B cells (obtained from "nude" athymic mice) stimulate as well as normal cells in MLC (*12*). It is possible that the B cell antigen ("β") which we have described previously may also be responsible for stimulation in MLC. In fact, "β" could be an allele of the antigen described in this report. Its map location is consistent with this, as is the observation that all strains thus far tested express either "β" or this MLC-associated antigen, but not both. There may also be a variety of other MLC loci elsewhere in the MHC, as has been previously suggested (*6*).

Functionally, the finding of a B cell antigen mapping in Ir may be significant, since the precise mechanism of Ir gene expression has not yet been resolved. Independent evidence exists for defects in the T and in the B populations of nonresponder strains (*2, 13*). Thus it is possible that the Ir region codes for a series of membrane antigens, some expressed on T cells (*14*) and others on B cells (*10*), governing responsiveness for a broad series of antigens. On the other hand, the genetic mapping of such lymphoid differentiation antigens could be fortuitous, and a definitive association with Ir must await demonstration of functional relationships.

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3 October 1973

Osmotic Gradients across Snail Epidermis: Evidence for a Water Barrier

Abstract. *Cryoscopic analysis of frozen sections provided indirect evidence for the presence of a waterproof layer limiting evaporation from living epithelial cells in dormant land snails.*

Although large water concentration differences commonly exist across the skins of terrestrial animals (*1*), living cells in these layers are usually protected from desiccation by waterproof stratified epithelia in vertebrates and by cuticles in the invertebrate phyla. However, the single-layered molluscan epidermis secretes no cuticle, bearing instead a zone of microvilli along its free apical surface (*2*). Those areas of a land snail's skin not covered by the shell are normally protected by the sustained secretion of watery mucus. When snails withdraw into the shell, a limited area of mantle tissue remains exposed to the air at the aperture. During dormancy, which sometimes lasts for periods exceeding a year (*3*), cutaneous secretion in this area almost completely ceases, and evaporative water loss is drastically decreased (*4–6*). Surprisingly, the levels to which evaporation is reduced are similar to those found in some insects (*7*). In a study involving surface temperature measurements (*6, 8*), evaporation appeared to be retarded by a waterproof barrier, not immediately at the mantle surface, but located beneath a superficial compartment in equilibrium with the ambient air and freely exchanging water with it. This superficial compartment behaves as if it were composed of mucus, which has been shown in earlier studies (*5, 6*) to be hygroscopic but not particularly impermeable to water, even when air-dried. The absence of an impermeable layer on the surface suggests that the barrier might be located within or beneath the epithelial cells. If this were the case, structures outside the barrier would be subjected to atmospheric dehydration.

As a means of locating the impermeable barrier by the depth to which dehydration extends into the tissue, I recorded the distribution of melting points in frozen transverse sections of mantle tissue. Dormant *Otala lactea* (Müll.) were kept several weeks without disturbance (*9*) before quick freezing in isopentane cooled to -140°C with liquid nitrogen. Sections 4 μm thick were placed on a perforated, temperature-controlled silver block and mounted in kerosene under a glass cover slip. After sufficient time for temperature equilibration to abolish thermal gradients, the frozen specimens were photographed by conventional transmitted light microscopy at regular temperature intervals from -10°C to final melting.

A superficial zone, similar to that found in frog skin (*10*), remained unfrozen even at -30°C, which suggests very high solute concentrations (*11*). This layer probably consists of dehydrated mucus since it is exterior to the epithelial cells and swells slightly when a significant proportion of the section

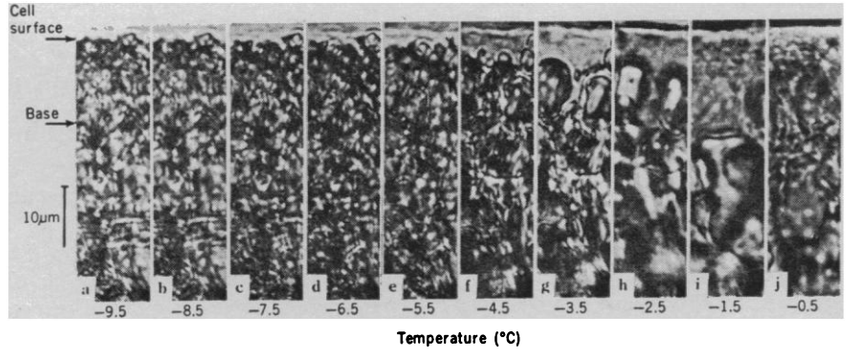

Fig. 1. Frozen section of mantle epithelium photographed at progressive stages of melting. Ice crystals appear as a semiopaque, granular mass beneath the superficial, transparent unfrozen zone. The ice front retreats gradually with temperature in (a) to (f) and more rapidly in (g) to (i). The ice in (j) is completely melted. Increase in individual crystal size just before melting is seen particularly in (f) to (i). The upper and lower limits of the epithelial cells are indicated by the arrows. Their position, determined by the slight change in ice texture at the base, was checked later with stained sections from the same tissue.

melts. Ice crystals of a much finer texture than the cells themselves indicate rapid intra- and extracellular freezing (*12*) in the tissue beneath. In these circumstances it seemed likely that melting points give a reliable indication of solute concentration in life. Warming the sections to about −4° produced a slight but perceptible recession of the ice from the unfrozen surface zone (Fig. 1, a to f). Above −4°C the ice front generally receded more rapidly with temperature, but with some irregularity. During this stage ice crystals became larger, rounded in outline, and more transparent (Fig. 1, g to i). Tissues deeper than about 20 μm from the surface melted simultaneously at temperatures close to the melting point of bulk hemolymph samples (0.22 to 0.29 osmole per kilogram).

A quantitative survey of the position of the ice front in relation to temperature (Fig. 2) showed a consistent pattern of progressive melting away from the surface, demonstrating considerable apical dehydration and solute concentration. A superficial zone exhibits a steep linear osmotic gradient from about 2.2 os/kg to an external concentration exceeding the limit of direct measurement: 5 os/kg. It is possible to calculate the osmotic concentration at the snail's surface from known atmospheric vapor pressures immediately before freezing. A value of 28 os/kg, equivalent to a vapor pressure of 9.9 mm-Hg (50 percent relative humidity at 22°C) was obtained by using a formula relating depression of vapor pressure to osmolality (*13*). A less steep osmotic gradient appears to extend across the bulk of the epithelial cells, ranging from close to hemolymph osmolality (0.3 os/kg) at the base to about 2.2 os/kg near the apical region. Gradients were absent in control sections of mantle tissue taken from areas which were covered with the shell in life, and hence not subjected to the dehydrating effects of the atmosphere.

Under the steady-state conditions existing when the mantle was first frozen, the permeability of an osmotic barrier is, by Fick's law, inversely proportional to the osmotic gradient across it. Results in Fig. 2 show the principal barrier to be located close to the epithelial cell's free edge, although the whole cell appears to impede water flow to some extent. Further confirmation that water penetrates the apical zone with some difficulty was provided by

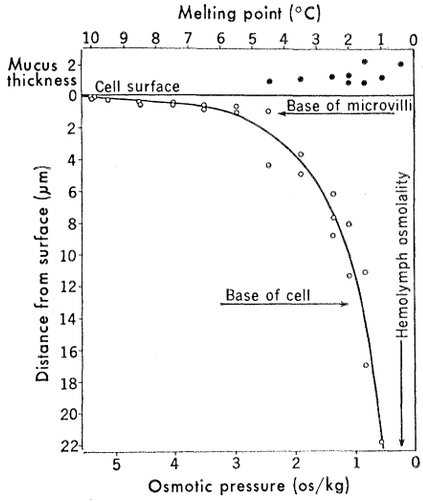

Fig. 2. Analysis of the location of melting points or osmotic pressures across the epithelium (open circles). The thickness of the unfrozen mucus layer (closed circles) at different stages of melting is also plotted. Each point is a mean of 15 measurements taken on a grid placed randomly on a photograph of the section. The points are based on data taken from several different animals.

experiments in which the intact mantle was exposed to a Ringer solution (0.3 os/kg) for varying lengths of time before freezing. It took 4 to 5 minutes to abolish dehydration, whereas most other cells reach osmotic equilibrium with external media much more quickly—of the order of a second in the case of mammalian erythrocytes (*14*). These observations, together with a steep osmotic gradient which appears to coincide with the apical microvilli, suggest that these structures might be the site of the waterproof barrier. However, microvilli are normally associated with increasing transport (*15*) and their role in retarding water movement requires further confirmation.

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7. Mantle permeability in the snail *Otala lactea* at 25°C was found to be 0.016 mg cm^{-2} hour^{-1} per mm-Hg vapor pressure difference (*6*). This value is almost identical to the permeability of cockroach nymph cuticle (0.017 mg cm^{-2} hour^{-1} mm-Hg^{-1}) at the same temperature calculated from data in J. W. L. Beament, *J. Exp. Biol.* **35**, 494 (1968).
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16. Supported by grants from the National Research Council of Canada.

3 October 1973; revised 12 November 1973 ■

Biological Responses of Atta texana to Its Alarm Pheromone and the Enantiomer of the Pheromone

Abstract. S-(+)-4-Methyl-3-heptanone *is the principal alarm pheromone of* Atta texana. *The dextrorotatory form of the ketone has also been identified from* Atta cephalotes. *Both enantiomers have been synthesized in high optical purity;* Atta texana *is more responsive to the (+) enantiomer than to the (−) form. These results implicate a chiral receptor system.*

S-(+)-4-Methyl-3-heptanone (**1**) has been identified as the principal alarm pheromone of the leaf-cutting ant, *Atta texana*. Both enantiomers have been synthesized with rigorous precautions to ensure chemical and optical purity, and bioassayed for threshold activity. Identification of the pheromone without specification of chirality has previously been reported, and the bioassay has been described (*1*). We now report the (+) enantiomer to be more effective in eliciting response, at the threshold level, from all sizes of workers of *A.*