based on the saline used by Hoyle (1953) and had the following composition:

Glucose	0·02 M/l.	$MgCl_2$	0·002 M/l.
NaCl	0·140	$KHCO_3$	0·004
$CaCl_2$	0·002	KH_2PO_4	0·006

When the concentration of the glucose was altered (i.e. to 0·002 and 0·20 M/l.), the total osmolarity of the solution was maintained by altering the NaCl concentration.

The technique used to determine the percentage absorption of glucose from the lumen of the gut was essentially similar to that described by Treherne (1957). With this technique the experimental solution contained the dye Amaranth (Azo-Rubin S) to which was added varying amounts of ^{14}C-labelled glucose. The dye, which was not absorbed from the lumen of the gut, was used as a marker and the net percentage absorption was determined by comparing the ratio of dye to glucose in the various parts of the alimentary canal. The concentration of the dye was determined in solution at pH 10·0 using a Unicam absorptiometer at an absorption maximum of 510 mμ. The fore-gut and caeca of *Schistocerca gregaria* contained a dark coloured material which tended to interfere with the colorimetric determination at the dye concentration previously used. In the present investigation, therefore, the dye concentration was raised from 0·008 to 0·05 M/l. which reduced the effects of the interfering substances. The radioactive glucose molecules were generally labelled with ^{14}C and were assayed in solution using a thin windowed G.M. tube (G.E.C. CV2139) as previously described (Treherne, 1957).

The separation and identification of ^{14}C-labelled substances from the haemolymph, gut contents and mid-gut tissue was accomplished by descending paper chromatography using Whatman no. 1 filter-paper. The samples were applied to the paper with silicone-lined micropipettes, approximately 2·0 μl. in volume. To separate the substances from the mid-gut tissue it was necessary to wash the surfaces of the gut wall free from surface-contaminating materials. The lumen was washed by inserting a steel hypodermic needle through the opening of the proventriculus and clearing the contents of the diverticula and mid-gut with a flow of distilled water. The surfaces were judged to be clean when all the dyestuff, which had been added to the experimental solution, had been washed away. The outer surface of the gut was washed free of haemolymph by quickly dipping the gut into two lots of distilled water. The tissue was then homogenized in 0·2 ml. of distilled water, centrifuged at 13,000 r.p.m. and the supernatant was applied to the base-line on the paper. The following four solvent systems were used in this investigation: ethyl acetate/acetic acid/water (Jermyn & Isherwood, 1949), methanol/formic acid/water (Bandurski & Axelrod, 1951), *n*-propanol/ammonia/water (Hanes & Isherwood, 1949) and *n*-propanol/ethyl acetate/water (Baar & Bull, 1953). Reducing substances on the chromatograms were detected by the silver nitrate method of Trevelyan, Proctor & Harrison (1950). To detect non-reducing carbohydrates the chromatograms were sprayed with 0·5% sodium-*meta*periodate in water (Evans & Dethier, 1957) before

treatment with silver nitrate. Control experiments showed that the various biological fluids caused no interference with any of the solvent systems used.

To assay the chromatograms for radioactivity the paper was cut into appropriate strips and then placed over a 1·0 cm. wide slit in a piece of Perspex beneath which was a thin-windowed G.M. tube. Counts were then made on 1·0 cm. wide portions of the paper until the whole length of the strip had been assayed.

To determine the identity of some unknown carbohydrates on the chromatograms these were eluted, subjected to acid hydrolysis and the products re-run on chromatograms. In the hydrolysis, acid was added to the solution to make 4·0N-HCl and the solution was then placed in a boiling water-bath for 20 min. Ion-exchange crystals were added, then centrifuged off and the liquid dried *in vacuo* over P_2O_5 before being re-run on a chromatogram.

RESULTS

To study the absorption of glucose from the fluid passing down the alimentary canal individuals were force-fed by mouth with 0·10 ml. volumes of an experimental solution containing 0·02M/l. glucose. The results of experiments giving the absorption after 0·25, 1·0 and 5·0 hr. are illustrated in Fig. 1. It will be seen that the bulk of the absorption occurred when the solution reached the mid-gut cacea. Some absorption of the remaining glucose occurred as the fluid passed along the rest of the alimentary canal.

The percentage glucose absorption was also investigated in experiments in which the alimentary canal was filled via the rectum with 0·15 ml. of a 0·02M/l. glucose solution. Fig. 2 shows the net percentage absorption after 5·0, 15·0 min. and 1·0 hr. The glucose disappeared most rapidly from the caeca and to a lesser extent from the ventriculus. There was no significant uptake from the hind-gut.

To study the effect of concentration on glucose uptake the net percentage absorption was measured at concentrations of 0·002, 0·02 and 0·20M/l. In these experiments the alimentary canal was filled via the rectum and all measurements were made after 0·25 hr. In Fig. 3 the absorption at 0·02M/l. is compared with that at 0·002M/l. and it will be seen that the absorption was similar at the two concentrations. When the concentration was raised to 0·20M/l. there was less percentage absorption from the caeca than at 0·02M/l. This difference was statistically significant ($P = < 0·01$). The disappearance of glucose from the ventriculus, however, was similar at the two concentrations.

The disappearance of glucose from the lumen of the gut was followed *in vitro*. The alimentary canal was removed from an anaesthetized insect and filled via the rectum with 0·15 ml. 0·02M/l. glucose. The canal was then closed by ligatures at the crop and rectum and suspended for 15 min. in 1·0 l. of saline, to which had been added KCN and iodoacetic acid, each to a concentration of 2·0 mM/l. A stream of air bubbles ensured an adequate circulation of saline around the suspended gut. The net percentage absorption was found to be similar to that from the intact insect (Fig. 4).

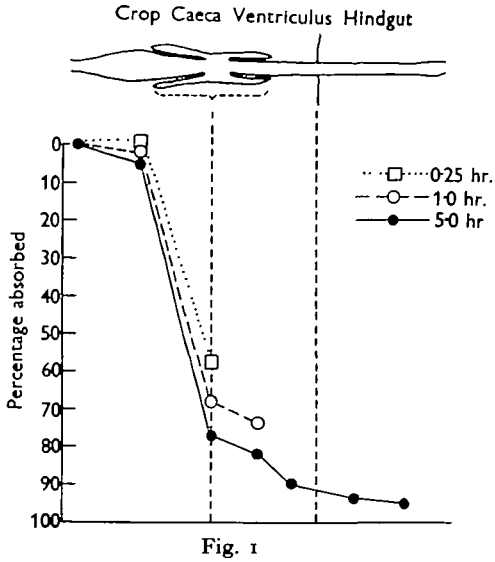

Fig. 1

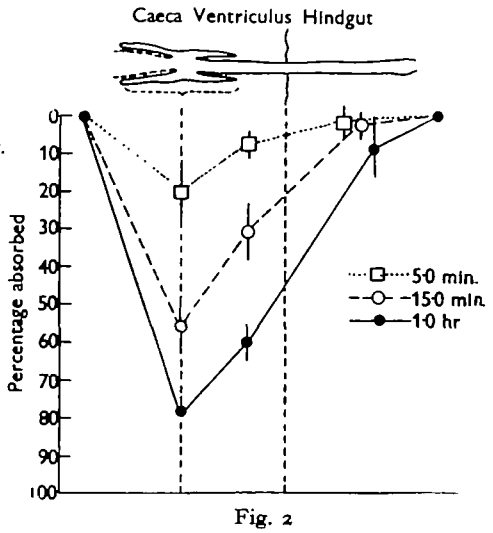

Fig. 2

Fig. 1. The net percentage absorption of glucose, from fluid passing along the alimentary canal, at three time intervals after force-feeding by mouth with 0·02 M/l. glucose solution.

Fig. 2. The net percentage absorption of glucose at varying times after the injection of a 0·02 M/l. glucose solution into the alimentary canal via the rectum. Each point is the mean of five determinations, the vertical lines represent the extent of the standard deviation.

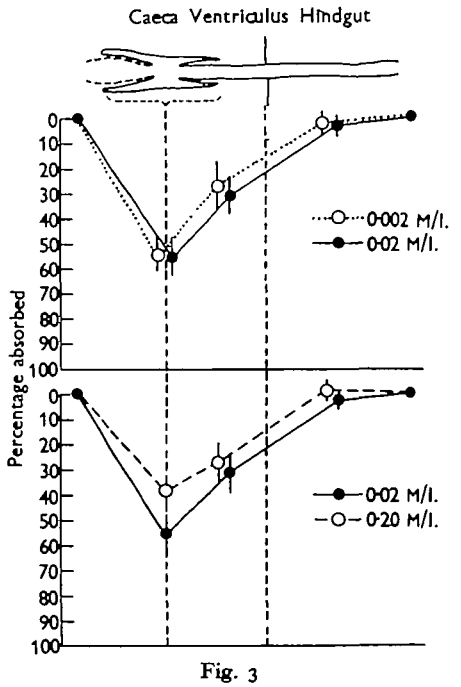

Fig. 3

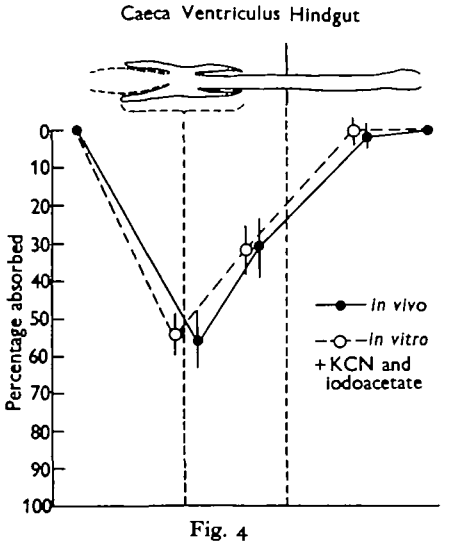

Fig. 4

Fig. 3. The effect of concentration on the net percentage absorption of glucose from the alimentary canal filled via the rectum. The symbols represent the mean and the extent of the standard deviation for five determinations.

Fig. 4. The net percentage absorption from a poisoned gut isolated in saline, compared with that from the alimentary canal of an intact insect. Each point is the mean of five determinations, the vertical lines representing the extent of the standard deviation.

The fate of the ^{14}C-labelled glucose was followed using the technique of paper chromatography. As in previous experiments 0·15 ml. vol. were injected into the alimentary canal via the rectum. Subsequently, 2·0 μl. samples of the haemolymph and gut contents were run on chromatograms, together with control spots of non-radioactive glucose, which were detected by spraying with silver. In Fig. 5 the

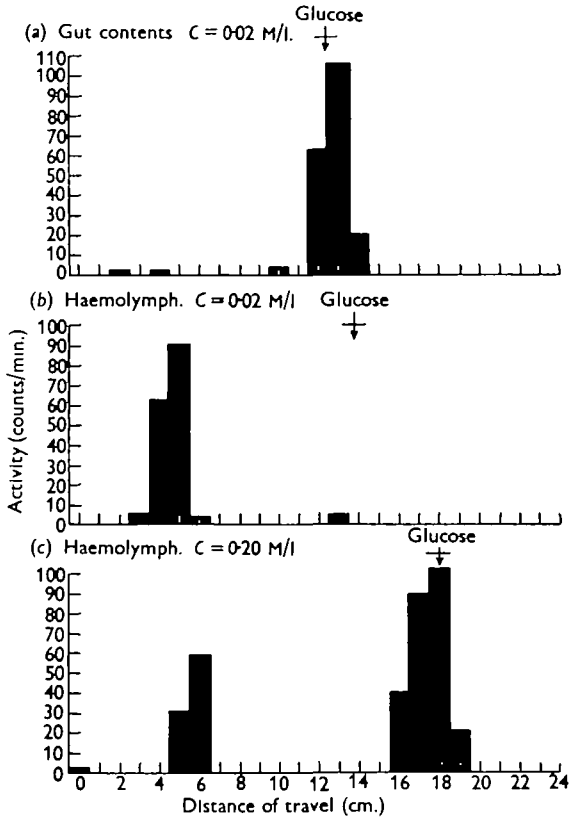

Fig. 5. Radioactivity on paper chromatograms of the caeca contents and haemolymph after injection of ^{14}C-labelled glucose into the alimentary canal. The symbols above the histograms represent the position and extent of control spots of glucose run adjacent to the experimental ones. C = glucose concentration in the gut lumen.

distribution of radioactivity in the contents of the caeca and the haemolymph is shown for chromatograms with ethyl acetate/acetic acid/water as the solvent. These results showed that the ^{14}C in the lumen of the gut was still incorporated in glucose molecules, for the radioactive peak invariably coincided with the control glucose spots. The ^{14}C recovered from the haemolymph was, however, no longer incorporated in the glucose molecule for the radioactive peak moved less than half the distance of the control glucose spot (Fig. 5*b*). This result was obtained with a glucose concentration in the experimental solution of 0·02 M/l. When the concentration in the alimentary canal was raised to 0·20 M/l. a second radioactive peak

appeared in the glucose position (Fig. 5*c*). Thus at this concentration much of the ^{14}C in the haemolymph remained as glucose.

To determine the identity of the substance causing the unknown peak of radioactivity in the haemolymph, chromatograms were run with four different solvents and control spots of possible substances were run beside the radioactive ones. Of the various substances tested the only one which consistently coincided with the radioactive peak was trehalose (α-D-glucopyranosyl α-D-glucopyranoside) (Fig. 6). This identification was also supported by the fact that the radioactive spot was strongly reducing only after treatment with 0·5% sodium *meta*periodate, indicating the presence of a non-reducing carbohydrate.

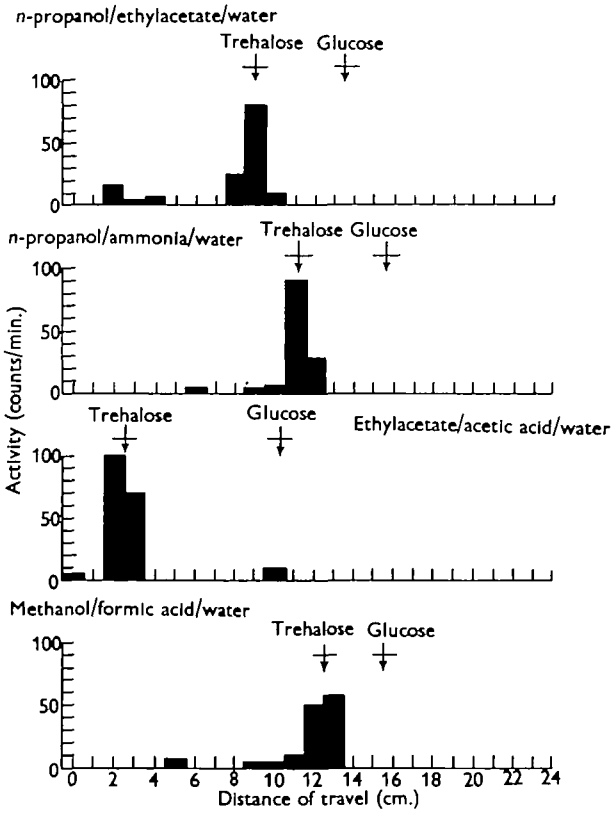

Fig. 6. The radioactivity of chromatograms of haemolymph after injection of 0·02 M/l. ^{14}C-labelled glucose into the alimentary canal. The symbols above the histograms represent the position and extent of control spots of trehalose and glucose run adjacent to the experimental ones.

^{14}C-labelled substances in the gut tissue were examined by paper chromatography. The alimentary canal was filled with a 0·02 M/l. glucose solution and after a suitable period the caeca tissues were washed and the tissue homogenate run on chromatograms. Control of trehalose and glucose were added for comparison. The results obtained were rather variable, but in most cases there was no peak of radio-

activity coinciding with the control trehalose spot. Several unknown radioactive peaks occurred in various positions on these chromatograms. Acid hydrolysis of a spot eluted from the base-line ($R_G = 0$) caused the radioactive products to appear as glucose when re-run on fresh chromatograms. This result suggests the presence of glycogen in caeca tissue.

DISCUSSION

It is evident that, as in the cockroach (Treherne, 1957), most of the glucose was absorbed from the lumen of the mid-gut caeca in the locust. The experiments in which individuals were fed by mouth showed, however, that a smaller proportion may be absorbed from the lumen of the ventriculus. A disappearance of glucose from the ventriculus was also demonstrated in the experiments in which the alimentary canal was filled via the rectum. This disappearance may not be entirely due to an uptake by the ventriculus, for it is possible that glucose may have diffused into the caeca as the concentration there fell. Thus it should be borne in mind that all of the glucose which disappeared from the lumen of the ventriculus may not have been absorbed into the haemolymph by this organ.

The chromatograms showed that the absorbed glucose had been incorporated as trehalose in the haemolymph. This non-reducing disaccharide was first reported for several species of insects by Wyatt & Kalf (1956). Its presence has since been demonstrated in the haemolymph of *Schistocerca gregaria* by Howden & Kilby (1956), and has recently been encountered in the blowfly *Phormia regina* by Evans & Dethier (1957). The present experiments showed that with an initial concentration of 0·02 M/l. all of the glucose was incorporated as trehalose, but that at 0·20 M/l. relatively large amounts remained unchanged in the haemolymph. Now the percentage absorption of glucose from the caeca was shown to be the same at concentrations of 0·002 and 0·02 M/l., but was depressed at 0·20 M/l., the level at which excess glucose accumulated in the haemolymph. These results are not inconsistent with the hypothesis that the limiting factor in the absorption of glucose is a diffusion process. Thus it would be expected that the amount of glucose leaving the lumen would be proportional to the concentration difference across the gut wall and for the net percentage absorption to be constant, as indeed it was at 0·002 and 0·02 M/l. glucose. At 0·20 M/l. the presence of appreciable amounts of glucose in the haemolymph would tend to reduce the concentration gradient so that the net percentage absorption would be reduced. The conversion of glucose would, therefore, operate to maintain a steep concentration gradient across the gut wall and thus to facilitate diffusion. The formation of trehalose involves a virtual doubling of the molecular volume which might tend to restrict back diffusion into the gut lumen.

The validity of the hypothesis outlined above was tested by comparing the disappearance of glucose from the gut of an intact insect with its disappearance from a gut isolated *in vitro*. In this experiment the isolated gut was suspended in a relatively large volume of circulating saline, so that the glucose concentration in the fluid bathing the gut was always at a very low level. Any possible effects of tissue metabolism on glucose movement were eliminated by adding iodoacetate (which is

known to depress glucose transport in mammals (Wilbrandt & Laszt, 1933)) and cyanide to the saline. This system approximates to the condition in which glucose is absorbed by diffusion through the gut wall, the steep concentration gradient being maintained by the rapid conversion of glucose to trehalose in the haemolymph. The fact that the amount of glucose absorbed in this model system was similar to that in the intact animal supports this hypothesis and suggests that the cell metabolism is not of primary importance in glucose absorption.

Howden & Kilby (1956) found that the concentration of glucose in the haemolymph of the locust was 50–100 mg %. Thus at the lowest glucose concentration used in these experiments (0·002M/l.) the glucose would initially have been at a lower concentration in the gut lumen than in the haemolymph. Yet the percentage absorption at this concentration was similar to that at 0·02M/l., both *in vivo* and in the isolated preparation. Thus, according to the above hypothesis, it must be assumed that even at low concentrations the conversion to trehalose is effective in maintaining an adequate local concentration gradient across some portion of the gut wall. Alternatively it can be postulated that there is a secretory mechanism which becomes effective at low concentrations, although in the absence of any evidence for such a process the former hypothesis would seem to be more acceptable.

It has often been suggested that the mechanism of hexose absorption in the mammalian intestine might be achieved simply by a conversion to some other compound, thus increasing the diffusion gradient into the mucosal cells. Such a reaction was first proposed by Höber (1899). This thesis was later developed by Verzár who suggested a possible conversion of glucose to glycogen (Verzár, 1931) or a phosphorylation of glucose in the mucosal cell (Verzár & MacDougall, 1936). More recent work on mammals has, however, not supported this hypothesis. Bárány & Sperber (1939), for example, demonstrated a true active transport of glucose against a concentration gradient, while Campbell & Davson (1948) have shown that a synthetic glucose derivative, 3-methylglucose, which is not phosphorylated *in vivo* is absorbed rapidly against a concentration gradient in the cat. Serious theoretical objections have also been raised by Wilbrandt (1954). Glucose absorption in this insect would thus seem to be fundamentally different from the process in the mammalian intestine. In fact, something like the simple facilitated diffusion mechanism originally suggested by Höber & Verzár would seem to be adequate to account for the facts observed in the absorption of glucose from the alimentary canal of *Schistocerca gregaria*.

The chromatograms of the caeca tissue showed that the ^{14}C originally present as glucose was found in a variety of compounds. These compounds were not identified, although there was good evidence that some of the ^{14}C was incorporated in glycogen. It seems most reasonable to suppose that these compounds represented various stages in the utilization of glucose by the cell's metabolism. It may thus be necessary to distinguish between that glucose which was translocated and that which was retained by the tissue for its own metabolic needs. This possibility has also been visualized in the absorption of glucose from the small intestine of mammals (Fisher & Parsons, 1950).

SUMMARY

1. The absorption of glucose from the alimentary canal of *Schistocerca gregaria* has been studied by filling the gut with a saline solution containing ^{14}C-labelled glucose together with a dye, Amaranth, which was used as a marker. The net percentage absorption was calculated from the glucose/dye ratio in the various parts of the alimentary canal.

2. The bulk of the glucose was absorbed from the mid-gut caeca, smaller amounts being absorbed by the ventriculus.

3. Glucose absorption was studied at concentrations of 0·002, 0·02 and 0·20 M/l. in solutions in which the total osmolarity was maintained by altering the NaCl concentration. The percentage absorption was similar at concentrations of 0·002 and 0·02 M/l., but was significantly less at 0·20 M/l.

4. The fate of the ^{14}C-labelled glucose was followed using paper chromatography. The glucose was shown to be rapidly converted to trehalose in the haemolymph. At a concentration of 0·20 M/l. this mechanism became saturated and excess glucose accumulated in the haemolymph.

5. The absorption of glucose *in vitro*, from a gut suspended in a relatively large volume of poisoned saline, was found to be similar to that in the intact insect.

6. From these observations it is suggested that glucose is absorbed by diffusion across the gut wall and that the process is facilitated by the rapid conversion of glucose to trehalose in the haemolymph, which tends to maintain a steep concentration gradient across the gut wall.

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