J. exp. Biol. (1978), 75, 189–201
With 5 figures
Printed in Great Britain

CONTROL OF SALIVATION IN THE BLOWFLY *CALLIPHORA*

BY CHRISTINA M. HANSEN BAY*

Department of Zoology, University of Cambridge, Downing St, Cambridge CB2 3EJ

(*Received* 15 *November* 1977)

SUMMARY

1. A technique has been developed for collecting saliva from the salivary duct of flies during feeding.

2. In newly emerged flies salivation is evoked by stimulation of the labellar taste papillae by crystalline sugar, by sugar in solution and by water. Only sugars which excite the sugar receptors are effective.

3. In flies which have previously fed, solid sucrose or meat induce regurgitation of the crop contents, or salivation if the crop is empty. Sucrose in solution provokes neither reaction.

4. Saliva always contains digestive enzymes, even if the ingested food requires no digestion.

5. Blood taken from salivating flies stimulates fluid secretion from isolated glands, whereas blood taken from non-salivating flies is ineffective.

6. Section of the cephalo-thoracic nerve cord abolishes salivation in response to feeding. Section of the ventral nerve cord posterior to the thoracic ganglion, or removal of the abdomen, severely reduces but does not abolish the salivatory response.

7. Saliva secreted by mature flies contains 75 mM-Cl^- whereas saliva secreted during the first meal after emergence has a higher Cl^- concentration (about 165 mM-Cl^-) which gradually declines during the first meal.

INTRODUCTION

There have been numerous studies on the neural basis of various components of the feeding behaviour of blowflies (see review by Barton Browne, 1975), but one aspect which has been neglected is the control of salivation. This paper describes a technique for collecting saliva from flies during feeding. The preparation has been used to study the stimulatory and inhibitory factors controlling salivation, some of the neural and hormonal pathways involved in this control, and the nature of secreted saliva.

Most of the food ingested by blowflies is in a liquid or semi-liquid form (Graham-Smith, 1930), and either saliva or regurgitated fluid may be used to dissolve solid food. The paired, tubular lingual salivary glands which extend throughout the body of the fly secrete a KCl solution containing digestive enzymes (Oschman & Berridge, 1970; Prince & Berridge, 1973; Hansen Bay, 1978*a*). The chitinous ducts of the two

* Present address: Department of Physiology and Biophysics, State University of New York at Stony Brook, Stony Brook, N.Y. 11794, U.S.A.

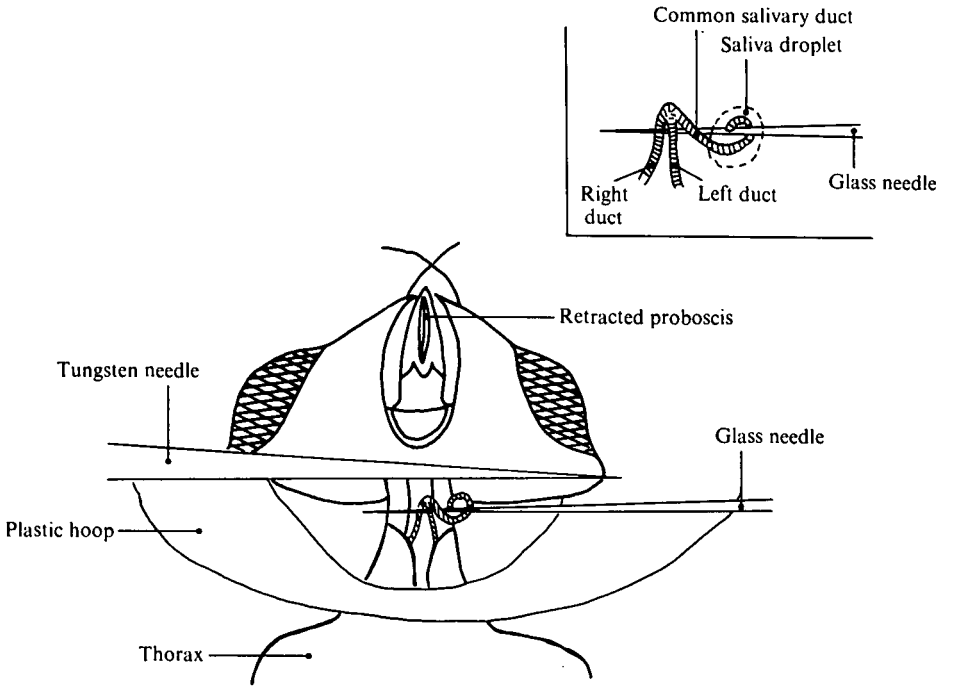

Fig. 1. The method of collecting saliva from the salivary duct of a fly as seen down the microscope. The legs of the plastic hoop fit into a slit in the cork upon which the fly is lying. The tungsten-and-glass needles are each held in a micromanipulator. The inset shows the position of the glass needle in relation to the salivary ducts. Saliva flows out of the cut end of the common duct and collects as a droplet (broken line) around the needle.

glands unite to form the common salivary duct which passes down the proboscis to join the alimentary tract in the distal region of the proboscis. Food entering the oesophagus may pass directly into the midgut or into the crop which is a diverticulum of the gut. Contraction of the crop forces fluid back into the foregut, from where it may go to the midgut or else back down the proboscis onto the food. This arrangement makes it impossible to collect saliva at the end of the proboscis, as it is contaminated either by food or by regurgitation. This difficulty is avoided in the new preparation by collecting saliva from the common salivary duct before it enters the proboscis.

METHODS

Adult blowflies, *Calliphora erythrocephala* Meig., were taken from the laboratory culture. Flies were collected after emergence, and either isolated with no food or water or maintained on sucrose, water and ox heart. Unless otherwise stated, flies were 1–3 days old and unfed prior to the experiment.

The preparation used for the collection of saliva is shown in Fig. 1. The fly was laid dorsal side down on a cork attached to the stage of a dissecting microscope, and held down with a plastic hoop inserted between head and thorax and fitting into a slit in the cork. Using a micromanipulator, a fine tungsten needle was inserted point foremost between the head and thorax and then moved sideways towards the head, gradually widening the gap between head and thorax. The salivary duct was then seen

beneath the transparent cuticle of the neck. This cuticle was removed, taking care not to damage the tracheae and muscles, to expose the salivary duct in the region where the ducts of the two glands unite to form the common duct. A second micromanipulator carried a fine glass needle, made from tubing drawn out to a fine point on a vertical pipette puller (model 700B, David Kopf Instruments). The point of this needle was inserted beneath the duct of one gland so that it passed through the fork where the two ducts unite. The needle was then raised slightly and the common duct cut anterior to the fork. The short length of duct attached to the fork was coiled around the glass needle.

Saliva passed down both ducts and accumulated as a drop around the glass needle. It was collected in a siliconed fine pipette containing a little liquid paraffin and ejected under liquid paraffin in a siliconed glass dish. The volume of the drop, and of all small samples of fluid, was determined by measuring the diameter of the drop using a calibrated graticule in the eyepiece of the microscope.

Normally a little salivation occurred during the operation but this soon ceased and could be prevented by chilling the fly in the refrigerator for a few minutes before the operation. Flies were fed solid food by placing the food between the lobes of the labellum. Liquid food was contained in a fine pipette and held as a drop in contact with the oral surface of the labellum.

Drugs for injection were dissolved in Ringer solution and 1 μl was injected through an intersegmental membrane in the abdomen using a fine glass pipette attached to the compressed air supply. For the collection of blood, a similar pipette attached to a plastic tube was inserted through an intersegmental membrane. Blood entered the pipette by capillary action and was ejected by gently blowing through the plastic tube.

Dissection of the salivary glands and crop was performed under Ringer solution of the following composition (mM): Na, 155; K, 20; Ca, 2; Mg, 2; Cl, 156; Tris, 10; phosphate, 2; malate, 2·7; glutamate, 2·7; citrate, 1·8. Phenol red (< 0·01 mM) was included to keep a constant check that the pH remained between 7·2 and 7·4. The dorsal cuticle over the abdomen was cut off and the salivary glands were gently grasped where they entered the abdomen, freed from the surrounding tracheae and removed from the fly. The crop duct was grasped with forceps and the whole crop lifted out of the fly, blotted gently to remove external fluid and burst under liquid paraffin.

In surgical experiments involving section of the ventral nerve cord, the nerve was severed using forceps. In the cephalo-thoracic region, the nerve lies just dorsal to the common salivary duct and it is possible to sever it without interrupting the collection of saliva. Care was taken not to damage the surrounding musculature and tracheae or the underlying recurrent nerve. The nerve cord was also cut just posterior to the thoracic ganglion where it lies beneath the leg musculature. Details of the operation are given in the Results section.

Isolated glands were set up as described by Oschman & Berridge (1971) for measurement of secretory rate. Chloride concentration was determined by the electrometric method of Ramsay, Brown & Croghan (1955). Sucrase activity was assayed using the amylase assay of Robyt, Ackerman & Keng (1972), with 2% sucrose solution as substrate.

Where results are shown as secretory responses of individual flies, they are representative of experiments on at least five flies all giving essentially similar responses.

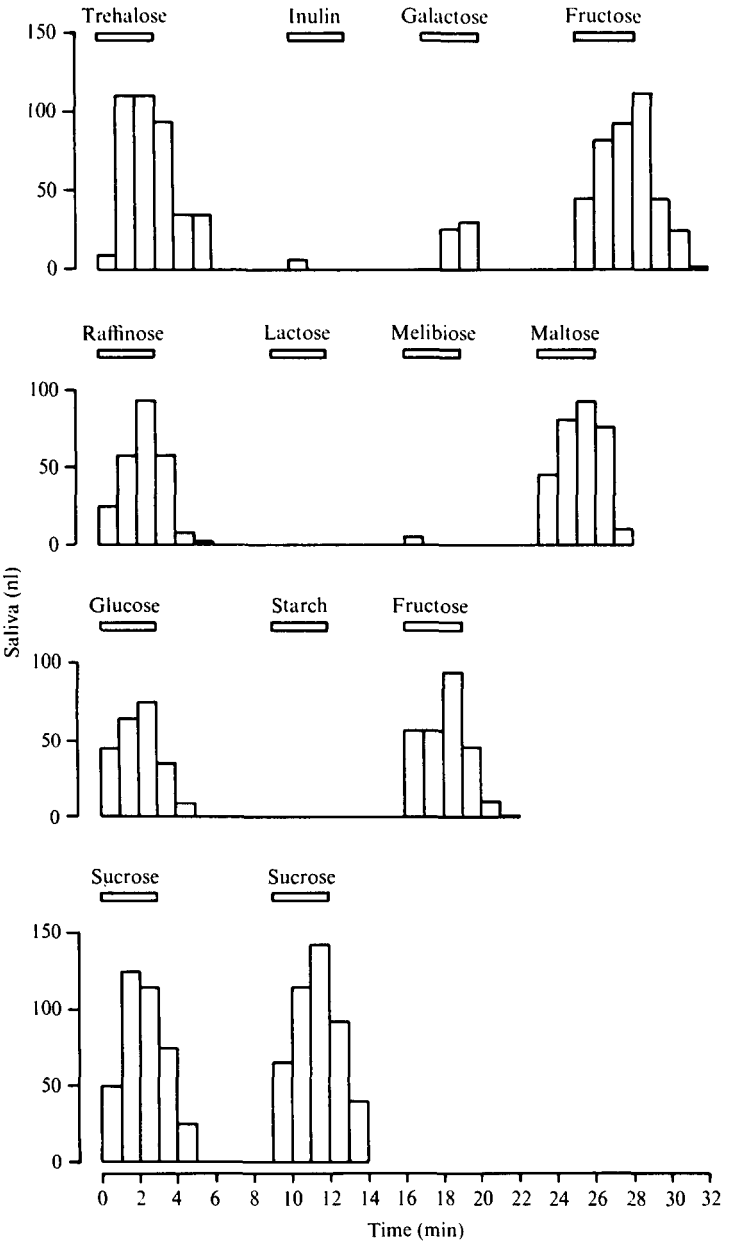

Fig. 2. The ability of various carbohydrates to stimulate salivation. Each line shows the response of one fly presented with a succession of substances. Each carbohydrate was held for 3 min (bar) between the labellar lobes, and at least 3 min were allowed to elapse before the next substance was presented.

RESULTS

Stimulation of salivation

Solid sugar. When a sucrose crystal was placed on the oral surface of the labellar lobes, the proboscis was extended and both sucking and salivation were initiated, a

were maintained for as long as the sugar remained on the labellum. Although no saliva could reach the food, the crystal became quite moist and was gradually ingested. Ingestion of a single crystal took about 10 min, compared to 2 min in flies with the salivary duct intact, indicating that saliva normally facilitates dissolution and ingestion of solid sugar. Removal of the sugar during ingestion caused vigorous searching movements of the proboscis for about 30 s and salivation continued for up to 2 min before ceasing. If sugar was then placed again on the lobes after a few minutes, the same response of salivation and sucking occurred (see Fig. 2).

There are three sets of receptors which mediate the response of a fly to food (Dethier, 1955). Salivation can be evoked only by direct stimulation of the interpseudotracheal, or taste, papillae on the oral surface of the labellum. Salivation never occurred in response to stimulation of the tarsal or labellar hairs by sucrose, either as a solid or in solution. Each of the taste papillae contains four sensory cells, including a sugar receptor and a mechanoreceptor (Dethier & Hanson, 1965), either of which could be excited by the presence of a sucrose crystal on the taste papillae. To determine which sensory modality was involved in causing salivation, a variety of carbohydrates were tested for their ability to induce salivation. Each substance was held for 3 min between the oral surfaces of the labellar lobes in contact with the taste papillae, and any saliva secreted was collected and measured. The results are shown in Fig. 2. Although all the carbohydrates provided the same mechanical input, they were not all equally effective in evoking salivation. Only sugars which are known to stimulate the sugar receptor (sucrose, glucose, trehalose, fructose, raffinose) induced salivation for longer than 2 min. Only a little salivation occurred in response to galactose which is a weak stimulant for the tarsal sugar receptors (Dethier, 1955), and the non-stimulating carbohydrates (inulin, lactose, starch, melibiose) did not evoke any secretion.

Sugar in solution. Flies were fed 10 μl of a 1 M sucrose solution and the rate of salivation measured. Ingestion appeared to occur normally, taking about 60–90 s, and was accompanied by secretion of up to 100 nl saliva. When the solution was presented to flies which had just been feeding on solid sucrose for 3 min, no salivation accompanied ingestion of the solution.

Meat. Protein-deprived flies were used to test the stimulating power of meat since newly emerged flies made no attempt to ingest meat until they had eaten some sugar. Flies were fed only on sucrose and water for 6 days after emergence and then starved overnight. A small scrap of meat was placed between the labellar lobes and the salivation rate determined. All the flies attempted unsuccessfully to ingest the meat, but the extent of salivation was very variable. In some flies salivation continued at a high rate (50–100 nl/min) until the meat was removed, while in others only 20–25 nl saliva was secreted altogether.

Inhibitory influences

When solid sucrose or meat is offered to a fly which has previously been fed and then starved for some hours, regurgitated fluid rather than saliva may be used to dissolve the food. It was possible to show that this fluid came from the crop and not from the midgut by feeding a fly an alkaline solution of sugar containing the pH indicator phenol red. The contents of the anterior region of the midgut were yellow

since the pH is maintained at about 5·4, and the crop contents were red since the remain at the pH of the ingested solution (Waterhouse, 1940). Regurgitated fluid was red, showing that it came from the crop. When an acid solution of sugar was used, all the fluids remained yellow.

Flies were fed, starved for some hours and offered solid sucrose. After determining whether salivation or regurgitation occurred, the flies were dissected and the crop volume measured. Salivation occurred only in flies whose crop contained less than about 0·5 μl fluid and therefore had nothing to regurgitate. However, when previously unfed flies were fed 10 μl 1 M sucrose solution and then offered solid sucrose 1–2 min after drinking had ceased, salivation did occur although the crop contained fluid. Neither sucrose in solution nor water ever evoked salivation unless the fly had never previously eaten.

Neural and hormonal elements of the salivatory response

In unfed flies, excitation of the taste papillae results in salivation. The role of the central nervous system in this response was investigated in various surgical experiments. Fig 3(*a*) shows the result of severing the cephalo-thoracic nerve cord which lies just dorsal to the common salivary duct in the neck region. Flies were fed sucrose and after 2 min the nerve was severed. Sham-operations involved movements of the forceps in the region of the nerve without actually cutting it. In every case, salivation ceased within 2 min after an operation but continued after a sham-operation. The flies continued to feed on the sucrose unconcernedly. Feeding after an operation never evoked salivation, but injection of 5-hydroxytryptamine (5-HT), which causes secretion by isolated glands, did cause salivation, indicating that the salivary glands were still intact. Injection of Ringer alone did not cause salivation.

Fig. 3(*b*) shows the result of a similar experiment in which the ventral nerve cord was severed just posterior to the thoracic ganglion. Cuticle was removed just anterior to the mesothoracic legs, and the leg-stumps and muscles gently pulled away to expose the ganglion. The salivary duct was then set up for the collection of saliva, and the fly fed solid sucrose for 3 min to determine the rate of salivation. When salivation ceased, the nerve cord was cut or a sham-operation performed, and the fly fed again. Although salivation was severely reduced by this operation it was not entirely abolished. Subsequent injection of 5-HT caused a normal rate of secretion, indicating that the decrease was not due to damage to the glands. The salivation rate in response to feeding was not affected by the sham-operation.

The role of the thoracic nervous system was further investigated using 'abdomenless' flies in which the abdomen was cut off and the wound sealed with wax. The operation appeared to have little effect on the feeding behaviour of the flies. As shown in Fig. 4, feeding these flies evoked a little salivation, but this was not maintained for long, although the flies continued to try and eat the sugar. Secretion was at a low rate owing to the absence of the abdomen which contains most of the secretory region of the glands. Subsequent injection of 5-HT provided an estimate of the possible rate of salivation in these 'abdomenless' flies.

Unlike most insect salivary glands, those of *Calliphora* are not innervated. The basal rate of fluid secretion by isolated glands is greatly increased by treatment with

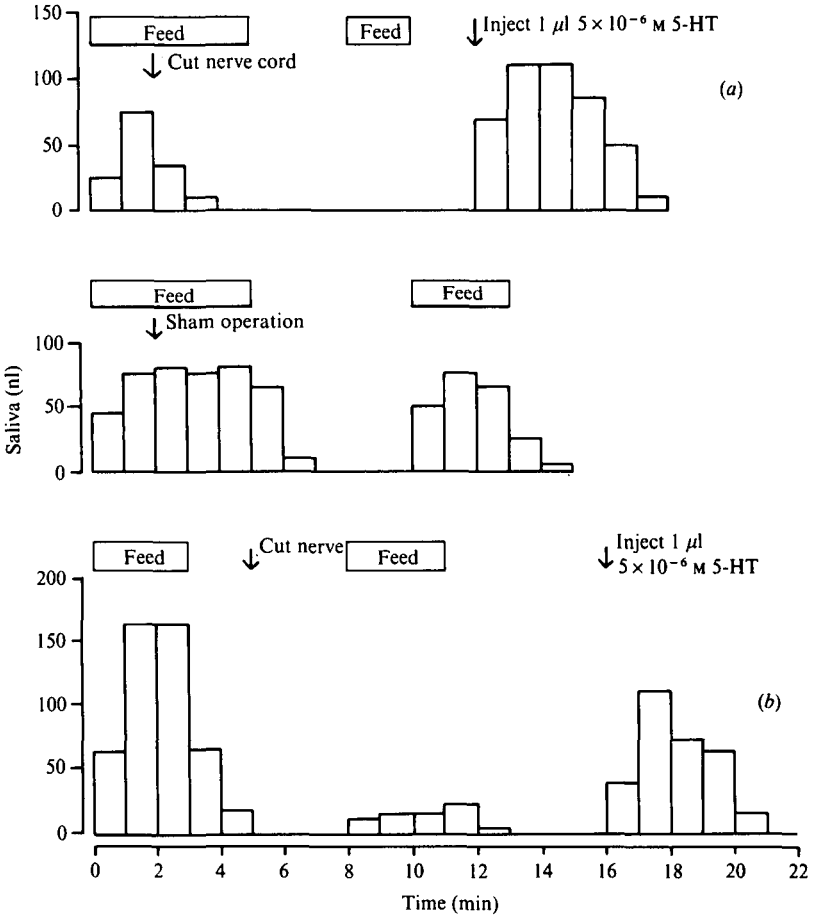

Fig. 3. The effect of severing the ventral nerve cord on salivation. Flies were fed solid sucrose as indicated (bar). Each line shows a typical result from an experiment on a single fly. (*a*) The cephalothoracic nerve was severed in the neck, or a sham-operation performed (arrow). (*b*) The ventral nerve cord was severed just posterior to the thoracic ganglion (arrow).

10 nM 5-HT, and it is possible that 5-HT may act as a hormone in the fly. If salivation is controlled hormonally, then blood taken from salivating flies should stimulate secretion by isolated glands, while blood from non-salivating flies should be ineffective. Results of the experiments shown in Fig. 3(*a*) indicate that there is a rapid removal mechanism for the hormone in the intact fly, since salivation ceased within 2 min of cutting the cephalo-thoracic nerve cord. Since this mechanism may involve degradation of the hormone by the blood, it is important that there be no delay between the collection of blood and its application to isolated glands.

In order to see if salivation was occurring during the collection of blood, flies were prepared as for the collection of saliva. In some flies salivation was induced by feeding sucrose, and in others salivation was prevented by severing the cephalo-thoracic nerve cord. About 1 μl blood was collected from the abdomen of a fly, immediately applied to an isolated salivary gland and the rate of fluid secretion measured over the next 10 min. Each gland was tested with blood from both salivating and non-salivating

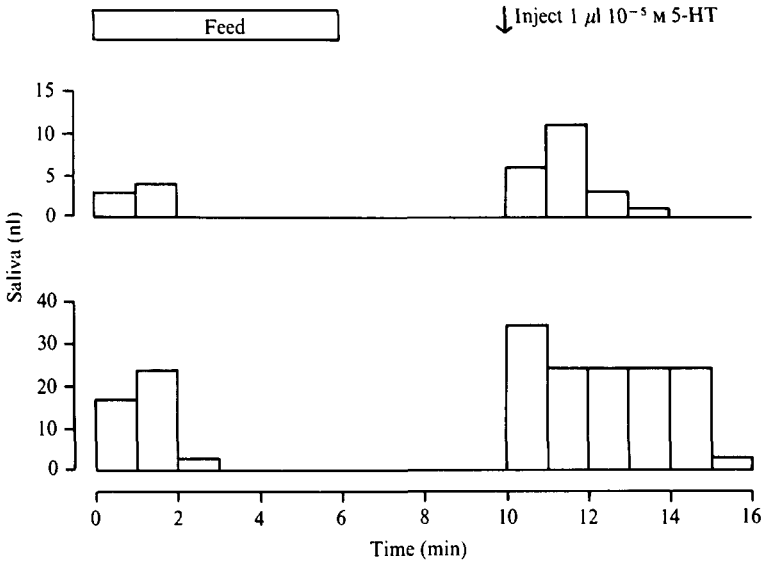

Fig. 4. Saliva secreted by two 'abdomenless' flies in response to feeding (bar) and to injection of 5-HT. Flies were prepared as described in the text. (Note the difference in vertical scales in Figs. 3 and 4.)

flies. The order of testing was reversed on alternate glands, and the glands were well rinsed between treatments. With blood from non-salivating flies the rate of fluid secretion was $0{\cdot}6 \pm 0{\cdot}1$ nl/min, while with blood from salivating flies the rate increased to $12{\cdot}6 \pm 0{\cdot}8$ nl/min (mean ± S.E.M., $n = 6$), confirming that a hormone is involved in the control of salivation.

The composition of saliva

Enzymes. Isolated abdominal regions of the salivary glands treated with 5-HT secrete fluid containing at least three digestive enzymes – α-amylase, α-glucosidase and α-galactosidase – and the same enzymes are found in saliva of flies fed solid sucrose (Hansen Bay, 1978*a*). Fig. 5 shows the rate of enzyme (sucrase) secretion induced by feeding flies either sucrose or glucose. The rate of secretion is very similar in each case, despite the fact that sucrose requires digestion whereas glucose does not. Sucrase was also found in the saliva of flies fed water, and in saliva secreted by flies in response to injection of 5-HT.

Ions. Fluid produced by the secretory region of isolated glands contains 130 mM-K^+, 20 mM-Na^+ and 166 mM-Cl^-. Some of these ions are reabsorbed in the proximal region of the gland leaving only 50 mM-K^+ and 20 mM-Na^+ (Oschman & Berridge, 1970; Prince & Berridge, 1973). The anionic composition of saliva produced by intact salivary glands was investigated by determining the concentration of Cl^- in saliva secreted in response to feeding or after injection of 5-HT. The results are shown in Table 1. Saliva secreted by 1-week-old flies in response to feeding contained about 75 mM-Cl^-, suggesting that reabsorption of Cl^- occurs in the proximal region of the gland. Saliva secreted after injection of 5-HT had a more variable Cl^- concentration. Of samples from 14 flies, 10 had levels of Cl^- comparable to fed flies, 1 had the

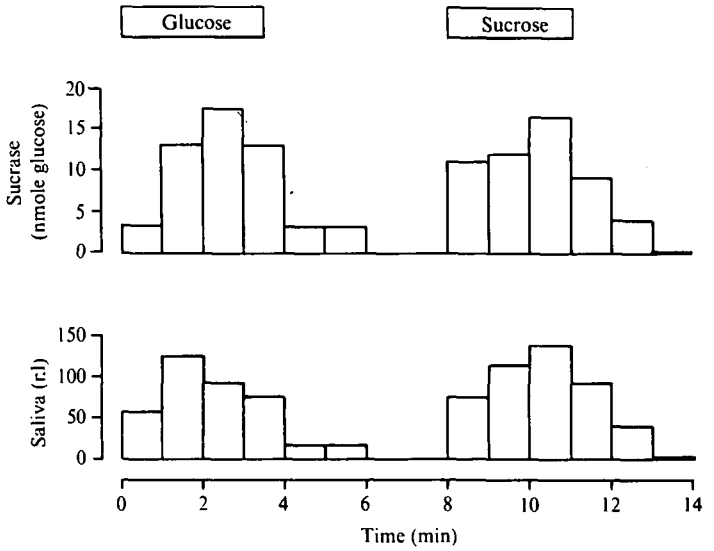

Fig. 5. The rate of fluid and enzyme secretion of a fly fed glucose and sucrose. Similar responses were obtained whatever the order of presentation of the sugars. Sucrase activity is expressed as nmol glucose equivalents produced in 10 min at 37 °C and pH 5·6.

Table 1. *The chloride concentration of saliva*

Age of fly (days)	Stimulus for salivation	[Cl^-] saliva (mM) (mean ± S.E.M.)	Range of [Cl^-] (mM)	*n*
7–10	feeding	74·5 ± 2·7	55–86	11
7–10	5-HT	84·6 ± 7·7	56–159	14
1–2	feeding	166·4 ± 5·8	143–208	12

Table 2. *The chloride concentration in successive drops of saliva secreted by unchilled, newly emerged flies during their first meal*

Fly 1		Fly 2		Fly 3	
Vol. saliva (μl)	[Cl^-] (mM)	Vol. saliva (μl)	[Cl^-] (mM)	Vol. saliva (μl)	[Cl^-] (mM)
341	175	322	143	352	144
230	127	310	143	310	125
268	90	268	116	268	106
310	87	381	95·5		

unreabsorbed level of 158 mM and 3 were intermediate. Thus in 10 out of 14 cases the reabsorptive mechanism was activated following injection of 5-HT.

Table 1 also shows the Cl^- concentration in the saliva secreted by 1- to 2-day-old flies which were fed to induce salivation. The flies were chilled before being prepared for the collection of saliva so that no salivation occurred before the flies were fed. The saliva contained about 166 mM-Cl^-, – a much higher concentration than in the older flies. In a similar experiment, saliva was collected from flies which had not been

chilled, so that the stress of the operation caused a little salivation before the flies were fed. When this spontaneous secretion ceased, the flies were fed and saliva collected for as long as the flies would salivate (about 20 min), and the Cl^- concentration of successive 230–400 nl volumes determined. The concentration of Cl^- decreased in successive samples of saliva, from the high value found in the chilled flies towards the lower value of older flies (Table 2).

The extent of salivation in intact flies

During the ingestion of sugar, a mixture of food and saliva passes partly to the midgut but mainly to the crop (Hansen Bay, 1978*a*). The Cl^- concentration of the crop contents should therefore provide an estimate of the amount of saliva secreted, assuming that all the chloride comes from saliva. (The only other possible source of Cl^- is the crop wall which is lined with cuticle and is thought to be impermeable.) Unfed 3-day-old flies were each fed 10 μl of either water or 1 M sucrose solution or were allowed to feed on solid sucrose for 20 min. The flies were then chilled to prevent regurgitation, dissected, and the volume and Cl^- concentration of the crop contents determined. The results are shown in Table 3, together with an estimate of the volume of saliva secreted by each of the intact flies. For comparison, the volume of saliva secreted by experimental flies under the same conditions is also included in Table 3.

In both intact and experimental flies more saliva is secreted during the ingestion of solid sucrose than of sucrose solution or water. Although the extent of salivation is similar for both classes of flies fed solid sugar, rather more salivation occurs in the intact flies during ingestion of liquid, especially of sucrose solution.

Table 3. *The measured volume and chloride concentration of the crop fluid of intact flies fed water, 1 M sucrose solution or solid sucrose*

Fed 10 μl water			Fed 10 μl sucrose			Fed solid sucrose		
Crop vol. (μl)	[Cl^-] (mM)	Saliva vol. (nl)	Crop vol. (μl)	[Cl^-] (mM)	Saliva vol. (nl)	Crop vol. (μl)	[Cl^-] (mM)	Saliva vol. (nl)
5·8	4·90	295	9·1	8·90	536	0·7	44	185
7·5	3·60	217	9·5	3·60	217	1·8	62	672
7·5	4·15	250	10·0	5·55	334	4·1	50	1234
4·1	4·05	244	9·1	4·75	286	4·7	38	1076
8·2	3·75	226	9·1	10·0	603	3·9	44	1034
			9·1	5·15	310	4·1	34	839
Estimated saliva vol. mean ± S.E.		246 ± 13·5			381 ± 62·3			840 ± 153
Range of saliva volumes measured directly (nl)		17–109			40–100			800–1280

The volume of saliva secreted during feeding was calculated from the relationship: saliva vol. = crop vol. × $[Cl^-]_{crop}/[Cl^-]_{saliva}$. $[Cl^-]_{saliva}$ was taken as 166 mM since the flies were eating their first meal (see Table 1). In these calculations a crop volume of 10 μl was used for flies fed liquid to allow for the fraction of food going to the crop. The bottom line shows the volume of saliva collected from experimental flies during ingestion of the three types of food.

DISCUSSION

With this experimental preparation it is possible to study the physiological stimuli causing salivation, to dissect the components of the control pathway, and to analyse saliva free from contamination. The method is applicable to other insects in which the salivary ducts unite in the region between head and thorax, provided that a suitable method of feeding can be found. The feeding behaviour of the flies was not apparently altered by the experimental manipulations, and flies released after an experiment lived for several days.

The first meal taken by a fly after emergence is accompanied by salivation whether the food is sugar, in either solid or liquid form, or water. In flies which have previously fed, liquid food never elicits salivation, and solid sugar or meat induce salivation only if there is no crop fluid available for regurgitation. It is not known how flies distinguish between solid sugar and sugar in solution. Sugar must be in solution to stimulate the chemoreceptors and it has been suggested that crystalline sugar dissolves in a very thin layer of fluid covering the surface of the sensillum (Dethier, 1955). Perhaps such a solution is a stronger stimulus for the receptor than the 1 M sugar solution used in these experiments. Solid sugar may also excite the mechano-receptor cells of the taste papillae to a greater extent than sugar solution does.

Provided that the fly is not satiated, excitation of the taste papillae by solid sucrose results in sucking movements accompanied either by salivation or by regurgitation. Sensory information from the taste papillae travels to the suboesophageal ganglion in the labial nerve. Action of the cibarial pump causing sucking or regurgitation is controlled from the brain via the labral nerve (Dethier, 1959), and the brain also appears to control salivation via the ventral nerve cord. Presumably, integration of excitatory and inhibitory information in the brain determines whether or not salivation occurs in response to stimulation of the taste papillae. Two sets of receptors, stretch receptors in the abdomen and foregut, are activated by the presence of fluid in the crop and gut (Gelperin, 1971) and these could provide inhibitory input. However, salivation can occur when the crop contains fluid, since flies fed 10 μl 1 M sucrose solution did salivate when presented with solid sucrose 1–2 min after drinking had ceased. In this case, inhibition from crop fluid did not overcome the excitatory input from sugar on the taste papillae, perhaps because of a general increase in the level of excitation of the central nervous system resulting from feeding, as suggested by Barton Browne, (1975).

This increased level of excitation is also apparent in flies interrupted from feeding on solid sugar. When previously unfed or starved flies are offered sucrose, salivation and sucking occur. If the sugar crystal is removed from the labellar lobes, the proboscis makes vigorous searching movements and salivation ceases. Touching the taste papillae or the labellar hairs with a plastic rod now evokes salivation, whereas this is not normally an effective stimulus. This abnormal state of excitation persists only for a minute or two after feeding has been interrupted.

From the measurements of crop Cl^- concentration after ingestion and from the collection of saliva during feeding, there is good agreement in the extent of salivation during ingestion of solid sucrose between intact and experimental flies, despite the reduced intake of food in the latter. Although no saliva could reach the food, it did

become moist and eventually dissolve. One possible source of this fluid is the small labial salivary glands which lie in the labellum beside the oral aperture and may secrete fluid to keep the labellar surface moist (Lowne, 1892). Ingestion of solid sugar results in a far higher level of Cl^- in the crop than does ingestion of liquid, showing that a greater proportion of the crop fluid is saliva, so that there are more digestive enzymes in the crop when there is more sugar for them to act upon. One of the enzymes, amylase, is maximally activated by Cl^- above 1·5 mM (Hansen Bay, 1978*a*), and the concentration of Cl^- in the crop exceeds this value under all conditions tested.

Secretion by the salivary glands is controlled hormonally, but the site of release of the hormone is not known. Salivation was severely reduced in the 'abdomenless' flies and after section of the ventral nerve cord posterior to the thoracic ganglion, and was abolished after section of the cephalothoracic nerve trunk. If the hormone were released from the neuroendocrine complex in the anterior thorax, these operations should not affect the release, since the complex is connected to the brain by the recurrent nerve which is intact, not by the ventral nerve cord. It seems more likely that the hormone is released from neurosecretory axons in the abdomen, and possibly also in the thorax, as a result of electrical activity in the ventral nerve cord. Depolarization of the thoracic and abdominal regions of the nerve cord with 60 mM-K^+ solution releases a factor capable of stimulating secretion in isolated salivary glands (Berridge, unpublished observations), which supports this idea. There appears to be only a limited amount of hormone available for release in the thorax, since salivation was maintained for only a few minutes after severing the nervous connection with the abdomen, and the main release site is probably in the abdomen.

The hormone has not yet been identified but it appears to act in an identical manner to 5-HT. Salivation in response to feeding is abolished by injection of > 2 nmol gramine (Hansen Bay, 1976) which is a known antagonist of 5-HT on salivary glands (Berridge & Prince, 1974), indicating that the hormone acts on the same receptor as 5-HT. Both 5-HT and the hormone cause secretion of both fluid and enzymes from the salivary glands (Hansen Bay, 1978*a*, *b*). Unlike the situation in mammalian salivary glands (Selinger, 1975), there appears to be no mechanism for stimulating fluid secretion without enzyme secretion. Saliva always contains enzymes, whatever the nature of the feeding stimulus.

The factor controlling reabsorption of K^+ and Cl^- ions in the proximal region of the glands has not yet been identified. Reabsorption of Cl^- always occurred in response to feeding except in newly emerged flies. The high level of Cl^- in saliva of these flies could be due to a delay in switching on the reabsorptive mechanism, or because the glands were used for the first time and it took time to establish the correct ionic gradients across the epithelium. As shown in Table 2, secretion of at least 1 μl saliva has to occur before the Cl^- level approaches that in mature flies. The decline in Cl^- was seen only in flies which had not been chilled and so had already secreted a little before they were fed. Flies which had been chilled did not secrete enough saliva during feeding for this effect to appear. Injection of 5-HT resulted in reabsorption in the majority of cases but it is not known whether 5-HT is acting directly, or indirectly by causing the release of some other factor. In isolated glands 5 nM 5-HT did not cause reabsorption of K^+ (Oschman & Berridge, 1970), but it may be effective at higher concentrations.

I am most grateful to Dr M. J. Berridge for his advice and encouragement, and to Professors A. D. Carlson and H. Lipke for their comments on the manuscript. I thank the Science Research Council for financial support.

REFERENCES

Barton Browne, L. (1975). Regulatory mechanisms in insect feeding. *Adv. Insect Physiol.* **11**, 1–116.

Berridge, M. J. & Prince, W. T. (1974). The nature of the binding between LSD and a 5-HT receptor: a possible explanation for hallucinogenic activity. *Br. J. Pharmac.* **51**, 269–278.

Dethier, V. G. (1955). The physiology and histology of the contact chemoreceptors of the blowfly. *Q. Rev. Biol.* **30**, 348–371.

Dethier, V. G. (1959). The nerves and muscles of the proboscis of the blowfly *Phormia regina* Meigen. in relation to feeding responses. *Smithson misc. Collns* **137**, 157–174.

Dethier, V. G. & Hanson, F. E. (1965). Taste papillae of the blowfly. *J. cell. comp. Physiol.* **65**, 93–100.

Gelperin, A. (1971). Abdominal sensory neurons providing negative feedback to the feeding behaviour of the blowfly. *Z. vergl. Physiol.* **72**, 17–31.

Graham-Smith, G. S. (1930). Further observations on the anatomy and function of the proboscis the blowfly, *Calliphora erythrocephala*. *Parasitology* **22**, 47–115.

Hansen Bay, C. M. (1976). Secretory control mechanisms in salivary glands of adult *Calliphora*. Ph.D. thesis, University of Cambridge.

Hansen Bay, C. M. (1978*a*). The secretion and action of the digestive enzymes of the salivary glands of the blowfly *Calliphora*. *J. Insect Physiol.* **24**, 141–149.

Hansen Bay, C. M. (1978*b*). The control of enzyme secretion from fly salivary glands. *J. Physiol. Lond.* **274**, 421–435.

Lowne, B. T. (1892). *The Anatomy, Physiology, Morphology and Development of the Blowfly* Calliphora erythrocephala. London: R. H. Porter.

Oschman, J. L. & Berridge, M. J. (1970). Structural and functional aspects of salivary fluid secretion in *Calliphora*. *Tissue & Cell* **2**, 281–310.

Oschman, J. L. & Berridge, M. J. (1971). The structural basis of fluid secretion. *Proc. Fedn Am. Socs exp. Biol.* **30**, 49–56.

Prince, W. T. & Berridge, M. J. (1973). The role of calcium in the action of 5-hydroxytryptamine and cyclic AMP on salivary glands. *J. exp. Biol.* **58**, 367–384.

Ramsay, J. A., Brown, R. H. J. & Croghan, P. C. (1955). Electrometric titration of chloride in small volumes. *J. exp. Biol.* **32**, 822–829.

Robyt, J. F., Ackerman, R. J. & Keng, J. G. (1972). Reducing value methods for maltodextrins. II. Automated methods and chain-length independence of alkaline ferricyanide. *Analyt. Biochem.* **45**, 517–524.

Selinger, Z. (1975). Diverse functions of calcium in the rat parotid acinar cell. In *Calcium transport in Contraction and Secretion* (ed. E. Carafoli *et al.*), pp. 139–146. North-Holland.

Waterhouse, D. F. (1940). Studies of the physiology and toxicology of blowflies. V. The hydrogen ion concentration in the alimentary canal. *Pamphl. Coun. scient. ind. Res. Aust.* **102**, 7–27.