with the aortico-pulmonary region indicate that the magnitude and continuity of such a system in the adult animal make any attempt at specific localization futile. Previously, Palme (*6*) had noted this diffuse distribution but drew attention to the organization of large glomera in specific locales, whereas Hollinshead (*1*) found a distribution of glomera from the left coronary artery to the ligamentum arteriosum confined to the region between the pulmonary artery and the ascending aorta.

The distribution appears to be so random that to give different names to the aggregations of glomus tissue found in arbitrary areas of this region is of little value unless, or until, significant anatomical or physiologic differences become apparent. Krahl (*2*) maintained that the glomus pulmonale was different by virtue of its position and blood supply. Our results in the adult animal have not confirmed these observations. The right and left coronary arteries have been shown to supply the aortico-pulmonic glomera in the adult animal (*1, 7*). The functional significance of this supply, or the role played by the vasa vasorum of the pulmonary trunk or ascending aorta is unknown. The vessel, deep in the media, which ended blindly, in one animal would appear to be a vestige of a vessel previously described and probably functionally significant in fetal and newborn animals. The proximal part of this vessel appears to close after birth in the same way as the ductus arteriosus (*1, 3, 8*).

What we see in the adult cat is the mature pattern in which no direct pulmonic arterial blood supply to glomus tissue exists. The rare occurrence of partially obliterated vessels (and perhaps even patent vessels) is a vestigial manifestation of an organization of greater significance to the fetus and neonate. Aortico-pulmonary glomera seems to be the most appropriate name until it is decided whether their predominant physiological role in the adult animal lies with the pulmonary artery or with the aorta.

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9. Partially supported by USPHS grant H.E.-08359.

23 March 1964

## A Primitive Heart in the Echinoid Strongylocentrotus purpuratus

Abstract. *A pulsating vessel and a compartmented contractile chamber have been found to move coelomic fluid from the perivisceral cavity into and throughout the hemal system of the sea urchin,* Strongylocentrotus purpuratus.

The question of whether echinoderms have a circulatory system has been disputed for nearly 150 years. Our study demonstrates the existence of a true circulatory system.

The axial gland, a little understood structure in echinoderms, is an elongated soft body, generally brownish or purplish in color, found near the stone canal. It has been referred to by a variety of names including heart (*1*), kidney (*2*), brown gland (*3*), ovoid gland (*4*), dorsal organ (*5, 6*), and septal gland (*6*), indicative of the considerable disagreement about its structural relationships and function. This study of the hemal system and axial gland complex of the purple sea urchin, *Strongylocentrotus purpuratus*, has yielded new information that provides a better understanding of the gland's function.

The axial gland is a hollow organ which tapers at both ends. In cross section it forms a circle, half of which consists of dense tissue and the other half is a membrane (Fig. 1).

We have found that there are two prominent and separate cavities, the axocoel and the axial gland lumen (axial sinus). The axial gland lumen is occupied by a pulsating vessel which extends the length of the axial gland with branches that form the dense spongy region. The pulsating vessel terminates aborally in an ampulla-like structure in the axocoel, directly under the madreporite. This ampullar structure is divided into two contractile chambers, which are visible in a live animal after removal of the rectum (Fig. 2). The first, a more conspicuous and opaque chamber, leads from the pulsating vessel; the second chamber, broader and thinner, extends around the inner surface of the aboral sinus.

Rhythmic contractions of the pulsating vessel were studied by time-lapse cinematography, intervals from 3 to 24 frames per second being used. Beat frequencies averaged six per minute, ranging from four to eight. Contraction of one chamber follows that of another, as in a two-chambered heart with auricular and ventricular beats. A contraction sequence begins with the second chamber, passes to the first, and continues peristaltically along the pulsating vessel within the axial gland lumen.

A connection between the stone canal of the water vascular system and lumen of the axial gland (Fig. 2), shown in serial sections, confirms earlier accounts (*3*) and clarifies confusion on this point (*7*). Separation between contractile chambers and the cavity of the axocoel is complete. The axocoel, however, communicates directly with the perivisceral coelom via a narrow slit (2 to 4 mm long) near the beginning of the stone canal. Perivisceral fluid with its contained cells can therefore move freely in and out of the axocoel.

The opening between the perivisceral coelom and axocoel forms a pathway in which coelomic fluid moves throughout the hemal system and to other tissues of the body, notably gonads and alimentary tract. Two percent fluorescein dye (0.01 ml) was injected into the coelomic cavity of an intact animal. After 15 minutes the animal was killed, the coelomic fluid with fluorescein dye was removed, and the exposed tissues were rinsed several times to remove excess dye. By means of ultraviolet light, the dye was shown to be distributed throughout the hemal system.

On the surfaces of the contractile chamber numerous microscopic ostia occur. These lead into an internal network of vessels of capillary dimension, the walls of which consist of numerous longitudinal contractile fibers. From the first chamber, these capillaries converge

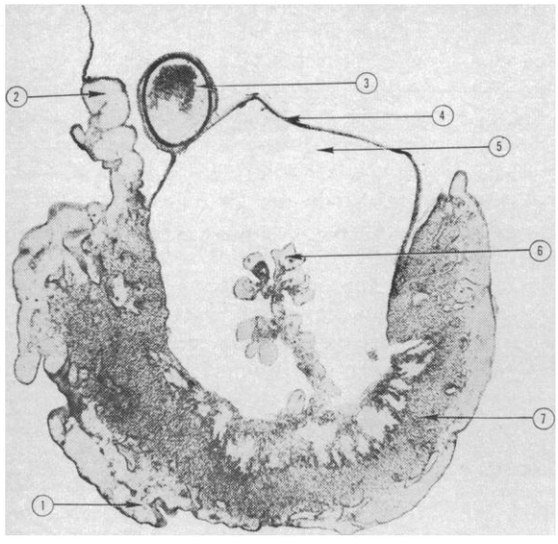


Fig. 1 (above). Cross section of axial gland near oral end. *1*, Surface vessel; *2*, vessel formed from surface vessels on the axial glands; *3*, stone canal; *4*, membrane; *5*, lumen of axial gland (axial sinus); *6*, pulsating vessel; and *7*, dense tissue of axial gland. (× 25)

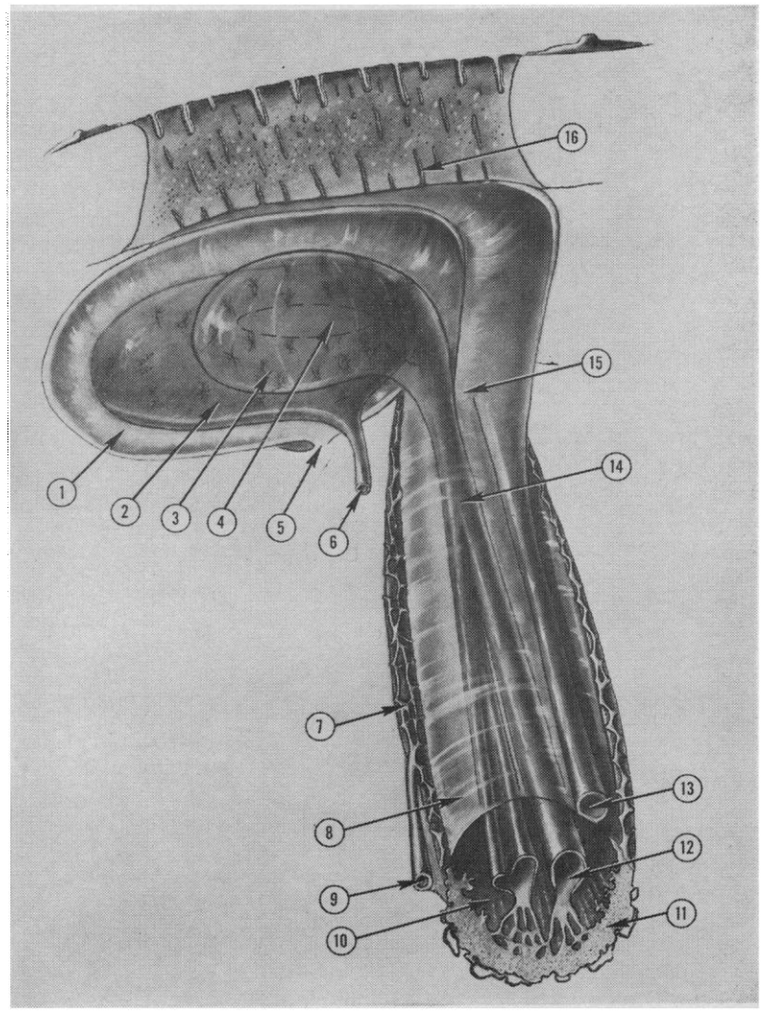


Fig. 2 (top right). A semischematic longitudinal illustration of the axial gland and pulsating vessel complex. *1*, Cavity of the axocoel; *2*, the broad flattened compartment; *3*, the small conspicuous compartment; *4*, opening between numbers *2* and *3* above; *5*, slit between axocoel and coelomic cavity; *6*, rectal hemal vessel from inner hemal sinus; *7*, surface vessels; *8*, membrane; *9*, axial oral vessel; *10*, lumen of axial gland (axial sinus); *11*, dense region; *12*, right branch of the pulsating vessel within lumen of axial gland; *13*, stone canal; *14*, the single pulsating vessel near the aboral end of the axial gland and within the axial lumen; *15*, opening between axial lumen and stone canal; and *16*, madreporite. (× 40)

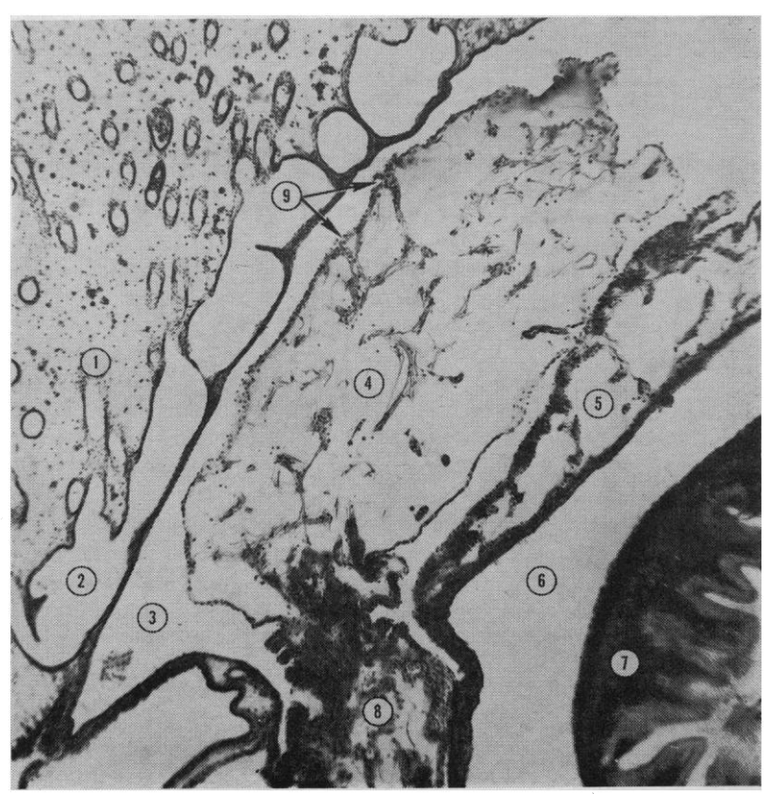


Fig. 3 (bottom right). Photomicrograph of a median longitudinal section of the main contractile compartments. *1*, Madreporite; *2*, cavity beneath the madreporite; *3*, axocoel cavity; *4*, the small conspicuous compartment—note the internal network of fibrous vessels which converge at the beginning of the pulsating vessel; *5*, the broad flattened compartment; *6*, coelomic cavity; *7*, rectum; *8*, pulsating vessel; *9*, ostia. (× 60)

into the pulsating vessel as it enters the axial gland. Motion picture analysis showed a unidirectional movement of pulsations. Since the converging vessels are so numerous at this point (Fig. 3), it is reasonable to attribute a valvular function to them. The pulsating vessel is much branched. The terminal processess of each branch appear to end blindly within the dense tissues of the axial organ. These penetrating branches create a multiplicity of small cavities, each communicating with the main axial gland lumen (Fig. 1). The gland surface is covered by a vascular network that eventually converges into a single vessel that leads to the periesophageal ring. From this ring another vessel leads along the surface of the esophagus. It eventually contributes to and forms the inner hemal sinus, which runs along the inner surface of the digestive tract, and leaves the rectum to terminate in the second contractile chamber. Thus, fluid can enter the contractile chamber by two routes, (i) the inner hemal sinus and (ii) the previously mentioned microscopic ostia.

Since microinjection of fluorescein dye into the cavity of the axocoel demonstrated movement of dye throughout the pulsating chambers, we conclude that injected fluid passes from the axocoel into the chambered vessels by way of the ostia and thence into the pulsating vessel within the axial gland lumen.

Injection of dye into the axial gland lumen was followed within seconds by its appearance in the surface network of vessels on the axial gland. The dye was traced into the stone canal and peripharyngeal ring and then throughout the water vascular system. This confirms our histological observation on the communication between the water vascular system and axial gland (*7*).

Analysis of motion pictures also revealed contractions of the stone canal. A long segment of this tube contracts simultaneously with the pulsating vessel. On close observation we noted a synchrony in the contraction of the stone canal and the pulsating chambers. When the latter contracts, the lumen of the stone canal is conspicuously open.

We found a direct communication between the lumina of the axial gland and stone canal. The rhythmic contraction of the pulsating vessel together with the pulsations of the stone canal may be important in moving fluids throughout the entire water vascular system.

Since acetylcholine inhibits pulsations and adrenaline accelerates them, the process appears to be myogenic in origin.

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7. The only evidence suggesting a connection between the water vascular system and the axial gland was reported by Hyman (*6*), who stated that colored water was forced through the madreporite and traced to the axial gland and through the water vascular system (*6*, p. 568). However, Hyman noted the disagreement of other workers on the existence of a connection between the water vascular system and axial gland (*6*, p. 473).
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## Ultraviolet Sensitivity of Escherichia coli Containing Heat-Inducible λ Prophages

Abstract. *The* c1-t *mutants of bacteriophage λ can form prophage at 36°C but cause lysis of sensitive bacteria at temperatures above 42°C. Growth of cultures at 42° to 46°C induces prophage replication and lysis in* Escherichia coli *K12* (λ c1-t); *lysogenic strains containing wild-type prophage are not induced to lyse at these temperatures. Heat induction is prevented by chloramphenicol. Strains containing heat-inducible prophage are much more sensitive to killing by ultraviolet light than is K12 (λ+).*

The *c1* gene of bacteriophage λ controls the production of a cytoplasmic factor that is required for the establishment and maintenance of prophage. This factor has been called "immunity substance" because it also prevents vegetative multiplication of related phages introduced into the lysogenic cell. According to Jacob and his co-workers (*1*) this cytoplasmic factor is a repressor that inhibits the synthesis of one or more of the "early" proteins essential for initiating growth of vegetative phage.

Sussman and Jacob (*2*) and Thomas and Lambert (*3*) have described mutants of bacteriophage λ that can be maintained as prophage at temperatures below 37°C but are induced to become vegetative phage at higher temperatures. Both groups of investigators reported that mutations in the *c1* gene of the bacteriophage λ can be suppressed by mutations of the host bacterium; these suppressors also ameliorate the action of bacterial mutations which affect the synthesis of certain enzymes. These findings suggest that the repressor is a protein and that temperature-sensitive λ mutants produce an unstable protein that becomes nonfunctional at temperatures that do not affect the structure of the normal repressor. Suppressor mutations in the host bacterium presumably supply some mechanism that allows wild-type protein to be produced on a mutant messenger RNA.

It has been generally assumed that ultraviolet light induces vegetative multiplication of prophage by interfering with the synthesis or function of the repressor. This report concerns the sensitivity of lysogenic strains that contain temperature-sensitive prophages of bacteriophage λ to induction by ultraviolet light.

Wild-type bacteriophage λ that had incorporated 5-bromouracil into its DNA was plated on strain M3, a nonlysogenic strain of *Escherichia coli* K12, and the plates were incubated overnight at 43°C. Phage from clear plaques was replated at 43° and 35°C. Seven mutants giving clear plaques at 43°C but turbid plaques at 35°C were isolated. Genetic tests of the phage mutants are incomplete, but they have been designated as "*c1-t* mutants." An additional mutant used in these experiments, λ *c1-t1*, was the gift of J. J. Weigle. Each mutant was used to produce a lysogenic strain of M3. Cultures of the lysogenic strains were grown with aeration in broth containing 1 percent Difco tryptone + 0.5 percent NaCl to a concentration of 2 to $5 \times 10^8$ per milliliter. For studies of heat induction, the bacteria were diluted in the broth at the