THE NUTRITION OF THE CENTRAL NERVOUS SYSTEM IN THE COCKROACH, *PERIPLANETA AMERICANA* L.

THE EXCHANGE AND METABOLISM OF SUGARS

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INTRODUCTION

The insect central nervous system is enveloped by a continuous cellular and fibrous membrane, the perilemma, which has been shown to be a barrier to the inward diffusion of sodium and potassium ions (Hoyle, 1952, 1953; Twarog & Roeder, 1956), acetylcholine (Twarog & Roeder, 1956) and dyes (Wigglesworth, 1960). On the other hand, this structure must also, as Wigglesworth (1959, 1960) has pointed out, be specialized to allow exchanges of nutritive and excretory substances to take place between the central nervous system and the haemolymph. This important role of the perilemma has been little studied and no quantitative observations have been made on the entry of nutritive substances into the insect central nervous system. In this investigation an attempt has been made to throw some light on these processes by studying the uptake of some $^{14}$C-labelled sugars in the abdominal nerve cord of the cockroach.

Relatively little is known of the biochemical events occurring within the insect central nervous system, and the opportunity has been taken to learn something of these processes by following the metabolic fate of the $^{14}$C-labelled compounds in the abdominal nerve cord of this insect. These observations form part of a wider investigation being carried out in this laboratory in collaboration with Prof. V. B. Wigglesworth. The parallel histological and histochemical aspects of this problem are dealt with in a separate study on the central nervous system of this insect (Wigglesworth, 1960).

METHODS

The adult male *Periplaneta americana* L. possesses a nerve cord which is relatively free from associated fat body, and for this reason is particularly suitable for use in an investigation of this kind.

In these experiments the uptake and subsequent metabolism of $^{14}$C in the nerve cord was studied after the injection of radioactive glucose into the haemolymph. The injected glucose, which was generally labelled with $^{14}$C, was dissolved in Hoyle's saline (Hoyle, 1953) and had a specific activity in the range 3·77–25·7 mc./mM. In each case 10·0 $\mu$l. of the radioactive solution was introduced into...
the haemolymph, with an Agla syringe, through an abdominal intersegmental membrane, the punctured cuticular surface being afterwards sealed with wax. After a given experimental period the insect was anaesthetized with CO₂, some haemolymph collected for analysis and the whole abdominal nerve cord quickly removed by dissecting through the ventral abdominal surface. Care was taken to ensure that all adhering tissues were removed from the nerve cord and those which showed excess amounts of fat body in the region of the ganglia were rejected. The cut ends of the isolated nerve cord were gripped with fine watch-maker’s forceps and the whole preparation was washed in ice-cold saline for an appropriate period, the duration depending on the nature of the experiment. On removal from the saline the nerve cords were lightly blotted, plunged into liquid nitrogen and then transferred to a glass homogenizer immersed in liquid nitrogen, for extraction with three changes of N formic acid in 50% aqueous ethanol (Heslop & Ray, 1958). Further extraction of the nerve cords with boiling 30% KOH for 6 hr. yielded only negligibly small amounts of radioactivity.

The uptake of ¹⁴C-labelled compounds was also studied in some isolated preparations. In these experiments whole abdominal nerve cords were quickly removed from the insects and placed in oxygenated saline containing trehalose and glucose in concentrations similar to those in the haemolymph. A connective, usually that between the fourth and fifth abdominal ganglia, was then gripped at both ends with automatically operated watch-maker’s forceps (Heslop & Ray, 1959) in which the lateral movements were obtained by sliding ground-glass plates lubricated with petroleum jelly (Goldacre, 1954) (Fig. 1). The isolated connective was then transferred to a radioactive solution by submerging the tips of the forceps beneath the surface of the liquid. The solution was stirred with a fine jet of water-saturated oxygen or compressed air. In other experiments the last abdominal ganglia and whole nerve cords were isolated in this way. With some isolated preparations a radioactive haemolymph solution was prepared by injecting ¹⁴C-labelled glucose into a cockroach and, after 40 min., removing the haemolymph from the base of the prothoracic leg with a silicone-lined pipette. The haemolymph was then precipitated with 2 vol. of absolute ethanol (to remove proteins), dried in vacuo over P₂O₅ and restored to its original volume by the addition of distilled water. The washing procedure used at the end of these experiments was similar to that used for the in vivo preparation.

The in vitro production of ¹⁴CO₂ by abdominal nerve cords was measured in radioactive haemolymph and in ¹⁴C-labelled glucose solutions using a procedure based on that of Villee & Hastings (1949). The apparatus used consisted of a microdiffusion cell with a ground-glass lid containing two 8·0 mm. diameter glass cups; the first held the radioactive solution and the nerve cord and the second 0·2 ml. of 5·0% NaOH with a small roll of filter-paper. The apparatus was gassed with a stream of oxygen for 10 min. before the commencement of each experiment. The radioactivity absorbed by the NaOH was subsequently measured with a scintillation counter.

The radioactivity extracted from the nerve cords was assayed using a thin-
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Windowed G.M. tube (Mullard MX/123) or, for low activities, a Panax liquid scintillation counter (SC/LP). With this latter instrument the radioactive sample (c. 0.2 ml.) was dissolved in 3.0 ml. of a dioxane liquid phosphor. The lead castle of the scintillation counter was cooled with tap water and at an operating voltage of 1200 V. the instrument showed a background activity of about 30 counts/min.

The separation and identification of compounds present in the nerve cord and haemolymph was achieved by paper chromatography. The freshly collected haemolymph was precipitated with 2 vol. of ethanol, dried in vacuo over P₂O₅ and then restored to twice its original volume with 60% ethanol. Whole nerve cords were removed from the insects, washed for 90 sec. in ice-cold saline, plunged into liquid nitrogen and then ground up in either N formic acid in 50% ethanol, dimethyl formamide or 5% trichloro-acetic acid. The trichloro-acetic acid was subsequently removed from the solution by washing seven times with ether. Essentially similar results were obtained with all three solutions. For the detection of compounds by conventional chemical sprays ten nerve cords were extracted for each chromatogram; with radioactive nerve cords only one was necessary per chromatogram. Successive 3-0 µl. aliquots of the extracts were transferred to Whatman no. 1 filter-paper for separation by descending chromatography. The solvents used for one-dimensional chromatography were: phenol-water, n-propanol/ethyl acetate/water (Baar & Bull, 1953), 70% n-propanol (Chen, 1958), n-propanol/ammonia/water (Hanes & Isherwood, 1949), butanol/acetic acid/water (Synge, 1951) and ethyl acetate/acetic acid/water (Jermyn & Isherwood, 1949). Two-dimensional
chromatograms were developed with 70% n-propanol followed by water-saturated phenol (Chen, 1958) and n-butanol/acetic acid/water (74/19/50) followed by phenol (Fowden & Steward, 1957). Ninhydrin-positive substances were detected by the ninhydrin-cobalt chloride spray of Wiggins & Williams (1952); reducing substances by the silver nitrate method of Trevelyan, Proctor & Harrison (1950); non-reducing carbohydrates by the sodium-metaperiodate spray of Evans & Dethier (1957) followed by silver nitrate; phosphorus compounds by the acid-molybdate method of Hanes & Isherwood (1949); and non-volatile aliphatic acids with the bromo-phenol blue spray of Lugg & Overell (1947). The concentrations of trehalose and glucose in the haemolymph were estimated by the method of Dimler, Schaefer, Wise & Rist (1952). Hydrolysis of oligo- and polysaccharides was effected by boiling in 3 N-HCl in a sealed tube for 3 hr., the products being re-run on fresh chromatograms.

Assay of radioactivity on chromatograms was carried out by moving the paper strips across a 1 cm. wide slit above a GM tube, successive counts being made until the whole paper was completed. With chromatograms of low activity a scintillation technique was employed in which the 14C was eluted from 1 cm. wide pieces of the chromatogram and counted in the liquid phosphor. In the technique which was finally employed each 1 cm. wide piece of chromatogram paper was placed in the 30 ml. glass container and soaked with 0.2 ml. of distilled water. After 1 hr. 3 ml. of dioxane phosphor were added and the paper was removed, care being taken to drain the liquid from the paper in doing so. With radioactive glycogen it was necessary to soak the chromatogram strip overnight or to extract the radioactivity by boiling in a sealed glass tube.

The insects were reared and all experiments were performed at a temperature of 28.0 ± 1.0°C.

RESULTS

Haemolymph carbohydrates

Chromatographic analysis of the haemolymph revealed the presence of traces of glucose together with massive amounts of a non-reducing substance, subsequently identified as trehalose. The identification of this compound was based on the fact that acid hydrolysis yielded only glucose molecules and on the very close similarity of its position to that of authentic trehalose on chromatograms developed with four different solvent systems.

The concentrations of these two carbohydrates in the haemolymph of adult male cockroaches are summarized in Table 1. These results show a very high concentration of the disaccharide as compared with the low and extremely variable glucose content.

Experiments were performed to determine the metabolic fate of 14C-labelled glucose introduced into the haemolymph. To do this 10-μl. of a 48.6 mM./l. solution were injected into individual cockroaches from which haemolymph samples were extracted after varying experimental periods. The radioactivity in the haemolymph was separated on paper chromatograms, some typical results being illustrated in Fig. 2. It was found that in all cases the injected glucose was rapidly
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converted to trehalose, which accumulated in the haemolymph, only very small amounts of glucose remaining in equilibrium with the trehalose.

Table 1. The concentrations of trehalose and glucose in the haemolymph of adult male cockroaches

<table>
<thead>
<tr>
<th>Serial</th>
<th>Trehalose (mg./100 ml.)</th>
<th>Glucose (mg./100 ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>990.3</td>
<td>18.0</td>
</tr>
<tr>
<td>2</td>
<td>1517.1</td>
<td>27.0</td>
</tr>
<tr>
<td>3</td>
<td>1196.7</td>
<td>18.0</td>
</tr>
<tr>
<td>4</td>
<td>1281.2</td>
<td>58.7</td>
</tr>
<tr>
<td>5</td>
<td>1273.8</td>
<td>39.9</td>
</tr>
<tr>
<td>6</td>
<td>1665.9</td>
<td>72.1</td>
</tr>
<tr>
<td>7</td>
<td>1815.5</td>
<td>43.2</td>
</tr>
<tr>
<td>Mean</td>
<td>1395.9</td>
<td>39.5</td>
</tr>
</tbody>
</table>

Washing and efflux experiments

In order to determine the amounts of 14C-labelled compounds contained in the experimental abdominal nerve cords it was necessary to devise some methods by which the surface radioactivity, from the haemolymph, could be separated from that contained within the nerve cords. A series of experiments was, therefore
performed in which radioactive abdominal nerve cords were removed from injected cockroaches and washed in successive 0.2 ml. amounts of Hoyle's saline. At the end of the series of washings the radioactivity remaining in the cords was extracted in the usual way. From the activities of the washings and the final extraction it was possible to construct graphs showing the amount of 14C remaining associated with the nerve cords throughout the experiment. Such a graph is illustrated in Fig. 3 and shows the decline in radioactivity associated with a nerve cord taken from an insect injected 1 hr. previously with 10-0 μl. labelled glucose solution. The curve in Fig. 3a when plotted on a logarithmic scale with respect to time (Fig. 3b) can
be resolved into two components: a rapid initial decline with a half time of 19 sec., and a second slow fall with a half time of about 580 sec. This effect is essentially similar to that obtained for the efflux of tritiated water from isolated lobster nerves (Nevis, 1958).

In some further experiments isolated abdominal nerve cords were soaked in a 4.9 mM./l. 14C-glucose solution for a period of 1 hr. and the subsequent efflux of radioactivity on washing in a succession of 0.2 ml. amounts of saline was recorded. These results were compared with the efflux obtained from isolated cords which were submerged in the radioactive solution for a period of only 1 sec. before washing in the usual way. Both sets of results are summarized in Fig. 4, from which it will be seen that the efflux from the nerve cord soaked for 1 hr. could be resolved into two components, which were similar to those for cords taken from intact insects (Fig. 3). The efflux from the cord exposed for 1 sec. showed, however, only a single rapid component which paralleled that for the cords soaked for 1 hr. It seems most reasonable to conclude from these results that the rapid component represented the washing of 14C-labelled material from the surface and the slow one the loss from the interior of the nerve cord. Thus to determine the total activity of the 14C-labelled components within the nerve cord it was only necessary to extrapolate the slow component back to zero time. In all experiments the total radioactivities in the nerve cords have been obtained and calculated in this way, the activity being expressed in terms of that contained in unit volume of nerve cord water.
The accumulation of radioactive material in the abdominal nerve cord

The increase in radioactivity in the nerve cord was studied following the injection of 10·0 μl. of the 14C-labelled glucose solution into the haemolymph. The individual cockroaches used in these experiments showed great variability in haemolymph volume, with a resulting variation of injected radioactivity expressed per unit volume of haemolymph. To minimize this source of variation all results for the accumulation of 14C-labelled material in the nerve cords are expressed as the ratio: activity in nerve cord/activity in haemolymph.

![Graph](image)

Fig. 5. The rate of conversion of injected 14C-labelled glucose to trehalose, which accumulated in the haemolymph (a), compared with the entry of radioactivity into the nerve cord expressed as the ratio: activity in nerve cord/activity in haemolymph (b).

The entry of radioactive material into the nerve cord following the injection of 14C-labelled glucose is illustrated in Fig. 5b, where it is also compared with the rate of conversion of glucose to trehalose in the haemolymph. It will be seen that there was an initial rapid rise of radioactivity in the abdominal nerve cord relative
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to that in the haemolymph. This activity reached a peak after about 10 min. and was followed by a fall, to a ratio of about 0.2 after 30 min. During this period the glucose was converted to trehalose, which accumulated in the haemolymph, reaching an equilibrium by about 30 min. After this the activity in the nerve cord rose again rather more slowly to a level close to that in the haemolymph.

The significance of the form of this graph will be discussed in a later section, it is only necessary here to mention that the second build-up of radioactivity is of interest because it represents the accumulation of $^{14}$C originating from trehalose which is in equilibrium with the small amount of glucose in the haemolymph.

A further series of experiments was performed in which the radioactivities in single ganglia and connectives were compared. Fig. 6 illustrates the increase in radioactivity, relative to that in the haemolymph, in the last abdominal ganglion and in the connective between the fourth and fifth abdominal ganglia. The form of this graph is essentially similar to that obtained for the whole nerve cord and it is clear that the radioactivity appeared at similar rates in the different parts of the central nervous system.

**CO$_2$ production by the isolated nerve cord**

The above experiments which recorded the accumulation of radioactivity within the abdominal nerve cord do not necessarily give an accurate picture of the movement of $^{14}$C-labelled compounds into the nerve cord, for no account could be taken with the intact insect of the $^{14}$CO$_2$ produced by the nervous tissue. Experiments were, therefore, performed to measure the rate of $^{14}$CO$_2$ production using isolated, ligatured nerve cords in the radioactive haemolymph solution. The $^{14}$CO$_2$ evolved was absorbed in 5.0% NaOH and its activity assayed using the scintillation counter. The $^{14}$CO$_2$ production was also measured in a $^{14}$C-glucose solution (75.99 mM/l.)
of concentration equivalent to the combined trehalose and glucose content of the haemolymph. The results are tabulated in Table 2, from which it will be seen that the $^{14}$CO$_2$ produced in vitro represented only a very small part of the total radioactivity contained within the nerve cord.

**Table 2. The $^{14}$CO$_2$ production of isolated abdominal nerve cords after a period of 1 hr.**

<table>
<thead>
<tr>
<th>Solution</th>
<th>Activity in nerve cord (counts/min.)</th>
<th>Activity as $^{14}$CO$_2$ (counts/min.)</th>
<th>$^{14}$CO$_2$ production (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radioactive haemolymph</td>
<td>2,992.0</td>
<td>27.0</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>3,357.4</td>
<td>42.1</td>
<td>1.25</td>
</tr>
<tr>
<td></td>
<td>3,889.8</td>
<td>49.1</td>
<td>1.30</td>
</tr>
<tr>
<td></td>
<td>3,962.8</td>
<td>44.4</td>
<td>1.11</td>
</tr>
<tr>
<td></td>
<td>1,949.8</td>
<td>12.5</td>
<td>0.64</td>
</tr>
<tr>
<td>Glucose (75.99 mM/l.)</td>
<td>6,095.4</td>
<td>117.3</td>
<td>1.92</td>
</tr>
<tr>
<td></td>
<td>12,078.0</td>
<td>140.0</td>
<td>1.16</td>
</tr>
<tr>
<td></td>
<td>21,281.0</td>
<td>360.3</td>
<td>1.69</td>
</tr>
<tr>
<td></td>
<td>19,588.0</td>
<td>126.3</td>
<td>0.64</td>
</tr>
<tr>
<td></td>
<td>25,236.0</td>
<td>203.3</td>
<td>0.80</td>
</tr>
</tbody>
</table>

**Sugar fluxes in the nerve cord**

At this stage it was essential to obtain a quantitative estimate of the entry of $^{14}$C-labelled sugars into the nerve cord. It was also necessary to discover the proportion of the radioactivity originating from the trehalose and from the small amount of glucose in equilibrium with the disaccharide in the haemolymph. Fortunately the apparently slow rate of $^{14}$CO$_2$ production by the nerve cord enabled the influx of the sugars to be calculated, with only negligible error, according to the equation given by Croghan (1958) for ion fluxes:

$$m \cdot t = C_t \log_x (x-y),$$

where $m =$ total sugar influx, expressed as mM. glucose units/litre of nerve cord water/min.;

$x = C_x / C_0$ (i.e. the activity in the nerve cord when exchange is complete);

$y =$ activity in haemolymph (counts/unit volume);

$C_t =$ concentration of $^{14}$C-labelled compounds in the nerve cord, expressed as equivalent glucose units (75.19 mM/l.);

$C_0 =$ concentration of $^{14}$C-labelled trehalose and glucose in the haemolymph, expressed as glucose units (75.99 mM/l.).

(In this section all trehalose concentrations are expressed as equivalent glucose concentrations in mM/l.)

The fluxes of the $^{14}$C-labelled sugars were compared as between:

(1) Intact insects, in which the nerve cord radioactivity originated as trehalose and glucose in the haemolymph.

(2) Isolated preparations, in which the trehalose and glucose were labelled with $^{14}$C in the haemolymph solution.
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(3) Isolated preparations, in which only the glucose (2·19 mM./l.) was labelled with $^{14}$C, the trehalose being non-radioactive.

The data for the flux in the intact insect are taken from Fig. 5 and represent the entry of radioactivity (after 30 min.) when the injected glucose had come into equilibrium with the trehalose. These values have been corrected to zero time for this calculation.

![Graph](image)

Fig. 7. The calculated influx of radioactivity originating as: (i) trehalose and glucose in the haemolymph of intact insects (closed circles); (ii) as trehalose and glucose for connectives isolated in radioactive haemolymph (open circles); (iii) for isolated connectives in saline in which only the glucose (2·19 mM./l.) was labelled with $^{14}$C, the trehalose being non-radioactive (open squares). The fluxes are expressed as equivalent mM. $^{14}$C-labelled glucose/litre nerve cord water/min.

From Fig. 7 it will be seen that on plotting $2·3 C_t \log_{10} [z/(x-y)]$ against $t$ approximately straight lines were obtained in all three cases. The flux in the intact preparation was, apparently, similar to that of the isolated connective immersed in the haemolymph solution, and was equivalent to 1·09 mM. glucose/l. nerve cord water/min. The influx when only glucose was labelled was approximately 0·08 mM./l. nerve cord water/min. Thus it seems that the radioactivity originating from the glucose accounted for about 7·3% of the $^{14}$C entering the abdominal nerve cord.

The metabolism of $^{14}$C-labelled sugars in the nerve cord

In order to follow the metabolic fate of the sugars entering the central nervous system the radioactivity was extracted, and subsequently separated on paper chromatograms, from nerve cords of cockroaches injected 3 hr. previously with 10·0 $\mu$l. of $^{14}$C-glucose solution. It was found that the extracted $^{14}$C could be separated into at least seven peaks of radioactivity. It seemed likely that some of
these radioactive peaks might represent $^{14}$C incorporated in phosphorus compounds. The phosphorus spots detected on the chromatograms with the acid-molybdate spray did not, however, invariably correspond with these peaks of radioactivity, while $^{32}$P injected as orthophosphate was not consistently associated with the $^{14}$C. Negative results were obtained also in tests for the incorporation of the $^{14}$C as lactic and pyruvic acids. Several of these zones of $^{14}$C activity were, however, found to be associated with some ninhydrin-positive spots obtained from nerve cord extracts. These ninhydrin-positive substances present in the nerve cord were separated on two-dimensional chromatograms (Fig. 8) which revealed the presence of glycine, alanine, serine, aspartic acid, glutamic acid and glutamine. These substances, together with glucose and trehalose, were run with nerve cord extracts on chromatograms developed in four different solvent systems (Fig. 9). From these chromatograms it was possible to identify the major radioactive components as: aspartic acid, glutamic acid, glutamine, occasionally small amounts of alanine, trehalose and glucose. The peak of radioactivity at the base line ($R_f = 0$) was found to be water-soluble, alcohol-insoluble and to yield only glucose on acid hydrolysis (Fig. 9g), indicating the presence of $^{14}$C-labelled glycogen in the nerve cords. None of the activity was found to be associated with added $\gamma$-amino-butyric acid.

The proportions of the various radioactive substances accumulating within the nerve cord following the injection of $^{14}$C-labelled glucose into the haemolymph...
Fig. 9. The distribution of radioactivity extracted from abdominal nerve cords 3 hr. after injection of $^{14}$C-labelled glucose into the haemolymph developed in four different solvent systems (a–d). A chromatogram of the radioactivity from the peak at the base-line (i.e. $R_f = 0$) after acid hydrolysis is illustrated in (a).
are summarized in Fig. 10a. This data, together with that illustrated in Fig. 5,
has been used to construct a diagram showing the concentrations of these com-
pounds in the nerve cord relative to that of the labelled sugars in the haemolymph
(Fig. 10b).

The in vitro metabolism of 14C-labelled glucose was studied by immersing isolated
nerve cords in 2·19 mm./l. glucose saline solution containing non-radioactive

![Graphs showing percentages of radioactivity in nerve cord and activity ratios over time.]

**Fig. 10.** (a) The proportions of the various 14C-labelled compounds which accumulated in the nerve cords following injection of 14C-labelled glucose into the haemolymph. (b) The estimated concentrations of the 14C-labelled compounds in the nerve cords (expressed as the ratio: activity in nerve cord/activity in haemolymph) calculated from the data given in (a) and Fig. 5 (b).
trehalose (36-90 mM./l.). After 2 hr. the preparations were washed and the extracted radioactivity was run on chromatograms developed with four different solvent systems (Fig. 11). In these preparations only occasional traces of aspartic acid, glutamic acid, glutamine and trehalose were found, but alanine accumulated in relatively large amounts together with massive concentrations of glucose. The appreciable amounts of radioactivity remaining at the base line yielded only glucose on acid hydrolysis and were presumed to be glycogen.

![Chromatograms](image)

**Fig. 11.** The distribution of radioactivity extracted from nerve cords isolated in 2.19 mM./l. ¹⁴C-labelled glucose and non-radioactive trehalose developed in four different solvent systems.

Some further experiments using isolated preparations were performed in which the nerve cords were immersed in the radioactive haemolymph solution or in a saline solution containing labelled glucose in equivalent concentration to that of
the trehalose and glucose in the haemolymph (75·99 mM./l.). In both cases substantial amounts of alanine were found to have accumulated with the isolated nerve cords (Fig. 12). In the cords isolated in the radioactive haemolymph glucose, together with some reduced amounts of glutamic acid, accumulated in addition to the trehalose; the cords placed in the high-glucose solution showed concentrations of alanine and glycogen in addition to the large amounts of glucose present.

**Fig. 12.** Chromatograms of radioactivity extracted from nerve cords isolated in radioactive haemolymph and in a solution of 75·99 mM./l. 14C-labelled glucose. These chromatograms were developed in ethyl acetate/acetic acid/water.

**DISCUSSION**

The cockroach can now be added to the number of insects which have been found to possess haemolymph containing appreciable amounts of the disaccharide trehalose (Wyatt & Kalf, 1956; Howden & Kilby, 1956; Evans & Dethier, 1957). The concentration of 1·39% in the haemolymph of this insect is of the same order
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as that found in the locust, *Schistocerca gregaria* (Howden & Kilby, 1956; Treherne, 1958b) and the blowfly, *Phormia regina* (Evans & Dethier, 1957). The rapid conversion of glucose to trehalose, which accumulates in the haemolymph, has also been previously demonstrated in the locust (Treherne, 1958a, b), the actual conversion apparently taking place in the fat body of this insect (Candy & Kilby, 1959). In the cockroach, as in the locust, a small amount of glucose (c. 39.5 mg./100 ml.) remained in equilibrium with the trehalose in the haemolymph.

The movement of 14C into the abdominal nerve cord after injection of a small amount of radioactive glucose into the haemolymph followed a rather complex course (Figs. 5, 6). Immediately after the injection of labelled glucose there was a very rapid increase in radioactivity within the nerve cord which, after about 10 min., fell sharply to about a fifth of the activity in the haemolymph. After approximately 30 min. the activity commenced to rise again, this time more slowly, to a level similar to that in the haemolymph. When considering these results it should be borne in mind that the specific activities of the haemolymph carbohydrates were changing rapidly during the first 30 min. of the experiments. The initial steep rise in activity represented the movement of relatively few glucose molecules, of very high specific activity, into the nerve cord. During this period the glucose was rapidly converted to trehalose which accumulated in the haemolymph, the whole system approaching equilibrium after about 30 min. The second slow increase in radioactivity thus represented the entry of 14C originating as trehalose or as the small amount of glucose remaining in the haemolymph. This interpretation is supported by the subsequent chromatographic analysis when it was found that during the first part of the experiment the 14C was largely incorporated as glucose in the nerve cord. Thus the initial rise and fall in activity can be regarded as an experimental artifact, the true movement of sugars into the abdominal nerve cord being represented by the second uptake curve when the 14C-labelled trehalose was in effective equilibrium with the small amount of glucose in the haemolymph.

The appearance of radioactivity in different parts of the nerve cord was shown to occur at closely similar rates and to parallel the uptake of the 14C-labelled sugars by the whole system. Such a result could be obtained either by the sugars passing through the different surfaces at similar rates or, if the passage through these surfaces is unequal, by a rapid movement of 14C-labelled compounds within the central nervous system.

The influx of 14C originating from the labelled trehalose and glucose in the haemolymph was calculated to be equivalent to about 1.09 mM. glucose/l. of nerve cord water/min. This apparently rapid flux could be brought about by an entry of trehalose or by a relatively rapid movement of molecules from the small amount of glucose in the haemolymph. Some light was thrown on the process by the measured influx in the isolated preparation when only the glucose was labelled with 14C, the trehalose being non-radioactive. Here the apparent flux was equivalent to 0.08 mM./l. nerve cord water/min., which suggests that only about 7% of the activity in the nerve cord originated from the small glucose pool in the haemolymph.
This means that one molecule of glucose passed into the nerve cord for approximately every seven molecules of trehalose. Trehalose molecules are, however, seventeen times more concentrated than those of glucose in the haemolymph, which implies that the individual glucose molecules were in fact passing into the nerve cord at about 2.5 times the rate of the trehalose molecules. The diffusion constant of glucose through water (0.68 × 10⁻⁶ cm²/sec. at 25°C, *International Critical Tables*) is, on the other hand, only 1.2 times that of a disaccharide (sucrose −0.55 × 10⁻⁶ cm²/sec.). Such apparent discrepancy between the rates of passage of these two sugars into the nerve cord and their free diffusion constants could be brought about, for example, by some sort of transport of glucose, or by the slowing down of trehalose due to steric or frictional hindrance of the larger disaccharide molecule in diffusing through some kind of pore membrane. It would be unwise, however, to press these conclusions too far at this stage. The glucose influx was calculated from data obtained with the isolated preparation in which these processes might be altered in some way. Against this should be set the fact that the influx from the radioactive haemolymph was similar in the intact insect, although this result is further complicated by the changed metabolism of the ¹⁴C-labelled compounds within the isolated nerve cord.

The prompt appearance of ¹⁴C, supplied as trehalose and glucose, in the carbon skeletons of aspartic and glutamic acids *in vivo* and in appreciable amounts of alanine *in vitro* represents circumstantial evidence for the presence of the Krebs tricarboxylic acid cycle enzymes in the central nervous system of this insect. The identification of glucose-6-phosphate and phosphoglyceric acid in the cockroach nerve cord (Heslop & Ray, 1958) suggests that the carbohydrate metabolism is linked to the tricarboxylic acid cycle by the conventional glycolytic pathway. Thus alanine could be produced by the amination of the resulting pyruvic acid and aspartic acid by the amination of oxalacetic acid derived from the pyruvic acid. Glutamic acid formation would result from the amination of α-ketoglutaric acid synthesized by the enzymes of the tricarboxylic acid cycle.

More than half of the administered ¹⁴C was found to be incorporated as glutamic acid and glutamine in the intact nerve cord, suggesting that the very reactive amino acid occupies a central position in the metabolism of the central nervous system in this insect. The linkage with glutamine is of especial importance for this substance must function as an important reservoir of amino-nitrogen in the nervous tissue. The high concentration of these two substances, together with the relatively low carbohydrate content, is essentially similar to the state of affairs in the mammalian brain tissue (cf. McIlwain, 1959). It is, therefore, of interest to note that in isolated rat and guinea-pig cerebral cortex administration of glutamic acid led to respiratory rates higher than those induced by glucose (Weil-Malherbe, 1936).

The presence of glycogen has been previously demonstrated histochemically in the central nervous system of the mosquito larva (Wigglesworth, 1942), where it occurs in granular deposits and vacuoles, and in the cockroach nerve cord (Wigglesworth, 1960). The synthesis of glycogen within the nervous systems of these insects was demonstrated by following its accumulation after feeding starved
individuals with various carbohydrates. A linkage of carbohydrate metabolism and glycogen synthesis with the amino acid metabolism can also be inferred from the observations of Wigglesworth (1942) that the feeding of starved larvae with alanine and glutamic acid resulted in an accumulation of glycogen in the ganglia and connectives of the nerve cord.

Finally, it is relevant to compare the distribution of $^{14}$C among the compounds in the cockroach nerve cord with that obtained for the mammalian central nervous system. Isolated rat-brain cortex slices can convert $^{14}$C-glucose into labelled glutamic acid, aspartic acid, alanine and glutamine (Kini & Quastel, 1959) as in the cockroach. The rat nervous tissue differed from that of the cockroach in the formation of appreciable amounts of $\gamma$-amino-butyric acid, a substance which was not detected among the labelled compounds in the insect nerve cord. Some other major differences in the metabolism of the $^{14}$C in the nervous system of these two animals were an apparent absence of glycogen formation, an accumulation of large amounts of lactic acid and a relatively high rate of $^{14}$CO$_2$ production in rat-brain slices (Beloff-Chain, Catanzaro, Chain, Masi & Pocchiari 1955). The possibility cannot of course be eliminated that some of the apparent differences in the metabolism of $^{14}$C may have been due to the different techniques employed, for the rat brain was studied in isolation and the cockroach nerve cord in vivo. It should be mentioned in this respect that in the present investigation the in vitro preparations did not accumulate the various $^{14}$C-labelled compounds in the same proportion as the nerve cords of an intact insect.

**SUMMARY**

1. $^{14}$C-labelled glucose injected into the cockroach was found to be rapidly converted to trehalose, only small amounts remaining in equilibrium with the disaccharide in the haemolymph. The entry of these sugars into the cockroach central nervous system was studied by following the increase in radioactivity within the abdominal nerve cord after the injection of radioactive glucose into the haemolymph.

2. The levels of radioactivity increased at closely similar rates in different parts of the abdominal nerve cord.

3. The influx of sugars into the nerve cord was calculated to be equivalent to 1.09 mM. glucose/l. of nerve cord water/min.

4. The greater part of the $^{14}$C entering the nerve cord originated from the trehalose, only about 7% being derived from the small amount of glucose in the haemolymph. The movement of the relatively small number of glucose molecules into the nerve cord occurred, nevertheless, at approximately 2.5 times the rate of the larger trehalose molecules.

5. Chromatographic analysis revealed that more than half of the absorbed $^{14}$C was incorporated as glutamic acid and glutamine in the nerve cord. Smaller amounts of glycogen, trehalose, glucose, aspartic acid and occasional traces of alanine were found. In the isolated nerve cord substantial amounts of alanine.
accumulated, the formation of the other amino acids being reduced. $^{14}$CO$_2$ production in vitro was found, after 1 hr., to represent only about 1% of the total activity within the nerve cord.

6. The results demonstrate a linkage of carbohydrate and amino acid metabolism and represent circumstantial evidence for the presence of the tricarboxylic acid cycle enzymes in the central nervous system of this insect.

REFERENCES


The exchange and metabolism of sugars in the cockroach


