tion of colchicine both as an arrester of metaphase in dividing cells and as an anti-inflammatory agent in acute gouty arthritis.

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References and Notes

1. E. D. Sabatini, K. G. Bensch, R. J. Barnett, *J. Histochem. Cytochem.* **10**, 652 (1962).
2. L. G. Tilney and K. R. Porter, *Protoplasma* **50**, 317 (1965).
3. L. G. Tilney, Y. Hiramoto, D. Marsland, *J. Cell Biol.* **29**, 77 (1966).
4. P. Harris, *ibid.* **14**, 475 (1962).
5. S. Inoue, *Exp. Cell Res. Suppl.* **2**, 305 (1952).
6. D. F. Bainton and M. G. Farquhar, *J. Cell Biol.* **28**, 277 (1966).
7. S. E. Malawista, *Arthritis Rheumat.* **8**, 752 (1965).
8. J. E. Z. Caner, *ibid.*, p. 757.
9. R. Penny, *Proc. Roy. Soc. Med.* **59**, 306 (1966).
10. S. E. Malawista and P. T. Bodel, *J. Clin. Invest.* **45**, 1044 (1966).
11. K. T. Rajan, *Nature* **210**, 959 (1966).
12. S. E. Goldfinger, R. R. Howell, J. E. Seegmiller, *Arthritis Rheumat.* **8**, 1112 (1965); R. Wechsler, S. L. Wallace, D. Gerber, J. Scherer, *ibid.*, p. 1104.
13. S. E. Malawista, *J. Exp. Med.* **122**, 361 (1965).
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Calcareous Septa Formed in Snail Shells by Larvae of Snail-Killing Flies

Abstract. *Larvae of 13 species of* Pherbellia *and* Colobaea *that feed in exposed aquatic snails uitilize a product of the Malpighian tubules before they pupate to form a plate-like structure within the shell or to reinforce the anterior end of the puparium. The substance is partly calcium carbonate, and carbonic anhydrase may be involved in its production.*

A few insects are known to utilize calcium carbonate collected in their Malpighian tubules. A cercopid, *Ptyelus*, makes a spiral shell of calcium carbonate; larvae of *Cerambyx* beetles form a calcareous plate to close their burrows; cuticle of stratiomyid larvae is reinforced by deposits of lime; chorion of eggs of phasmids is composed partly of lime derived from the Malpighian tubules; and the inner surface of the puparium of the celery fly, *Euleia* (=*Acidia*) *heraclei* (L.), is strengthened with a hard layer of lime (*1*). The puparium of the face fly, *Musca autumnalis* De Geer, also is strengthened with calcium carbonate (*2*).

Pupating larvae of several species of snail-killing flies (Diptera: Sciomyzidae) utilize a product of their Malpighian tubules to form a plate-like partition, the "septum," in shells of their food snails (Figs. 1–3) or simply to coat the anterior end of the puparium with a layer of the material. The occurrence, formation, morphology, chemical composition, and possible involvement of an enzyme are discussed here.

Although larvae of various Sciomyzidae feed on aquatic and terrestrial snails, snail eggs, slugs, and fingernail clams, septa are produced only by a few species that routinely feed on and pupate within the shells of exposed aquatic snails (Lymnaeidae, Physidae, Planorbidae). The phenomenon was studied first during rearings of *Pherbellia dorsata* (Zetterstedt) (*3*). Later it was observed during rearings of 11 other species of *Pherbellia* [*P. argyra* Verbeke, *P. griseola* (Fallén), *P. idahoensis* Steyskal, *P. nana* (Fallén), *P. obtusa* (Fallén), *P. quadrata* Steyskal, *P. seticoxa* Steyskal, *P. similis* (Cresson), *P. trabeculata* (Loew), *P. trivittata* (Cresson), and *P. vitalis* (Cresson) (*4*)] and *Colobaea americana* Steyskal.

Production of septum material is not correlated strictly with the taxonomic affinities of the species concerned, and the feature apparently has evolved several times (*4*). All of the 12 species of *Pherbellia* that have been studied do not belong to the same species groups (which are based on morphological characters), and some other members of these groups do not produce septum material. Seven of the producing species are Nearctic, two are Palearctic, and *P. argyra, P. griseola,* and *P. nana* are Holarctic in distribution. Of the four reared species of the Holarctic genus *Colobaea*, only the Nearctic *C. americana* produces septa. Species that produce septa overwinter as pupae in habitats that become flooded during spring. The integument of the puparia of some species that form septa is characteristically thinner and weaker than the integument of species that form puparia outside of shells.

The septum or coating material is produced in the Malpighian tubules. These tubules are white, extend almost the length of the body, and are easily visible through the transparent integument during the entire larval period. They become enlarged several days before formation of the puparium, and their color often changes to light pink or yellow a few hours before excretion of the septum material.

Formation of complete septa requires 1 to 2 hours. The larva shortens, thickens, and extends its anterior spiracles (as do pupating larvae of other reared Sciomyzidae) just before elimination of the milky material through its anus. A rather large amount of this liquid is squeezed anteriorly between the shell and larva by peristaltic movements of the body wall. By the time this material reaches the anterior end of the larva it has a frothy texture. The hardening material is then formed into the plate-like structure by movements of the anterior end of the larva. These movements consist primarily of a side-to-side waving and occur most frequently at the margin, where in most species a hole eventually develops in the septum. It is not known whether any other substances, such as salivary gland secretions, are added to the septum material after it reaches the anterior end of the larva.

The calcareous material dries and hardens quickly on exposure to air, which indicates that moisture conditions within the shell are important for construction of well-formed septa. Species that optimally produce complete septa in slightly damp containers formed imperfect septa when rearing containers were wet. Pupating larvae of a few species (*P. idahoensis, P. nana,* and *P. quadrata*) do not form complete septa even in drier containers. They produce only a rim of the material on the inner wall of the shell or a thick coating on the anterior end of the puparium, or both. Although puparia of these and other species whose larvae occasionally leave the shell to pupate become encrusted with septum material, the anterior and posterior spiracles remain clean. This is probably due to a hydrofuge substance secreted by glands around the spiracular openings.

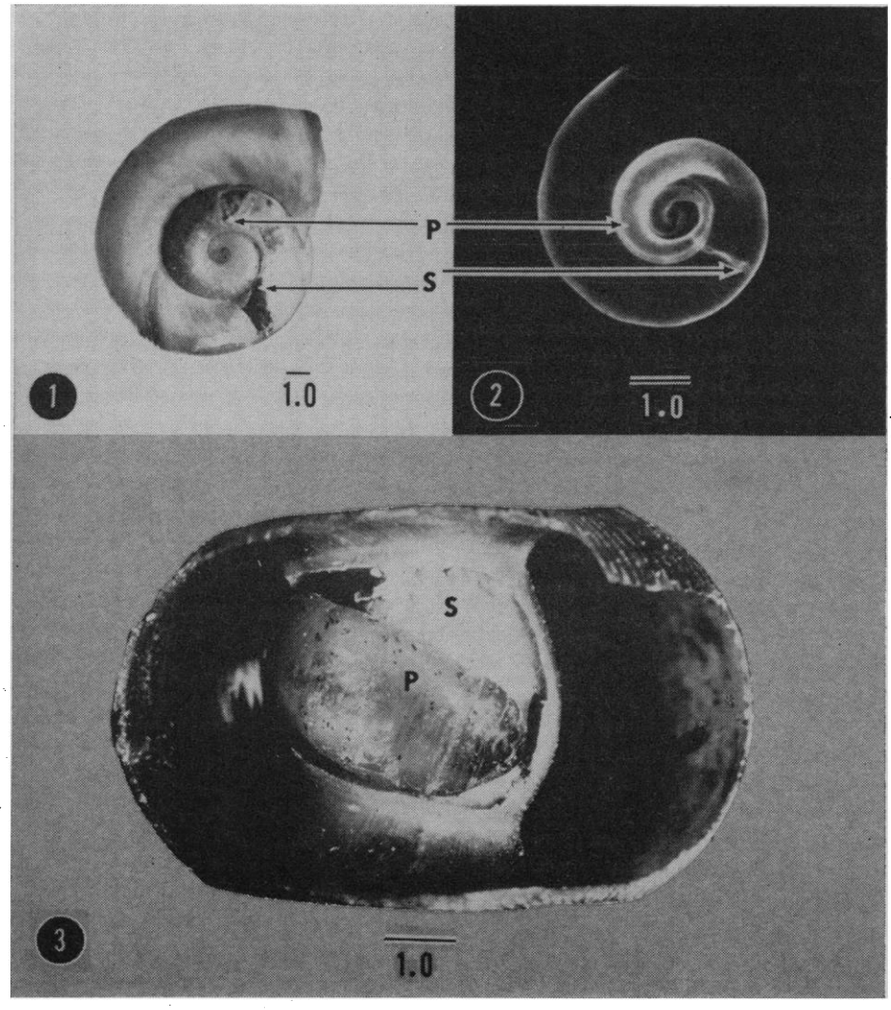
Septa are formed in discoidal (such as *Helisoma*) as well as pyramidal (such as *Lymnaea*) shells. Septa and puparia of some species (for example, *P. seticoxa*) have been found in both types of shells in nature. When the shell of the food snail is several times larger than the puparium, the larva pupates

and the septum is formed in the innermost whorls. The septum or septum material necessarily occurs at the aperture when the shell is only slightly larger than the puparium. The puparium of *C. americana* is formed with its anterior end exposed at the aperture of the small shells of *Gyraulus*, and the uneaten tissue, which has been pushed to the aperture before pupation, is incorporated into the septum. Because larvae of most *Pherbellia* pupate farther in from the aperture and in larger shells, septa of these species usually do not include such uneaten tissue.

Complete septa are white, yellowish, or pinkish. They are dry and rather brittle, about as hard as chalk, and the surface is somewhat water-repellent. They range from 0.5 to 1.0 mm in greatest thickness and often are thinnest along a narrow circumferential line about 1.0 mm from the inner wall of the shell. A small hole, about 0.1 mm from the outer wall of the shell whorl, is present in even the most perfectly formed septa (Fig. 3), and the anterior spiracles of the puparium usually are in or near it, which suggests that this hole may be important in permitting air exchange. The hole and the circumferential line of weakness apparently facilitate action of the eversible head-sac (ptilinum) which the fly uses to break the septum and to emerge from the shell. Morphology of septa of various species of *Pherbellia* is described in a separate paper (*4*).

Spectrographic analyses of septa from two species have been made (*5*). A 13.7-mg sample from *P. dorsata* contained: (i) in percentages, Ca, 50; Mg, 3.2; P, 3.2; and K, 1.2; (ii) in parts per million (ppm), Na, 1310; Fe, 685; Sr, 510; Mn, 280; Al, 100; B, 88; Zn, 44; and Cu, 29. A 97.6-mg sample from *P. seticoxa* contained: (i) in percentages, Ca, 32.7; P, 1.10; Mg, 1.06; and K, 0.62; (ii) in ppm, Na, 1310; Al, 355; Mn, 180; Fe, 164; Sr, 139; Cu, 29; B, 25; and Zn, 21. The percentages of $CO^{=}_3$ in three samples of septa of *P. trivittata* were 22, 27, and 44 when calculated as $CaCO_3$ from figures for CO_2 evolved with acid treatment. Vigorous bubbling was observed when septa of various species were placed in HCl.

Occurrence of large amounts of carbonate in the septum material raises the question of whether carbonic anhydrase is involved. This enzyme catalyzes the hydration reaction during formation of carbonic acid from carbon dioxide and water; carbonate ion results from the loss of two hydrogen ions from carbonic acid at alkaline *p*H (*6*). Thirteen whole larvae of *P. trabeculata* were shown to possess carbonic anhydrase by the gel-indicator method (*7*). In addition, the more sensitive naphthyl acetate esterase technique (*8*), modified by changing the coupler dye to 80 mg of diazo blue B, revealed strong activity at the expected position of carbonic anhydrase in the gel. Within the genus *Pherbellia* there appear to be excellent experimental animals for study of the association between carbonic anhydrase and carbonate secretion.



Figs. 1–3. Fig. 1. Septum and puparium of *Pherbellia seticoxa* in shell of *Helisoma trivolvis* (Say). Fig. 2. Septum and puparium of *P. trabeculata* in shell of *Australorbis glabratus* (Say); x-ray photograph. Fig. 3. Septum and puparium of *P. dorsata* in shell of *H. trivolvis*. *S*, septum; *P*, puparium; scales in millimeters.

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References and Notes

1. V. B. Wigglesworth, *The Principles of Insect Physiology* (Methuen, London, 1965), p. 519.
2. G. Fraenkel and C. Hsiao, *Bull. Entomol. Soc. Amer.* **12**, 294, abstr. (1966).
3. L. V. Knutson, thesis, Cornell University (1963).
4. A. D. Bratt, L. V. Knutson, B. A. Foote, C. O. Berg, *Mem. Cornell Univ. Agr. Exp. Sta.*, in press.
5. Analyses made by T. Greweling, Department of Agronomy, Cornell University.
6. I. M. Kolthoff and N. H. Furman, *Volumetric Analysis* (Wiley, New York, 1929), vol. 2, p. 140.
7. L. J. Edwards and R. L. Patton, *Stain Tech.*, in press.
8. D. V. Tappan, M. J. Jacey, H. M. Boyden, *Ann. N.Y. Acad. Sci.* **121**, 589 (1964).
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