

containing 11, 13, 15, 17, 19 and 21% oxygen and the number of bar-presses was recorded.

**Results and discussion.** The results obtained (table) indicate that, by increasing the amount of oxygen in the gas mixture entering the helmet, the number of bar-presses was decreased. As the oxygen concentration increased from 9% to 11%, there was already a marked reduction (about 50%) in bar-presses. The subsequent stepwise increase in oxygen concentration resulted in a further decrease in bar-presses. At 21% oxygen, this number was decreased by about 77% as compared to the initial value. This response pattern suggested that the animals were sensitive to changes in oxygen concentration, and modified their bar-press rates accordingly. Consequently, another experiment was carried out in which the trained monkeys were exposed to a mixture containing 21% oxygen for a period of 30 min. The oxygen concentration was then reduced to 9% (equili-

bration of gas mixture in the helmet was attained within 1 min), and the animals were maintained at 9% oxygen for another 30 min. The number of bar-presses under these 2 conditions was recorded. The mean number of bar-presses at 21% oxygen was 94 and increased to 379 during exposure to 9% oxygen, which corresponds to a relative increase of over 300%.

These findings indicate that squirrel monkeys respond to hypoxia in their environment and readily learn to change the noxious conditions appropriately. The results also suggest that oxygen itself has no positive reinforcing effect, since the number of bar-presses decreased when the oxygen concentration was increased.

Since lack of oxygen can adversely affect cerebral circulation and metabolism<sup>5</sup>, resulting in impaired performance, this experimental model may be of value in testing drugs assumed to influence cerebral metabolism processes.

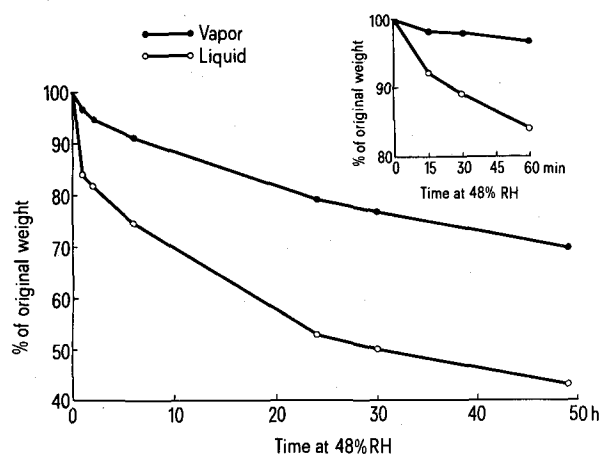
## Changes in water permeability of an insect egg in response to level of water in the environment

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**Summary.** The water permeability of eggs of the southern corn rootworm, *Diabrotica undecimpunctata howardi* Barber, varied depending on the previous experience of the eggs. Eggs acclimated in a 'water vapor' system were seen to be less permeable than eggs acclimated in contact with moist paper, as measured by the rate of loss of water from the eggs and the susceptibility of the eggs to death from desiccation. This is the first known report of the ability of an insect egg to adapt to the level of water available in the environment.

During the course of studies of the moisture relations of eggs of the southern corn rootworm, *Diabrotica undecimpunctata howardi* Barber, some observations suggested the level of water available to young eggs influenced water permeability as the eggs became older<sup>1</sup>. This phenomenon has apparently not been reported. However, it would be of obvious significance for studies of egg shell permeability and for evaluations of water availability as



Loss of weight (= water) from southern corn rootworm eggs preconditioned in 2 levels of water availability, vapor and contact with moist paper expressed as the percentage of original weight. Inset in upper right of figure indicates the pattern of weight loss in the first 60 min. Eggs were 0–4 h old when placed at the 2 conditions. After 96 h of acclimation, they were placed at 48% RH, and weighings were begun within 2.5 min.

a mortality factor in field populations. I therefore designed an experiment to test whether the apparent phenomenon was real. The results are described here.

**Materials and methods.** Eggs were obtained from a colony maintained at this laboratory by previously described methods<sup>2</sup>. The methods of measuring egg weight and determining egg hatch have been described<sup>1</sup>. Eggs were acclimated to 2 different levels of water availability, one, termed 'moist paper', in which the eggs rested on moist blotter paper in a plastic petri dish sealed with parafilm, and another, termed 'water vapor', in which eggs rested on 80-mesh stainless steel screen in glass hygrometers through which air (100% RH) was pumped continually (see Krysan<sup>1</sup> for details of the method). It has been demonstrated that eggs hatched equally well when left to complete embryogenesis in either of these 2 systems<sup>1</sup>. The water amount present in the 'moist paper' system is the amount that provides optimum oviposition in our laboratory colony, allows optimum hatch in our standard laboratory procedures, and probably does not differ markedly from the amount of water in the soil in natural oviposition sites. Eggs were acclimated in the systems for 96 h and then removed and placed in a balance room maintained at 45–48% RH, 25°C.

The first weighing of the treated eggs took place 2.5 min after they were removed from the acclimating environment. Thereafter the eggs remained in the balance room and were weighed at intervals until they had been in the

1. J. L. Krysan, *Entomologia exp. appl.* 30, 154 (1976).
2. T. F. Branson, P. L. Guss, J. L. Krysan and G. R. Sutter, in: *Corn rootworms: Laboratory rearing and manipulation*, p. 18. U. S. Dept. Agric., ARS-NC-28, 1975.

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  $25 \pm 0.1^\circ\text{C}$ .

**Results.** The figure summarizes the pattern of loss of weight (weight loss equals water loss<sup>1</sup>) 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  $85.3 \mu\text{g}$  (SD =  $3.3 \mu\text{g}$ ) at the first weighing and  $37.4 \mu\text{g}$  (SD =  $7.7 \mu\text{g}$ ) after 48 h; those from water vapor weighed  $74.8 \mu\text{g}$  (SD =  $6.4 \mu\text{g}$ ) at the start and  $52.7 \mu\text{g}$  (SD =  $6.6 \mu\text{g}$ ) 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<sup>1</sup>. 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 paralleled 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 hygostat that purports to be at 100% RH has been reviewed<sup>3,4</sup>. As Edney<sup>3</sup> pointed out, it is probably not possible to have a hygostat 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 hygostat.

I assume therefore that water was more readily available to eggs on moist paper than to those in the hygostat 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 insects*, 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<sup>2-4</sup>. Micro-electrode recording from acinar cells in the cockroach salivary gland has revealed that nerve stimulation causes a hyperpolarisation<sup>5</sup> that can be mimicked by bath applications of several biogenic amines, the most potent being dopamine<sup>6</sup>. In order to study fluid secretion evoked by nerve stimulation and dopamine in this tissue we have adapted the technique originally used by Ramsay<sup>7</sup> 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<sup>8,9</sup> 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;  $\text{NaH}_2\text{PO}_4$ , 1;  $\text{NaHCO}_3$ , 1;  $\text{CaCl}_2$ , 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.

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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).