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Feeding preference by the male blowfly *Phormia regina* for some natural foods and its relation to carbohydrate, amino acid, and alcohol content

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Abstract

The male blowfly, *Phormia regina* Meigen shows preference among a variety of natural and artificial foods. In a laboratory study preference was observed among sweet and fermenting juices as well as between liver and fish. The preferences between fresh and fermenting juices were based not only on component sugars, but also on other components, namely, amino acids, alcohol salts and presumably other chemical compounds. The preference between decaying liver and fish was probably based partly on the composition and concentration of amino acids in these foods. A variety of amino acids were found in foods and some of these amino acids were shown to be excitatory, some inhibitory and others neutral. Salts were also observed to be present in the juices; and in a laboratory study NaCl at lower concentrations acted synergestically with 0.1 M sucrose and at higher concentrations repulsive. It is suggested that feeding preference is based on several chemicals which behave either in an excitatory, additive, neutral, inhibitory or synergistic manner. The ratio of excitants to inhibitants is important in deciding the direction of preference.

Introduction

The adult blowfly in the laboratory can exist on a diet of carbohydrate and water (Dethier, 1969), but normally it feeds on a chemically diverse and complex variety of foods. In the wild adult blowflies feed on various nectars, fruits and honey dews from which they obtain the necessary carbohydrate (Webber, 1958). Presumably they obtain proteins from decaying meat and other decaying materials. Proteins are required for oocyte development, and proteins ingestion varies cyclically with ovarian events (Strangways-Dixon, 1961; Belzer, 1970).

The chemical diversity of natural foods is very broad. Some investigation of nectars (Finch, 1974) has revealed that carbohydrates vary in composition from plant to plant of a given species. The composition of carbohydrates in individual fruits of different species appear to be different quantitatively and qualitatively. The preferences of blowflies for different carbohydrate and protein source in nature have not been studied, and little is known about sensory responses. The only work available on sensory responses to natural foods is that of Dethier (1974) in which electrophysiological responses of labellar chemoreception to a few natural foods were recorded.

The purpose of the present study is to determine quantitatively some feeding preferences of male *Phormia regina* for selected natural foods and to relate the results to receptor physiology.

Material and methods

The flies used in this study were from a stock culture maintained on artificial diet (Hill *et al.*, 1947; Orr, 1964). Newly emerged adults were kept in an incubator at 26 °C and r. h. of about 85%. Three-to-4-day-old male flies were used. Prior to testing they had fed only on distilled water.

Preparation of food. Two types of foods were used: sweet foods and decaying or fermenting materials. The former were honey and juices of orange and apple; the latter were decaying liver, fish, and beer.

Orange juice was squeezed from 1000 g fresh orange, and 100 ml of distilled water added. Apple juice was prepared from 1000 g peeled, blended fresh apples. 200 ml of distilled water were added, the contents mixed, and filtered in a Buchner funnel. The filtrate was kept frozen until used. In the case of honey, 20 ml were diluted with 60 ml of hot distilled water mixed thoroughly and stored in a refrigerator at 4 °C. About 20 ml of each juice were kept frozen for later use in electrophysiological investigation. In later tests apple and orange juices were allowed to ferment for at least 5 days before use. A portion of each fermenting juice was kept frozen for later use in electrophysiological studies.

1000 g liver and fish were allowed to decay for about 5 days and blended; distilled water was added, filtered, and kept frozen.

Basically the method is similar to that of Belzer (1970); also see Dethier, (1976), but the volume of juice sucked by the fly was computed from the reading obtained from a graph paper, underneath the transparent capillary tubes (internal diameter 0.1 cm). Since the size of the capillary tube was known, the ingested volume could be calculated. Each fly was put in a tube of about 8 × 3 cm. One end was covered by a nylon mesh (see Belzer, 1970; Dethier, 1976, p. 43). A two-hole rubber cork to receive the two capillary tubes was inserted in the other end. Each set of capillary tubes was fastened to a wooden board with rubber bands. The board was tilted at an angle of about 5° from the bench. The angle depended on the type of liquid in the capillary tubes, because the surface tension of the various juices of liquids is different. In this set-up each fly had two choices. For each set of tubes there was a blank to control for evaporation. The loss due to evaporation was subtracted from the loss due to the volume sucked by the fly. The fluids were introduced in the capillary tubes by a syringe. Care was taken not to introduce bubbles. Recordings were taken twice a day and all tests were run in continuous light from a 15 w tube held 1 m above the capillary tubes. The fresh juices were changed once a day after the second reading. The decaying solutions, fermenting juices and beer were changed after every 6 h because they tended to form bubbles in the capillary tubes. Stale beer also appeared to change colour and taste if kept too long in the capillary tubes. The capillary tubes were therefore drained thoroughly, cleaned with a mixture of sulphuric acid and potassium dichromate, rinsed with tap water first, then distilled water and finally alcohol. They were allowed to dry before new solutions were introduced. Meanwhile the flies were kept in their respective tubes turned upside down. The flies were tested for five days unless death occurred.

0.1 M sucrose was used as standard in the comparisons of fresh juices and decaying foods because *P. regina* ingests larger quantities of 0.1 M sucrose than of any other concentration, though it is not necessarily the most stimulating concentration (see Dethier, 1968). Choice ratio was calculated as a percentage of the total fluid ingested in each day.

Food samples were prepared by adding 1 ml each of apple juice, orange juice and honey into 15 ml round bottomed flasks and freeze dried. Standard solutions were made by putting 10 mg of each of pure sucrose, fructose and glucose (the sugars which were found in orange, honey and apple juice) into a small vial and 1 ml Tri-sil added to each. Each of the vials was shaken for 30 s to dissolve the sugars. Sucrose was found to be difficult to dissolve and had to be warmed to about 75 °C. Better results were obtained when the vial was left to stand for about 6 h or overnight. The other two sugars dissolved easily.

For food samples, 1 ml Tri-sil was added to the freeze dried food samples and shaken for about 2 min. The samples were left to stand overnight so that the sugars dissolved.

Gas chromatography. I used a Perkin-Elmer 3200 gas chromatograph. The carrier gas was argon at a flow rate of 60 ml/min. The chart speed was 600 mm/h.

Column for the analysis of trimethlsily (TMS) ethers was 3% SE-54 on 80–100 mesh (Taschromatography P, 90 cm). The column was conditioned by heating to 250 °C for 24 h with the outlets disconnected from the detectors, and an argon flow rate of 80 ml/min. Separations were achieved as follows: 10 min isothermal holds at 160–165 °C followed by 3°/min increase to 295 °C final hold of about 15 min if necessary.

With each analytical batch, the same volume of

aliquots in all of the silylated extracts from the samples and from a set of standards were injected on the same day. Peak areas were calculated by fitting triangles in the peaks and calculating the area of each and summing the total. Due to anomerisation of the various sol sugars in solution, the subsequent chromatograms sometimes showed two or more peaks for each monosaccharide, but these summed to produce totals for each sugar. Since the sugar concentration in each of the standard solution was shown, it was easy by simple proportion to determine the amount of sugar and hence the concentration in the food samples.

Determination of free amino acids was done with a Model 120B Amino Acid Analyzer fitted with a calculating integrator. To deproteinizate the samples, 5 ml of each of certain food extracts (apple juice, decaying liver extract solution, decaying fish extract solution and honey solution) were pipetted into flasks. 50 ml of 1% picric-acid were added to each flask and shaken. The suspension was centrifuged for 10 min. The supernatant was poured off and passed through a Dowex 2 × 10 resin bed. The colourless filtrate was put in a 15 ml diam. bottomed flask and freeze dried. This material was dissolved in a 5 ml 0.2 N HCl and pH adjusted. The solution was transferred to a 5 ml volumetric flask and made to the volume mark using pH 2.2 buffer. Various volumes of aliquots usually in μl depending on the concentration of free amino acids, were pipetted on each of column 44 15PA35.

Alcohol concentration was determined by gas chromatography using a copper column in Perkin-Elmer gas chromatography machine. The temperature range was 30–200 °C and the programme rate was 2.5 ml/min. 5 μl ethyl alcohol of known concentration was used as a standard. The same volume of each of fermenting orange juice, apple and stale beer was injected into the machine with a hypodermic syringe. The percentage of alcohol in each sample was calculated using peak areas as already described. The concentration of alcohol was calculated by using the formula: % by weight or volume = (M) × (E)/10R, were M = mol concentration, E = molecular weight and R = density.

Effect of colour on feeding preference. Some preference tests were carried out in a dark room and others in light. Both tests were run concurrently. Red light was used when taking readings in the dark room. The readings in both situations were compared.

Salt/sugar and amino acid/sugar mixtures were prepared by weighing sucrose in g and put into a volumetric flask. Salt was also weighed in g and added to sugar in the flask. Distilled water was added to the flask about half way and the flask was shaken until the sugar and salt dissolved. Since sucrose dissolves slowly in cold water, the contents were therefore warmed to about 70–80 °C and allowed to cool completely. Cold water was then added to the flask up to the mark. Amino acid/sugar mixtures were also prepared in the same way. The different amino acids in each group were weighed in turn and added to sucrose in the flask. To determine the amount to be weighed, the following formula was used: A 1.0 molar solution of a substance is prepared by dissolving 1 molecular weight of substance in g in 1000 ml of water. Since the molecular weights are known, it is easy to calculate the weights required for each substance.

Observations and results

Preferences between juices. These varied from sample to sample. Generally they were most pronounced during the first day of testing, and the amount of fluid ingested was more on the first day (Table 1).

Table 1. Comparisons of preferences between juices. N = 5. Period for testing in days: 3, but at arrow (→)*: 2. Ap = apple; Or = orange; Ho = honey; sa = sample; ju = juice; fr = fresh; ferm = fermenting; sucr = sucrose.

Comparisons	Amount μl ingested	Choice Ratio
Ap sa I *vs* 0.1M sucr	133±4	63± 2 :37±0.6*
Ap sa II *vs* 0.1M sucr	565±6	65± 0.2:35±9*
Ap sa III *vs* 0.1M sucr	69±4	82± 3 :18±0.1*
Or sa I *vs* 0.1M sucr	233±5	86±12 :14±2*
Or sa II *vs* 0.1M sucr	175±5	60± 3 :40±3*
Ho *vs* 0.1M sucr	330±5	68± 7 :32±2*
Or ju *vs* ap ju	91±4	62± 5 :38±0.7*
Ho *vs* ap ju	71±4	90± 1 :10±0.1*
Or ju *vs* ho	101±5	61± 6 :39±0.7*
Fr ap *vs* ferm ap	161±6	88± 8 :11±1*
→ Fr or *vs* ferm or	56±2	95± 3 : 5±0.04*
Ferm app *vs* ferm or	131±4	51± 4 :49±0.3 n.s
Ferm ap *vs* beer	11±2	51± 0.2:49±0.2 n.s
Ferm or *vs* beer	127±5	57± 7 :43±5 n.s

* = significant $P < 0.01$.
n.s. = not significant $P > 0.05$.

Table 2. Comparisons for preferences between fermenting foods. N = 5. Periods for testing in days: 3, but (arrows): 2.

Comparisons	Amount µl ingested	Choice ratio
0.1M sucrose *vs* beer	562 ± 11	81 ± 10 :19 ± 1*
0.1M sucrose *vs* liver	463 ± 6	76 ± 7 :24 ± 4*
0.1M sucrose *vs* fish	177 ± 5	73 ± 4 :27 ± 3.6*
Apple juice *vs* liver	560 ± 9	68 ± 5 :32 ± 1*
Liver *vs* fish	496 ± 12	90 ± 12 :10 ± 0.03*
→ Liver *vs* water	55 ± 3	90 ± 0.4:10 ± 0.2*
→ Fish *vs* water	84 ± 6	80 ± 0.9:20 ± 0.2*
Liver *vs* beer	728 ± 11	62 ± 7 :38 ± 6*
Liver *vs* honey	660 ± 6	62 ± 5 :38 ± 4.5*

* = significant $P < 0.01$.

Preferences between fermenting foods. Generally, as in juices, more fluid was ingested during the first day (Table 2). In case of sucrose *vs* liver, the amount of liver ingested remained almost constant. In case of apple juice *vs* liver, the drop in the fluids ingested was the same in both cases.

Relation between preference and sugar and alcohol composition. With the exception of honey which lacks sucrose the analyses show that apple and orange juices are qualitatively the same in sugar composition, but quantitatively there are big differences (Table 3). The amounts of sugar in orange and apple vary considerably from sample to sample. Honey was constant, since a standard volume was used all the time from the same sample.

The preference tests between juices and sugar mixtures (Table 4) show that in all cases the juices were preferred to the corresponding sugar mixture. The colour of the juices may influence the preferences since the sugar mixtures are colourless. This was tested and the results showed that in the dark *ca.* 71 ± 1.7% of orange juice was taken against

Table 3. Sugar composition and variability of sugar concentration in two samples of juices used in some of the preference tests.

Sample	Juice	Type Sugar	Wt (mg/l ml)	Conc	Total conc. sugar
		Fructose	8.12	0.05M	
1	Apple	Glucose	2.94	0.02M	0.076M
		Sucrose	4.98	0.02M	
		Fructose	15.21	0.08M	
	Honey	Glucose	4.97	0.03M	0.112M
		Fructose	8.75	0.05M	
1	Orange	Glucose	8.07	0.05M	0.211M
		Sucrose	40.34	0.12M	
		Fructose	23.21	0.13M	
2	Apple	Glucose	4.98	0.03M	0.165M
		Sucrose	3.20	0.01M	
		Fructose	7.39	0.04M	
2	Orange	Glucose	6.27	0.04M	0.145M
		Sucrose	23.66	0.07M	

Table 4. Comparisons of juices with their own sugar components mixed at the same concentrations they occur in the juices. N = 5, Period for testing and abbreviations as Table 1.

Comparisons	Amount µl ingested	Choice Ratio
Ap ju *vs* apple-sugar mix	316 ± 9	85 ± 14:15 ± 7*
Or ju *vs* orange-sugar mix	160 ± 4	60 ± 4:40 ± 2*
Ap-sugar mixture *vs* ho-sugar mix	760 ± 13	53 ± 9:47 ± 7 n.s
Or-sugar mix *vs* ap sugar mix	692 ± 9	76 ± 5:24 ± 3*
Fr ap sugar mixture *vs* ferm ap-sugar mix	547 ± 9	66 ± 5:34 ± 3*
Ferm ap *vs* ferm ap sugar mix	81 ± 4	86 ± 4:24 ± 1*
Ferm ap sugar mix *vs* fr ap sugar mix plus alcohol	47.2 ± 4	52 ± 3:48 ± 2 n.s

* = significant, $P < 0.01$.
n.s = not significant, $P > 0.05$.

Table 5. Sugar composition and concentrations in fermenting juices.

Juice	Type	Sugar	Wt (mg/l ml)	Concentration	Tot conc sugar/juice
		Fructose	23.21	0.15M	
Apple[1]	Fresh	Glucose	4.98	0.03M	0.19M
		Sucrose	3.20	0.01M	
		Fructose	15.95	0.09M	
Apple	Fermenting	Glucose	4.18	0.02M	0.12M
		Sucrose	1.72	0.01M	
		Fructose	7.39	0.04M	
Orange[1]	Fresh	Glucose	6.27	0.04M	0.15M
		Sucrose	23.66	0.07M	
		Fructose	-	-	
Orange	Fermenting	Glucose	-	-	
		Sucrose	21.57	0.06M	0.06M

[1] From sample 2, in Table 3.

only 29 ± 2% of orange sugar mixture. There was no significant difference between the results of tests carried out in light and those in darkness.

The preferences of fresh juices to fermenting ones may be due to a lower concentration of sugars in fermenting juices. The results for the sugar concentration in fresh and fermenting juices (Table 5) indicate a lower concentration of sugar in fermenting juices, the alcohol concentrations in fermenting juices are given in Table 6). In the preference tests, between sugar mixture with alcohol and without, all the flies orientated to the sugar mixture with 0.42 M alcohol and shortly afterwards they started moving randomly.

Relation between preference and amino acid concentration. On the basis of proboscis extension/retraction studies of the blowfly, some amino acids have been found to be excitatory, some inhibitory and others neutral (Goldrich, 1973). Shiraishi and Kuwabara (1970) classified 19 amino acids on the basis of their electrophysiological responses thus: Class 1 acids: (alanine, cystine, glycine, serine, threonine and tyrosine) neither stimulate nor inhibit any receptor. – Class 2 acids: (arginine-HCl, aspartic acid, glutamic acid, histidine-HCl and lysine-HCl) inhibit all three receptors at high concentrations. – Class 3 acids: (hydroxyproline, proline) stimulate the salt receptor and inhibit the water receptor. – Class 4 acids: (isoleucine, leucine, methionine, phenylalanine, tryptophan and valine) stimulate the sugar receptor.

Table 6. Alcohol content in fermenting juices after 5 days of fermentation (Sample I) at room temperature and after 10 days (Sample II).

Sample	Juice	% alc content	Alc conc
I	Ferm or	3.0	0.8M
	Ferm ap	1.9	0.5M
	beer	7.3	2.0M
	Ferm or	1.7	0.5M
II	Ferm ap	1.6	0.4M
	Stale beer[1]	2.9	0.8M

[1] After 2 days in an open tin at room temperature.

Table 7. Free amino acids in some of foods used in preference tests.

Type amino acids	Conc free amino acids in μ moles			
	Apple	Honey	Fish	Liver
Lysine	-	1.3	-	71.2
Histidine	-	0.4	-	11.9
Arginine	-	0.2	-	-
Aspartic acid	9.9	0.8	3.6	26.6
Threonine	1.2	0.6	3.4	27.5
Serine	-	3.9	5.4	52.6
Glutamic acid	4.6	0.4	5.3	59.4
Proline	0.6	13.5	-	13.9
Glycine	1.7	1.8	8.7	62.6
Alanine	6.3	1.5	46.5	64.1
Cystine	-	-	1.8	5.9
Valine	1.3	0.5	14.9	32.6
Methionine	0.2	-	2.0	14.8
Isoleucine	1.6	0.3	7.1	20.3
Leucine	0.8	0.5	14.5	64.1
Tyrosine	-	0.3	0.7	-
Phenylalanine	-	1.2	4.8	23.2

Table 8. Feeding preference between (A) water, and (B) between 0.1M sucrose and mixture of 0.1M sucrose with the four classes of amino acids. N = 4.

A Class of amino acids (0.1M of each)	Total μl ingested	%	% of amino acid
I (serine, glycine, cystine	A 67.6±4.5	19±0.4	81±1**
tyrosine & threonine)	B 374 ±7	45±3	55±0.2 n.s
II (aspartic acid, glutamic	A 35.4±4.5	94±2.2	6±3**
histidine, lycine & arginene)	B 190 ±8	88±2	12±0.5**
III (proline)	A 37.7±3.4	50±0.1	50±4 n.s
	B 204 ±4	42±2	58±4*
IV (isoleucine,	A 1.3±0.8	0	100*
methionine, phenylalanine and valine)	B 245 ±8	53±4	47±3.0 n.s

** = significant $P < 0.01$; * = significant $P < 0.05$; n.s = not significant $P > 0.05$.

Amino acids were present in the foods used in this study, and it is possible that they played a part in the preferences observed. The free amino acids were therefore determined (Table 7).

These acids were behaviourally tested according to the classification above as follows: group I amino acids (serine, glycine, alanine, cystine, tyrosine and threonine. – Group II amino acids (aspartic acid, glutamic acid, histidine, lysine and arginine. – Group III (Proline). – Group IV (isoleucine, methionine, phenylalanine and valine). – All the acids in each group were mixed together at a concentration of 0.1 M each.

(i) Water *versus* amino acids. Group I amino acids (all mixed together) were preferred to water, that water was preferred to group II acids, and that there was no preference between group III amino acids and water. With group IV amino acids there was a very small uptake of the acids and water was not taken at all (Table 8A).

(ii) 0.1 M sucrose *versus* a mixture of 0.1 M sucrose and amino acids. Preferences for group I, II and III were in the direction obtained in tests without sugar (Table 8B), though with a barely significant preference for group III amino acids with sugar present rather than indifference without it. With sugar present, group IV amino acids were perhaps slightly inhibitory.

Effect of salt on preference. (i) 0.1 M sucrose *vs* 0.1 M sucrose plus various concentrations of NaCl (Table 9). For all concentrations of NaCl mixed with sucrose up to 0.1 M NaCl the salt/sugar mixture was preferred to the sugar alone, significantly so for all salt concentrations above 0.005 M. At 0.175 M NaCl there was no preference, and at 0.25 M NaCl the preference reversed strongly in favour of sucrose alone.

Table 9. Feeding preference between 0.1M sucrose alone and a mixture with various concentrations of NaCl. N = 5.

Conc NaCl	Total μl ingested	% of 0.1M sucrose	% of sucrose/ salt mixture
0.005M	145±6	47±1	53±0.5 n.s
0.01M	215±6	40±1	60±3*
0.025M	247±6	38±3	62±5*
0.1M	255±5.6	37±2	63±9*
0.175M	190±5.6	47±2	53±7 n.s
0.25M	253±7	77±7	23±1*

* = significant, $P < 0.01$.
n.s = not significant, $P > 0.05$.

(ii) Water *vs* a mixture of 0.25 M NaCl/0.1 M sucrose and higher concentration of NaCl. At a concentration of 0.25 M NaCl/0.1 M sucrose mixture, the mixture was preferred to water and at a concentration of 0.5 M NaCl/0.1 M sucrose mixture, there was no preference between water and the mixture. At 1 M NaCl, one fly out of five survived after one day of testing. Water was preferred to the mixture.

(iii) 0.2 M sucrose *vs* a mixture of 0.2 M sucrose and various concentrations of salt (Table 10). A similar pattern of preference to that in Table 8B, is shown here with minor variations at a few concentrations of NaCl.

Table 10. Feeding preference between 0.2M sucrose and a mixture of 0.2M sucrose with various concentrations of NaCl. N = 5.

Conc NaCl	Total µl ingested	% sucrose	% of sucrose/ NaCl mixture
0.01M	97 ± 7	38 ± 0.7	62 ± 3*
0.025M	127 ± 6	35 ± 1	65 ± 4*
0.1M	183 ± 3	36 ± 1	64 ± 1*
0.175M	181 ± 6	51 ± 7	49 ± 1 n.s
0.25M	163 ± 7	73 ± 2	27 ± 1*
0.5M	130 ± 5	98 ± 2	2 ± 0.1*

* = significant, $P < 0.01$.
n.s = not significant, $P > 0.5$.

Discussion

The results show that *Phormia* is capable to discriminate between natural foods. The discrimination, however, is variable. The variation observed in preference from sample to sample of juices could be due to fluctuations in carbohydrates and perhaps amino acids in the fruits. Sucrose and amino acids are known to fluctuate seasonally in plant sap (Mittler, 1958; Zimmerman, 1960; Ziegler, 1962).

As to what extent can the absolute and relative stimulatory powers of these foods be explained in terms of their sugar content can be explained by the following comparisons: The observation that all juices were preferred to the standard concentration of 0.1 M sucrose (Table 1) might suggest that the concentrations of sugar in the juices were higher than in the standard solution in the following order, orange ∂ honey ∂ apple ∂ 0.1 M sucrose. However, the sugar analyses (Table 3) show that this is not necessarily the case, since even sample 1 apple juice was preferred to 0.1 M sucrose, inspite of the lower total sugar concentration in that sample. That it is not sugars alone on which the preferences are based can be seen in Table 4. In all cases the natural foods were preferred to the mixture of sugars alone. This indicates the existence of other feeding stimulants. That sugar plays a major role in these preferences, can however be seen from the results for the comparisons of sugar mixtures of the juices (Table 4). The order of the preference is the same as in the juices themselves: Orange sugar mixture ∂ apple sugar mixture ∂ fermenting apple mixture.
Additional evidence for the magnitude of the role of stimulants other than the major sugars can be obtained between the comparison of apple – sugar mixture with honey – sugar mixture. In this case there was no preference between the two comparisons, whereas in the natural foods, honey was preferred to apple juice, indicating that the stimulating effects of juice were far more complex than could be explained simply by their sugar mixtures. It is possible that the juices were preferred to their sugar – mixture, basing on colour differences since the sugar mixtures are colourless. This possibility of visual cues was ruled out, since there was no significant difference in feeding preference between tests carried out in light or in darkness.

Dethier (1961) showed that low concentrations of alcohol attract and high concentrations repel the fly from the food source. Depending on the concentration, alcohol in the fermenting juices (Table 6) could either act as an attractant or repellant. However, in all cases fresh juices were preferred to fermenting juices confirming that sugar (Table 5) plays a major role in these preferences. A better picture could probably be obtained with antennectomised flies when all olfactory cues are eliminated.

Salts are known to be present in various plant juices and NaCl used in these investigations is known to be a micronutrient in plants. Dethier (1968) found that at very low concentrations NaCl can be accepted by the fly, and can enhance the response to sugar (Kuwabara, 1961; Morita *et al.*, 1965). $CaCl_2$ on the other hand can inhibit the sugar and salt receptors by hyperpolarising them (Morita, 1959). Since mixtures of chemical compounds are the rule in the natural foods of *Phormia* and other insects, it is likely that there would be deterrents as well as excitants in many foods. Because of this Ishikawa (1966) hypothesised with respect to the silkworm that rejection of a plant does not depend solely on deterrents, but possibly on ratios, between amount of stimulants and deterrents. Jermy (1961) had earlier pointed out that the host range of a given phytophagous insect is primarily determined by the number and concentration range of chemicals to which the chemoreceptors are tuned in a positive and negative sense. These hypotheses have not been tested.

My results with mixtures of sucrose and various concentrations of salt are in accordance with the above hypotheses even though the high concentrations beyond 0.1 M NaCl are unlikely to be present

in the juices. The higher concentrations just demonstrate the role of deterrents in feeding behaviour. In all salt/sucrose mixtures where salt concentration was below 0.175 M, the mixture was preferred to sucrose alone, confirming that salt at low concentrations enhances response to sugar. Above this concentration sucrose alone was preferred to the mixture, showing that the ratio of NaCl as an inhibitant to that of sugar was higher than in previous mixtures. At 0.175 M NaCl, however, the excitatory effects of sugar neutralized the inhibitory effects of salt. It is interesting to note that at a concentration of 0.25 M NaCl in the salt/sucrose mixture, very little of the mixture was taken, meaning that at this concentration, the inhibitory property of NaCl overides the excitatory effect of sucrose. However, the finding that when the same mixture was compared to water, it was highly preferred to water, showed that feeding preference is rarely absolute.

These results might also explain why there was no single sensory pattern signifying acceptance or rejection in Dethier's (1972, 1974) investigations with caterpillars and *P. regina*, and with locusts by Winstanley and Blaney (1978). Further, the results show that in situations where behaviour patterns are similar, the sensory patterns may not necessarily be the same. For instance, there was no preference between 0.175 M NaCl/0.1 M sucrose mixture and 0.1 M sucrose alone just as there was no preference between water and 0.5 M NaCl/0.1 M sucrose mixture. The behaviour of these two tests is the same. It would be interesting to find the sensory neural correlates for those behavioural patterns.

In nature, blowflies are captured around dead or decaying matter where females oviposit (Dethier, 1976) and possibly also feed. Our observations in these investigations have shown (Table 2) that male blowflies are capable of discriminating between sweet foods and decaying meat as well as different kinds of decaying meat. The results also show that decaying liver and fish are both stimulating since they were preferred to water. It is possible that amino acids and perhaps other chemical components act as a basis for the preference since amino acids are known to have an important role on feeding responses of several insects from several orders (see Dethier, 1966; Mitchell, 1974 for reviews). Table 7 shows the composition and concentration of free amino acids in the foods in these preference tests and indicates that liver has a higher concentration of the total concentration of these acids than fish. It is possible that the discrimination between fish and liver was at least partly based on the concentration of amino acids. Olfactory cues too could play part.

Not all amino acids are excitatory; some are inhibitory and others neutral (Shiraishi & Kuwabara, 1970). Goldrich (1973) found water satiated flies to be responsive to alanine and valine and suggested that alanine might stimulate the sugar cell. The preference test data (Table 8A) support Goldrich's findings since group I acids were highly preferred to water. The results for the tests with group II acids were in agreement with the predicted behavioural responses (Shiraishi & Kuwabara, epic. cit.) since more water was taken than the acids. Group III acids, however, behaved differently from what would be expected in light of the electrophysiological responses which showed that this group stimulates the salt receptor implying that this group is inhibitory as far as feeding behaviour is concerned. Since the results showed no preference between the acids and water, this group would be considered to be neutral. The discrepancy here could be due to the differences in concentrations of the acids used in the two cases. Dethier (1968) found that salt is acceptable to the fly at low concentrations. This may well be the case with regard to these acids. Our findings with Group IV amino acids were puzzling as very little of the acid was taken and no water at all.

The results with sucrose/acid mixtures confirm earlier findings with respect to the properties of those acids. Group III may be mentioned particularly as it behaves like salt. At low concentrations these acids synergise with sucrose, just like salt. Group IV amino acids, although they stimulated the sugar receptor, appear to be neutral in our behavioural tests, confirming our earlier findings in this study. Hence, stimulation of the sugar cell may not necessarily mean acceptance.

These findings with amino acids and salts explain at least partly why the juices were always preferred to the sugar-mixtures of the same concentration as those occurring in the juices themselves. In nature, mixture is the rule and hence feeding preference is based on a number of compounds some of which are excitatory, others inhibitory, and some neutral. The ratio among these combinations ap-

pear what really matters. This ratio may vary from species to species or even from strain to strain of an insect. Sucrose and amino acids are essential for larval growth and adult oviposition in *Phormia.* It appears that common nutrients are substances with stimulating properties; though the converse is not necessarily true as many allelochemics are strongly stimulating but their nutritive value is low or nil (Schoonhoven, personal communication). In the absence of reasons to the contrary, this correlation and nutritiveness would seem to support the idea that insects choose what is nutritionally good; if they did not, they would not servive too well.

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Résumé

Relation entre les teneurs en glucides, acides aminés et en alcool et les préférences alimentaires de Phormia regina

P. regina Meigen mâle préfère certains aliments naturels ou artificiels. Au laboratoire ses préférences parmi les jus sucrés sont les suivantes: orange > miel > pomme > sucrose 0.1 M; et parmi les aliments en décomposition: sucrose 0.1 M > foie > poisson. Les jus frais sont préférés aux jus fermentés. Les préférences ne dépendent pas seulement de la nature des sucres, mais aussi des acides aminés, de l'alcool, des sels et probablement d'autres substances chimiques. Le foie contient à la fois le plus grand nombre et la concentration la plus élevée en acides aminés, ce qui explique partiellement le goût pour le foie. Expérimentalement on peut classer les acides aminés en stimulants, inhibiteurs et neutres. Des sels ont été notés dans les jus, mais aucune analyse qualitative et quantitative n'en a été faite. Mélangé à du sucrose à 0.1 M, NaCl agit en synergie aux faibles concentrations, devient neutre à 0.175 M et à des concentrations supérieures le sucrose seul est préféré au mélange avec NaCl. Cependant, un mélange de NaCl à 0.25 M (bien qu'une telle concentration soit peu probable dans des jus végétaux) avec du sucrose à 0.1 M était préféré à l'eau. Il en est déduit que les préférences alimentaires dans la nature sont orientées par plusieurs substances chimiques qui se comportent d'une façon stimulante, additive, neutre, inhibitrice ou synergique, et que les préférences alimentaires sont relatives. Le rapport de stimulants sur inhibiteurs est important dans la détermination du sens de la préférence.

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