THE EVAPORATION OF WATER FROM HELIX ASPERSA

I. THE NATURE OF THE EVAPORATING SURFACE

By JOHN MACHIN

Department of Zoology, Queen Mary College, University of London*

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INTRODUCTION

It is known that the activity of terrestrial pulmonates is connected in some way to the extensive and often rapid fluctuations in water content which are characteristic of these animals (Howes & Wells, 1934; Wells, 1944). The work of Hughes & Kerkut (1956) and Kerkut & Taylor (1956) has further shown that the activity of the pedal ganglion of slugs changes with the osmotic pressure of the bathing solution. The experiments described in this series of papers are concerned with evaporative water loss, which can cause marked changes in blood concentration. The common garden snail, Helix aspersa, was chosen as the experimental animal.

Although important advances have been made in the field of evaporation from arthropods by Beament (1958, 1959, 1961), detailed studies of evaporation from moist-skinned animals have apparently never been made. Strictly speaking the term 'evaporation' describes two processes, evasion and diffusion. Evasion refers to the vapourization of molecules at the evaporating surface and diffusion to their movement through the adjacent layers of air (Ramsay, 1935). The work presented here is relevant to the vapourization of water molecules at the outer surface of the snail. Since the skin is covered by a thin layer of mucus, it is necessary to consider the properties of this mucous layer and the means by which it is maintained.

NATURE AND MAINTENANCE OF THE MUCOUS LAYER

Observations

Microscopic examination of active snails revealed that the surface layer of mucus is spread thinly on the raised areas (tubercules) of the skin, and tends to collect in the grooves. An indication of this distribution is given by the reflected light patterns shown in Pl. 1, fig. 1. Clear-cut, rounded highlights are produced in the grooves where the mucus is relatively deep and its surface smooth (arrow A). On the other hand, thinly covered areas over the tubercules are distinguished by irregular patches of many reflected pin-points of light (arrow B), scattered by minute irregularities of the skin's surface.

Attempts were made to dry the skin of freshly drowned specimens by blowing air over them with an electric fan. Drying was indicated by an increase in the area thinly covered by mucus. Observations on recently drowned animals showed that it was possible to continue this drying until the mucus in the grooves was completely dried.
up. It was found that as the mucus receded in the grooves it was progressively more
difficult to dry it up. The almost dried skin of a drowned snail is shown in Pl. 1, fig. 2.
The scattered highlights along the grooves indicates that the mucus has been almost
completely removed. If drying was continued beyond this stage, the skin became dull
and leathery. It was possible, however, to relubricate it with fresh mucus by lightly
rubbing the animal with a finger or by artificially increasing the internal haemocoelic
pressure of the animal.
When the drying process was repeated with living snails, it was found that the
mucous layer could never be extensively removed from the skin, even when very rapid
air currents were used. Incipient drying of the skin appeared to cause local muscular
undulations which spread mucus from the grooves out over the affected areas. It was
thought that the undulations might form part of a large-scale mechanism of mucus
movement along the grooves, similar perhaps to the regular waves of contraction which
are reported by Nisbet (1961) in the skin of some members of the Achatinacea. How-
ever, no significant movement or spreading of small patches of carmine or Lycopodium
powder placed in different regions of the body was observed, suggesting that a mechan-
ism of this type does not exist in Helix aspersa.

Experiments with isolated skin preparations

The effects of drying out and of hydrostatic pressure increases were further in-
vestigated using isolated skin preparations. Simultaneous surface-temperature
measurements by means of thermocouples provided an assessment of the evaporation
rate of the preparation.

The sequence of events in which living integument from the dorsal part of the
snail’s body was first isolated and then stretched in specially designed clamps is
illustrated in Text-fig. 1. Several methods failed because muscular activity made it
difficult to keep the preparation sufficiently stretched during clamping. This prob-
lem was finally solved by using a toothed metal ring which gripped the preparation
inside a thick rim of foot and mantle tissue left attached to the dorsal integument.

The clamp and preparation were then incorporated into one wall of a small con-
tinuous-circulation wind tunnel to be described in detail in Machin (1964). In these
experiments the wind tunnel was filled with dried air which was circulated at a
constant speed over the preparation. The completely assembled apparatus, which en-
abled one side of the isolated integument to be bathed in Ringer solution, is shown in
section in Text-fig. 2. It consists of three units: the preparation clamp, a glass Ringer
vessel, and a reservoir. The clamp is constructed of ‘Perspex’ in two halves, secured
by four brass screws. It can be reversed easily, being externally symmetrical. The glass
vessel which contains the bathing fluid is sealed with silicone grease and a neoprene
gasket behind the clamp, and is held in position by a brass plate retained by four
adjustable nuts. In these experiments a 0.7 Locke solution (Cardot, 1921) was gravity-
fed from the reservoir which allowed hydrostatic pressures of up to 40 cm. of water
to be exerted on the inside of the membrane. This pressure was measured directly in
a vertical graduated tube. To make sure that the skin could not be remoistened by the
Locke solution leaking through it, each preparation was separately tested to maximum
hydrostatic pressure before each experiment.

Text-fig. 3 shows typical simultaneously recorded depressions in surface tempera-
Evaporation of water from Helix aspersa. I

tures of isolated integument and of a thin porous earthenware plate soaked in distilled water, expressed against time. The membranes were held in the opposite walls of the wind tunnel. The surface temperature rapidly dropped at first in both cases to a steady minimum, appropriate to the temperature, humidity and wind speed maintained in the tunnel. Once the supply of superficial water had been depleted, and the rate of evaporation decreased, the temperature began to rise again. With the porous plate, as expected, little resistance to water loss was evident and the temperature rise took place relatively rapidly, except in the very last stages where water had to be removed from within the plate itself. The slow regular temperature rise of the skin preparation, on the other hand, indicated that desiccation was a gradual process. At the end of the experiment the skin was thin, brittle and parchment-like in the centre, where it had been exposed, but still moist and flexible where the clamp had gripped it. Complete immersion in snail Ringer for a few hours restored the dried area's flexibility.

The effect of increasing the hydrostatic pressure of the Locke solution was studied with similar preparations. The preparations were placed in position on the wind tunnel and subjected to a hydrostatic pressure of 2.5 cm. water. Text-fig. 4 shows a typical surface-temperature record in this type of experiment. It can be seen that a short time after the initial equilibration period the temperature began to rise as the supply of surface mucus was depleted. At this point the hydrostatic pressure was increased to 10 cm. water for one minute and then reduced to 2.5 cm. again. The increased hydrostatic pressure involved of course the addition of extra Ringer which was
Text-fig. 2. Diagrammatic cross-section of apparatus used in experiments with isolated body-wall preparations.

Text-fig. 3. Simultaneously recorded surface-temperature curves of an isolated skin preparation (●) and a thin porous earthenware plate soaked in distilled water (○).
Evaporation of water from Helix aspersa. I

slightly warmer than the evaporating surface of the preparation. The surface temperature was therefore raised, but again quickly decreased to the equilibrium minimum. This indicated that the original rapid evaporation rate had been continued and the mucous coating of the skin restored. This could be repeated again and again. After several times, however, the preparation was allowed to dry more thoroughly, for about 1 hr., before increasing the internal pressure again. Text-fig. 4 shows that, as a result of continued surface desiccation, extensive relubrication was no longer possible.

Text-fig. 4. Surface-temperature curve of an isolated skin preparation, to show the effect of hydrostatic pressure on evaporation.

PROPERTIES OF ISOLATED MUCUS

Collection of fresh uncontaminated mucus in sufficient quantities was difficult. The mucus usually found as a thin layer covering the snail's surface could be collected by gently scraping with a coverslip or glass rod, but then only in small quantities. These samples will be referred to below as 'normal' mucus. Since some experiments required relatively large samples, alternative sources of snail mucus had to be exploited. Campion (1961) has shown that the electrical stimulation of Helix will cause the animal to produce large quantities of mucus rather different in appearance and consistency to 'normal' mucus. However, in the present investigation, it was found that large samples of a bright yellow mucus could be easily obtained by pressing the snail with a flat spatula, especially in the mantle region. Samples collected in this way will be referred to in future as 'stimulated' mucus.
Estimates of the water content of isolated samples were obtained by weighing immediately after collection and then allowing them to evaporate to dryness. The samples were suspended on small wire loops on a torsion balance reading to 0.1 mg. The water content of ‘normal’ and ‘stimulated’ mucus, expressed as a percentage of the original net weight of the sample, is given in Table 1. It will be seen that the amount of evaporable water is very high in both cases, being in excess of 88%.

Table 1. The water content of ‘normal’ and ‘stimulated’ mucus samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>% of wet weight</th>
<th>Mean ± s.e.</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Normal’ mucus</td>
<td>88.0</td>
<td>91.0</td>
</tr>
<tr>
<td>‘Stimulated’ mucus</td>
<td>92.3</td>
<td>96.4</td>
</tr>
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</table>

Measurements of the rate of water loss in dry air at room temperature of 20°C were made by suspending the sample just above the surface of concentrated sulphuric acid which filled a 3 in. x 1 in. specimen tube to a depth of ½ in. Direct comparisons were made in similar conditions with distilled water drops of identical size. Three typical weight loss curves of distilled water, ‘normal’ and ‘stimulated’ mucus droplets are given in Text-fig. 5. Over 70 similar determinations of rate of water loss were made, with sample drops of the same weight range. No significant difference between water loss from distilled water, ‘normal’ and ‘stimulated’ mucus was found.

Rate of water loss in dry air

Preliminary experiments indicated that the vapour pressure of mucus was high, therefore the margin of error in maintaining an excess atmospheric vapour pressure would be narrow. The local temperature differences which could easily turn a saturated atmosphere into one in which evaporation from the mucus could occur were eliminated in a specially designed apparatus. This consisted of a massive brass cell, fitted with an airtight lid and insulated by an inverted vacuum flask, all of which were kept in a constant-temperature room maintained at 10°C. After thorough cleaning a central depression in the cell was filled with distilled water, the apparatus sealed and left for 24 hr. to equilibrate. Weighed samples or standard solutions of NaCl held in shallow stainless steel dishes were then introduced into the cell alongside the distilled water and left for 1 week. At the end of this the samples and standards were reweighed. Appropriate corrections were made for the inevitable weight loss before saturation in the cell could be re-established. These were based on the weight losses of identical samples of distilled water treated in the same way.

In Table 2, the percentage weight increases of a number of mucus samples are given, together with the calculated NaCl concentration equivalent, determined from measurements made with the NaCl standards. It can be seen that the behaviour of the mucus corresponds on the average to a NaCl solution of 5.4 g/l. Since the same
weight increase in saturated air represents an identical vapour pressure deficit, it has been possible to calculate that the vapour pressure of the mucus is 0.3% less than that of distilled water under the same conditions.

Text-fig. 5. Typical weight loss curves of distilled water (●), 'stimulated' mucus (○) and 'normal' mucus (+) in still dry air at 20°C.

Table 2. The concentration of 'stimulated' mucus samples, calculated from water uptake from saturated air

<table>
<thead>
<tr>
<th>% wt. increases per week</th>
<th>NaCl equiv. (g./100 ml.)</th>
<th>Mean ± s.e.</th>
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<tbody>
<tr>
<td>4.2</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>5.3</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>1.4</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>4.2</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>11.8</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>5.8</td>
<td>0.50</td>
<td></td>
</tr>
<tr>
<td>10.3</td>
<td>0.90</td>
<td></td>
</tr>
<tr>
<td>7.0</td>
<td>0.60</td>
<td></td>
</tr>
</tbody>
</table>

Mean ± s.e. 0.54 ± 0.09 g./100 ml.

Depression of freezing point

The method of freezing-point determination used was adapted from that originally described by Jones (1941) and subsequently improved by Freeman & Rigler (1957). The size of mucus samples necessary for saturated air and freezing-point experiments
permitted the use of stimulated mucus only. Measurements of the depression of freezing point of ‘stimulated’ mucus samples are in agreement with the results previously discussed. The figures given in Table 3 are similarly expressed—NaCl concentration equivalents. An average equivalent concentration of 7.4 g. NaCl/l. is calculated to correspond to a 0.4% lowering of the vapour pressure of pure water.

Table 3. Equivalent concentrations of stimulated mucus samples calculated from depression of freezing-point determinations

<table>
<thead>
<tr>
<th>Mucus concn. (g./100 ml.)</th>
<th>NaCl equivalent</th>
<th>Mean ± s.e.</th>
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<tbody>
<tr>
<td>0.74</td>
<td></td>
<td></td>
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<tr>
<td>0.76</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.74</td>
<td></td>
<td>0.74 ± 0.01</td>
</tr>
<tr>
<td>0.77</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.68</td>
<td></td>
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DISCUSSION

Evaporative water loss from terrestrial snails takes place not from the skin directly but from a superficial layer of secreted mucus. Since the mucous layer has to be continuously replaced from beneath, its nature and distribution are not necessarily constant; neither are its properties as an evaporating surface. Therefore it is not sufficient to draw conclusions from experiments with isolated samples of mucus, without taking into account the efficiency of the mucus replacement mechanism and the changes which may occur in the mucous layer as a whole, under natural conditions.

It has been shown that even in high wind speeds the skin of an active, healthy individual remains moist. Experiments with isolated skin preparations and freshly drowned snails indicate that the hydrostatic pressure of the blood plays an important part in the skin-lubrication mechanism. Although direct haemocoelic pressures of Helix aspersa have not been recorded, Picken (1937) has shown that the haemocoelic pressure of Lymnaea stagnalis, a snail of comparable size and musculature, varies between 3 and 11 cm. H₂O. It seems likely, therefore, that the 10 cm. H₂O pressure which was required to remoisten isolated skin preparations is well within the capabilities of the common garden snail. Campion (1961) and Machin (1962) have shown that the mucous glands of the foot and body wall are surrounded by extensive blood spaces. Therefore increases in haemocoelic pressure due to the muscular tonus of the whole body would result in the more or less continuous extrusion of mucus on to the skin. Probably under normal conditions this mechanism is adequate. However, when higher wind speeds are experienced, a more efficient relubricating mechanism, capable of keeping pace with the more rapid removal of water, comes into play. First, mucus extrusion is increased, when the rapidly drying areas of the skin begin undulating by local muscular contractions, apparently under some form of nervous control. The secretion of a thick mucous layer by the mantle during the formation of the epiphragm is similarly due to intense muscular activity (Machin, 1962). Secondly, fresh mucus is transferred from the grooves, which seem to act as reservoirs, to the adjacent tubercules exposed to the full force of the moving air.
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By experimenting with dead animals and isolated skin preparations it has been possible to investigate the effect of complete surface desiccation by eliminating the relubricating mechanisms found in the whole active snail. Once superficial mucus is dried out, the skin becomes very dry and brittle. Surface temperature and other indicators of evaporation rate show that, as more and more water is now removed from the skin itself, it becomes increasingly impermeable to water. This phenomenon is known also for non-living membranes, for example gelatin (Barrer, 1941). It is important to realize that the surface desiccation occurs under normal atmospheric conditions even when the inside of the skin is freely bathed in blood or physiological saline. Further cases in which mucus production and then water diffusion are unable to keep pace with evaporation losses are indicated in the work of Gray (1928) and Manton & Ramsay (1937). Gray found that the rate of evaporation from the skin of a living newt in air decreased with time. However, as soon as the skin was remoistened by immersing in water, the original evaporation rate was restored. Manton & Ramsay found a reduction in water loss as the skin of an earthworm, subjected to high temperatures and low humidities, became more and more desiccated. Although very little of the mechanism of mucus secretion in the earthworm is known, it is probable that the observations described above are due to the severity of the experimental conditions. It must therefore be emphasized that measurements of evaporation from normally moist skins are meaningless in conditions which cause the skin to become superficially dry.

In spite of the fact that the water content of the desiccated skin usually recovers on complete immersion in water it is probable that severe desiccation will result in permanent damage. Histological preparations of superficially dried skin support this view, since the epidermis, mucous glands and other tissues become very much compressed, all histological detail being obscured. The skin of snails and other terrestrial pulmonates would be particularly susceptible to this type of injury, because the epidermis, ciliated in patches, consists of a single-layered epithelium. It is suggested therefore that superficial drying out is a very real danger to the animal, and thus a highly efficient protective mechanism of mucus secretion has been evolved.

Experiments described in the second half of this paper have shown that the water content of mucus drops removed from the snail have a high water content, which is very readily removed in dry air. Preliminary experiments indicated that there was no significant difference in the rate of evaporation from mucus and that from distilled water under the same conditions. More refined measurements of depression of freezing point and equilibrium in saturated air showed that there is a slight vapour pressure deficit on the part of the mucus, which is never greater than 0.4%. This would correspond to that of a NaCl solution of 7.4 g./l. For practical purposes and over the greater range of natural environmental conditions the evaporation of water molecules from mucus can be considered identical in rate to that of a free water surface. However, when atmospheric humidity approaches saturation point, it would be theoretically possible for the snail to take up water vapour from the air by way of the mucus. Since in saturated atmospheric conditions liquid water would also certainly be present in the environment, in the form of precipitated dew or rain, the means of water absorption outlined above is likely to be unimportant. The present author (Machin, 1962) has shown that snails immersed in water increase their weight 30%/hr. due to the
uptake of water through the skin, whereas the equivalent uptake in saturated air is only 5%/week. The fact that moist-skinned animals take up liquid water through the skin very readily has also been demonstrated by Adolph (1927, 1932) and by Sawyer (1956), both working with Amphibia.

**SUMMARY**

1. Observations of intact specimens of *Helix aspersa* together with experiments with isolated skin preparations are described.

2. Under normal atmospheric conditions increases in haemocoelic pressure, probably due to general muscular activity, are sufficient to maintain the superficial mucous coating of the skin.

3. Under conditions of rapid water loss more intense muscular undulations serve to spread mucus which collects in the grooves to more exposed areas of the skin.

4. The water content, the rate of water loss in dry air, the equilibrium in saturated air and depression of freezing point of isolated mucus samples have been measured.

5. The vapour pressure of mucus has been shown to be within 0.4% of that of distilled water under the same conditions.

6. The significance of the above findings is discussed in relation to evaporative water loss and water uptake of an intact snail.

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**REFERENCES**


Evaporation of water from *Helix aspersa*. I


EXPLANATION OF PLATE

Close up photographs of the dorsal body surface of *Helix aspersa*.

Fig. 1. A normal active animal. A clear-cut, rounded highlight (see arrow A), indicates a thick mucous covering in the grooves. On a raised area of the skin, where the mucous covering is thin (see arrow B), surface irregularities break up the reflections into pin-points of light.

Fig. 2. A freshly drowned specimen after partial drying of the skin. Reflected light scatter by surface irregularities extends to the grooves.