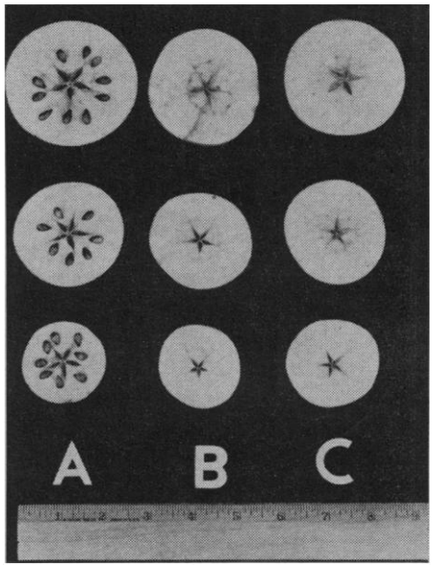

Fig. 2. Seeded and parthenocarpic Wealthy apple fruits. (A) Seeded fruits from open-pollinated flowers, with seeds removed from locules and placed on cut surface of fruit; (B) seedless fruits produced by treating unpollinated flowers with lanolin paste containing KGA_3 ($5 \times 10^{-3}M$); (C) seedless fruits produced by treating unpollinated flowers with lanolin paste containing apple-seed extract (1 kg-eq of fraction 1 per gram of paste). Paste applied 26 May; fruits photographed 7 September 1966.

The source of the extract (immature seeds) and its high gibberellin-like activity provide strong support for the hypothesis that gibberellins produced in the ovule after fertilization are responsible for fruit-set in the apple.

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Heat Inactivation of the Relaxing Site of Actomyosin: Prevention and Reversal with Dithiothreitol

Abstract. *Adenosine triphosphate and magnesium (MgATP) inhibit contraction by binding to a specific relaxing site on natural actomyosin gel. This inhibitory control site is distinct from the active sites where MgATP causes contraction. In high concentrations of MgATP, calcium triggers contraction by releasing the protein from substrate inhibition, allowing the contractile reactions to occur. Heating the protein for 5 minutes at 43°C selectively inactivates the relaxing site. After this treatment, actomyosin with MgATP contracts as well without calcium as with it. That this effect of heat is prevented and reversed by dithiothreitol (an agent that reduces disulfide bonds) indicates that the structure of the relaxing site depends on certain labile sulfhydryl groups, which may be those of tropomyosin. When these are oxidized to disulfide bonds, the site loses its activity; when the disulfide bonds are reduced, the site regains its activity.*

It is now generally believed that muscular contraction is controlled by changes in the amount of calcium available to the contractile filaments (*1*). Even though adenosine triphosphate and magnesium (MgATP), which cause contraction, are always present in high concentration (about $0.005M$), the filaments cannot contract so long as the concentration of calcium in the sarcoplasm is held below some required level (about $10^{-7}M$). The sarcoplasmic reticulum maintains this relaxed condition by actively pumping calcium out of the sarcoplasm (*2*). Activation of the muscle allows the calcium concentration in the sarcoplasm to rise; calcium then binds to the filaments, and this binding causes them to contract.

Natural actomyosin is extracted directly from muscle; it contains tropomyosin and certain other proteins in addition to the main constituents—actin and myosin. Like the contractile filaments in muscle, such a gel in a high concentration of MgATP cannot contract or hydrolyze ATP without a trace amount of calcium (*3*). Without this calcium, under these conditions, the substrate, MgATP, inhibits hydrolysis of ATP and contraction by binding to a specific relaxing site. This site is separate and distinct from the active sites where MgATP causes contraction (*4*). When calcium is added to a gel inhibited by MgATP, it triggers contraction by overcoming this substrate inhibition and allowing the contractile reactions to occur (*3, 4*).

The sensitivity of the gel to substrate inhibition and to calcium is lost after a number of different treatments: aging (*4*), partial titration of SH groups (*4, 5*), mild digestion with trypsin (*6*), special washing procedures (*7*), and heat (*8*). Certain of these treated preparations have been shown to regain their calcium sensitivity when tropomyosin is added to them (*6*). This has led to the view that the function of the relaxing site probably depends on tropomyosin in association with one of the main protein constituents—most probably with the F-actin polymer (*9*). Other less well-defined proteins have also

Table 1. Effects of dithiothreitol on heat inactivation of the relaxing site of natural actomyosin. The reaction mixture contained 0.005 mole of MgCl, 0.03 mole of KCl, and 0.06 mole of tris(hydroxymethyl)aminomethane (*p*H 7.4) per liter at 25°C. The procedure for inactivating the protein was as follows. The above solution was heated to 43°C. Actomyosin, as prepared in this laboratory (*13*), was added to a final concentration of 0.12 mg/ml. After 5 minutes, the reaction solution was cooled to 25°C and tested. To reverse inactivation, the reaction solution, heated as above, was cooled to 4°C, and DTT was added as indicated. At intervals, portions were removed and tested at 25°C. The rate of contraction was measured by recording the increase in turbidity of the actomyosin gel suspension at 545 mμ (*13*). All rates were measured at $0.002M$ ethylene glycol *bis*(aminoethylether)-*N,N'*-tetraacetic acid (EGTA) and $1 \times 10^{-4}M$ ATP in addition to the conditions described above.

Conditions	Rate of contraction (optical density change at 545 mμ per second)
Heat inactivation and protection by DTT	
Unheated protein	0
Unheated protein + 0.5 m*M* DTT	0
Unheated protein + $0.002M$ Ca^{++}	0.460
Unheated protein + 0.5 m*M* DTT + $0.002M$ Ca	.450
Heated protein	.370
Heated protein + $0.002M$ Ca^{++}	.340
Protein heated with 0.5 *mM* DTT	
Reversal by DTT	
Heated protein	0.390
Heated protein incubated with 0.5 m*M* DTT for 24 hours	0
Heated protein incubated with 5 m*M* DTT for 2 hours	0

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

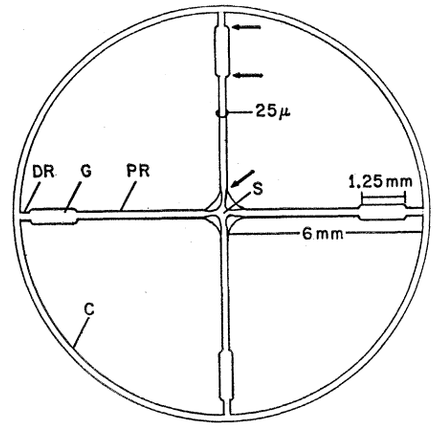

Fig. 1. Diagram of the "pipes" constituting the circulatory system of *Phialidium*. *S*, Stomach; *PR*, proximal radial canal; *G*, gonadal cavity; *DR*, distal radial canal; *C*, circumferential canal. The arrows point to the locations of functional "sphincters."

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."

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, spermato-