Glucose Aversion in the German Cockroach, *Blatella germanica*

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A recently discovered strain of German cockroaches (*Blatella germanica* L.) avoids ingesting toxicant/diet mixtures because of an aversion to D-glucose in the diet rather than to the toxicant. D-fructose stimulated feeding in glucose-averse cockroaches. However, only fructose–glucose mixtures with a molar ratio $\geq 9:1$ fructose:glucose stimulated feeding. Rather than being learned, glucose aversion is inherited as an autosomal incompletely dominant trait, which appears to be controlled by a single major gene. This is the first report of nutrient avoidance as a resistance mechanism and of aversion to this typically phagostimulatory molecule recognized as the universal metabolic fuel.

**Blatella germanica**  
German cockroach  
Glucose aversion  
Nutrient avoidance  
Behavioral resistance  
Genetics

**INTRODUCTION**

Feeding behavior in animals is frequently initiated upon stimulation by various carbohydrates. Sugars, particularly mono- and disaccharides, are generally regarded as phagostimulatory to many insects (Bernays, 1985). The sugar receptor of flies has been studied intensively and is reported to contain a pyranose (P) site sensitive to D-glucose, sucrose, maltose, etc., and a furanose (F) site sensitive to D-fructose, D-fucose etc. (Shimada, 1987; Shimada *et al.*, 1974; Wieczorek and Köppl, 1982). Also, the water receptor of *Protophormia terraenovae* is stimulated by some sugars (Wieczorek and Köppl, 1978). Behavioral mutants of *Drosophila melanogaster* with altered taste perceptions to several sugars have been described (Falk and Atidia, 1975; Isono and Kikuchi, 1974; Tanimura *et al.*, 1982), but these differ only in degree of threshold phagostimulation: none of these sugars were deterrent or inhibited feeding.

Many sugars stimulate feeding in cockroaches (Tsuji, 1965), and sensilla on the maxillary palps of *Periplaneta americana* contain sugar-binding sites (Becker and Peters, 1989). Consequently, sugars are frequently incorporated into insecticide-dosed diets to enhance toxin intake by cockroaches.

While investigating the efficacy of a fructose–glucose corn syrup diet dosed with the insecticide hydramethylnon against the German cockroach, *Blatella germanica*, we identified two field locations (T-164 and H-905) where the performance of this diet declined over time. Initially, in 1983, T-164 and H-905 cockroach populations were reduced by over 90% with this diet. However, by 1988, population increases exceeding 39% were recorded with this same diet. Furthermore, we observed that the diet was rejected by the field cockroaches, yet it was consumed by a laboratory strain with no history of exposure to a toxicant–sugar mixture.

It is generally recognized that insects and other animals will avoid consuming a nutrient if they detect toxic substances within the nutrient matrix (Garcia and Brett, 1977; Hartmann, 1991; Feeny, 1992). We report here a novel behavioral adaptation, nutrient aversion, which serves to protect an insect from ingesting a toxicant. Furthermore, the aversion described is to D-glucose, a molecule recognized as the universal metabolic fuel and one which is ubiquitous within the habitat of the German cockroach.

**MATERIALS AND METHODS**

**Experimental insects**

Two strains of *B. germanica* were used throughout these studies. The Orlando normal was maintained in the laboratory for $>40$ yr without insecticide exposure. The T-164 strain was collected in 1989 from an apartment in Gainesville, Fla. This location had been treated for c. 5 yr with a toxic diet containing the electron transport inhibitor, hydramethylnon. Laboratory T-164 insects were reared in the presence of toxic diet and nontoxic food (dog chow, Purina®) to maintain the aversive trait. Prior to experiments, the T-164 insects were moved to a clean container apart from the toxic diet for at least one generation.

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Chemicals
Technical grade hydramethylnon (97%) was obtained from the Clorox Co. (Pleasanton, Calif.), oleic acid was supplied by Henkel Co. (Los Angeles, Calif.), corn syrup, CornSweet 55® was obtained from Archer Daniels Co. (Cedar Rapids, Iowa), all crystalline sugars were obtained from Sigma Chemical Co. (St Louis, Mo.), and agar (Bacto) was supplied by Difco (Detroit, Mich.).

Identification of aversive component
Diet pairs were set 3 cm apart in positions within the T-164 apartment and in another apartment (H-905) where the hydramethylnon diets also were previously rejected. In each of the three trials each member of the diet pair lacked one of these three components: hydramethylnon, oleic acid (a co-solvent for hydramethylnon) or corn syrup (CornSweet 55; 55% fructose, 42% glucose, 3% higher saccharides). Diet consumption was measured gravimetrically, correcting for water loss.

Feeding on corn syrup and its major components, D-fructose and D-glucose, was determined in the laboratory. Separate populations of 1000–2000 Orlando normal and 1-164 insects were maintained in replicate (n = 4) containers (20 cm dia, 20 cm high) with harborage, food (Purina® dog chow) and water. The populations were of mixed age and sex. Both the food and water were removed 48 h prior to feeding trials to facilitate a rapid response to the diets and to minimize diet weight loss due to evaporation. Each container received two preweighed dishes (34 cm³), one with a given sugar mixed with 1% agar and one containing plain 1% agar. The sugar diets were prepared by bringing 1% agar to the boil, allowing it to cool to c. 60°C and then adding either 40% w/w corn syrup (the same concentration used in the toxic diet), D-glucose, D-fructose, or mixtures of fructose and glucose. The concentration of the fructose–glucose mixtures equalled 1 M. The dishes were reweighed after a 2 h feeding period, and a water loss correction was factored into the diet consumption calculations. An excess of diet remained following the feeding period. A feeding index was calculated to distinguish stimulation, non-detection, and aversion:

Feeding index = sugar–agar diet consumed (mg) - agar-only diet consumed (mg)

sugar–agar diet consumed (mg) + agar-only diet consumed (mg)

Positive, zero, and negative feeding indices reflect stimulatory, neutral (no detection), and deterrent responses, respectively, to each sugar diet. A feeding index of 1 = 100% consumption of the sugar-containing agar, whereas an index of −1 = 100% consumption of plain agar.

A second set of experiments was designed to detect strain-dependent responses to hexoses similar to glucose, and disaccharides containing a glucose molecule. Adult male B. germanica were inverted and dorsally restrained on narrow (1.5 cm) glue strips. Then, droplets (1 μl) of 1 M sugar solutions were placed on the mouthparts. On each of the 2 days, four of eight sugars plus water were tested (n = 100). Therefore, each insect received a random sequence of five solutions, with 1 h between applications. A positive response was recorded if the droplet was imbibed within 5 s. Solutions and strains were tested blind.

Inheritance of glucose aversion
The mode of glucose aversion inheritance was determined. Orlando normal (glu + /glu + ) × 1-164 (glu/glu) reciprocal crosses were performed. The F₁ offspring of one of these crosses (glu/glu+) was mass backcrossed to the glu+/glu+ parent. Male cockroaches were collected 7–10 days after adults emerged from the various crosses. They were deprived of food and water for 48 h, then offered a glucose solution prepared with 8 mM amaranth (Treherne, 1957). A choice assay performed earlier indicated that amaranth was not detected at this concentration. The dye solution was left in place for 5 min, after which water was also provided for an additional 15 min. Individual insects were placed in 1.5 ml microcentrifuge tubes and triturated in 0.5 ml ethanol:water (1:1) with a Teflon pestle, then centrifuged (12,000 rpm). An equal volume of cold (0°C) acetone was added to the supernatant and centrifuged (12,000 rpm) to precipitate proteins. The supernatant was measured at 520 nm in a spectrophotometer (DU-7, Beckman Instruments). Dye (glucose) intake was determined by comparing the A520 readings from each insect against a calibration curve established by feeding known volumes (1–16 μl) of 8 mM amaranth to individual insects. For F₁ reciprocal cross offspring, 15 insects were assayed per concentration per genotype. Based on results of the reciprocal cross study, a concentration of 2 M glucose was selected for the backcross experiment because it provided the greatest F₁ (glu/glu+) vs parent (glu+/glu+) discrimination and least variation. Fifty-five males of each backcross parental genotype, and 201 backcross offspring were assayed for glucose intake.

Toxicant/diet studies
We determined the effect of substituting fructose for glucose in a 2% hydramethylnon-based diet on the mortality of T-164 insects. Eighty male T-164 and Orlando normal B. germanica were fed hydramethylnon diets with either 27.2% w/w of fructose, or diets with 15.4% fructose plus 11.8% glucose. Diets, water and alternate food (dog chow) were provided ad lib LT-50's (± 95% C.I.) were compared for each diet and strain.
TABLE 1. Feeding responses of *B. germanica* in T-164 and H-905 apartments to paired diets containing or lacking three components

<table>
<thead>
<tr>
<th>Diet component</th>
<th>Diet consumed (mg/day)</th>
<th>n</th>
<th>Present</th>
<th>Absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydramethylnon (2%)</td>
<td>29</td>
<td>15.8 ± 1.3</td>
<td>17.4 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>Oleic acid (2%)</td>
<td>13</td>
<td>10.0 ± 1.3</td>
<td>8.4 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>Corn syrup (40%)</td>
<td>17</td>
<td>14.8 ± 1.0*</td>
<td>70.7 ± 14.2</td>
<td></td>
</tr>
</tbody>
</table>

*Row mean diet consumption different at P < 0.05, Wilcoxon signed-ranks test.

RESULTS

Identification of aversive diet component

In the field, diets lacking corn syrup had nearly 5-fold greater consumption (P < 0.05, Wilcoxon signed ranks test) than diets with this material. Removal of the toxicant or co-solvent from the diet did not affect feeding (Table 1). In laboratory studies, corn syrup presented in an agar matrix stimulated feeding in the Orlando normal insects but deterred feeding in the T-164 field strain (P < 0.0001, t-test) (Fig. 2). All stages of T-164 *B. germanica* were observed walking on the corn syrup-agar surface, but they left this surface within 3 s. Feeding by T-164 cockroaches on nutritionally inert plain 1% agar was sustained for up to 2 mins. Presumably, these agar-feeding insects were responding to water since they had been deprived of both food and water prior to testing. In another trial that tested the two major components of corn syrup, 1 M D-fructose stimulated feeding in the two strains (P > 0.05, t-test) (Fig. 2). D-glucose, however, stimulated feeding in the Orlando normal strain, but deterred feeding in the T-164 insects (P < 0.0001, t-test) (Figs 1 and 2). 95% of the total feeding (glucose-agar + agar) by the T-164 cockroaches occurred on unsupplemented agar. Cockroaches collected from the H-905 apartment were also deterred by glucose (Feeding index = -0.08).

Decreasing the concentration of glucose from 1 M to 1 mM reduced consumption in the Orlando normal strain but increased it in the T-164 strain; apparently, neither strain detected 1 mM glucose (Fig. 3). Although fructose stimulated feeding in both strains, its level in the above corn syrup (55%) did not overcome the deterrent effect of glucose. A fructose:glucose molar ratio of at least 9:1 was necessary to overcome glucose aversion in the T-164 strain (feeding index > 0) (Fig. 4). T-164 feeding indices were significantly different at all molar ratios (P < 0.05; ANOVA; Fishers LSD). All ratios stimulated feeding by Orlando normal cockroaches approximately the same (P > 0.05; ANOVA).

The sugars eliciting the most positive responses from individuals of both cockroach strains were fructose, maltose, and sucrose (Fig. 5). These 1 M sugar solutions were imbibed immediately (within 2 s) when placed on the mouthparts, despite the test insects being food and water satiated. Both strains imbibed D-mannose (C-2 epimer of glucose) as frequently as water, but they responded to D-galactose (C-4 epimer) less than water (P < 0.05, t-test).

The number of Orlando normal positive respond to the α and β anomers of glucose was similar. Less than 20% of the T-164 males accepted either anomer of D-glucose, significantly fewer than those responding to water (P < 0.001, t-test). Significantly fewer (< 10%) Orlando normal and T-164 *B. germanica* imbibed nutritionally inert L-glucose than water (P < 0.001, t-test).

Inheritance of glucose aversion

Individual male Orlando normal (*δ*glu+/*δ*glu+) and T-164 (*δ*glu/*δ*glu) strains (P1) and reciprocal
FIGURE 2. Consumption of corn syrup and its major components by T-164 and Orlando normal strains of *B. germanica*. Cockroaches were each offered sugar prepared in 1% agar and unsupplemented 1% agar.

Feeding index (± SE) = \( \frac{\text{sugar-agar diet (mg)} - \text{agar-only diet (mg)}}{\text{sugar-agar diet + agar-only diet (mg)}} \).

+, 0, and − feeding indices reflect stimulatory, neutral (no detection), and deterrent responses, respectively, to each sugar diet. A feeding index of 1 = 100% consumption of the sugar-containing agar, whereas an index of −1 = 100% consumption of plain agar. *Indicates a significant difference in feeding index between the strains (P < 0.0001, t-test).

FIGURE 3. Glucose concentration dependent feeding indices for T-164 and Orlando normal strains. Interpretation as in Fig. 2.
GLUCOSE AVERSION IN THE GERMAN COCKROACH, B. GERMANICA

FIGURE 4. Effect of fructose-glucose mixtures of various ratios on T-164 B. germanica feeding indices. Interpretation as in Fig. 2. All bars are significantly different from each other (P < 0.05, ANOVA, Fisher’s LSD).

DISCUSSION

This is the first report of aversion to a typically phagostimulatory sugar by an insect. The rejection of glucose-agar mixtures by T-164 cockroaches in favor of nutritionally inert plain agar (95% of total feeding), despite prior food deprivation, illustrates the intensity of the aversion. Mutant strains of Drosophila melanogaster (Falk and Atitia, 1975; Isono and Kikuchi, 1974; Rodrigues and Siddiqi, 1981) and mice (Lush, 1991) were identified which fed less and/or displayed a loss of electrical response in taste receptors to several sugars, including glucose, but glucose aversion in B. germanica is clearly distinct from prior descriptions of attenuated responses to sugars.

Food aversion learning was demonstrated in mice (Etscorn, 1980), terrestrial mollusks (Gelpitin and Forsythe, 1975) and in grasshoppers fed substandard diets (Bernays and Lee, 1988) following injection with a malaise-inducing compound. We ruled out responses due to associative learning because we used insects which were not exposed to the toxicant-diet mixture anytime prior to the experiments. We determined that glucose aversion in B. germanica, like decreased sensitivity to sugars in D. melanogaster (Taniruma et al., 1982; Rodrigues and Siddiqi, 1981), was the result of a chemosensory mutation. It appears that the mutation may be in a single major gene.

Some general mechanisms for glucose aversion were considered. First, if reduced sensitivity to glucose were observed then one might expect a mutation in a glucose receptor site resulting in altered binding capacity.
However, it is unlikely that altered binding of glucose to its receptor site would cause an aversive reaction. Receptors for glucose-bound deterrent allelochemicals such as glucosinolates and glycosides have been identified in phytophagous insects (Schoonhoven, 1987). Therefore, the mutation may be in a receptor for a deterrent cell (or pathway) which has been altered to bind D-glucose. Finally, rather than altered binding at the peripheral level, the mutation may be elsewhere in the chemosensory pathway, with coding for aversive rather than phagostimulatory behavior.

The percentage of positive responds in both strains to D-glucose and its epimers differed: the glucose response was highest in Orlando normal and lowest in T-164. D-galactose received fewer positive responses than water, supporting findings by Tsuji (1965). Furthermore, Jakinovich et al. (1981) determined the response ranking of Periplaneta americana to several methyl glycosides as glucose > mannose > galactose, with the α anomer being generally more effective than the β anomer. Thus, there would appear to be chiral center recognition of monosaccharides in both cockroach species at the sensory and/or CNS level.

Both B. germanica strains rejected L-glucose. L-Glucose is nutritionally inert (Casati, 1974), although, at least in humans, it is indistinguishable in sweetness from D-glucose (Shallenberger et al., 1969). Electrophysiological responses of labellar sugar and water receptors to L-glucose in two fly species were interpreted as stimulatory (Jakinovich et al., 1971). However, both aversive and stimulatory responses could reveal similar impulse patterns at the peripheral receptor level though the CNS may interpret them differently. Perhaps D-glucose aversion in B. germanica is a consequence of a mutant chemosensory pathway that recognizes D-glucose as its enantiomer, L-glucose.

Fructose was phagostimulatory to both Orlando normal and T-164 cockroaches. However, a ratio of at least 9:1 fructose:glucose was necessary to overcome the deterrent effect of glucose to T-164 insects. At this ratio, 100 mM glucose was present in the mixed diet. 100 mM glucose alone deterred feeding (Fig. 3). Based on
FIGURE 6. Ingestion of glucose by parental and reciprocal cross (F₁) male offspring. Individuals previously deprived of food and water for 48 h were offered 8 mM amaranth dye with various glucose concentrations. The amount imbibed per insect was measured at A520. All genotypes imbibed equivalent amounts of amaranth without glucose. As glucose concentration increased, intake by glu/glu (T-164), glu+/glu, and glu/glu+ decreased.

herbivore studies, sensory coding of stimulatory and inhibitory data is centrally processed based on simple arithmetic rules with the CNS evaluating impulses from different receptors differently (Schoonhoven, 1987). If T-164 B. germanica uses a similar mechanism, then feeding decisions could be based on data weighted more heavily from inhibitory glucose pathways than from stimulatory fructose pathways. In caterpillars, impulses from deterrent cells appear to have informational value which is c. 2.5 times higher than impulses from stimulatory cells (Schoonhoven, 1987).

Cockroaches pre-adapted to reject glucose survived in the presence of toxic diets containing glucose (corn syrup). Initially, in 1983, T-164 populations were reduced by over 90% using glucose-containing toxic diets. By 1988, population increases of 39% were recorded with these same diets. Removal of glucose from these diets again resulted in high population reductions (85%) (unpubl. data). In laboratory studies reported here, substitution of fructose for glucose in the toxic
Glucose Ingested (µL)

FIGURE 7(b)

FIGURE 7. Frequency distributions of amounts of 2 M glucose imbibed by male B. germanica. (a) Open bars indicate quantities imbibed by individual glu+/glu+ (Orlando normal). Solid bars for glu/glu+ (F1). (b) Consumption by offspring of glu+/glu+ * glu/glu+ backcross. Bimodal and nearly identical distributions of known genotypes in (a) and unknown backcross offspring (b) indicate that a single gene may code for this trait.

diets reduced the LT-50 nearly 5-fold for T-164, a level similar to that of Orlando normal. The role of behavior—in insecticide resistance was reviewed recently by Sparks et al. (1989). They challenged the interpretation of many resistance studies by offering behavioral explanations for responses to toxicants attributed to physiological and biochemical mechanisms. Ross (1992) identified an indirect insecticide avoidance behavior in German cockroach strains which were deterred by the carriers and diluents for pyrethroids. Our studies provide the first evidence of nutrient avoidance as a behavioral resistance mechanism.

Glucose aversion has ecological consequences for the German cockroach. This behavior diminishes the variety of food available. Foraging time would increase, thereby increasing both energy expenditure and risks due to exposure. However, an omnivore such as B. germanica, can probably compensate for the absence of this nutrient. It has adapted to nitrogen-poor diets by restricting uric acid excretion and by male-to-female transfer of stored urates during spermatophore deposition (Mullins and Cochran, 1987; Mullins and Keil, 1980). This cockroach has evolved an association with humans, and is now nearly completely confined within human dwellings and dependent on human foods. Although glucose is widely distributed within the cockroach’s habitat (most sweetened products), there are many foods without it. Furthermore, the consequences of ingesting diets lacking glucose are probably minimal; other carbohydrates and lipids could satisfy the insect’s energy requirements. Thus, glucose aversion would not rapidly disappear naturally and would thrive in the presence of glucose-containing toxic diets.

REFERENCES


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