room for 48 h. At this time they were placed on moist blotter paper and the percentage hatch subsequently determined. Each experiment consisted of 6 replicates of 10 eggs per replicate, and the experiment was duplicated. The temperature was maintained at 25 ± 0.1 °C.

Results. The figure summarizes the pattern of loss of weight (weight loss equals water loss[1]) of eggs exposed to 48% RH after 96 h in water vapor or on moist paper. Eggs preconditioned in water vapor did not lose a significant amount of weight until the weighing at 24 h; those preconditioned on moist paper lost significant weight (5% level) in the first 15 min. Eggs from moist paper weighed 85.3 µg (SD = 3.3 µg) at the first weighing and 37.4 µg (SD = 7.7 µg) after 48 h; those from water vapor weighed 74.8 µg (SD = 6.4 µg) at the start and 52.7 µg (SD = 6.6 µg) at 48 h. Thus eggs from moist paper weighed significantly more (0.5% level) at the outset of the experiment, but they weighed significantly less (0.5% level) at the finish. This could be interpreted to mean that the measured loss was simply evaporation of water from the outside surface of the eggs, but it has been demonstrated that, given the weighing procedure used, superficial water does not contribute to changes in weight[1]. Furthermore, the weight at the end of 48 h was significantly lower in the eggs acclimated on moist paper so they had lost more weight.

When both sets were placed on moist paper and subsequent hatch was determined, hatch was significantly higher (70% SD = 13%) for the eggs acclimated at 100% RH than for eggs acclimated on moist paper (10% SD = 10%).

I conclude therefore that eggs acclimated in the water vapor system are less susceptible to water loss than are those acclimated on moist paper. Furthermore, this difference in water loss was parallelled by higher mortality in the group that lost more water.

For purposes of description, I have referred to water states as 'vapor' and 'moist paper'. The moist paper system is easy to describe: microscopic examination of the eggs and paper showed water on the paper fibres on which the eggs rested. The precise condition of water in the so-called vapor state is unknown. The difficulty of knowing the actual state of water in a hygrostat that purports to be at 100% RH has been reviewed[3,4]. As Edney[3] pointed out, it is probably not possible to have a hygrostat with 100% RH without some droplets of liquid water some place therein. I did not see water droplets on eggs or in containers nor on the walls of the hygrostat.

I assume therefore that water was more readily available to eggs on moist paper than to those in the hygrostat but my observations do not permit precise definition of the state of the water in this water vapor system. I conclude that in this species, the water conservation mechanism of the egg is adaptable. The information has obvious significance for laboratory studies of egg shell permeability and also for evaluation of water availability as a mortality factor in field populations.

3 E. B. Edney, in: Water relations of terrestrial arthropods, p. 55. Cambridge Univ. Press, Cambridge 1957.
4 E. Bursell, in: Physiology of insecta, vol. 2, p. 343. Ed. M. Rockstein. Academic Press, New York 1973.

Fluid secretion by isolated cockroach salivary glands

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Summary. An isolated preparation of the innervated cockroach salivary gland has been developed to study secretion. This gland secretes a fluid rich in Na and Cl in response to nerve stimulation or bath application of dopamine.

The salivary glands of a number of insects receive an innervation which is probably dopaminergic[2–4]. Microelectrode recording from acinar cells in the cockroach salivary gland has revealed that nerve stimulation causes a hyperpolarisation[5] that can be mimicked by bath applications of several biogenic amines, the most potent being dopamine[6]. In order to study fluid secretion evoked by nerve stimulation and dopamine in this tissue we have adapted the technique originally used by Ramsay[7] for urine collection from insect Malpighian tubules. It is notable that previous reports of the composition of insect salivas have shown that the principal cation is potassium[8,9] while that in vertebrates is sodium. Our results demonstrate that cockroach saliva is sodium rich and resembles more closely that of certain mammals than of the insects studied so far.

Materials and methods. Whole paired glands, consisting of reservoirs, reservoir ducts, acini and acinar ducts, were dissected from adult cockroaches (Nauphoeta cinerea) of either sex, allowed free access to food and water. Dissection was carried out under the perfusion medium (pH 7.6) which had the following composition (mM): NaCl, 160; KCl, 10; NaH_2PO_4, 1; $NaHCO_3$, 1; $CaCl_2$, 5; Tris, 5; HCl, 4; glucose, 20. Each main acinar duct was freed from its adherent reservoir duct and the glands were placed in a perspex chamber (volume 1.5 ml). One of the acinar ducts was ligatured near its cut end with enamelled Ag wire and pulled into a pool of liquid paraffin, separated from the perfusion chamber by a perforated celluloid barrier. The tissue was anchored in the chamber by a ligature round the reservoirs (figure 1). The chamber was perfused at a rate of 2.5 ml/min by a Watson-Marlowe flow inducer (MHRE 200); the rate was increased to 10 ml/min when solutions were changed.

1 Acknowledgments. We are indebted to the Science Research Council for financial support.
2 N. Klemm, Comp. Biochem. Physiol. *43A*, 207 (1972).
3 K. P. Bland, C. R. House, B. L. Ginsborg and I. Laszlo, Nature New Biol. *244*, 26 (1973).
4 H. A. Robertson, J. exp. Biol. *63*, 413 (1975).
5 C. R. House, J. exp. Biol. *58*, 29 (1973).
6 F. Bowser-Riley and C. R. House, J. exp. Biol. *64*, 665 (1976).
7 J. A. Ramsay, J. exp. Biol. *31*, 104 (1954).
8 F. C. Kafatos, J. exp. Biol. *48*, 435 (1968).
9 J. L. Oschman and M. J. Berridge, Tissue Cell *2*, 281 (1970).

Ionic composition of saliva

Species	Ionic concentration (mmoles/l) Na	K	Cl	HCO_3
Cockroach (Nauphoeta)*	140.9±4.5	25.7±1.5	151.7±6.5	–
Moth (Antherea)[8]	1	190	19	180
Blowfly (Calliphora)[9,10]	20	160	160	–
Human parotid[11]	90	20	50	60
Horse parotid[12]	56	15	50	52
Sheep parotid[13]	170	13	11	112

* Values given are means ± SE.

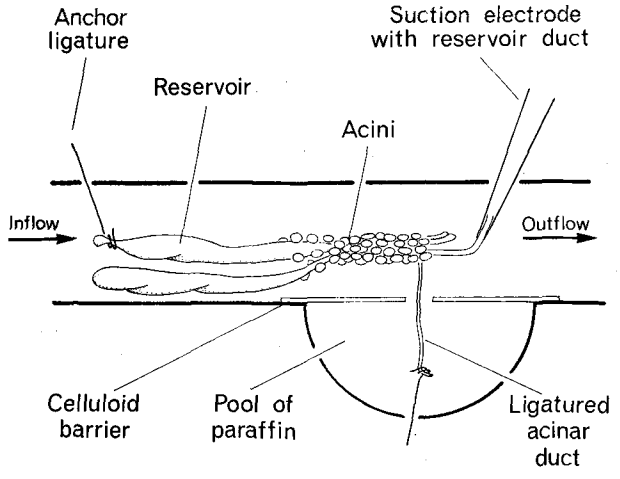

Fig. 1. Plan view of experimental arrangement for investigating fluid secretion in isolated cockroach salivary glands. Tissues were stimulated either by delivering electrical pulses to the salivary nerve held in the suction electrode or by perfusing the chamber with dopamine solutions.

For electrical stimulation, the reservoir duct (bearing the salivary nerve) on the same side as the ligatured acinar duct was drawn into a suction electrode. Square-wave stimuli (0.5 ms duration) were delivered either by a Grass stimulator (SD 5) or by isolated stimulator (Devices Type 2533) driven by a gated pulse generator (Devices Type 2521) and a Digitimer.

Chemical secretagogues were made up as concentrated (1 mg/ml) stock solutions kept at 4 °C and diluted into the perfusion medium immediately before use. The secretory rate was determined by removing secreted fluid at suitable intervals to a second paraffin pool with silicone-treated micropipettes where the diameter of the droplet was measured optically. Unstimulated tissues were found to secrete at 0–3 nl/min. Secretory responses to nerve stimuli and dopamine were observed for at least 5 h after isolation. Sodium and potassium concentrations in the secreted fluid were estimated by atomic absorption spectrophotometry (Pye Unicam SP90A) and chloride by coulombometric titration (Radiometer CMT10).

Results and discussion. Figure 2 shows typical secretory responses to nerve stimulation (upper panel) and dopamine (lower). With electrical stimuli the secretory rate rose to a maximum within 3–5 min and after 10–15 min declined toward the basal value. The magnitude of the response increased with stimulus voltage over the range 2–60 V. Usually stimuli of 30 V were used since these gave near maximal responses and were thought less likely to exhaust the tissue. Dopamine also increased the rate of secretion. Above a threshold of about 3×10^{-9} M dopamine produced secretory responses graded with concentration up to a maximum at 10^{-6} M. Each secretory re-

10 W. T. Prince and M. J. Berridge, J. exp. Biol. *58*, 367 (1973).
11 J. H. Thaysen, N. A. Thorn and I. L. Schwartz, Am. J. Physiol. *178*, 155 (1954).
12 F. Alexander, J. Physiol., Lond. *184*, 646 (1966).
13 R. N. B. Kay, J. Physiol., Lond. *150*, 515 (1960).

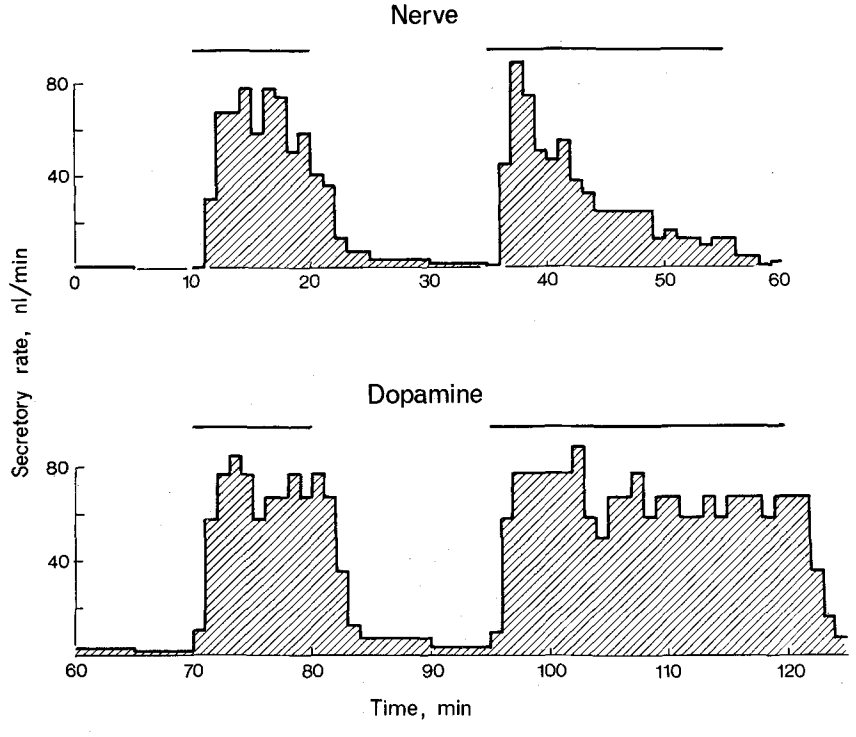

Fig. 2. Typical secretory responses evoked by nerve stimulation and dopamine. Periods of stimulation are indicated by horizontal bars. Electrical stimuli (50 V, 0.5 ms) were delivered at a frequency of 10 Hz. After the secretory responses to nerve stimulation dopamine solutions (2×10^{-6} M) were passed through the chamber for 10 min and subsequently for 25 min. Prolonged nerve stimulation (upper right) was accompanied by a fall in the secretory rate whereas prolonged exposure to dopamine (lower right) was not.

sponse reached a peak within about 5 min and during prolonged exposure to dopamine the secretory rate fell usually to a maintained lower value after about 10 min. In the same preparation the amplitudes of the maximal responses to dopamine and nerve stimulation were identical (figure 2) and in the range 60–100 nl/min.

Glands were stimulated for 20–30 min periods with dopamine (10^{-7}–10^{-6} M) and the secreted fluid collected for either chloride (14 samples) or sodium and potassium analysis (9 samples). The results given in table indicate that the major cation in the saliva is sodium and that chloride is present at a concentration almost equal to the combined concentrations of sodium and potassium. The composition of certain other salivas is also shown in the table for comparison. It can be seen that cockroach saliva is different from both insect salivas and is somewhat similar to the mammalian ones. Other experiments have shown that salivary secretion evoked by dopamine in Nauphoeta glands is reversibly abolished in sodium-free medium. Coupled with the analytical results this suggests that fluid secretion in this tissue is driven by active sodium transport.

The effects of emetine hydrochloride on brain protein synthesis and on dark avoidance response in the goldfish

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Summary. Emetine hydrochloride at 100 μg injected intracranially blocked more than 80% of protein synthesis of the brain in the goldfish for at least 4 days, but was not lethal. Testing the effect of emetine hydrochloride on dark avoidance go-no go learning, the fish injected with emetine hydrochloride showed poorer performance than those injected distilled water throughout the experiment, except on the 1st day, suggesting potentialities of emetine as a new blocking agent of memory consolidation.

It is generally believed that memory consolidation is inhibited by the blockage of protein synthesis in animals[3]. The mechanisms of chemical actions of protein synthesis blockers, however, are neither simple nor yet completely clarified. Cycloheximide[4–7] and acetoxycycloheximide[8–11], for example, have been said to block directly memory consolidation through the interruption of protein synthesis, whereas the inhibitory actions of puromycin[12,13] are less clear than that of other antibiotics. Thus, the relation between protein synthesis and memory consolidation has been suggested by means of only a few specific drugs, but is not generalized up to now. So, in the present experiments, emetine hydrochloride (E-HCl), which is an ipecac alkaloid and is reported to block the protein synthesis for long duration[14], was used for the first successful attempt to clarify relations between its inhibitory effects on protein synthesis and blocking actions on memory consolidation.

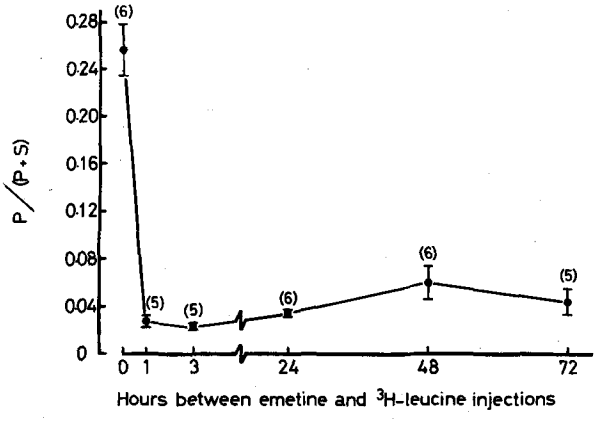

Fig. 1. The time course of protein inhibition in goldfish brain resulting from an intracranial injection of emetine hydrochloride (100 μg/10 μl). Abscissa 0 is the control injection of 5 μCi of ³H-leucine. Number of fish for each point is shown in parentheses. Dots and bars represent, respectively, means and standard errors of P/(P+S).

Materials and methods. The subjects used were goldfish, 9.5–11.0 cm long, in these experiments. Time course of inhibition of protein synthesis caused by 100 μg/10 μl of E-HCl was determined in goldfish brain. At 1, 3, 24, 48 or 72 h after the injection of E-HCl, 5 μCi/5 μl of L-leucine-4, 5-³H (specific activity 58 Ci/mmole, The Radiochemical Center, Amersham, England: ³H-leucine) was injected. Fish were decapitated 30 min later, the whole brain was quickly removed and protein was isolated as the trichloroacetic acid (TCA) precipitate fraction. The degree of protein synthesis was determined by the following formula:

$$P/(P+S) = \frac{\text{cpm in TCA precipitate (P)}}{\text{cpm in TCA precipitate (P)} + \text{cpm in TCA supernate (S)}}$$

When ³H-leucine was injected intracranially, the temperature of the water in the home tank was 18 °C.

Goldfish were given dark avoidance go-no go training for 3 days. For intracranial injections, a small hole was drilled in the centre of the skull between the 2 eyes, 3 days before the beginning of training trials[15]. Fish were not fed. On the previous day of training, fish were divided randomly into experimental and control groups, and the experimental group was injected with E-HCl 100 μg/10 μl in distilled water, while the control group was given 10 μl of distilled water.

For 3 days of training, a fish was given 2 sessions of 20 trials of dark avoidance go-no go training in each day which consisted of 10 go training tirals and 10 no-go training trials, randomly ordered. Intersession interval was 2 min.

The apparatus used was a gray plastic shuttle box with a trapezoidal barrier[16]. The temperature of water in both apparatus and home tanks was 16.0–18.0 °C. An electric light bulb (20 W/100 V a.c.) was placed over each compartment at 20 cm from surface of the water. These bulbs were ordinarily lighted and the CS was to put off either of the 2 bulbs for 10 sec. Which bulb was put off was determined in accordance with both the sequence of go and no-go trials and where the fish was, and it was con-