Effect of Diet on the Amount and Composition of Locust Blood Carbohydrates

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Blood sugar concentrations found for the migratory locust *Locusta migratoria migratorioides* reared in this Laboratory under strictly controlled conditions were in marked contrast with findings reported for the closely related *Schistocerca gregaria*, especially with the statement that trehalose is the obligatory major carbohydrate in the blood of that insect (Howden & Kilby, 1956; Treherne, 1957, 1958 *a-c*).

There were indications, however, that the free access to growing wheat of our locusts provided optimum feeding conditions. Cannibalism, generally a considerable problem in work with migratory locusts, was not observed, and the heavy mortality reported among hoppers did not occur. It is often considered necessary to breed three to four times as many hoppers as the number of adults demanded, but under our standard conditions several thousands of locusts were bred with practically all hoppers developing satisfactorily.

Since among other differences the total blood carbohydrate concentrations of our locusts were much higher than any value hitherto reported, we examined whether migratory locusts are subject to variation of concentration and composition of blood carbohydrates with alteration of feeding conditions.

MATERIALS AND METHODS

Locusts. The migratory locusts *Locusta migratoria migratorioides* and *Schistocerca gregaria* were kept under identical conditions at a relative humidity of 45%. From 9 a.m. to 10 p.m. the cages were brightly lit and the temperature was $34 \pm 0.5^\circ$. The night temperature was $28 \pm 1^\circ$.

Eggs collected in moist sand and kept at the temperatures stated hatched in 10 days. Unless otherwise stated, the locusts had free access to growing green wheat, dry wheat bran and water. Hoppers hatched in 3 consecutive days were kept together, no greater difference in age being allowed in any experiment.

Both locusts were gifts from the Anti-Locust Research Centre of London. Colonies of *Locusta migratoria* were maintained for 2 years, but *Schistocerca* was used in a few comparative experiments only.

Sampling of haemolymph. Locusts reared at the recommended humidity are rich in blood, and samples are easily

obtained. On lateral puncture of the cervix, haemolymph will rise into a micropipette placed at the wound. Generally 0.2 ml. can be drawn in this way when some experience has been gained. When it was necessary to preserve the insect, the wound was covered with silicone grease to prevent loss of blood. The wax commonly used for such purposes is apt to be contaminated by carbohydrates, but silicone grease is obtainable in very pure quality and is easily heat-sterilized.

Determination of glucose. A sample (50 μ l.) of haemolymph was deproteinized by transfer to 2.0 ml. of 95% (v/v) ethanol followed by centrifuging for 15 min. at 2050 g_{av} . Glucose was determined on 0.20 ml. of the supernatant by the colorimetric glucose oxidase-peroxidase-o-dianisidine method (Hansen, 1962), which was shown to be specific.

The average glucose recovery for twenty-four additions of different known amounts of carbohydrate to locust haemolymph was 98.8 (s.d. ± 4.2)%, and the average deviation of twenty duplicate haemolymph glucose values was found to be ± 4.69 (s.d. ± 2.94)%, with s.e.m. ± 0.93 .

Determination of total carbohydrate. Total carbohydrate (expressed as glucose) was determined by the colorimetric anthrone method (Hansen, 1960), and as 0.01 ml. of haemolymph, resulting in a final average protein concentration (perchloric acid-precipitable material) in the analysis medium of only 0.000003%, allowed for sufficient extinctions, total carbohydrate values obtained with undeproteinized haemolymph did not differ from those obtained with perchloric acid-treated haemolymph and no protein influence could be seen on the sugar-anthrone spectrum.

Extinctions at 620 m μ obtained with fed animals were in the range 0.3–0.7. Reproducibility with undeproteinized haemolymph was just as satisfactory as with tungstic acid-deproteinized human and other mammalian blood (Hansen 1960). Thirty-two duplicate determinations of total carbohydrate ranging from 1775 to 3377 mg./100 ml. showed an average deviation of the duplicate values of ± 0.53 (s.d. ± 0.35)%, with s.e.m. ± 0.09 .

Chromatographic separation of carbohydrates. Carbohydrates were identified by paper chromatography with the system of Wyatt & Kalf (1957).

Phenylosazone crystallization. Sodium acetate (300 mg.) was ground in a mortar, carefully mixed with 200 mg. of phenylhydrazine hydrochloride and transferred to a test tube with 5 ml. of water (Hawk & Bergeim, 1937). A sample (200 μ l.) of haemolymph (work with that small amount based on the results of Garard & Sherman, 1918) was blown into the phenylhydrazine mixture and for 45 min. heated on a boiling-water bath. The tubes were allowed to cool on the bath.

Identification of different classes of carbohydrates by a modification of the anthrone method. As Koehler (1952) observed, under controlled heating with anthrone, colour peaks will occur at characteristic times for sugars of different classes. The oligo- and poly-hexoses, examined by Mokrasch (1954) and Hansen (1960) and in the present investigation, yield colour intensity-time curves of the same form as those obtained with the constituent hexoses. Further, boiling with the anthrone reagent will distinguish aldoses from ketoses as seen both from Mokrasch (1954) and from Fig. 1. Extinctions were measured in a Beckman model DU spectrophotometer.

Fig. 1. The extinction at 620 $m\mu$ of different aqueous carbohydrate solutions (10 mg./100 ml.; 2.0 ml.) treated with the anthrone reagent (10.0 ml.) was measured (Beckman model DU spectrophotometer, with matched 1 cm. Corex cells) against corresponding blank solutions. $\circ$, Glucose; $\bullet$, mannose; $\triangle$, trehalose; $\blacktriangle$, glycogen; $\square$, dextran; $\blacksquare$, fructose; ∇ , sucrose. The theoretical values, slightly higher than for an equivalent amount of glucose, were obtained with highly purified cellobiose, maltose, trehalose, glycogen and dextran; D(+)-mannose [C.P., Pfanstiehl; sp. rotation stated to be $+14.25^\circ$; $[\alpha]_D^{20.6}$ found to be $+14.7^\circ$ (c 8 in water); Sowden (1957) gives $+14.6^\circ$] gave values amounting to only 64% of those obtained with glucose. The actual higher extinctions of cellobiose, maltose, trehalose, glycogen and dextran, and the lower ones of mannose, were multiplied by the factor necessary to obtain maximum extinction identical with that of glucose. For fructose and sucrose the actual extinctions are stated.

RESULTS AND DISCUSSION

Glucose and total carbohydrate content of locust haemolymph

In numerous experiments with immature as well as mature adult *Locusta migratoria* fed on the standard wheat diet, the apparent blood glucose values found by the anthrone method and the true blood glucose established by the glucose-oxidase method invariably were of the order seen from the experiment reported in Table 1.

Twelve immature adult *Schistocerca*, six males and six females, gave values similar to those with *Locusta migratoria*, namely 2490 (s.d. ± 365) mg. of glucose/100 ml. by the anthrone method and 246 (s.d. ± 25) mg./100 ml. by the glucose-oxidase method. The anthrone spectrum obtained with six *Schistocerca* on wheat in no case differed from that found for *Locusta migratoria*.

Weis-Fogh (1952) reported reducing values, taken to represent glucose, of 220–240 mg./100 ml. of haemolymph from *Schistocerca*. Howden & Kilby (1956) and Treherne (1957, 1958a-c) obtained blood glucose concentrations only 10–40% of those obtained by Weis-Fogh (1952). If locusts do not possess a system for regulating blood sugar similar to that in vertebrates, it is possible to account for the discrepancies as being due to optimum feeding conditions because in our experiments growing wheat was always available in the cages.

Fig. 2 shows the effect of starvation. The amount of food available may very well explain the different carbohydrate concentrations reported by various workers.

Nature of the carbohydrates other than free glucose of locust haemolymph

That material other than carbohydrate should be responsible for the high values found by the anthrone method was not likely, since the absorption spectrum obtained with haemolymph was in agreement with that of glucose (Hansen, 1960). By application of the glucose-oxidase method to hydrolysed haemolymph it is evident that almost

Table 1. *Total carbohydrate content of haemolymph of adult Locusta migratoria*

Values are expressed as glucose of undeproteinized haemolymph determined by the anthrone method and glucose by the glucose-oxidase method. The haemolymph was deproteinized with ethanol immediately it was drawn. The locusts were fed on wheat.

Animal no. ...	...	...	...	1	2	3	4	5	6	7	8	9	10	Average
Sex ...	...	...	...	♂	♂	♂	♂	♂	♀	♀	♀	♀	♀	
Anthrone value (as mg. of glucose/100 ml. of blood)				2108	1936	2010	1679	3064	1740	1838	1887	1226	1685	1917
Glucose (mg./100 ml. of blood)				369	257	323	282	406	244	228	228	261	207	281
Glucose (% of anthrone values)				18	13	16	17	13	14	12	12	21	12	15

the entire anthrone values are due to glucose or material hydrolysable to glucose.

Samples (25 μ l.) of haemolymph were drawn from five fed adult males and five fed adult females on the standard wheat diet and transferred to test tubes with 2.0 ml. of water. Then 1.0 ml. of each of the dilutions was transferred to centrifuge tubes, which on the addition of 1.0 ml. of 12 % perchloric acid (Hansen, 1962) were centrifuged for 15 min. at 2100 g_{av} . The clear supernatants were transferred to ampoules, which were sealed and kept at 100° for 17 hr. The final concentration of acid (6 %) was the highest that could be used without risk of influencing the glucose-oxidase reaction, but it meant that considerable time was required for the hydrolysis of some carbohydrates. Treatment for 17 hr. at 100°, however, ensures complete

hydrolysis of even pure cellulose. At 100° the procedure is safe, since destruction of glucose, as measured by reaction in the glucose-oxidase assay, was negligible.

In the experiment described the hydrolysates were cooled and duplicate glucose-oxidase determinations made on 0.20 ml. samples. Anthrone determinations were carried out on the remaining portions of the hydrolysates. The results obtained by the two methods are compared in Table 2.

Except for the higher concentrations, these findings are in agreement with the reported composition of the blood sugar of *Schistocerca* of up to 100 mg. of glucose/100 ml., and trehalose in amounts approaching 1000 mg./100 ml. (Howden & Kilby, 1956; Treherne, 1957, 1958*a-c*), since trehalose, a non-reducing disaccharide composed of two molecules of glucose, would be measured as glucose by the anthrone method (Fig. 1).

Trehalose was apparently also identified by the chromatographic system of Wyatt & Kalf (1957), but we failed to isolate crystalline trehalose by the method of Wyatt & Kalf (1957) even from large samples of blood taken not only from *Locusta migratoria* but also from *Schistocerca*.

Phenylosazones. Freshly drawn haemolymph from *Locusta migratoria*, as well as from *Schistocerca* feeding on wheat, when blown into the phenylhydrazine mixture, yielded small amounts of the osazone formed from glucose, mannose and fructose. Additionally, a massive crystallization occurred of an osazone characteristic of reducing disaccharides that are composed solely of glucose units.

Crystalline blood carbohydrates. Haemolymph from adult *Locusta migratoria* and *Schistocerca* was transferred to 20 vol. of 95 % ethanol and the precipitated protein removed by centrifuging for 15 min. at 2650 g_{av} . The supernatant was evaporated almost to dryness and then rediluted with ethanol (final ethanol concentration not below 94 %), when fractional precipitation of two different crystalline materials occurred in the cold (3°). In a few hours large needles formed and overnight fine ones. On recrystallization the large crystals had m.p. 224–225° and the small crystals had m.p. 100–102°. Both crystalline materials were

Fig. 2. Effect of starvation (water allowed) on total blood carbohydrates (---) and free blood glucose (—) of adult *Locusta migratoria migratorioides*. Each point represents two males and one female.

Table 2. Total carbohydrate content of haemolymph of adult *Locusta migratoria*

Values are expressed as glucose determined by the anthrone method and the corresponding glucose values found by the glucose-oxidase method after perchloric acid hydrolysis.

Animal no.	...	...	...	...	...	1	2	3	4	5	6	7	8	9	10
Sex	...	...	...	...	...	♂	♂	♂	♂	♂	♀	♀	♀	♀	♀
Anthrone value (as mg. of glucose/100 ml. of blood)						2151	1954	1650	Spoilt	1914	2271	3169	2561	2363	2482
Glucose (mg./100 ml. of blood)						2020	1821	1500	—	1777	2029	2757	2069	1864	1977
Glucose (% of anthrone values)						94	93	91	—	93	89	87	81	79	80

water-soluble, reducing and gave aldose reactions with anthrone (Fig. 1), but reacted with glucose oxidase only after acid hydrolysis. The total crystal crop, i.e. both carbohydrates precipitated together, was 53 mg. from 3.6 g. of haemolymph from *Locusta migratoria* and 24 mg. from 1.8 g. of haemolymph from *Schistocerca*.

In the absence of the non-reducing sugar trehalose, chromatographic findings by the system of Wyatt & Kalf (1957) did not exclude maltose and cellobiose (R_G values: trehalose, 0.38; maltose, 0.39; cellobiose, 0.39). The needle forms and melting points of the isolated crystals were those of these disaccharides (Hassid & Ballou, 1957: maltose, m.p. 102–103°; cellobiose, m.p. 225°; trehalose, m.p. 97°).

The possibility of obtaining individual derivatives was limited by the general identity of the final products of maltose and cellobiose hydrolysis, but enzymic hydrolysis seemed feasible because of the different linkages in maltose (4- O - α -D-glucopyranosyl-D-glucose) and in cellobiose (4- O - β -D-glucopyranosyl-D-glucose).

A mould cellulase (obtained from Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.) produced glucose sensitive to the glucose-oxidase method from cellulose, authentic cellobiose and the crystals with m.p. 224–225°, but did not attack authentic maltose or the crystals with m.p. 100–102°.

By fractional precipitation from water of the mould cellulase a crystalline material (long pointed needles with a marked tendency to form brushes) was obtained which did not produce glucose from cellulose or maltose, but which did so from both authentic cellobiose and the crystals with m.p. 224–225°.

A cellobiase from emulsin (obtained from Bios Laboratories, New York, N.Y., U.S.A.) produced glucose from authentic cellobiose and the crystals with m.p. 224–225°, but not from authentic maltose or the crystals with m.p. 100–102°.

A yeast maltase (also obtained from Bios Laboratories) produced glucose from authentic maltose and the crystals with m.p. 100–102°, but not from authentic cellobiose or the crystals with m.p. 224–225°.

With the exception of Wyatt & Kalf's (1957) actual isolation of crystalline trehalose from diapausing pupae of *Telea polyphemus*, all the findings on trehalose in insects that have been reported in the literature rest on the systems developed by Wyatt & Kalf (1957). Further, Fairbairn (1958) reported trehalose findings among the crustaceans, whereas Hu (1958) found in the crab *Hemigrapsus nudus* maltose, maltotriose and a polysaccharide fraction containing glucose, galactose and fucose, but was unable to detect trehalose in the animal.

Influence of diet on the composition of blood sugar

The selective feeding of many insects is a recognized fact, the significance of which is discussed at length by Fraenkel (1958). Much difficulty was experienced in experiments with different foods, but finally it was found that transfer to pears, an apparently satisfactory diet, provided an uncomplicated demonstration of the possible influence of diet on the composition of locust blood sugar.

Besides the anthrone curves obtained with haemolymph from locusts fed on pears, Fig. 3 also shows the result obtained with transfer to Brussels sprouts, another satisfactory food. The changed curve was not the only difference induced by the pear diet. Whereas for locusts fed on Brussels sprouts no deviation from the findings with animals on the standard wheat diet was seen at all, the total carbohydrate concentration (on the assumption that it was accounted for by glucose and fructose molecules) for locusts with constant access to pears was about 100% higher than for animals on wheat. In the experiment reported in Fig. 3 the blood sugar findings for the locusts fed on pears were 4685 (s.d. $\pm$ 539) mg./100 ml. and 2328 (s.d. $\pm$ 235) for the locusts on wheat.

The anthrone analysis would not detect the presence in the haemolymph of monosaccharides such as ribose, arabinose and xylose, which yield only a few per cent of the extinction produced by an equivalent amount of glucose, but it is evident from Fig. 3 that fructose cannot play any considerable role in determining the form of the curve

Fig. 3. Curves obtained by the anthrone analysis (Fig. 1) of glucose (○), fructose (■), sucrose (▽), and haemolymph of *Locusta migratoria* feeding on wheat (●), on Brussels sprouts (△) or on pears (□). Each blood sugar curve is an average value for six adult locusts, three males and three females.

obtained in the anthrone reaction for the locusts fed on wheat or Brussels sprouts. Fructose was also excluded by the high percentage of the blood carbohydrates hydrolysable to glucose (Table 2). It was therefore not surprising that the chromatographic system of Wyatt & Kalf (1957), so often applied to insect blood, did not show the presence of fructose. The same chromatographic system, however, established the presence of fructose in the haemolymph of locusts fed on pears. A sucrose spot also appeared, which again had not been found with locusts fed on wheat.

It may be significant that the 58 (S.D. $\pm$ 12 %) of the total blood sugar of the locusts fed on pears which could be accounted for as glucose after hydrolysis approximates to the quantity found for animals fed on wheat, since Kerly & Leaback (1957) were able to demonstrate hexokinase activity in *Locusta migratoria* muscle whereas there was no evidence of the presence of a locust ketohexokinase.

The experiments reported in the present paper demonstrate the possible influence of diet on the amount and composition of locust blood sugar and thus throw light on discrepancies between the results of previous workers. Since fructose may be present in the haemolymph under certain circumstances, Humphrey & Robertson's (1949) findings for *L. migratoria* may be valid, even if Levenbook (1949) was unable to confirm their results.

However, in accordance with the statement that 'carbohydrates are presumably absorbed only in the form of monosaccharides in insects, as in other animals' (Gilmour, 1961), some authors have considered the findings of trehalose in insects to be explained by synthesis of the disaccharide by the animals. The presence in the blood of maltose, cellobiose and possibly sucrose would probably mean that absorption of intact disaccharides occurred.

SUMMARY

1. Migratory locusts with free access to growing green wheat had total blood carbohydrate concentrations averaging 2 %, values about twice any hitherto reported.

2. About 90 % of the total blood carbohydrate consisted of glucose or material hydrolysable to glucose, the free glucose averaging 15 % of the total blood sugar.

3. Trehalose could not be obtained from haemolymph of migratory locusts feeding on wheat, but crystalline maltose and cellobiose were isolated and identified.

4. Both total carbohydrate and glucose concentrations were influenced by the amount of food and could be considerably lowered by starvation.

5. The carbohydrate composition of the haemolymph was greatly influenced by the diet. Fructose was not present in the haemolymph of locusts feeding on wheat or Brussels sprouts but accounted for more than half of the 100 % increase in blood carbohydrate concentration of locusts transferred to a fructose-rich food (pears).

6. There was some chromatographic indication that part of the blood fructose was bound in sucrose.

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