rally similar to the cuticular lipids[22,23]. Cuticular hydrocarbons are synthesized by tissue associated with the cuticle[13,24-26]. Therefore, it is likely that the biosynthesis of the hydrocarbon sex pheromone components by abdominal epidermal tissue may be common in Dipteran species.

1 Acknowledgment. This work was supported by the Science and Education Administration of the U.S. Department of Agriculture under grant No.7801064 from the Competitive Research Grants Office. Contribution of the Nevada Agricultural Experiment Station, Journal Series No 524. Housefly pupae were kindly supplied by Dr Ted Shapas, S.C. Johnson and Son, Inc., Racine, Wisconsin.

2 Author for correspondence.
3 W.M. Rogoff, A.D. Beltz, J.O. Johnsen and F.W. Plapp, J. Insect Physiol. *10*, 239 (1964).
4 D.A. Carlson, M.S. Mayer, D.L. Silhacek, J.D. James, M. Beroza and B.A. Bierl, Science *174*, 76 (1971).
5 D.R. Nelson, J.W. Dillwith and G.J. Blomquist, Insect Biochem. *11*, 187 (1981).
6 E.C. Uebel, P.E. Sonnet and R.W. Miller, J. econ. Ent. *5*, 905 (1976).
7 W.M. Rogoff, G.H. Gretz, P.F. Sonnet and M. Schwarz, Envir. Ent. *9*, 605 (1980).
8 E.C. Uebel, M. Schwarz, W.R. Lusby, R.W. Miller and P.E. Sonnet, Lloydia *41*, 63 (1978).
9 I. Richter, H. Krain and H.K. Mangold, Experientia *32*, 186 (1976).
10 D.A. Carlson and M. Beroza, Envir. Ent. *2*, 555 (1973).
11 F.A. Carroll, D.W. Boldridge, J.T. Lee, R.R. Martin, M.J. Turner and T.L. Venable, J. Agric. Fd Chem. *28*, 343 (1980).
12 I. Richter, Naturwissenschaften *61*, 365 (1974).
13 J.W. Dillwith, G.J. Blomquist and D.R. Nelson, Insect Biochem. *11*, 247 (1981).
14 Y. Schlein, R. Galun and M.N. Ben-Eliahu, Experientia *36*, 1174 (1980).
15 T.T. Blailock, G.J. Blomquist and L.L. Jackson, Biochem. biophys. Res. Commun. *68*, 841 (1976).
16 E.G. Bligh and W.J. Dyer, Can. J. Biochem. Physiol. *37*, 911 (1959).
17 D.A. Carlson, P.A. Langley and P. Huyton, Science *201*, 750 (1978).
18 E.C. Uebel, M. Schwarz, R.W. Miller and R.E. Menzer, J. Chem. Ecol. *4*, 83 (1978).
19 E.C. Uebel, P.E. Sonnet, B.A. Bierl and R.W. Miller, J. Chem. Ecol. *1*, 377 (1975).
20 E.C. Uebel, P.E. Sonnet, R.W. Menzer, R.W. Miller and W.R. Lusby, J. Chem. Ecol. *3*, 269 (1971).
21 E.C. Uebel, P.E. Sonnet and R.W. Miller, J. econ. Ent. *5*, 905 (1976).
22 G.J. Blomquist and L.L. Jackson, Prog. Lipid Res. *17*, 319 (1979).
23 D.R. Nelson, Adv. Insect Physiol. *13*, 1 (1978).
24 M.T. Armold and F.E. Regnier, J. Insect Physiol. *21*, 1581 (1975).
25 G.J. Blomquist and G.J. Kearney, Archs Biochem. Biophys. *173*, 546 (1976).
26 D.R. Nelson, Nature, Lond. *222*, 854 (1969).

Sex recognition pheromone in the tsetse fly *Glossina pallidipes* Austen[1]

P.A. Langley, T.W. Coates and D.A. Carlson[2]

Tsetse Research Laboratory, University of Bristol, School of Veterinary Science, Langford, Bristol, BS18 7DU (England),
8 April 1981

Summary. Sexual responses of adult male *G. pallidipes* towards baited decoys show that a contact sex pheromone for this species is present in the hydrocarbon fraction of the adult female cuticle. Results are consistent with the view that the pheromone is a C_{35} compound and is present in sufficient quantity in newly emerged females to elicit maximum responses from males. Thus, maturation of sexual responsiveness is considered to be behavioural in females of this species.

Glossina pallidipes Austen is an important vector of trypanosomiasis and in the dry savannah regions of central southern Africa shares its habitat with, and will feed on the same hosts as, *G. morsitans morsitans* Westwood[3]. Both *G. m. morsitans* and *G. pallidipes* are attracted to stationary models in proportions which enable the sex and species present in an area to be estimated[3]. The numbers of flies attracted visually to such models are greatly enhanced by the provision of an olfactory stimulus such as ox odour[3] or a mixture of CO_2 and acetone[4].

It is generally accepted that locally high concentrations of tsetse around host animals provide the situation for mating to occur[5], while the attraction of non-hungry *G. pallidipes* females to hosts and a variety of baits resembling hosts has been interpreted as indicating a mate-seeking response by females of this species[3,6]. Nevertheless, the presence of specific chemicals in the cuticles of female *G. m. morsitans* which elicit copulatory behaviour by males on contact[7] suggests that correct species identification is achieved by the male. In *G. m. morsitans* the contact sex pheromone or aphrodisiac is a single hydrocarbon component which has been identified, characterized and synthesized[8]. However, it is recognised that the chemical signal will only be effective when presented to the male on an object which possesses certain physical attributes of a female fly, namely its shape and size[9]. Evidence has also been obtained that no 2 species of *Glossina* are likely to share a sex pheromone structure in common[10], and this is important for species which share the same environment and hosts.

In the laboratory, *G. pallidipes* do not mate as readily as *G. m. morsitans* and only recently have techniques been developed to overcome this problem[6,11]. Female *G. pallidipes* are most receptive at the time of ovulation of the 1st mature oocyte, when they are 9 days old at a maintenance

Table 1. Sexual responses, scored subjectively as 0, 1, 2, 3 of ⩾ 9-day-old male *G. pallidipes* to decoys consisting of killed male *G. morsitans* and *G. pallidipes* or killed female *G. pallidipes* of different ages

Decoy Species	Sex	Age (days)	N	Test male responses (No.) 0	1	2	3
G. m. morsitans	♂	7	24	24			
G. pallidipes	♂	7-8	10	10			
G. pallidipes	♀	0	21	1	1	1	18
G. pallidipes	♀	2-4	15			4	11
G. pallidipes	♀	8-10	35				35
G. pallidipes	♀	52	10			1	9

N = number of males tested. Between 3 and 5 males used per decoy in individual tests.

Table 2. Sexual responses, scored subjectively as 0, 1, 2, 3 of ⩾ 9-day-old male *G. pallidipes* to dead male decoys baited with whole extracts or various components of female *G. pallidipes* surface cuticular waxes

Fraction	Female age (days)	Dose (♀ equivalent decoy^{-1})	N	Test male responses (No.) 0	1	2	3
Virgin laboratory ♀♀							
Whole cuticular extract	7–9	10	5		3	2	
		1	10	1	7	2	
1. Total hydrocarbons		1	10	2	4	2	2
Cholesterol esters	>9	1	10	10			
Methyl esters		1	10	10			
Triglycerides and free fatty acids		1	10	10			
Wild caught ♀♀							
Total hydrocarbons	?	5	20	3	4	10	3
2. Total hydrocarbons		4	6		1	3	2
Cholesterol esters	?	4	6	6			
Triglycerides		4	6	6			
Tri-, diglycerides and free fatty acids		4	6	6			

1. and 2. are sets of cuticular components, each obtained from the fractionation of a single cuticular extract. N = number of males tested. Between 1 and 10 males used per decoy in individual tests.

temperature of 25 °C and it has been suggested that changes in the hydrocarbon composition of the cuticles of females during the 1st 9 days of adult life indicate a delay in the appearance of the sex pheromone in this species[10].

Results have now been obtained which contradict this view and show that the sex pheromone of *G. pallidipes* is present in very young females in sufficient quantity to elicit full copulatory responses from males.

Adult *G. pallidipes* emerging in the laboratory from puparia collected in the Zambezi Valley near Makuti, Zimbabwe were used in our experiments, as well as the offspring of these flies maintained in the laboratory. Males and females were maintained separately at 25 °C and 80% relative humidity in groups of 15 or 20 flies, in gauze-covered circular cages made of PVC, and fed on pig blood[12]. Males were at least 9 days old when used in mating tests. Decoy objects were female *G. pallidipes* of different ages or 7–8-day-old males of either *G. m. morsitans* or *G. pallidipes* freshly killed by freezing. Initially the male decoys were washed with 1 ml fly^{-1} diethyl ether to remove surface cuticular hydrocarbons before dosing with candidate pheromone extracts. However, this was later found to be unnecessary (see table 1) where quantitation of materials was not required. All tests were conducted in a uniformly illuminated cabinet at 25 °C[9]. A single test male was brought into physical contact with a decoy and its response scored subjectively as 0, 1, 2, 3 according to the vigour of its copulatory attempts on the decoy[8,9]. Killed male decoys were baited with 1 µl hexane containing 5 female equivalents of dried ether extracts of cuticles of females, or baited with 1 µl hexane containing various fractions of such cuticles separated by liquid or preparative gas chromatography[8].

Table 1 shows that male *G. pallidipes* responded to dead females of any age but did not respond to dead male *G. pallidipes* or *G. m. morsitans*. Responses to components of the surface waxes of the female were therefore evaluated using dead males of either species as decoys dosed with the appropriate fractions. Results (table 2) demonstrate that the sex pheromone is a hydrocarbon. Separated cuticular hydrocarbons are described by their appropriate Kovats Indices (3700 and 3800 refer to unbranched paraffin standards having 37 or 38 carbons respectively: the 2nd pair of numbers in each index refers to internal methyl branching)[13]. Bioassay of these compounds showed that optimum pheromone activity was located in the KI3550 fraction

Table 3. Sexual responses scored subjectively as 0, 1, 2, 3 of ⩾ 9-day-old male *G. pallidipes* to dead male decoys baited with 5–10 female equivalents of cuticular hydrocarbon fractions

Kovats[13] index	N	Test male responses (No.) 0	1	2	3
2865	5	5			
2965	5	5			
3065	25	25			
3165	16	16			
3265	16	16			
3365	16	16			
3465	16	15		1	
3550	25	5	7	11	2
3650	20	17		3	
3750	22	17	3	2	

Fractions obtained from 2-day-old female *G. pallidipes* surface cuticular waxes. N = number of males tested. Between 2 and 6 males used per fraction in individual tests.

(table 3). Some activity was recorded in KI3465 and KI3650 fractions but this is thought to be due to incomplete separation and thus contamination of adjacent fractions with KI3550. The few low level responses of *G. pallidipes* males to KI3750 were probably due to its structure: it is probably a homologue of the majority compound at KI3550, with 2 more methylene groups. Our evidence suggests that the sex pheromone of *G. pallidipes* is a C_{35} hydrocarbon, and that the compound with most activity will prove to be a straight-chain, methyl branched alkane, similar in structure to that of *G. m. morsitans*. However, formal evidence for its identity has not yet been obtained. The cross-reactivity of *G. m. morsitans* pheromone[10] reflects the close phylogenetic relationships of the 2 species, and emphasizes the importance of behavioural mechanisms in preventing inter-specific matings in the field where the 2 species occur together. The copulatory responses of male *G. pallidipes* are often not as vigorous as those of *G. m. morsitans* even towards live females of their own species in the laboratory, and this is reflected in the depressed responses of *G. pallidipes* males to baited decoys in the laboratory when measured on our subjective 3 point scale. This is thought to indicate genuine behavioural differences between the 2 species, suggesting that the optimum conditions for mating have not been defined for *G. pallidipes* in our

laboratory bioassay. Results (table 1) suggest that female maturation[6] involves behavioural changes (increased receptivity) independent of any changes in the amount of sex pheromone, which is present in sufficient quantity on the day of emergence to elicit full copulatory responses from mature males.

1 We thank Dr A.M. Jordan for his helpful comments on the manuscript. The financial support of the Overseas Development Administration of the U.K. Government (Research Scheme R3450) and of the U.S. Department of Agriculture is gratefully acknowledged.

2 Address: U.S.D.A., S.E.A., A.R., Insects Affecting Man and Animals Research Laboratory, Gainesville (Florida 32604, USA).

3 G.A. Vale, Bull. ent. Res. *64*, 545 (1974).
4 G.A. Vale, Bull, ent. Res. *70*, 563 (1980).
5 J.P. Glasgow, The Distribution and Abundance of Tsetse. Pergamon Press, Oxford 1963.
6 T.G.T. Jaenson, Bull, ent. Res. *70*, 49 (1980).
7 P.A. Langley, R.W. Pimley and D.A. Carlson, Nature *254*, 51 (1975).
8 D.A. Carlson, P.A. Langley and P. Huyton, Science *201*, 750 (1978).
9 P.M. Huyton, P.A. Langley, D.A. Carlson and T.W. Coates, Physiol. Ent. *5*, 243 (1980).
10 P.M. Huyton, P.A. Langley, D.A. Carlson and M. Schwarz, Physiol. Ent. *5*, 253 (1980).
11 M.E. Leegwater van der Linden, Entomologia exp. appl. *29*, 169 (1981).
12 A.R. Mews, P.A. Langley, R.W. Pimley and M.E.T. Flood, Bull. ent. Res. *67*, 119 (1977).
13 E. Kovats, Adv. Chromatogr. *1*, 229 (1966).

Effects of D-glucose anomers on afferent discharge in the hepatic vagus nerve

T. Sakaguchi and M. Iwanaga

Departments of Physiology and Internal Medicine, Niigata University School of Medicine, Niigata City (Japan), 2 June 1981

Summary. Intraportal injections of α-D-glucose, optically equilibrated D-glucose and β-D-glucose reduced afferent discharges in the hepatic vagus nerve of anaesthesized rats. β-D-Glucose was most potent in decreasing the discharge.

Behavioral studies have presented good evidence for the existence of a neural glucosensitive mechanism in the hepatic portal vein[1]. Electrophysiologically, it has been shown that D-glucose injected into the portal vein affects afferent discharges taken from the hepatic vagus nerve in the isolated liver-vagus preparation[2]. D-glucose in the blood is known to exist as an optically equilibrated mixture of its 2 anomers; 36% α-D-glucose and 64% β-D-glucose[3], but no report has yet been published concerning the effect of D-glucose anomers on afferent discharges in the hepatic vagus nerve. Recently, it was observed that the vagus discharge was differentially changed by each of the glucose anomers injected into the portal vein in the rat.

Material and methods. 50 male rats weighing about 300 g were used. They were fasted for 22 h before each experiment, although allowed free access to water. The experiments were performed in the morning and animals, anaesthetized with pentobarbital sodium (45 mg/kg, i.p.), were adrenalectomized bilaterally 30 min before the beginning of nerve discharge recording to reduce variation in plasma levels of glucose in the blood[4]. Rectal temperature was maintained at about 36 °C by a heating lamp. Using elastic electrodes[5], nerve discharge was recorded from the distal cut end of the hepatic vagus nerve and expressed by the method described in the previous papers[2,6]. 5% glucose and 0.9% saline solutions were injected through a catheter inserted in the portal vein. The α or β form of crystallized D-glucose was dissolved in distilled water and kept at 36 °C immediately before use in order to eliminate mutarotation of the glucose. The purity of the 2 anomers was more than 98% and the rate of mutarotation of the 2 anomers in the water was negligible. Equilibrated D-glucose solutions consisting of 36% α-D-glucose and 64% β-D-glucose were obtained by keeping either α- or β-D-glucose solution for 22 h at room temperature (22 °C). The amount used for a portal injection was 0.2 ml and an injection was done for 15 sec via an infusion pump. Data were collected from the 1st response of each animal to a certain drug and differences were evaluated by Student's t-test.

Results and discussion. In a recording, reliable decreases in the afferent discharge of the hepatic vagus nerve were observed after the portal injections of 5% α-D-glucose, 5%

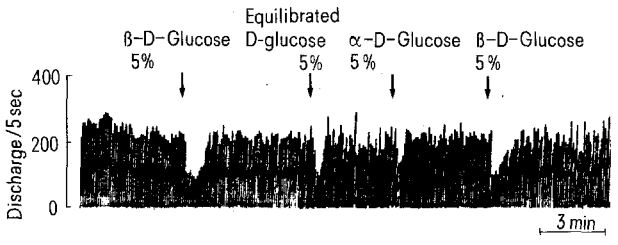

Figure 1. A recording showing the effects of D-glucose and its optical anomers on the afferent discharge of the hepatic vagus nerve. Arrows show the time of portal injection.

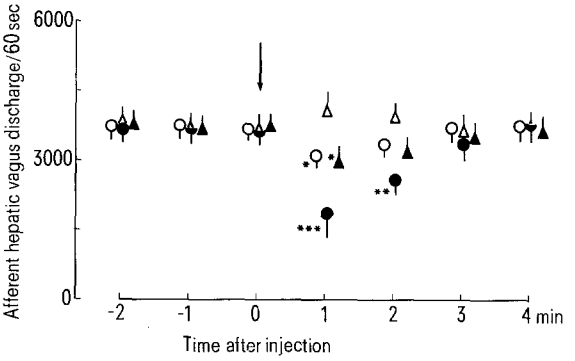

Figure 2. The effect of 5% α-D-glucose (○, n = 14); 5% equilibrated D-glucose (▲, n = 14); 5% β-D-glucose (●, n = 12); and 0.9% saline (△, n = 10) on afferent discharges of the hepatic vagus nerve. An arrow indicates the time of portal injection. Values are means ± SEM. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$: significantly different from the value before injection.