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GAS PERMEABILITY OF THE EPIPHRAGM OF A TERRESTRIAL SNAIL, *OTALA LACTEA*¹

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Permeability of the epiphragm of *Otala lactea* (Helicidae) to O₂, CO₂, and water vapor was determined and used to assess the significance of the epiphragm as a barrier to evaporative water loss and respiratory gas exchange during dormancy. A distinct region of the epiphragm, known as the *Kalkfleck*, occupies 6% of the area but accounts for 98% of the diffusive conductance of the epiphragm for O₂ and CO₂. The high gas permeability of the *Kalkfleck* results from air channels that penetrate a loose aggregate of crystalline CaCO₃ granules. The rest of the epiphragm is relatively impermeable and includes a nonporous layer which is absent from the *Kalkfleck*. Rates of water loss and respiratory gas exchange of *Otala* were incorporated with epiphragm permeability measurements in diffusion equations to determine the relative contribution of epiphragms to resistance to diffusion of gases from *Otala*. These calculations indicate that epiphragms provide up to 20% of the total resistance to evaporative water loss during dormancy and that the *Kalkfleck* is necessary to permit respiratory gas exchange.

INTRODUCTION

When exposed to dehydrating conditions, pulmonate land snails retract into their shells and enter a quiescent state in which they may survive for months or years (Nopp 1974). The physiology of this dormant condition is not well understood but clearly involves a dramatic reduction in evaporative water loss. Although the moist integument of an active snail offers essentially no resistance to evaporation, dormant snails, particularly species from xeric habitats, are among the most waterproof of organisms (Machin 1975).

Several structures help reduce evaporative water loss from the dormant snail. The shell prevents evaporation from most of the snail's surface, but water loss at the aperture of the shell must be controlled by other means. The primary barrier to evaporation from the body at the shell aperture is the mantle collar, a specialized organ

which occludes the aperture when the snail is retracted and which becomes highly impermeable to water during dormancy (Machin 1972; Newell and Machin 1976). Evaporation from the lung is controlled by an occludible breathing pore, the pneumostome. The final barriers to diffusion of water vapor through the aperture of the shell are one or more epiphragms, which are membranes formed from dried secretions of the mantle collar. Epiphragms consist of a mucoprotein matrix reinforced by deposits of calcium carbonate (Campion 1961; Block 1971). Often a series of epiphragms is secreted, with each successive epiphragm separated from the others by a narrow air space. The thickness, degree of calcification, and number of epiphragms produced vary with species, season, and environmental conditions (Block 1971; Rokitka and Herreid 1975a).

Epiphragms have often been assigned a role in the reduction of water loss during dormancy, but their importance in this regard is not well documented. Studies in which water loss from dormant snails is measured before and after epiphragm removal give variable results and are equivocal because disturbance of the dormant snail results in increased water loss (Nopp 1974; Van der Laan 1975). Few studies have been made of the properties of the epiphragms themselves. Machin (1968) mea-

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sured the permeability of isolated epiphragms to water vapor and found that permeability increased with increased humidity. The relative contribution of epiphragms to the total resistance to loss of water vapor from the dormant snail has not been determined.

If epiphragms are a significant diffusion barrier, they could potentially restrict O_2 and CO_2 exchange as well as water loss. All known organic membranes are less permeable to O_2 and CO_2 than to water vapor (Waggoner 1966). Moreover, the epiphragms of many species include perforations adjacent to the pneumostome which are thought to provide a path for respiratory gas exchange (Block 1971; Barnhart 1979). In the family Helicidae, a specialized area of the epiphragm known as the "ventilator" or "*Kalkfleck*" ("lime-spot") is believed to be an area of increased gas permeability, although it is not macroscopically porous (Block 1971; Picher 1972). However, the permeability of epiphragms to O_2 and CO_2 and the role of the *Kalkfleck* in gas exchange have not been quantified.

In the present study, the permeability of the epiphragm of *Otala lactea* (Helicidae) to O_2 , CO_2 , and water vapor was determined. The results were compared with previously published measurements of the rates of oxygen consumption and evaporative water loss of *Otala* to test the significance of epiphragms as a diffusion barrier. The epiphragms were also examined by scanning electron microscopy (SEM) to compare the structure of the *Kalkfleck* with that of the rest of the epiphragm.

MATERIAL AND METHODS

Dormant *Otala* were collected in June from an introduced population at Playa del Rey in Los Angeles County, California (Gammon 1943). The average mass of the specimens used was 7.4 g (see fig. 1A). Rather than sever the attachment of the epiphragm margin from the shell, each shell was cut in half above the aperture, leaving the epiphragms in situ. If multiple epiphragms were present, all but one were removed, leaving a single intact epiphragm in each preparation. Cracked or dirty specimens were discarded.

PERMEABILITY TO O_2 AND CO_2

A diffusion-chamber method was employed (fig. 2). The end of a 60-ml syringe barrel was cut off and a half-shell with intact epiphragm was carefully sealed over the cut end using a vinyl-wax sealing compound. A stopcock valve was mounted on the side of the syringe barrel near the epiphragm and a small hole drilled through the barrel near the opposite end. With the plunger withdrawn just past the hole, the syringe chamber was flushed with gas (0% O_2 , 24% CO_2 , 76% N_2) via the stopcock. After flushing for 1 min, I quickly advanced the plunger past the hole to a standard volume and closed the stopcock. The gas in the syringe chamber was stirred vigorously by a vaned magnetic stir bar, and the air outside the chamber was moved by a fan in order to minimize boundary layers of still air on both sides of the epiphragm. Temperature was controlled to 25.0 ± 0.2 C as oxygen diffused into the chamber across the epiphragm and CO_2 diffused outward. After a known length of time the gas in the chamber was analyzed with a Scholander gas analyzer (Scholander 1947) to determine PO_2 and PCO_2 .

The time course of the equilibration of partial pressure of O_2 and CO_2 in the chamber with that in the ambient air was used to calculate the diffusive conductance of the epiphragm. The diffusive conductance for a gas is directly related to the constant k , where

$$k = (d\Delta P/dt) \cdot (1/\Delta P); \quad (1)$$

ΔP is the difference in partial pressure of the gas between the chamber and the ambient air and t is the elapsed time. Because the relation between $\ln \Delta P$ and t is linear, rearranging and integrating (1) yields the following:

$$k = (\ln \Delta P_i - \ln \Delta P_f)/t, \quad (2)$$

where ΔP_i is the initial ΔP and ΔP_f is the final ΔP . In other words, k can be conveniently determined as the slope of the line relating the logarithm of ΔP to time. Conductance (G) is calculated as follows:

$$G = k \cdot V \cdot (1/22.4) \cdot (1/760) \cdot (273/T), \quad (3)$$

where V is the volume of the chamber, 22.4 is the molar volume of an ideal gas, and T is the absolute temperature. Units of G are mol/min · torr.

Three replicate tests were performed

with each epiphragm, using sufficient time to permit 70%–90% equilibration between the gas in the syringe chamber and the air outside of it. Slopes calculated from the replicates never differed by more than 5%,

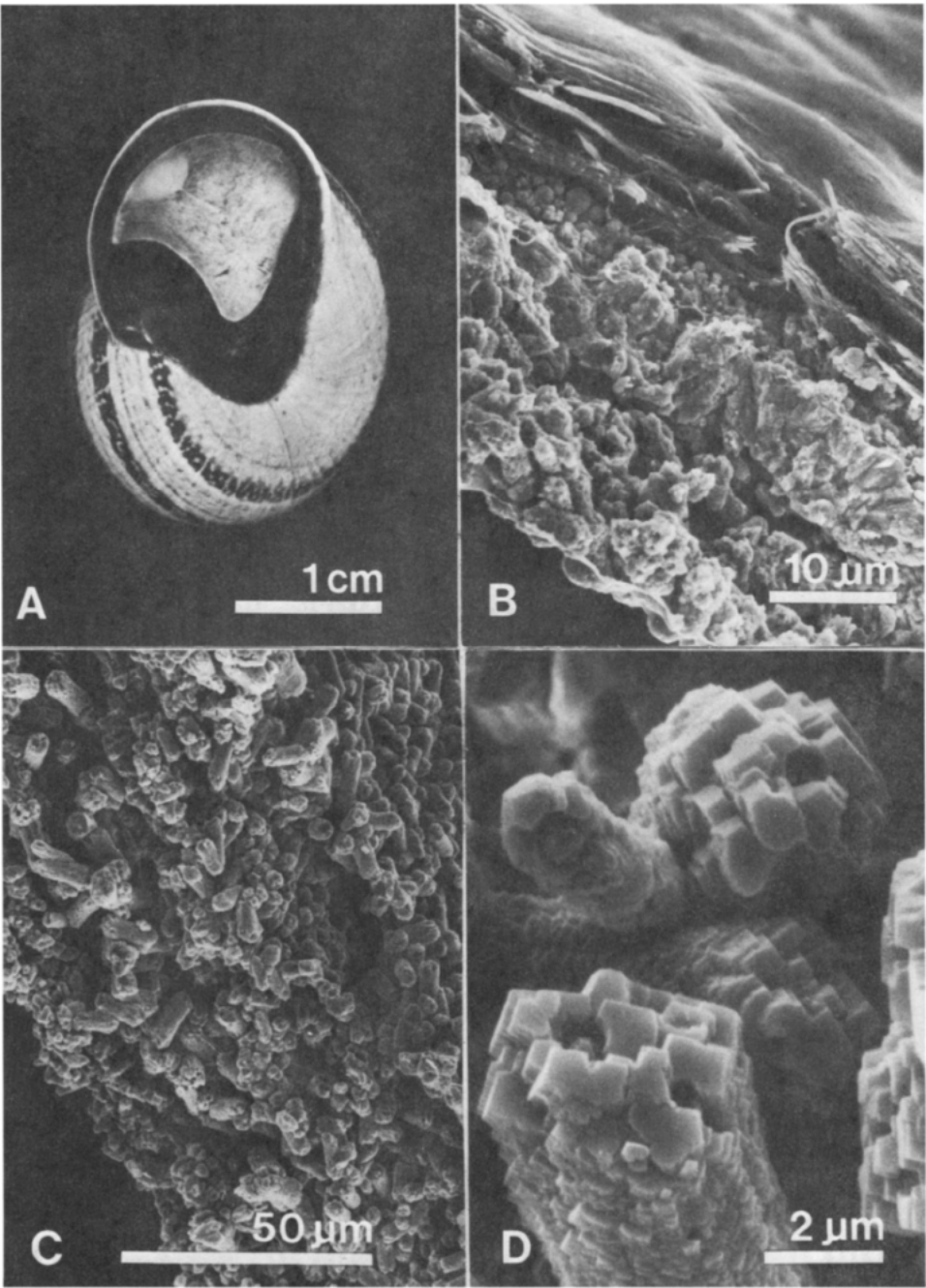


FIG. 1.—A, Dormant *Otala lactea* showing epiphragm across shell aperture. The *Kalkfleck* is the white spot at the upper left margin of the epiphragm. B, Torn edge of the non-*Kalkfleck* portion of the epiphragm. The surface which faces the mantle collar is visible at the upper right. C, Broken edge of the *Kalkfleck*. The surface which faces the mantle collar is at the upper right. D, Higher magnification of the *Kalkfleck* granules.

and the results from all three replicates for an epiphragm were averaged to determine k . Permeability of the syringe barrels to O_2 and CO_2 was found to be less than 1% of the observed diffusive conductance and was considered to be negligible.

The contribution of the *Kalkfleck* to the diffusive conductance of the epiphragm was measured by sealing the *Kalkfleck* with vinyl wax and then repeating the measurements. The difference between G observed before and after sealing the *Kalkfleck* was assumed to equal G of the *Kalkfleck*.

PERMEABILITY TO WATER VAPOR

A gravimetric method was used. The epiphragm preparations were sealed with vinyl wax over the mouths of vials containing either anhydrous $CaSO_4$, saturated $MgCl_2$ solution, or a water-soaked wick. These contents served to maintain 0%, 25%, or 100% relative humidity (RH), respectively, within the vials (Winston and Bates 1960). The vials were stored in a temperature-controlled cabinet at 25.0 ± 0.2 C. Relative humidity in the cabinet was determined to within 1% RH by a battery-powered psychrometer (Psychrodyne) and controlled by the addition of pans of water. Humidity in the temperature cabinet was checked frequently and did not vary by more than 2% RH during a test. After an initial 6-h equilibration period, the vials were weighed five times at 12–18-h intervals to determine the rate of water vapor uptake or loss. Capped vials weighed as controls showed that water flux through the walls of the vials was negligible.

Epiphragm permeability to water vapor is influenced by relative humidity, presum-

ably because of changes in the water content of hygroscopic material in the epiphragm (Machin 1968). Because different relative humidities must be maintained on opposite sides of the epiphragm in order for a net flux of water vapor to occur, the relationship between humidity and the water vapor conductance of the epiphragm must be expressed in terms of the average of the two values of relative humidity existing on the opposite sides of the epiphragm (see Machin 1968). This "average humidity" is symbolized as RH_{av} . The rate of weight change of the vials was measured at six different values of RH_{av} obtained by different combinations of humidity inside and outside of the vials. The effect of sealing the *Kalkfleck* on water vapor conductance was tested at a low and a high value of RH_{av} , in order to test whether water vapor conductance (G_{H_2O}) of the *Kalkfleck* changes with humidity.

The air outside the vials was moved by a fan. The air in the vials was not stirred, and a layer of still air 0.5 cm thick separated the desiccant or wick surface from the epiphragm. The diffusion resistance of this air space was a significant fraction of the total measured resistance to vapor diffusion and required correction in the calculation of epiphragm water vapor conductance. Assuming a diffusivity in air of $0.27 \text{ cm}^2/\text{s}$ (List 1971), the calculated water vapor diffusion resistance of the air layer is $0.31 \text{ min} \cdot \text{torr}/\mu\text{mol}$.² This resistance was in series with the resistance of the epiphragm and therefore was subtracted from the observed resistance to diffusion of water vapor from the vials (resistance = $1/G$). The correction was less than 4% of the largest resistance measured but was about 17% of the lowest resistance.

DIMENSIONS AND STRUCTURE

After permeability tests, each epiphragm was carefully removed from its shell with a scalpel. Thickness was measured by caliper micrometer at four points on each epiphragm, and the four values were averaged. Because of the fragility of the *Kalkfleck*, its thickness was measured

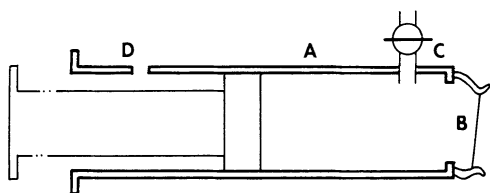


FIG. 2.—Apparatus for the measurement of epiphragm permeability to O_2 and CO_2 . The diffusion chamber is a 60-ml polyethylene syringe (A) with an epiphragm preparation (B) sealed over the open end. A stopcock valve (C) and a hole (D) permit the chamber to be filled with a known gas mixture (see Material and methods).

² The unit cm^2/s is equivalent to $3.326 \mu\text{mol} \cdot \text{cm}/\text{cm}^2 \cdot \text{min} \cdot \text{torr}$ at 25 C. See Piiper et al. (1971) for a discussion of units.

separately by examining fractured edges with a dissecting microscope and ocular micrometer. Total area of each epiphragm was determined using tracings and a Licor Leaf Area Integrator. Length and width of the *Kalkfleck* were measured by ocular micrometer, and *Kalkfleck* area was calculated from these measurements using the formula for the area of an ellipse. Fragments of four epiphragms of varying thickness were coated with 20 Å gold-palladium and examined by SEM, and the inner and outer surfaces and fractured edges of the *Kalkfleck* and non-*Kalkfleck* portions were compared.

RESULTS

PERMEABILITY TO O₂ AND CO₂

The O₂ and CO₂ permeability are presented as diffusive conductance and as permeation coefficients (conductance per unit area and unit thickness) in table 1. Comparison of conductance with and without the *Kalkfleck* blocked shows that, on the average, the *Kalkfleck* accounts for 98.3% of the total epiphragm conductance for O₂ and CO₂. Although the average *Kalkfleck* occupies only 6.1% of total epiphragm area and is 2.8 times thicker than the rest of the epiphragm, the *Kalkfleck* permeation coefficient is over 2,000 times greater than that of the rest of the epiphragm. The mean value of the ratio G_{CO_2}/G_{O_2} for the *Kalkfleck* is $0.85 \pm .003$ SE. Although this ratio is nearly identical to that predicted by Graham's Law on the basis of molecular weight ($\sqrt{32/44} = 0.853$), experimental determinations of the ratio of diffusivities (D_{CO_2}/D_{O_2}) in nitrogen are somewhat lower, ranging from 0.76 to 0.80 (Paganelli, Ackerman, and Rahn 1978; Piiper and Worth 1980).

PERMEABILITY TO WATER VAPOR

Diffusive conductance for water vapor is a sigmoidal function of RH_{av} (fig. 3). Sealing the *Kalkfleck* has relatively less effect on water vapor conductance at high humidity, reducing G_{H_2O} by an average of 63% at 25.5% RH_{av} but by only 7.5% at 66.7% RH_{av}.

EPIPHRAGM STRUCTURE

Although the average *Kalkfleck* is 2.8 times thicker than the rest of the epiphragm, it is much more fragile and is easily fractured or crushed. When magnified by SEM, the *Kalkfleck* appears to consist of granules, most of which are short cylinders from 2 to 10 μm long (fig. 1C). Under higher magnification these granules appear to be stacks or aggregates of smaller tabular crystals (fig. 1D). The *Kalkfleck*

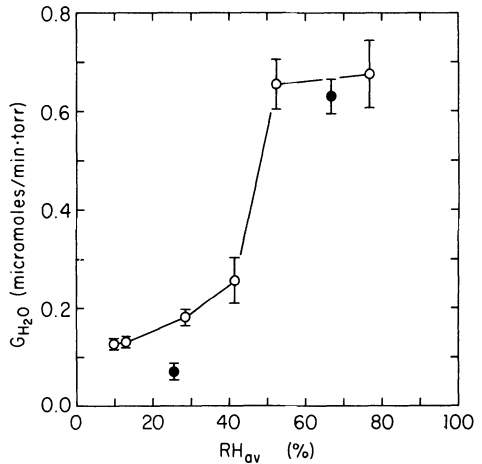


FIG. 3.—Water vapor conductance of *Otala lactea* epiphragms as a function of humidity at 25 C (see text for further explanation). No. = 10 for each group. Symbols indicate mean \pm 95% confidence interval. The closed symbols indicate that the *Kalkfleck* was blocked with wax.

TABLE 1

PERMEABILITY OF THE EPIPHRAGM OF *Otala lactea* TO CO₂ AND O₂.

	CONDUCTANCE (μmol/min-torr)			PERMEATION COEFFICIENT (μmol·cm/cm ² ·min-torr)	
	Total	Total Minus <i>Kalkfleck</i> ^a	<i>Kalkfleck</i> ^b	<i>Kalkfleck</i>	Non- <i>Kalkfleck</i>
CO ₂0827 ± .0053	$1.43 \times 10^{-3} \pm .44 \times 10^{-3}$.0813 ± .0055	.114 ± .018	$5.60 \times 10^{-5} \pm 2.87 \times 10^{-5}$
O ₂0972 ± .0061	$1.61 \times 10^{-3} \pm .55 \times 10^{-3}$.0955 ± .0063	.134 ± .022	$6.41 \times 10^{-5} \pm 3.48 \times 10^{-5}$

NOTE.—No. = 6. All values are mean \pm standard error of mean.
^a Conductance with *Kalkfleck* sealed.
^b Conductance of *Kalkfleck* estimated as difference after sealing *Kalkfleck*.

granules are loosely consolidated so that air spaces are present between them. Although the irregular form of these air spaces prevents the tracing of continuous channels through the *Kalkfleck*, such channels almost certainly exist.

Unlike the *Kalkfleck*, which appears to be homogeneous, the rest of the epiphragm consists of at least two distinct layers (fig. 1B). The outer layer (facing away from the mantle collar) is granular, but the granules are smaller and less clearly crystalline than those of the *Kalkfleck*. The granular layer is disproportionately reduced in thin epiphragms and is absent from the transparent, noncalcified epiphragms which are often formed initially by recently active snails. The second or inner layer, which faces the mantle collar, lacks granules and appears to be a homogeneous sheet with no perforations. When torn, this inner layer has a fibrous appearance (fig. 1B), and it is presumably equivalent to the mucopolysaccharide "matrix" described by Campion (1961). The inner layer remains intact when the granular outer layer is dissolved by acid and becomes pliable and elastic when soaked in water.

DISCUSSION

PERMEABILITY MEASUREMENTS

The diffusive conductance G (mol/min · torr) has several advantages over other parameters which might be used to describe the permeability of epiphragms or other structures to gases. The diffusion coefficient (cm²/s) and the permeability coefficient (cm/s) are more commonly used, but both are defined in terms of the concentration of the diffusing substance in the membrane and cannot be determined from partial pressure measurements alone (see Piiper et al. 1971). Furthermore, these parameters are area specific; therefore, their use is appropriate only if the structure through which diffusion is occurring is of uniform permeability over its entire area. Diffusive conductance, however, can be properly used to describe the flux of a gas across a heterogeneous structure such as an epiphragm in relation to a difference in partial pressure.

The O₂ and CO₂ permeation coefficients in table 1 emphasize the difference in permeability between the *Kalkfleck* and the

rest of the epiphragm. However, these permeation coefficients should not be interpreted as characteristics of the materials forming the epiphragm. The *Kalkfleck* is clearly a porous structure, and its permeability to gases must depend primarily on the dimensions and number of air channels which traverse it. The permeability of the rest of the epiphragm is probably determined mainly by the nonporous inner layer, but the permeation coefficient of this layer has not been determined separately.

The increase in permeability to water vapor with increased humidity is apparently not due to a change in the *Kalkfleck*. Sealing the *Kalkfleck* did not reduce water flux more in high humidity than in low. It appears that the rest of the epiphragm does become far more permeable to water vapor at high humidity. The hygroscopic character of epiphragms was described by Block (1971). Increased permeability to water vapor at higher humidity is characteristic of many hygroscopic materials, including the keratin of vertebrate epidermis (Machin 1968; Mautz 1982). This effect is presumably related to change in water content, which may affect several parameters that influence permeability. The most important of these parameters is thought to be the mobility of the diffusing water molecules. As water content of the material increases, hydrophilic sites may become saturated, and the average mobility of the water molecules in the material may increase (King 1944).

Epiphragm permeability to water vapor was previously measured by Machin (1968), who used isolated epiphragms clamped in a flow-by apparatus. *Kalkfleck* permeability was not distinguished. Converting Machin's measurements to area-specific conductance gives a mean value of 0.14 $\mu\text{mol}/\text{cm}^2 \cdot \text{min} \cdot \text{torr}$ at 25 C and 50% RH_{av}. The corresponding mean from the present study is 0.06 $\mu\text{mol}/\text{cm}^2 \cdot \text{min} \cdot \text{torr}$. The difference between the two means may be insignificant, since an interval of 2 SE from the former mean overlaps all of the values from the present study.

EPIPHRAGMS AND WATER LOSS

Do epiphragms reduce water loss from the dormant snail? Studies which compare the rate of water loss from snails with and

without epiphragms have given variable results (Nopp 1974), but it is evident that the mantle collar, not the epiphragm, is the primary barrier to water loss from the aperture of the shell (Machin 1975). This fact does not preclude a significant role for epiphragms in reducing water loss, particularly if there are periods during dormancy when the mantle collar resistance to water loss is "short-circuited" by mucus secretion or by prolonged opening of the pneumostome.

The relative importance of epiphragm resistance to diffusion of water vapor can be investigated with a simple circuit equation that defines the total resistance to water loss from the shell aperture. Water loss through the shell is small (Machin 1975) and can be neglected for simplicity. The average rate of water loss (\dot{M}_{H_2O}) from the shell aperture of a dormant *Otala* is 0.112 $\mu\text{mol}/\text{min}$ at 22 C in dry air (Machin 1967). If saturation vapor pressure is assumed to exist at some point in the tissues of the snail, the vapor pressure difference (ΔP_{H_2O}) driving the diffusive flux of water vapor to the dry air is 20 torr. These parameters can be used to define the total resistance to water vapor flux through the aperture (R_{H_2O}):

$$\begin{aligned} R_{H_2O} &= \Delta P_{H_2O} / \dot{M}_{H_2O} \\ &= 177 \text{ min} \cdot \text{torr}/\mu\text{mol}. \end{aligned} \quad (4)$$

The use of resistance (inverse of conductance) is convenient because the components of diffusion resistance through the aperture are in series, so that R_{H_2O} represents the sum of the diffusion resistance of the mantle collar, epiphragm, and the air space separating the epiphragm and mantle collar.

Calculation of R_{H_2O} permits the effect of the epiphragm on water loss to be evaluated in two ways. First, the measured epiphragm resistance to water vapor diffusion (inverse of G_{H_2O} ; fig. 3) can be compared with R_{H_2O} . A typical epiphragm in dry air provides a resistance of about 7 $\text{min} \cdot \text{torr}/\mu\text{mol}$, which is about 4% of R_{H_2O} . For comparison, the calculated water vapor diffusion resistance of the layer of still air between the epiphragm and mantle collar is 1.1 $\text{min} \cdot \text{torr}/\mu\text{mol}$, assuming dimensions of $1 \text{ cm}^2 \times 1 \text{ cm}^2$ and a diffusivity of $0.27 \text{ cm}^2/\text{s}$ (List 1971).

Second, R_{H_2O} can be calculated from the rate of water loss of snails with and without epiphragms, and the difference in R_{H_2O} determined. The water loss rates of dormant *Otala* with and without epiphragms (Machin 1967) yield R_{H_2O} of 177 and 114 $\text{min} \cdot \text{torr}/\mu\text{mol}$, respectively. The difference between these values is 63 $\text{min} \cdot \text{torr}/\mu\text{mol}$, a much larger change than can be accounted for by the epiphragm diffusion resistance. This discrepancy indicates that the epiphragm has other effects relevant to water loss. Possibly the presence of the epiphragm minimizes the disturbance to the snail during handling and weighing, which are known to increase mantle mucus secretion and water loss (Machin 1975). It is noteworthy that the variability of water loss from dormant snails is greatly reduced when an epiphragm is present (Machin 1967).

Many land snails, including *Otala*, produce multiple epiphragms during prolonged dormancy (Block 1971; Rokitka and Herreid 1975a, 1975b). The resistance of a series of five epiphragms (commonly observed in dormant *Otala*) is about 35 $\text{min} \cdot \text{torr}/\mu\text{mol}$ in dry air, or about 20% of the R_{H_2O} calculated in (4).

The resistance of epiphragms to diffusion of water vapor declines with increased ambient humidity. It could also be reduced by an increase in the rate of water loss from the mantle collar or pneumostome, although in this case the relative contribution of the epiphragms to R_{H_2O} might still be high. Further consideration of this problem will require more information on the variability of the rate of water loss and the conditions experienced by dormant animals in a natural situation.

EPIPHRAGMS AND RESPIRATION

An analysis similar to that above can be used to evaluate epiphragms as barriers to respiratory gas exchange. As a first approximation, a constant rate of oxygen consumption (\dot{M}_{O_2}) is assumed and used to calculate the difference in P_{O_2} which would be necessary to drive the diffusion of O_2 at that rate across an epiphragm, according to the equation

$$\Delta P_{O_2} = \dot{M}_{O_2} / G_{O_2}. \quad (5)$$

The average \dot{M}_{O_2} of a dormant *Otala* at

20 C is less than 4 $\mu\text{mol/h}$ (Herreid 1977). At that rate of flux the calculated ΔPo_2 is only 0.7 torr. That is, Po_2 behind the epiphragm would be only 0.7 torr lower than ambient. With a series of five epiphragms present, Po_2 near the mantle collar would be about 3.4 torr below ambient; PCO_2 would be elevated by a similar pressure. Thus, it appears that epiphragms slightly modify the atmosphere in which the dormant snail respires. It is somewhat unrealistic to assume a constant rate of gas exchange, because the pneumostome opens only periodically during dormancy and external gas exchange is intermittent (Nopp 1974; Kratochvil 1976). Instantaneous rates of gas exchange must therefore exceed the average respiratory rates, and the estimates of partial pressure differences across the epiphragms during gas exchange are therefore conservative.

The importance of the *Kalkfleck* in facilitating gas exchange can be calculated using equation (5). If the *Kalkfleck* is blocked, the drop in Po_2 across a single epiphragm necessary to drive the diffusion of O_2 at the rate observed in dormancy would be 41 torr. With five epiphragms

present, a difference of 207 torr would be necessary, which is impossible to achieve since ambient Po_2 is only about 150 torr. It therefore appears that the *Kalkfleck* is essential for normal gas exchange during dormancy.

The localization of epiphragm permeability to gases in the *Kalkfleck* may be related to the habit of attachment. On entering dormancy, *Otala* typically secrete the first few epiphragms in such a way that they form an attachment to some substrate such as the branch of a shrub (Machin 1967). These epiphragms do not span the entire aperture but, rather, are in the form of rings or "skirts" that connect the margin of the shell aperture to the substrate. The position of the *Kalkfleck* near the margin of the epiphragm insures that it will be included in these skirts and permits the aperture to be rather closely appressed to an impermeable substrate without appreciably affecting conductance for O_2 and CO_2 . Conductance for water vapor, however, should be reduced by attachment, since the rest of the epiphragm is significantly permeable to water vapor and its area will be reduced by attachment.

LITERATURE CITED

- BARNHART, M. C. 1979. Notes on the winter epiphragm of *Pupoides albilabris*. *Veliger* **21**:400-401.
- BLOCK, M. R. 1971. Epiphragms: some observations. *J. Conchology* (London) **26**:388-409.
- CAMPION, M. 1961. The structure and function of the cutaneous glands in *Helix aspersa*. *Quart. J. Microscop. Sci.* **102**:195-261.
- GAMMON, E. T. 1943. Helicid snails in California. *Bull. California Dep. Agr.* **32**:173-187.
- HERREID, C. F. 1977. Metabolism of land snails (*Otala lactea*) during dormancy, arousal and activity. *Comp. Biochem. Physiol.* **56A**:211-215.
- KING, G. 1944. Sorption of vapors by keratin and wool. *Trans. Faraday Soc.* **43**:552-559.
- KRATOCHVIL, H. 1976. Long-term measurements of respiration and heart rate on estivating terrestrial Pulmonata. *Zool. Anzeiger* **196**(5/6):289-317.
- LIST, R. J. 1971. Smithsonian meteorological tables. 6th rev. ed. Smithsonian Institution Press, Washington, D.C.
- MACHIN, J. 1967. Structural adaptation for reducing water loss in three species of terrestrial snail. *J. Zool.* (London) **152**:55-65.
- . 1968. The permeability of the epiphragm of terrestrial snails to water vapor. *Biol. Bull., Woods Hole* **134**:87-95.
- . 1972. Water exchange in the mantle of a terrestrial snail during periods of reduced water loss. *J. Exp. Biol.* **57**:103-111.
- . 1975. Water relationships. Pages 105-163 in V. FRETTER and J. PEAKE, eds. *The Pulmonates*. Academic Press, New York.
- MAUTZ, W. J. 1982. Patterns of evaporative water loss. Pages 443-481 in C. GANS and F. H. POUGH, eds. *Biology of the Reptilia*. Vol. **12**. Academic Press, New York.
- NEWELL, P., and J. MACHIN. 1976. Water regulation in aestivating snails. *Cell and Tissue Res.* **173**:417-421.
- NOPP, H. 1974. Physiologische Aspekte des Trockenschlafes der Landschnecken. *Sitzungsberichte-Oesterreichische Akad. Wiss., Math.-Naturwiss. Klasse, Abt. 1*, **182**:1-75.
- PAGANELLI, C. V., R. A. ACKERMAN, and H. RAHN. 1978. The avian egg: in vivo conductances to oxygen, carbon dioxide and water vapor. Pages 212-218 in J. PIIPER, ed. *Respiratory function in birds, adult and embryonic*. Springer, New York.
- PICHER, O. 1972. Atmung und Herzschlag einiger Landpulmonaten in Abhängigkeit von der Sauerstoffversorgung. *Sitzungsberichte-Oesterreichische Akad. Wiss., Math.-Naturwiss. Klasse, Abt. 1*, **180**:195-215.
- PIIPER, J., P. DEJOURS, P. HAAB, and H. RAHN. 1971. Concepts and basic quantities in gas exchange physiology. *Respiration Physiol.* **13**:292-304.
- PIIPER, J., and H. WORTH. 1980. Value and limits of Graham's Law for prediction of diffusivities of gases in gas mixtures. *Respiration Physiol.* **41**:233-240.

- ROKITKA, M. A., and C. F. HERREID II. 1975a. Position of epiphragms in the land snail *Otala lactea*. *Nautilus* **89**:23-26.
- . 1975b. Formation of epiphragms in the land snail *Otala lactea* under various environmental conditions. *Nautilus* **89**:27-32.
- SCHOLANDER, P. F. 1947. Analyzer for accurate estimation of respiratory gases in one-half cubic centimeter samples. *J. Biol. Chem.* **167**:235-250.
- VAN DER LAAN, K. L. 1975. Aestivation in the land snail, *Helminthoglypta arrosa* (Binney). *Veliger* **17**:360-368.
- WAGGONER, P. E. 1966. Moisture loss through the boundary layer. Pages 41-52 in S. W. TROMP and W. H. WEIHE, eds. *Biometeorology III. Proceedings of the 4th Biometeorological Congress*. Swets & Zeitlinger, Amsterdam.
- WINSTON, P. W., and D. H. BATES. 1960. Saturated solutions for the control of humidity in biological research. *Ecology* **41**:232-237.