been implicated in the relaxing mechanism (*10*). Therefore, treatments that desensitize actomyosin gel to calcium may do so by destroying (for example, with trypsin), removing (for example, washing procedures), or modifying (for example, with *p*-chloromercuribenzoate) these proteins.

We have found that heating the natural actomyosin gel for 5 minutes, under the conditions given in Table 1, overcomes the calcium sensitivity in a highly selective way. The heated protein is not inhibited by $10^{-4}M$ MgATP; its rate of contraction—the same with or without calcium—is about 80 percent that of native protein with calcium. Moreover, as shown in Table 1, dithiothreitol (DTT), an agent that prevents and reverses sulfhydryl oxidation (*11*), prevents and reverses this effect of heat. The data indicate that heat inactivation of the relaxing site occurs because certain labile SH groups are oxidized to the disulfide form.

Mueller has shown that tropomyosin has such labile SH groups and that tropomyosin sensitizes actomyosin to calcium only when these groups are in the reduced form (*12*). In the light of Mueller's findings, it appears that heat may desensitize natural actomyosin by oxidizing these labile SH groups of tropomyosin.

Other procedures for desensitizing the protein to relaxation may break covalent bonds, remove some of the protein, or irreversibly modify the protein. The heat treatment leaves the protein components intact, and the effect is easily reversed. The procedure is simple, reproducible, and highly selective, not affecting other apparent properties of the gel to any significant extent. For these reasons, it may prove useful in studies on the function and structure of the relaxing site.

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Gastrovascular System of Small Hydromedusae: Mechanisms of Circulation

Abstract. *Small medusae possess a circulatory system of narrow tubes subdivided into several compartments by functional "sphincters." Flow is activated by gastrodermal flagella twice as long as the diameter of the tubes. The flow may be reversed in any part of the system through pressure waves generated by muscular action of the gastric pouches. The combination of flagellar and muscular action provides an adjustable, low-pressure circulation.*

A gastrovascular system (coelenteron) is characteristic for Cnidaria in general (*1*). In small leptomedusae of the genus *Phialidium* Leuckhart, the system is represented in a form typical of hydromedusae (Fig. 1): the mouth, at the end of a tubular manubrium in the center of the subumbrella (Fig. 2), leads into a "stomach" (*S* in Fig. 1; Figs. 3 and 4), which, through four perradial pouches, opens into four radial canals (*PR* in Fig. 1; Figs. 8 and 9). These are continuous, peripherally, with a circumferential canal (*C* in Fig. 1). In their course through the gonads, the radial canals widen to form the gonadal cavities (*G* in Fig. 1; Fig. 7). The problem of how this system functions has not received much attention. In fact, I found no discussion of the principles of its dynamics in the literature. The prevailing view (*2*), supported by very few investigations, has been that the digestive material is driven through the canals, which are essentially open throughout, by the contractions of the umbrellar muscles. "At each contraction, the material moves back and forth or swirls about." Hyman (*2*) did not observe "any definite currents in the gastrovascular canals, although the flagella could be seen in active motion."

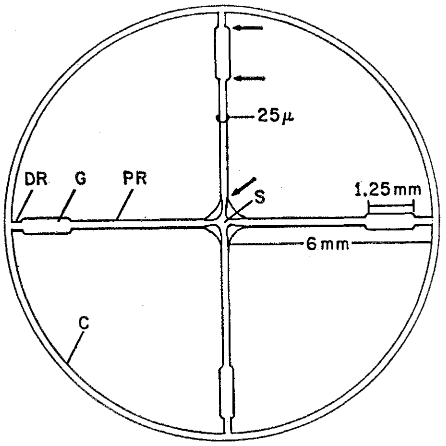


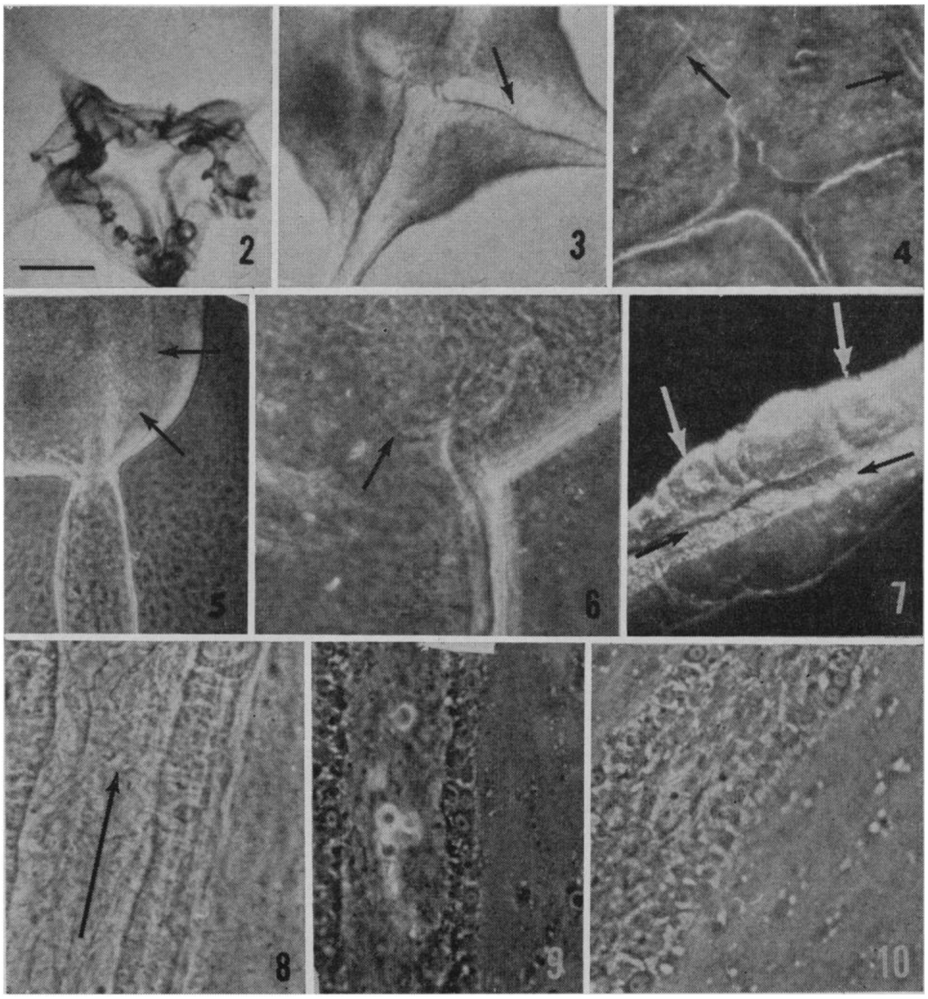
Fig. 1. Diagram of the "pipes" constituting the circulatory system of *Phialidium. S*, Stomach; *PR*, proximal radial canal; *G*, gonadal cavity; *DR*, distal radial canal; *C*, circumferential canal. The arrows point to the locations of functional "sphincters."

In medusae of *Phialidium*, two species of which exist in Puget Sound (*3*), the system can be more thoroughly investigated. Whole living specimens, usually of *P. hemisphericum*, were mounted upside down in depression slides and observed by ordinary light and phase microscopy on a cool stage at temperatures between 11° and 15°C. The animals were kept in fresh sea water and anesthetized whenever necessary with a 7.5 percent solution of $MgCl_2$ added in drops. They remained in excellent condition for many hours. Motion pictures at magnifications of up to 1000 times were taken of strategic regions in unfed individuals and at various times after the animals were fed with brine shrimp. The presence of particles and granules in the system facilitated observations of the flow. In an unfed animal, there were usually a few particles, which might have been detritus, minute organisms, spermatozoa, and so forth. After the animal was fed, the system filled more or less with digestive debris depending on the size of the meal. The speed of the moving particles varied greatly. Measurements of linear flow on strips of motion-picture film revealed that particles, ranging from 1 to 4 μ in diameter, moved with speeds of the order of 100 μ/sec. The estimated speed of blood flow in human pulmonary capillaries is 1 mm/sec (*4*); in capillaries of the human bulbar conjunctiva, only 26 μ/sec (*5*).

The following observations were made on anesthetized, motionless animals. Currents seen in the canals were as directed and as rapid as those in unanesthetized animals. Obviously, umbrellar contractions are not the driving force. The direction of flow, however, is quite variable. The gastric and gonadal cavities are relatively wide and very irregular in shape. The flagella, springing from their walls, often are oriented in opposite directions on two opposing sides (Fig. 7), and the currents in the cavities appear to be essentially circular in large parts of the lumina. In the narrow radial canals, three basic patterns of flow occur: (i) a more or less rapid current moves along the canal in either direction, with the flagella bent sharply near their origin and pointing in the direction of flow (Fig. 8); (ii) a circular flow, a "whirlpool," occurs in small areas in which the flagella lie looped and more or less randomly oriented in the lumen; and (iii) the canal appears collapsed (Fig. 10), there is little or no flow, and the flagella make no major excursions, although they vibrate. These conditions can slowly change one into the other. Occlusion of canals occurs for no apparent reason; the thin walls of the canal simply become flattened against the subumbrellar surface, sometimes within a few seconds, and the flow subsides simultaneously. Often the canals open again after a few minutes.

In the unanesthetized or incompletely anesthetized animals, all these conditions are also seen, but they are more changeable. The muscular activity of the stomach frequently influences the pattern of flow. In each radial canal, the pattern is set to a certain extent by the behavior of the particular gastric pouch from which it takes its origin. As the lumen of the pouch diminishes or widens with muscular activity, the flow in the canal is accelerated or retarded. Successive contractions or relaxations of the gastric pouch may stop the current and eventually set it in motion in the opposite direction. When this occurs, the flagella flip so that they once again point in the direction of the flow.

At the exit from the stomach pouches into the radial canals, flow is often discontinuous. Granules are prevented from floating into the canal; instead, they return speedily toward the center of the gastric cavity. The block appears to be under the control of two factors: (i) the width of the opening, which changes with the state of contraction of the local muscle but is hardly ever narrowed to less than 5 to 7 μ, and (ii) the direction of the flagella, which appear to form a collar at the gastric exit. When the exit is narrow and the flagella point into the stomach, hardly



Figs. 2–10. Photomicrographs from 16-mm motion-picture film, of parts of living medusae (*Phialidium* Leuckhart). The black bar in Fig. 2 represents 500 μ in Fig. 2, 250 μ in Figs. 3 and 4, 60 μ in Fig. 5, 320 μ in Fig. 7, and 16 μ in Figs. 6, 8, 9, and 10. Fig. 2. View into the stomach through open mouth with crenated lips. Three of four canals can be seen emerging from gastric pouches. Fig. 3. The "crossroads" of the stomach. A few granules are visible in the passageways (arrow). The dark triangles represent the folded muscular wall. The shadow at the left is the manubrium completely out of focus. Fig. 4. Somewhat flattened preparation of stomach in near darkfield. The muscular folds were slowly moving and showing waxing and waning creases (arrows). Fig. 5. Proximal radial canal (below) opening into gonadal cavity (outline at arrows) above through sphincter region, which is here partly contracted. Fig. 6. Same region at higher magnification, 5 minutes later, less contracted. Arrow points to flagella and indicates direction of flow. Fig. 7. The gonadal cavity in an ovary with many granules in the lumen. Flow was in opposite directions at opposite walls (black arrows). White arrows point to nuclei of egg cells. Fig. 8. Radial canal focused on flagella oriented in direction of flow (arrow). Fig. 9. Radial canal focused on center of lumen. The light halos indicate outlines of granules. Nuclei (light) and nucleoli (dark) are seen in the wall. Fig. 10. The same canal a few minutes later, collapsed. The lumen is not clearly outlined, but some flagella are visible.

any granules pass into the canal. When the opening is wide and the flagella point outward, there is a steady flow into the periphery in anesthetized as well as in unanesthetized animals. When the entrance is wide and the flagella point into the pouch, the flow is into the stomach. The entrance into and the exit from the gonadal cavities show very similar conditions (Figs. 5 and 6).

The stimuli affecting the muscles are not known. However, 1 to 2 hours after feeding, when the stomach is full of digestive material, two or three exits are usually wide open, and flow out of the stomach is almost continuous. At this time, the entrance into the gonads is usually open, but the exit is sometimes open and sometimes closed. An hour or two later when the stomach is nearly empty, it is common to see digestive matter circulating within the gonadal cavity, with the "sphincters" on both sides of the cavity closed. The net effect of sphincter control on the gastric side appears to be such that coarse material such as the exoskeletons of brine shrimp never get into the circulation at all. Large cellular clumps are broken up and almost squeezed into the canals by the muscular action of the stomach. On the peripheral side, the marginal canal receives much less granular material than the gonads do, and it begins to show a distinct increase in granules long after the gonadal cavities become filled with them.

Observations and measurements on the living system and various supportive findings give rise to the following conclusions.

1) The gastrovascular system of *Phialidium* is truly circulatory in that material is circulated in it, but the system has a complex and highly variable pattern, partly because it is subdivided functionally into compartments and partly because the possibility for reversal of flow exists in any part of the system.

2) The essential and main driving force is delivered by the action of long flagella (minimal length 55 μ). This is demonstrated by the fact that flow continues in anesthetized animals and that it occurs in the canals of isolated immobile pieces of animals in the absence of gastric or umbrellar musculature (*6*). The flow is always in the direction of the flagella. Where the flagella are looped or disordered, the currents are irregular and display whirlpools. In addition, the motion pictures show that peripheral granules commonly overtake central ones, an indication that the currents are fastest at the periphery.

3) Muscular action plays a twofold role. Slow and seemingly erratic contractions in the walls of the gastric pouches create pressure and suction at their exits into the canals. The positive or negative pressure waves produced are usually weaker than the pressure continually generated by the flagella. Occasionally, however, they are strong enough to stop and reverse the flow, and when this happens the flagella flip, reversing their net effect. Unless muscular action interfers with the flow, the flagella remain in their orientation and the currents, once set in motion, continue. Muscle also appears to widen or narrow the lumen in three well-defined locations, the exit from the gastric pouches and the proximal and distal ends of the gonads. The rhythmic contractions of the umbrellar musculature, previously thought to play the major role in driving the circulation, appear to have little influence on it. They may cause very brief local interruptions of flow through bending and clamping the canals, and they may help in preventing large clumps of debris from clogging the passages. The functional sphincters described still do not fully account for the flexibility of the system. In addition to them, the "whirlpools" arising anywhere in the canals in regions of fluctuating pressure will serve as temporary blocks to linear flow in either direction.

4) The system is under low pressure. With the dimensions of the system (Fig. 1) and the speed of flow one can, by assuming the viscosity of the circulating fluid to be that of sea water, estimate the pressure by applying Poiseuille's law. At a speed of 100 μ/sec, the pressure gradient must be of the order of 0.12 mm-Hg.

Gray (*7*) has pointed out that the movement of fluids in tubes by means of flagella is reasonably effective only if the diameter of the tube is not much more than twice the length of the flagella. In the radial canals of *Phialidium*, the flagella are twice as long as the tubes are wide, and they are very effective in driving the contents in either direction. Flagellar action in the wider compartments is bound to be less effective in promoting linear flow, but it creates circular currents well suited to the relatively slow digestive processes occurring in the gastric and gonadal cavities. In most animals, the function of cilia is taken over in circulatory systems by muscular action (*8*). Even in hydromedusae the inherent inability of cilia to change their direction actively must be overcome by muscle. It is the combination of flagellar and muscular motive forces which makes this system unusual, if not unique.

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Adenosine Triphosphate Usage by Flagella

Abstract. *Comparison of beat frequencies with rates of dephosphorylation of adenosine triphosphate by glycerinated sea urchin spermatozoa as functions of adenosine triphosphate concentration suggests that each molecule of the flagellar adenosine triphosphatase, dynein, dephosphorylates one adenosine triphosphate molecule during each beat cycle.*

Dynein, the adenosine triphosphatase isolated from the cilia of *Tetrahymena pyriformis* (*1*), has a molecular weight of approximately 600,000 and a specific activity of 1.3 to 3.5 micromoles of phosphorus per milligram of protein per minute at 20°C, depending on the degree of depolymerization and the conditions of cation activation (*2*). This enzyme, which is believed to be re-