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Ion and Water Transport by Isolated Cockroach Salivary Glands

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Summary. When the isolated salivary glands of the cockroach *Nauphoeta cinerea* Olivier are stimulated by dopamine, the putative neurotransmitter, they secrete a fluid containing (mM): Na, 121; K, 47; Cl, 143. Stimulation of glands by 5-hydroxytryptamine or the neurotransmitter evokes a secretion identical in Na composition to this. Dopamine-evoked secretion is abolished in the absence of extracellular Na. The relationship between the rates of fluid secretion and Na transport is linear. However, at very low rates of secretion the Na concentration falls. Calcium, K and Cl ions can be removed from the bathing solution without abolishing fluid secretion. Our evidence suggests that (i) the primary secretion is formed by active transport of Na in the acini, and (ii) the ionic composition of this secretion is modified by re-absorption of Na and an independent secretion of K in the ducts.

The accessibility and relatively large size of cells in insect tissues have encouraged the study of cellular processes in these animals. In particular, investigations of fluid transport mechanisms in salivary glands (Berridge, 1977; Prince, 1977), Malpighian tubules (Maddrell, 1977) and guts (Blankemeyer & Harvey, 1978) of a number of species have proved fruitful.

Cockroach salivary glands have a structure reminiscent of some mammalian exocrine glands with distinct acini and ducts composed of a variety of cell types (Bland & House, 1971; Whitehead, 1971). The innervation of this gland in the cockroach *Nauphoeta cinerea* (Olivier) has been examined microscopically by several techniques (Bland *et al.*, 1973; Bowser-Riley, 1978; Maxwell, 1978). The functional properties of the salivary nerves have been investigated by electrophysiological methods (House, 1973; Ginsborg & House, 1976; Blackman, Ginsborg & House, 1979), and it is evident that the putative neurotransmitter, dopamine, causes membrane permeability changes in acinar cells (Ginsborg, House & Silinsky, 1974). To complement these studies, information on the

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secretory function of the gland was desirable. Therefore an isolated perfused preparation was developed to allow the collection of saliva elaborated under controlled conditions. Preliminary observations suggested that the inorganic composition of the saliva resembled that of mammalian saliva (Smith & House, 1977); further observations on this subject are reported here.

Materials and Methods

The isolated perfused cockroach salivary gland has been described fully elsewhere (Smith & House, 1977). Briefly, the whole salivary apparatus (including the reservoirs) was removed from adult insects of either sex allowed free access to food and water. The preparation was mounted in a perspex chamber (vol. 1.5 ml). Then the secretory ducts were carefully separated from the adherent reservoir ducts and transected at the anterior end. One of these ducts was ligated at its free end which was then pulled into a pool of liquid paraffin B.P. A small hole just posterior to the ligature permitted escape of secreted fluid which was removed at intervals to a second paraffin pool for optical determination of droplet diameter and hence volume. Samples of saliva could then be taken into constant-volume micropipettes (volume 50–150 nl) for analysis.

The normal perfusion medium consisted of (mM): NaCl, 160; KCl, 10; $CaCl_2$, 5; glucose, 20; HCl, 4; Tris, 5; pH 7.6. The chamber holding the glands was perfused continuously at 2 ml/min except when solutions were changed at a rate of 10 ml/min for brief periods. Stock solutions of agonists were made up fresh for each experiment, stored at 4 °C and diluted into 50–100 ml of perfusion medium immediately before use. During prolonged stimulation the agonist solution was renewed every 10–20 min. Modifications of the normal perfusion solution are detailed in text and figure legends. The agonist drugs (dopamine, noradrenaline, adrenaline, and 5-hydroxytryptamine) were purchased from Sigma and other chemicals from BDH.

Nerve stimulation was carried out by drawing the reservoir duct into a suction electrode and delivering rectangular pulses (0,5 msec, 40 V) at 5 Hz for the desired period (House & Smith, 1978).

Sodium and potassium ions were determined in saliva by atomic absorption spectrometry (Pye Unicam SP 90 A). Samples of saliva were allowed to fill a constant volume micropipette and discharged into 2 ml of diluting fluid (water for sodium analysis, 2 mM NaCl for potassium analysis). Samples of standard solutions taken with the same pipette gave calibration signals in each experiment. Chloride was estimated directly by coulombometric titration (Radiometer CMT 10 chloride meter).

Results

I. Requirements for fluid secretion

Sodium. The secretory response of cockroach salivary glands to dopamine requires Na ions. The effect on such responses of reducing extracellular Na concentrations to low levels is reversible. In the experiment

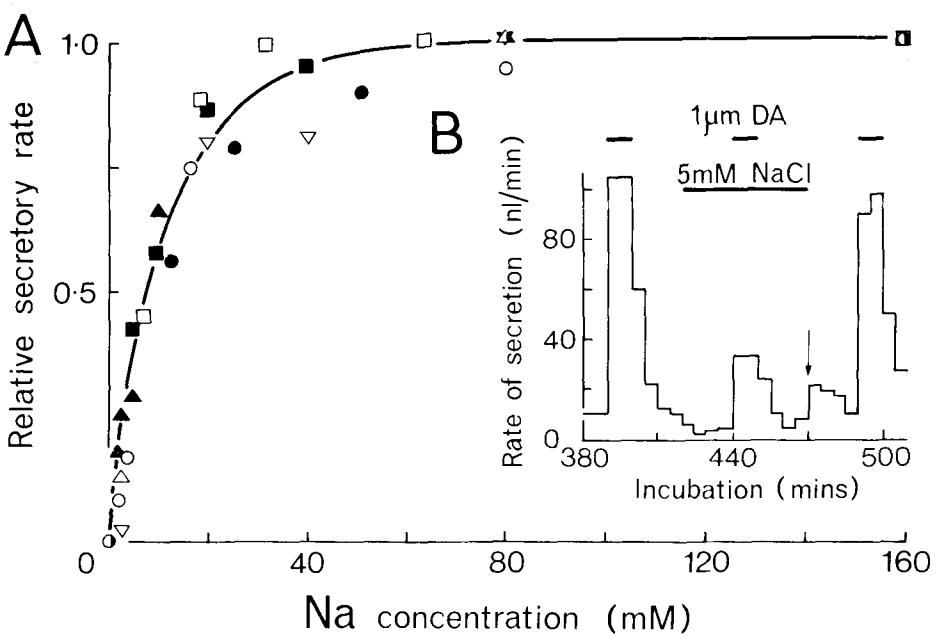

Fig. 1. (*A*): Variation of dopamine-elicited secretory rate with the Na concentration of the bathing fluid. Glands were bathed in a mixture of normal solution and a choline solution (in which 160 mM choline chloride was substituted for NaCl, the other components being identical to those of normal solution) in the desired proportions. Dopamine (1 μM) was applied in the bathing fluid for 10-min periods and the secretory rate was determined at 5-min intervals. Each rate was normalized by dividing it by the value observed in the same gland bathed in normal solution. Different symbols represent results from different glands. Triangles represent results obtained in the presence of 90 mM KCl in the bathing solution. (*B*): Effect of low Na concentration on the response to dopamine. Normal solution was passed through the chamber before and after exposure to the low Na solution (bar). Dopamine (1 μM) was applied for 10-min periods (short bars). A transient increase in basal secretory rate on readmission of normal solution is marked with an arrow

illustrated in Fig. 1 *B*, a maximal response to 1 μM dopamine was initially obtained in the normal perfusion medium. Subsequently dopamine was washed out and fluid transport returned to the basal rate. The bathing solution was then changed to one containing 155 mM choline chloride and 5 mM NaCl. After an equilibration period the gland was then exposed to 1 μM dopamine which evoked a much smaller response than before. When the low Na solution was replaced by the normal medium there was an immediate increase in the unstimulated secretory rate, which declined over the next 20 min (Fig. 1 *B*, arrow). (It is interesting to note that Petersen and Ueda (1976) have reported a similar transient increase in amylase output from mammalian pancreas caused by Na restoration

after acetylcholine stimulation.) The suppression of salivary secretion in low Na solutions was not due to the permanent impairment of secretory ability since maximal responses could be obtained within 20 min of return to the normal solution (Fig. 1 *B*).

Experiments on several glands showed that the inhibition of secretory responses to dopamine was related to the degree of Na depletion. Results are shown in Fig. 1 *A* in which the responses at different Na concentrations are normalized with respect to the rate elicited from each gland in normal medium. The curve suggests that below about 50 mM the rate of Na transport can limit fluid secretion. Let us therefore assume that water movement is driven by NaCl transport into the lumen of acini. Entry of Na into transporting cells might be entirely passive. The electrochemical gradient driving this Na influx could be substantial since the membrane undergoes a large hyperpolarization when dopamine is applied (*see*, e.g., Bowser-Riley & House, 1976). Thus it is possible that the relation between the rate of fluid secretion and Na concentration could be altered by severely depressing or abolishing the electrical response to dopamine. We have tested this idea by examining the relationship in preparations bathed in a high K solution which should virtually abolish the hyperpolarization (*cf.* House, 1973). Salivary glands were exposed to a medium containing 90 mM KCl and various concentrations of NaCl, choline chloride being its replacement. No change in the dependence of secretory rate on Na concentration could be detected (Fig. 1 *A*, triangular symbols). It thus seems likely that the normal Na electrochemical gradient is not dominated by the electrical component. Consequently, we suggest that there is a steep Na concentration gradient across the membrane and, moreover, the intracellular Na concentration must reach very low values when the extracellular Na concentration is lowered as observed in toad urinary bladder (Frazier, Dempsey & Leaf, 1962) and frog skin (Rick *et al.*, 1978).

It is clear from the results in Fig. 1 that the absence of Na prevents fluid secretion even although K and Ca were still present in the external medium. Furthermore, high concentrations of K did not affect the concentration of Na in the secreted fluid. This is quite unlike the behavior of other insect tissues such as Malpighian tubules (Maddrell, 1977) which can transport fluid as a result of both Na and K secretion, their rates of movement depending on ambient concentrations.

Potassium. The effects of variation of extracellular K concentration on the secretory response to dopamine were studied by bathing glands

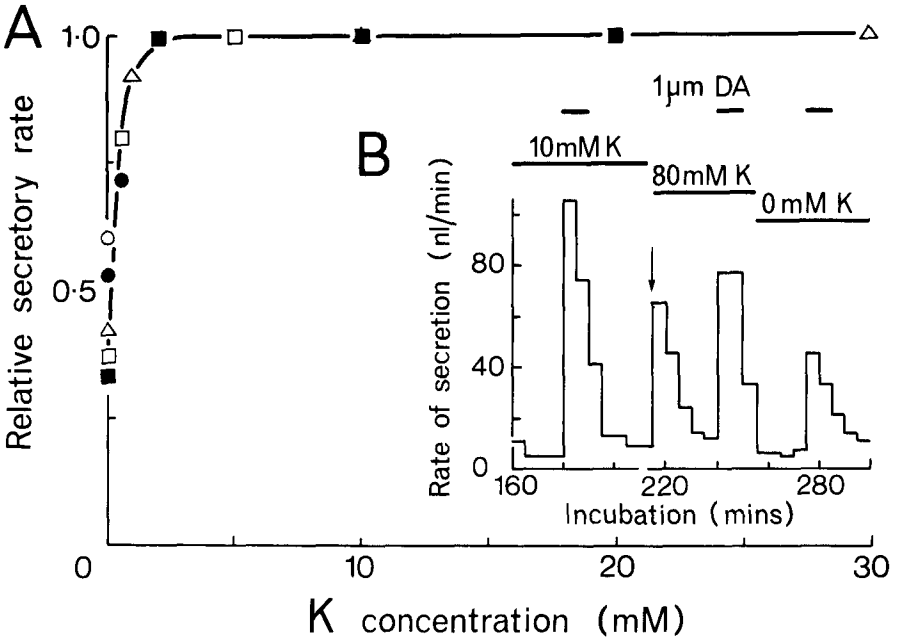

Fig. 2. (*A*): Variation of dopamine-elicited secretion with the K concentration of the bathing fluid. For K concentrations above 30 mM the normal solution was mixed with a KCl solution (in which 160 mM KCl was substituted for NaCl, the other components being identical to those of normal solution) in the desired proportions. To obtain final K concentrations between 10 and 30 mM, appropriate KCl was added to normal solution. For concentrations below 10 mM normal solution was mixed with a K-free solution (in which 10 mM choline chloride was substituted for KCl, the other components being identical with those of normal solution) in the desired proportions. Glands were stimulated for 10-min periods with 1 μM dopamine and secretory rate determined at 5-min intervals. Each rate was normalized as described for Fig. 2. Different symbols represent results from different glands. (*B*): Effect of varying K concentration on dopamine-elicited secretion. During exposure to a series of K concentrations the gland was stimulated by 1 μM dopamine for 10-min periods. The arrow marks a transient increase in basal rate observed on admitting the high K solution to the chamber

in different media in which KCl was substituted for NaCl or choline chloride for KCl. Admission of K concentrations greater than 30 mM to the perfusion chamber often caused a transient increase in the basal secretory rate. These responses to high K solutions probably arose from depolarization of acinar nerves and concomitant transmitter release because the presence of phentolamine, an inhibitor of transmitter action (Bowser-Riley, House & Smith, 1978), significantly suppressed these transients.

An example of an experiment in which the extracellular K concentration was varied is illustrated in Fig. 2*B*. In this case a gland was stimu-

lated with 1 μM dopamine in normal medium containing 10 mM KCl and 160 mM NaCl, and a large response occurred. After the rate of secretion had returned to basal level the perfusion solution was changed to one with 80 mM KCl and 90 mM NaCl. This solution elicited a secretory transient (Fig. 2*B*, arrow). Within 25 min the secretory rate had returned to basal level and 1 μM dopamine was administered; a somewhat smaller response that that in normal medium occurred. Immediately after exposure to dopamine, the gland was bathed in a K-free solution. Adminstration of dopamine after 20 min gave a response only 40% of the control in normal medium.

Figure 2*A* shows the results of several experiments compared by normalizing the secretory rates relative to those in standard medium. Complete K removal from the medium reduced responses to dopamine by 40–70%. The relative magnitude of the responses in different K solutions rose with K concentration up to 2 mM and was maximal above this value. Reduced responses to dopamine were obtained with K concentrations above 30 mM although this was not invariably the case.

Chloride. The secretory response to dopamine was reversibly abolished in the presence of a Cl-free solution in which NaCl had been replaced by Na methylsulphate and the other chlorides by sulphates. (It was necessary to reduce Ca concentration to 1 mM in the Cl-free solution, but this modification itself causes no change in secretory response.) With increasing Cl concentration the secretory response rose and reached a maximum above 100 mM; normalized results relative to responses in normal solution are shown (Fig. 3*A*). Use of NO_3 instead of methylsulphate to replace Cl did not abolish fluid secretion, responses being reduced by about only 40% (Fig. 3*B*).

Re-admission of normal solution after Cl omission led to a transient increase in the basal rate of secretion.

Calcium. Douglas and Poisner (1963) found that Ca-free solution appreciably depressed the rate of fluid secretion from mammalian salivary glands within 20 min and nearly abolished it after 40 min. Corresponding experiments on cockroach salivary glands, however, did not indicate a similar dependence of fluid secretion on extracellular Ca. An example of such an experiment is illustrated in Fig. 4. Test concentrations (0.1 μM) of dopamine were applied throughout for 10-min periods (short bars). After constant responses were obtained in normal medium containing 5 mM $CaCl_2$ (upper long bar) the chamber was perfused with a solution

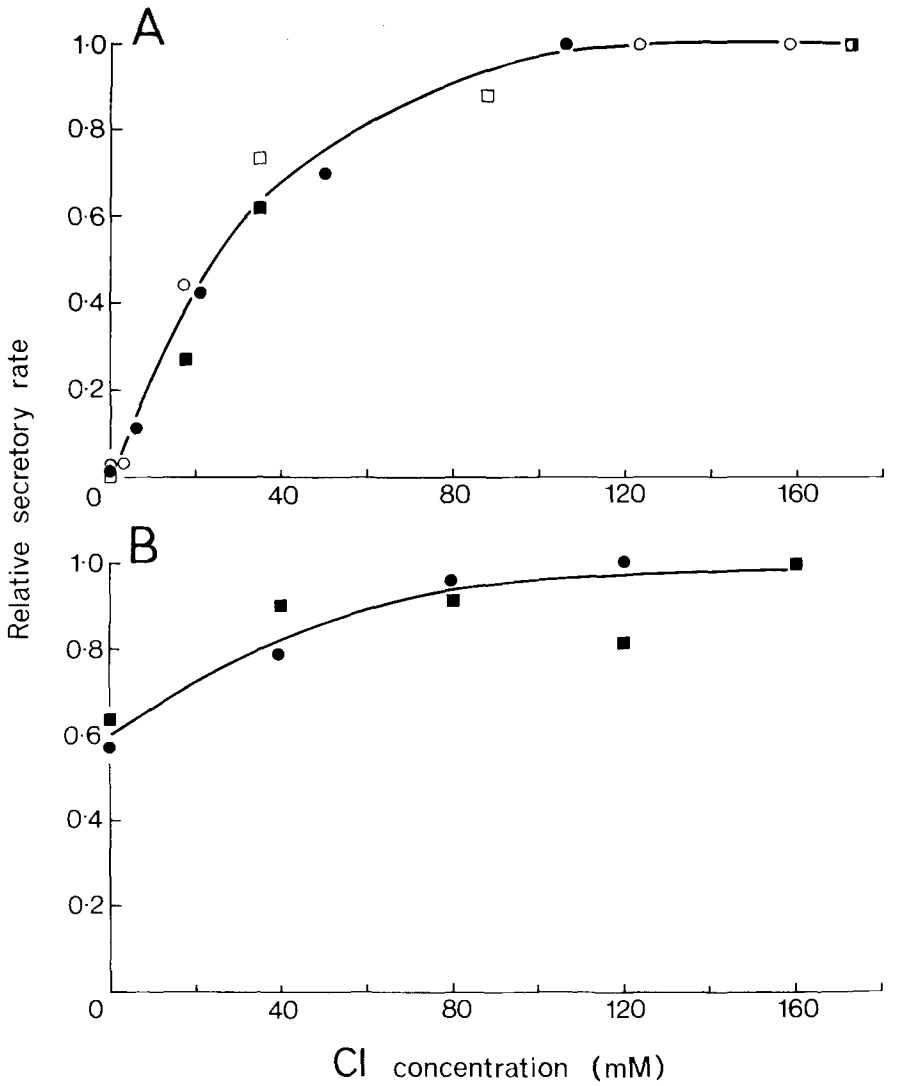

Fig. 3. (*A*): Variation of dopamine-elicited secretory rate with the Cl concentration of the bathing fluid. The Cl concentration was changed by mixing normal solution with a sulphate solution containing (mM): Na methylsulphate, 160; K_2SO_4, 5; $CaSO_4$, 1; Tris, 5; H_2SO_4, 2. Experiments were carried out as described for Fig. 1. (*B*): Variation of dopamine-elicited secretion with the Cl concentration of the bathing fluid when the Cl replacement was NO_3. The solution that was mixed with normal solution was identical to the sulphate solution except that 160 mM $NaNO_3$ was used instead of Na methylsulphate

containing in addition 5 mM $MgCl_2$ (lower long bar). Within 20 min the response to dopamine was reduced by 20%. A slight inhibitory effect of high Mg concentrations on mammalian salivary glands has also been

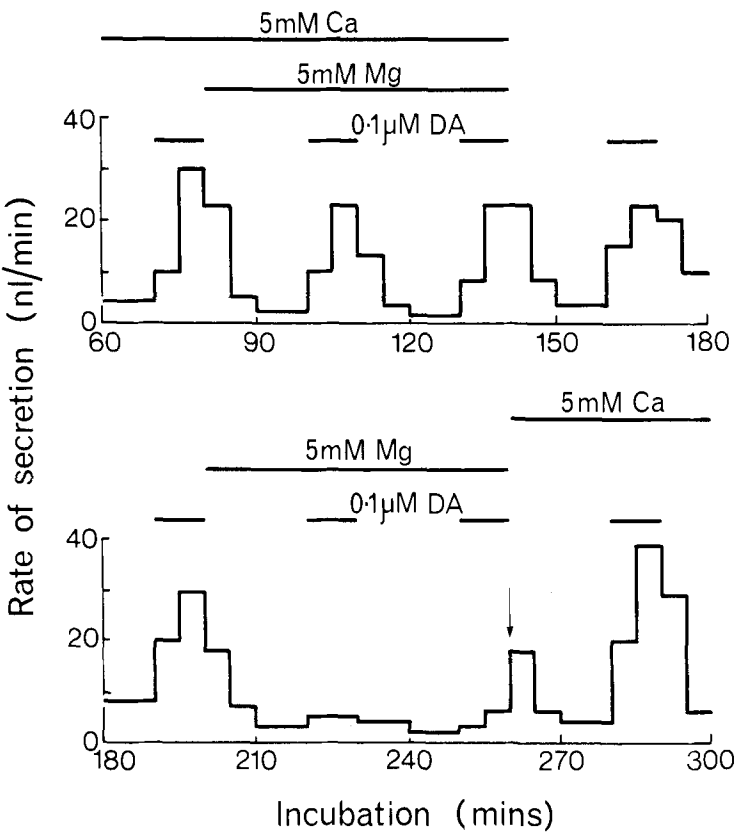

Fig. 4. Effect of Mg on responses to dopamine in the presence or absence of Ca in the bathing fluid. In this experiment the gland was bathed first in normal solution and stimulated with 0.1 μM dopamine for 10-min periods (short bars). The presence of Ca and Mg is indicated by the long bars. The arrow marks an increase in the rate of secretion observed on re-admission of Ca to the chamber

observed by Douglas and Poisner (1963). After one hour in this solution the gland was bathed in a solution lacking both Ca and Mg. Responses persisted in these conditions and after 50 min returned to the amplitude observed in normal medium. The perfusion solution was then changed to one containing 5 mM $MgCl_2$ without Ca and the response to dopamine was virtually abolished within 20 min. After one hour the gland was again bathed in normal solution whereupon the response to dopamine regained its magnitude within 20 min. Indeed, a transient increase in the secretory rate immediately occurred on re-admission of Ca. This transient could have been due to the persistence of dopamine from the preceding stimulation period or may have been caused by release of endogenous neurotrans-

mitter; in either case it argues strongly for a rapid reversal by Ca of the inhibitory effect of Mg.

II. Effects of Ouabain

The cardiac glycoside ouabain at 0.1 mM had no observable effect on the secretory response to dopamine of glands bathed in the standard solution or in one containing reduced K concentration (1 mM). However, it did cause progressive inhibition of the secretory response to electrical stimulation of the salivary nerve.

III. Composition of Saliva

Sodium. Evidence for ductal modification of saliva in mammals was obtained when it was noted that the ionic composition of saliva varied with flow rate (Thaysen, Thorn & Schwartz, 1954). The principal finding was that the Na concentration increased with rate whereas the K concentration fell. Since this might also hold for the cockroach, whose salivary glands have a similar structure, we stimulated isolated glands with various concentrations of dopamine to obtain fluid secreted at different rates for analysis. In each experiment the rate of secretion in the presence of dopamine was measured at 5-min intervals. The fluid secreted during the first 10 min was discarded but subsequently duplicate samples were taken for atomic absorption spectrometry. At low rates of secretion it was necessary to pool consecutive 5-min samples to obtain sufficient volume for analysis. In five experiments the salivary Na concentration (mean $\pm$ SEM $= 120.7 \pm 2.4$ mM) was independent of secretory rate above 15 nl/min (Fig. 5). Some variation between tissues was apparent (Table 1) and in some other experiments lower Na concentrations were observed (*see* Table 2). Saliva secreted at rates less than 15 nl/min had significantly lower Na concentrations (mean $\pm$ SEM $= 51.1 \pm 5.7$ mM; $P < 0.001$ according to Student's t test) than that formed at higher rates (Fig. 5).

The concentration of Na in cockroach saliva appears to be lower than that of the bathing medium. To determine whether the extracellular concentration of Na could affect its salivary concentration, several glands were bathed in solutions with different Na concentrations, osmolarity being maintained by choline. Samples were obtained first in normal medium and then in low Na solution after an equilibration period of

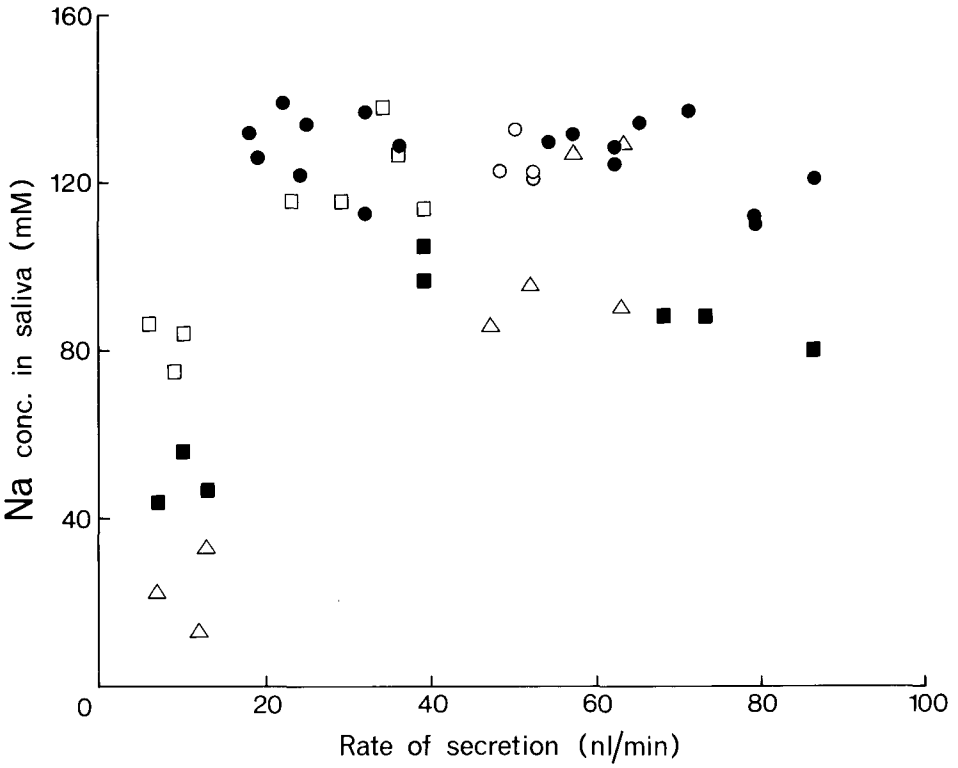

Fig. 5. Sodium concentration of cockroach saliva secreted at different rates in response to different dopamine concentrations in the bathing fluid. Different symbols represent observations on different glands

Table 1. Sodium composition of cockroach saliva

Experiment	Mean conc. (mM)	SEM	*n*
240	125.3	2.6	4
242	127.5	2.1	17
243	122.2	4.6	5
244	92.2	4.1	5
245	120.5	5.0	13

20 min. Collection of samples of saliva was as described above. We have expressed the results in terms of net transport rate of Na given by:

Net transport rate = ion concentration × volume secreted per min.

A similar procedure was adopted for K and Cl transport (*see* below).

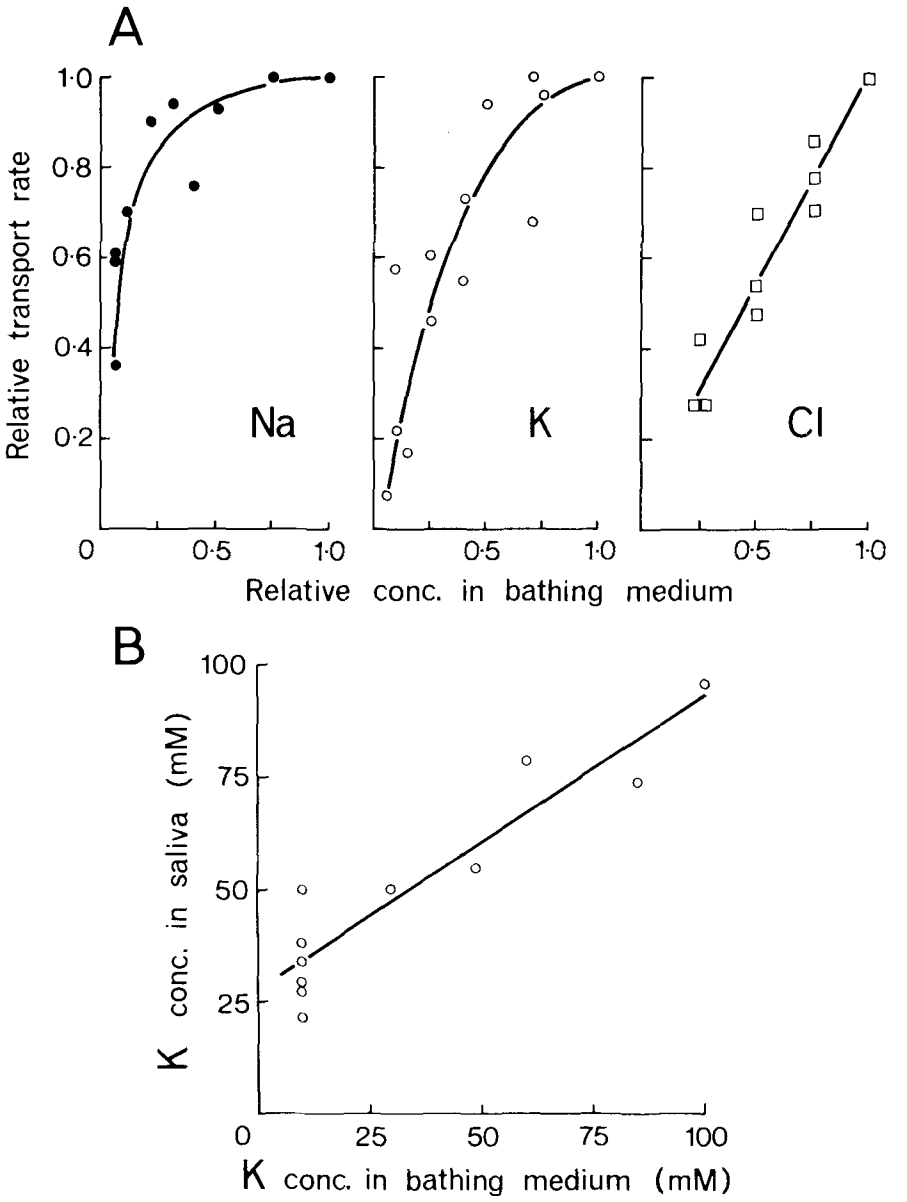

Fig. 6 (*A*): Transport of Na, K and Cl in salivary glands exposed to various concentrations of these ions in the bathing fluid. To obtain reduced concentrations of these ions in the solution, NaCl in normal medium was replaced by choline chloride (or $NaNO_3$) and KCl by choline chloride. Results from a number of experiments with each ionic species were normalized relative to the secretory rate and ion concentration in the normal solution. (*B*): Concentration of K in saliva from glands bathed in elevated K solutions. The increase in K concentration was achieved by replacing proportions of NaCl in the normal solution with KCl

Values for net Na transport in normal conditions were 5–15 nmol/min and rates observed at different Na concentrations were normalized relative to the rate in normal conditions. The rate of Na transport was weakly dependent on Na concentration in the bathing solution over a wide range (50–160 mM). Below 30 mM the rate fell steeply with Na concentration to reach zero when Na was absent in the external medium (Fig. 6*A*). This resembles the behavior of other epithelia known to transport Na actively (e.g., Frazier *et al.*, 1962; Ussing, 1949).

In mammalian salivary glands there is evidence for an exchange of Na for K during the passage of saliva along the ducts (Schneyer, 1969). This might also occur in cockroaches since the Na concentration is lower in saliva than in the bathing solution, and the opposite is true for K. However, the fall in salivary Na concentration that happens at low flow rates is not accompanied by a corresponding rise in K concentration as observed in mammalian glands. It has already been noted moreover that a drop in K concentration causes a fall in the rate of fluid secretion (Fig. 2*A*). This seems at variance with the properties of a Na/K exchange system in the ducts which merely modifies a primary secretion of NaCl and water.

The effect of a reduction in K concentration on the Na transport system in the cockroach salivary gland was examined in several experiments, an example being illustrated in Fig. 7. A gland was continuously stimulated by dopamine and for a period of 80 min (upper bar) the K concentration in the bathing medium was reduced from 10 to 0.1 mM. This reduction produced a decline in the secretory rates of fluid and Na into the saliva and this behavior might suggest that the low K solution rate-limits active Na transport in some way. This point will be taken up again in the Discussion along with the question of the observed rise in salivary Na concentration under these conditions.

It has been suggested elsewhere (House & Smith, 1978) that separate receptors for dopamine and 5-hydroxytryptamine exist in cockroach salivary gland while those for the neurotransmitter cannot be distinguished at present from dopamine receptors. It was of interest therefore to examine the ionic composition of saliva evoked by these agonists to see if differences occur. Table 2 shows the concentrations of Na in fluid secreted in response to nerve stimulation and bath applications of dopamine and 5-hydroxytryptamine. Evidently the Na concentrations were identical under those different stimulating conditions, and this suggests that the receptors for dopamine, 5-hydroxytryptamine, and the neurotransmitter activate the same secretory mechanism.

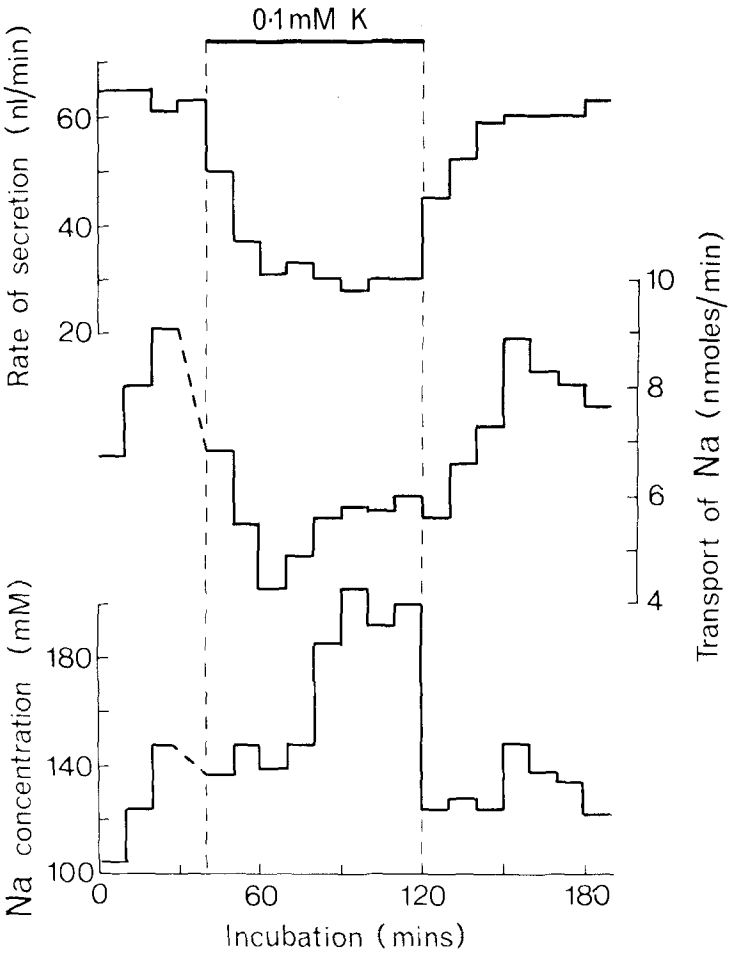

Fig. 7. Effect of reducing K concentration in the bathing fluid on rate of secretion, Na transport, and salivary Na concentration. This gland was exposed continuously to 1 μM dopamine, and after 20 min samples were taken for Na determination. After a further 40 min the K concentration was reduced from 10 to 0.1 mM (denoted by bar) for a period of 80 min. The effects of this low K solution on both fluid secretory rate and the Na concentration in saliva are shown; the transport rate for Na was calculated as described in the text

Table 2. Sodium composition of saliva

Stimulus	Mean conc. (mM)	SEM	*n*
Dopamine (1 μM)	100.9	5.4	12
5-hydroxytryptamine (1 μM)	107.5	2.4	10
Electrical (5 Hz, 60 V)	106.5	2.7	11

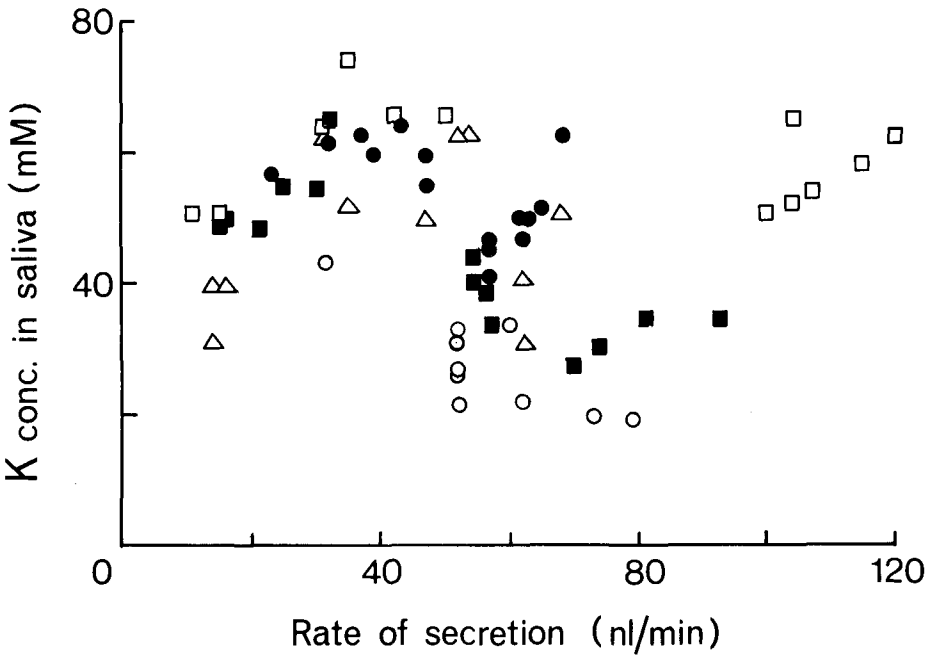

Fig. 8. Potassium concentration of saliva secreted by glands at various rates in response to different concentrations of dopamine. Different symbols represent results from different glands

Potassium. Samples for K analysis were collected in the same way as for Na analysis except that since the K assay was relatively insensitive it was impossible to take duplicate samples at high rates of secretion or to analyze saliva at rates below 15 nl/min.

Results from five experiments are shown in Fig. 8 and Table 3. No variation in K concentration over the rate 15–115 nl/min was detected (mean $\pm$ SEM $= 46.8 \pm 1.7$ mM). In view of the pronounced fall in Na concentration of saliva secreted at slow rates (Fig. 5), it was somewhat surprising not to record a prominent rise in K concentration at the low end of the flow range. As observed in the Na analysis, there was variation in the salivary K concentration between different glands (Table 3). The rate of K transport, calculated as above for Na, was dependent over a wide range on the K concentration in the bathing medium (Fig. 6*A*). Indeed, the transport characteristics for K were markedly different from those for Na. At concentrations above 10 mM the K concentration in saliva increased in direct proportion to the concentration in the bathing solution (Fig. 6*B*).

Table 3. Potassium composition of cockroach saliva

Experiment	Mean conc. (mM)	SEM	*n*
247	23.1	5.5	10
248	53.5	1.8	16
249	59.7	2.3	12
252	43.1	2.9	14
253	45.1	3.5	11

Table 4. Composition of reservoir contents

Ion	Mean conc. (mM)	SEM	*n*
Na^+	5.2	0.6	10
K^+	11.8	1.0	9
Cl^-	14.5	1.5	15

Chloride. The pattern of sample collection for Cl analysis had to be modified from that described for Na and K to suit the sensitivity of the method used. Saliva was collected over long periods of up to 30 min during continuous dopamine stimulation. For glands bathed in the normal bathing solution containing 185 mM Cl, the Mean $\pm$ SEM values of salivary Cl concentration were 143 ± 6.0 mM (6 experiments). Chloride was also assayed in saliva produced by glands bathed in media in which Cl was progressively replaced by NO_3. Over the range 40–160 mM the transport of Cl was closely dependent on its concentration in the bathing medium (Fig. 6*A*).

IV. Composition of Reservoir Fluid

On dissection of *N. cinerea* the reservoirs are frequently found to be full of a colorless liquid. For ionic analysis the reservoirs were ligated at the anterior end, dissected from the animal and punctured by a micropipette under liquid paraffin. Samples of up to 20 μl were taken for Cl, Na and K analyses. The reservoir fluid was found to be very dilute compared with saliva obtained from the end of the salivary duct, and the relative abundance of the cations differed, K being present at higher concentration than Na (Table 4). Our findings confirm observations made on the reservoir fluids of other cockroaches (Laird, Winston & Braukman, 1972; Wall, 1970). It has been suggested that the reservoirs act

as water stores. Since thirsty cockroaches drink water by immersing the whole of the mouthparts it is possible that water could be pumped directly into the reservoirs up the common duct where it would dilute the saliva. We have found that when thirsty cockroaches drink colored water to repletion the liquid is confined to the gut. Furthermore, since the ratio of Na/K concentrations is different in the reservoirs from that in saliva, it is likely that the reservoirs are filled slowly from the common salivary duct or that they are capable of modifying saliva when it has entered them.

Discussion

The results described above have led us to adopt the following working hypothesis (Fig. 9) for the elaboration of cockroach saliva. A basic assumption of this hypothesis is that the primary secretion of ions and

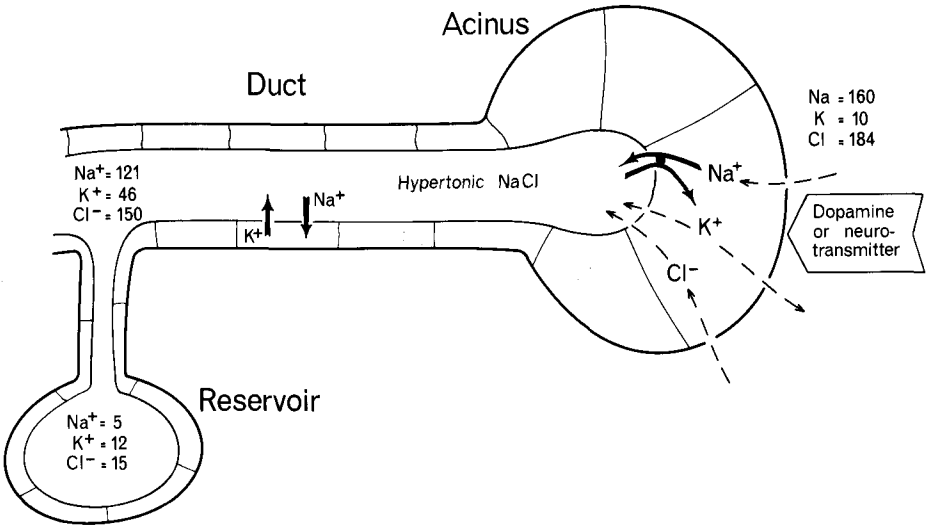

Fig. 9. Model scheme for the elaboration of saliva by cockroach salivary gland. Broken arrows indicate passive movements and solid arrows represent active transport. It is suggested that nerve or dopamine stimulation causes an increase in the permeability of the basal membrane to K and Na. As a result K moves from acinar cells into the bathing medium and possibly into the saliva if the signal for the K permeability increase is an intracellular one. The Na electrochemical gradient will move Na into the acinar cells across the basal membrane and the rise in intracellular Na concentration will stimulate active Na extrusion into the acinar lumen. It is possible that Cl ions enter cells in a coupled form with Na and pass into the lumen down an electrochemical gradient

water occurs in the acini and that some modification of the ionic composition of this fluid occurs in the ducts. Although we have no compelling evidence for this view, it seems the most attractive approach at present.

Binding of neurotransmitter, dopamine, or 5-hydroxytryptamine to their respective receptors on acinar cells causes changes in the ionic conductance of the basal membrane. An increase in K conductance is apparently responsible for the hyperpolarization of acinar cells in these circumstances and this is accompanied by a secondary change in conductance to another ion (Ginsborg *et al.*, 1974). It is interesting that seemingly homologous electrical responses can be recorded in mammalian salivary glands where a K-dependent hyperpolarization masks an increase in Na permeability (Petersen, 1970*a*). The existence of a K permeability change is compatible with the finding that mammalian salivary gland cells lose K to the extracellular fluid shortly after stimulation (Burgen, 1967). Presumably there are similar movements of K from cockroach acinar cells into saliva and external medium upon activation of acinar receptors. Thus we may expect some K in the primary secretion in the acinar lumen. It is highly unlikely that this kind of K transport can generate a significant water flow since in Na-free solutions containing 10–90 mM KCl no salivary secretion was evident. The cockroach salivary gland is clearly quite unlike those of *Calliphora* (Oschman & Berridge, 1970) and *Antherea* (Kafatos, 1968) in which K transport provides the driving force for fluid movement. In fact our evidence points to Na transport as the likely source of fluid secretion and we suspect that the additional conductance change that occurs in cockroach acinar cells is due to an increase in Na permeability. Thus activation of acinar receptors would lead to a Na influx down the electrochemical gradient from the external medium into the acinar cytoplasm. This gradient will be enhanced over that of the resting membrane due to the presence of the hyperpolarizing response to stimulation. However, this enhancement is probably not substantial since high K solutions known to suppress the hyperpolarization reduced the rate of fluid secretion only marginally in some glands. A more marked effect of elevated K solutions was observed on fluid secretion from mammalian salivary glands (Petersen, 1970*b*). In cockroach salivary glands it seem unnecessary to postulate active transport of Na into acinar cells from the external medium. Alternatively, we suppose that a Na/K pump on the luminal membrane of the acinar cells extrudes Na from the cells into the lumen at a rate that increases with intracellular Na concentration. It seems possible from our results that the cytoplasmic Na concentration can reach very low

values and that a powerful Na extrusion mechanism operates as has been suggested for other transporting epithelia (e.g., Spring & Hope, 1979).

Glands bathed in low K solutions secreted fluid at a reduced rate, and this was attributed to the depressed rate of Na transport. It is conceivable that under these conditions the luminal K concentration falls below normal limits and thereby inhibits the rate at which Na can be transported into the lumen of the acini. A further consequence of low luminal K concentration would be some kind of compensatory rise in luminal Na concentration necessary to maintain the osmolarity of the secretion at its usual value. It would be worthwhile to examine this question with an appropriate microtechnique for determining Na and K concentrations in the acinar lumen. Much of the above speculation depends in the postulated site of the Na pump. In keeping with our suggestion of a luminal membrane location is the failure to obtain inhibition with ouabain in the external medium. Apparently ouabain is notoriously unreliable as an inhibitor of Na transport in insect tissues for a variety of reasons, the most notable being the high K concentrations found in the bathing media for insects (Anstee & Bowler, 1979). However, our experiments with ouabain were made in solutions containing either 10 or 1 mM K and thus seem free from that criticism. Furthermore, in the same experimental conditions ouabain was capable of abolishing the neurally evoked secretory response and electrical response (C.R. House, *unpublished*). At present, therefore, it seems reasonable to conclude that the Na pump site is inaccessible to ouabain applied in the external medium.

It seems unlikely that Cl is actively transported across acinar cells into the saliva. The rate of transport of this ion is closely related to its concentration in the bathing solution and, moreover, fluid movement can be completely abolished in Cl solutions where Na is replaced by by choline. Furthermore, NO_3 can accompany Na ions into saliva whereas methylsulphate cannot, and this resembles the behavior of *Calliphora* Malpighian tubules where it is probable that Cl moves by passive diffusion (Berridge, 1969). We are unable to draw a conclusion about the route of chloride movement in cockroach salivary glands although it is possible that Cl crosses the basal acinar membrane in a coupled form with Na as has been suggested for rabbit ileum (Nellans, Frizzell & Schultz, 1973) and gallbladder (Frizzell, Dugas & Schultz, 1975).

Undoubtedly the net transport of NaCl provides the driving force for water movement between the bathing medium and saliva. In a variety

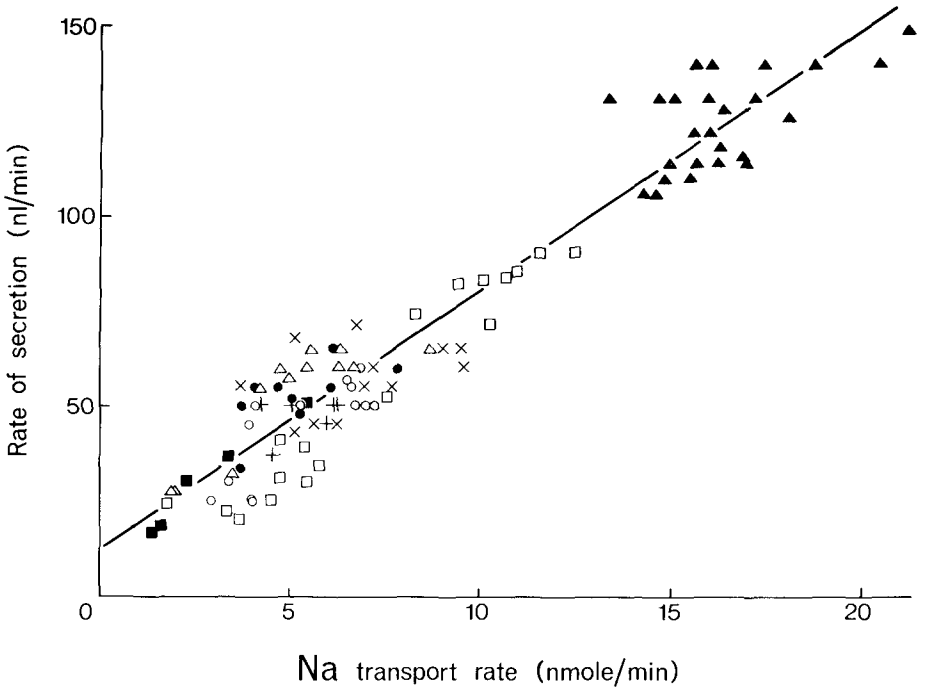

Fig. 10. Relationship between the rate of fluid secretion and the net transport rate of Na into the saliva from the bathing solution. The transport rate for Na has been calculated as described in the text. Different symbols represent results from different glands

of ionic environments and over a wide range of secretory rates the Na concentration in the saliva is generally constant. In experiments such as that shown in Fig. 7 fluid transport and net Na movement changed in parallel. The constant relationship between water and Na transport is brought out clearly in Fig. 10 where results of a number of experiments covering several ionic conditions, some of which drastically altered the secretory rate, are plotted. A linear relationship between fluid and Na transport rates prevails. This reflects the constancy of the salivary Na concentration observed over a wide range of secretory rates obtained under different conditions. The regression line in Fig. 10 has a slope of 6.76 nl/nmol which corresponds to an average Na concentration in saliva of 148 mM.

The mechanism described so far is capable of producing hyper- or isotonic NaCl solutions. The fluid collected from the end of the salivary duct is clearly different from this and some modification processes must be proposed. We suggest that modification occurs in the ductal tree

where Na is re-absorbed and K is secreted. Evidence for Na re-absorption is suggested by two facts. Firstly, the saliva has a lower Na concentration than the bathing solution. Our evidence indicates that K transport into the primary secretion is unlikely to be an important driving force for water transport. Thus it seems highly probable that the Na concentration in the primary secretion exceeds, or at least nearly equals, that of the bathing solution and is subsequently reduced by re-absorption. Secondly, the decline in Na concentration of saliva elaborated at very low rates implies that the primary secretion has been exposed to a re-absorption system longer. In mammalian salivary glands a similar fall in Na concentration is accompanied by a rise in K. This was not evident in our results. Moreover, when the concentration of K was raised above 10 mM its salivary concentration also rose in a roughly linear fashion over a wide concentration range. This is quite unlike the saturable variation in Na transport observed in corresponding experiments where the external Na concentration was changed. Since the salivary Na concentration remains unaffected by high concentrations of K in the bathing medium, it appears that some K movement into saliva can occur independently of Na re-absorpiton.

Our model of salivary secretion by the cockroach is rather similar to the mechanisms believed to operate in mammalian salivary glands (Petersen, 1971), but it differs in one important respect. Fluid secretion by mammalian glands is gradually reduced in Ca-free solutions to virtual abolition within 40 min (Douglas & Poisner, 1963). In contrast, the cockroach salivary gland readily responds to dopamine even after 60 min in Ca-free medium, and there is no prominent reduction in the magnitude of responses. However, Mg is a potent inhibitor of the secretory response to dopamine in the absence of Ca. The rapid reversal of the effect of Mg when Ca is re-admitted suggests that these ions compete for a superficial site important in stimulus-secretion coupling.

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