THE PERMEABILITY OF THE SHELL OF THE EGG OF *ACHETA COMMODUS* WALKER* (ORTHOPTERA, GRYLLIDAE)

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(*Received* 11 *July* 1959)

INTRODUCTION

During development the eggs of *Acheta commodus* almost double their original weight by absorbing water. Virtually all this increase occurs during the third and fourth day of incubation at 27° C. (Browning, 1953). In all insect eggs that have been studied and found to absorb water, the period during which water is taken in is always restricted to a specific, brief stage of embryogenesis (Edney, 1957).

The eggs of *Phyllopertha horticola* absorb water only between the third and eighth days at 20° C. and Laughlin (1953, 1957) explains this by assuming that the membranes, having been impermeable to water at first, become permeable on the third day and water enters the eggs in response to an osmotic gradient. On the eighth day the membranes again become impermeable, or alternatively water ceases to flow in because of the increased hydrostatic pressure in the egg. Laughlin supported this theory with measurements of the rate at which eggs lost water in dry air at different stages of development and by observations on the morphology of the membranes of the egg. But he added that his physical explanation would not account for the whole process. Slifer (1958 and earlier papers) also considers the entry of water into the eggs of *Melanoplus differentialis* to be controlled by impermeable layers in the shell which later become permeable.

Most authors accept an alternative theory that the movement of water into and out of eggs is under the control of specialized cells (e.g. the hydropyle cells of Acrididae (Slifer, 1938)). The evidence on which this theory rests is that: (i) the eggs of *Locustana* will not absorb water if left in an atmosphere of nitrogen (Mattée, 1951); (ii) the rate of water uptake in some eggs is markedly influenced by temperature (Banks, 1949; Browning, 1953); (iii) the course of water absorption seems to be closely related to the stage of development of the embryo (cf. Edney, 1957); and (iv) dead or injured eggs either do not swell or in some cases may swell until they burst. But in our opinion none of this evidence necessarily conflicts with Laughlin's theory.

* Previously called *Gryllulus commodus* (Browning, 1952, 1953).

We have tested Laughlin's theory using deuterium oxide, and assuming that an egg that is permeable to deuterium oxide may reasonably be considered to be permeable to water also.

METHODS

Freshly laid eggs of *Acheta* were kept at 12·5° C. for about a month to permit diapause development to be completed (Browning, 1952). During this period the eggs do not change appreciably in weight (Browning, 1953). The eggs were then carefully dried by rolling them on paper tissue and leaving them exposed to the air for about 20 min. They were then put on moist filter-paper in a sealed Conway vessel and incubated at 27° C. When water from a sample of eggs was to be collected the eggs were removed from the Conway vessel, dried as before, washed quickly in three changes of distilled water, dried again, placed in the chamber of the apparatus and crushed with a blunt seeker.

The apparatus used for collecting the water consisted of a spherical chamber connected via a ground glass joint and a high-vacuum stopcock to a long, narrow U-tube cold trap. This led, via another high-vacuum stopcock to a 'Speedivac' pump capable of producing a vacuum of 0·1 μ Hg. A cold trap and a McLeod pressure gauge were also placed in the vacuum line. When a sample of water was to be collected the cold trap in the vacuum line was frozen with liquid air and the system evacuated to the stopcock separating the sample chamber from the collecting U-tube. The sample chamber was sealed on to the apparatus and frozen. The stopcock was then opened, the whole system was evacuated, and the stopcock was turned off again. The collecting U-tube was then frozen, the stopcock was opened and the sample chamber was allowed to thaw at room temperature. Water evaporating from the sample chamber was collected for about an hour. About 1 mg. of water was condensed in the U-tube. Both stopcocks were then closed, the apparatus removed from the vacuum line and within 24 hr. the water was analysed in a mass-spectrometer.

The mass-spectrometer used was one designed for handling samples of gas for biological assays (Cooke-Yarborough & Russell, 1953) with a designed accuracy of $\pm 2\%$ in determinations of isotope ratios. With samples of water the accuracy was poor for two reasons. First, water reacts with the tungsten filament of the ion source, and secondly, the instrument has a high water background. In this type of design it is not possible to remove water adsorbed on the surface of the vacuum chamber, so that exchange between the sample and this adsorbed layer may give rise to very large 'memory' effects.

The procedure in handling the sample was designed to minimize this; the sample was admitted to the ion source of the spectrometer for some minutes before taking a reading, so that the water adsorbed in the chamber approached the composition of the sample and constant readings could be obtained. The sample tube was attached to the gas inlet of the mass-spectrometer and the gas handling lines were evacuated, the sample meanwhile being frozen with liquid air. When the sample tube was opened to the gas reservoir of the instrument any rise in pressure in the reservoir was

attributed to air; if this was considerable the sample was discarded, otherwise it was allowed to vaporize into the reservoir, from where it was admitted to the instrument.

We measured the ratio of the peak heights at masses 18, 19 and 20 atomic mass units. The instrument was calibrated with solutions of known dilution from an ampoule of 99% deuterium oxide. The calibration curve (Fig. 1) shows the large scatter of the results. However, the precision was high enough for the purpose of our argument.

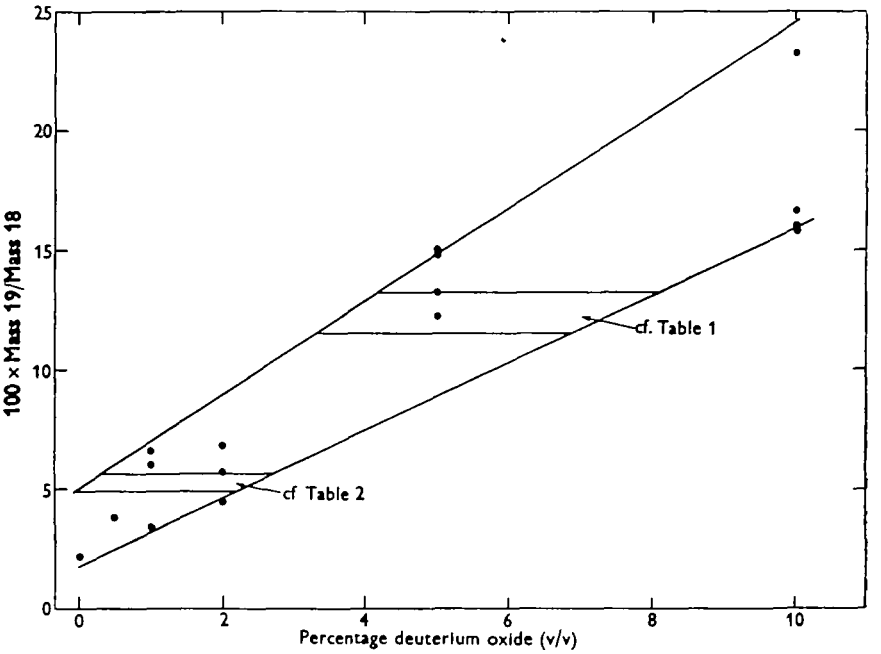

Fig. 1. Calibration diagram used for estimating deuterium-oxide concentrations in this study. All values in Tables 1 and 2 lie in the areas indicated in the figure.

RESULTS

A large number of eggs was taken and placed in a Conway vessel on filter-paper well moistened with a 10% solution of deuterium oxide. Samples of eggs were removed each day for 4 days and the water was extracted and analysed. From Table 1 it can be seen that the deuterium-oxide content of the eggs increased during

Table 1. *Changes in concentration of* D_2O *in eggs incubated in* 10% *solution of* D_2O

Days at 27° C.	Observed ratio*	% D_2O in eggs†	Mean weight of eggs: mg.
0	—	—	0·60 ± 0·04
1	12·7	3·8–8·0	0·61 ± 0·04
2	11·5	3·3–7·2	0·64 ± 0·03
3	13·2	4·0–8·3	0·81 ± 0·21
4	12·5	3·7–7·8	1·14 ± 0·23

* Ratio of 100 × mass 19/mass 18. † Read from Fig. 1.

the first 24 hr. when their weight was remaining constant, and that after this there was little change in the concentration of deuterium oxide in the eggs. This experiment was repeated with essentially similar results, except that the mass ratio of the water extracted from the eggs fluctuated around 10 (cf. Fig. 1) over a period of 7 days.

On the second day of the first experiment twenty-five eggs were removed, washed and placed in another Conway vessel on filter-paper which was moistened with just sufficient water to soak it. They were then replaced in the incubator and at intervals one-quarter of the filter-paper was removed and the water from it collected and analysed for deuterium oxide. At the same time four or five eggs were removed and weighed. The results set out in Table 2 show that during the period when the water content of the eggs was increasing rapidly deuterium oxide was appearing in the water outside the eggs.

Table 2. D_2O *content of water bathing eggs during period of water absorption. Eggs previously bathed for* 2 *days in* 10% D_2O

Days after transfer of eggs	Observed ratio	% D_2O in filter-paper	Mean weight of eggs: mg.
0	—	—	0·64 ± 0·03
1	4·9	0·6–2·3	0·81 ± 0·12
2	5·6	0·9–2·8	1·10 ± 0·27

In another experiment a group of eggs was placed in a Petri dish on moist filter-paper and incubated for 4 days at 27° C. At the end of this time they were removed and eggs weighing less than 1·0 mg. were discarded. A sample was removed and the remainder were placed in a Conway vessel, moistened with 10% deuterium oxide and replaced in the incubator. The water from the first sample was extracted and analysed. At the end of 1 day and 4 days, further samples of eggs were taken and the water from them collected and analysed. The results of this experiment showed that after 1 day the mass ratio of the water from the eggs had risen from 2·2 to 6·5 and had reached 10·3 by the end of 4 days. During this time there was no significant change in the weight of the eggs.

DISCUSSION

The objection may be raised that no net transfer of water had taken place when the eggs were not gaining weight, and that the deuterium found inside the eggs had got there by exchange with hydrogen atoms in the membrane. This cannot be disproved but seems unlikely.

Linderstrøm-Lang (1955) has shown that the hydrogen atoms in proteins in solution may exchange with deuterium from deuterium oxide very rapidly, but that the exchange is limited to labile hydrogen atoms. The membrane of the egg is a tough, modified protein, insoluble, chemically inert and of very high molecular weight. Such a protein has very few reactive groups, and the number of labile hydrogen atoms which could exchange with deuterium would be so low that

exchange could hardly account for the observed rate of movement of deuterium oxide across the membrane. The membrane is certainly permeable to O_2 and CO_2 and the simple explanation of our results is that it is permeable to water also. Apparently water may move across the membranes of the egg in both directions whether the water content of the egg is increasing or not.

SUMMARY

1. The passage of deuterium oxide across the egg membranes of *Acheta commodus* has been studied.

2. Deuterium oxide enters the egg at times when its weight is constant and leaves the egg at times when its weight is increasing as a result of uptake of water.

3. It is considered unlikely that these findings can be accounted for by simple exchange with hydrogen atoms in the membrane.

4. It is concluded that the shell membranes are permeable to water at all times.

REFERENCES

BANKS, C. J. (1949). The absorption of water by the eggs of *Corixa punctata* Illig. (Hemiptera–Corixidae) under experimental conditions. *J. Exp. Biol.* **26**, 131–6.

BROWNING, T. O. (1952). The influence of temperature on the completion of diapause in the eggs of *Gryllulus commodus* Walker (Gryllidae–Orthoptera). *Aust. J. Sci. Res.* B, **5**, 112–27.

BROWNING, T. O. (1953). The influence of temperature and moisture on the uptake and loss of water in the eggs of *Gryllulus commodus* Walker (Orthoptera–Gryllidae). *J. Exp. Biol.* **30**, 104–15.

COOKE-YARBOROUGH, E. H. & RUSSELL, M. C. B. (1953). A simple mass-spectrometer for analyses of stable tracer elements. *J. Sci. Instrum.* **30**, 474–80.

EDNEY, E. D. (1957). *Water Relations of Terrestrial Arthropods.* Cambridge University Press.

LAUGHLIN, R. (1953). Absorption of water by the egg of the garden chafer, *Phyllopertha horticola* L. *Nature, Lond.*, **171**, 577.

LAUGHLIN, R. (1957). Absorption of water by the egg of the garden chafer, *Phyllopertha horticola* L. *J. Exp. Biol.* **34**, 226–36.

LINDERSTRØM-LANG, K. (1955). Deuterium exchange between peptides and water. *Chem. Soc. Lond. Spec. Publ.* **2**, 1–20.

MATTÉE, J. J. (1951). The structure and physiology of the egg of *Locustana pardalina* Walk. *Dep. of Agric. Union of South Africa Sci. Bull.* no. 316, pp. 1–83.

SLIFER, E. H. (1938). The formation and structure of a special water-absorbing area in the membranes covering the grasshopper egg. *Quart. J. Micr. Sci.* **80**, 437–57.

SLIFER, E. H. (1958). Diapause in the eggs of *Melanoplus differentialis* (Orthoptera, Acrididae). *J. Exp. Zool.* **138**, 259–82.