percent of the cardiac cells are contracting at rates ranging from 20 to 120 beats per minute with a mean rate of 30 to 40 beats per minute. Approximately one-half of the cells that are resuspended in 0.9 percent sodium chloride solution or Krebs-Ringer buffer (minus Ca^{2+}) containing glucose lose contractile activity over a 2-hour period at room temperature. This does not appear to be related to a limitation of an energy source, since the further addition of glucose or albumin-bound palmitic acid to the medium does not have marked influence on contractile activity. Furthermore, with the conditions employed at present, addition of adenosine triphosphate (5 to 100 μM) does not affect either the initiation or maintenance of cardiac cell contractions.

Alterations in the osmolality of the suspending medium (0.9 percent NaCl) had marked effects on the proportions of contracting cardiac cells to total cells. Increasing the osmolality from 305 to 320 meq/liter with sucrose resulted in a sharp decrease (from 55 to 18 percent) in the percentage of beating cells. Any further increase in osmolality resulted in complete cessation of contractile activity; it could be restored to the original level by reducing osmolality to 305 meq/liter.

The absence of potassium in the suspension medium resulted in a rapid loss in both total viable cells and contracting cells (Fig. 3). At concentrations of 5 and 15 meq/liter of K^+, the percentage of contracting cardiac cells was initially high and remained constant throughout the 100-minute experimental period. During this same period, the percentage of viable cells decreased from 55 percent initially to approximately 30 percent. The presence of calcium (0.5 to 5.0 meq/liter) caused immediate cessation of cardiac cell contractions.

The procedure for tissue dissociation reported here results in a cell suspension containing a high percentage of viable cells, of which approximately half exhibit contractile properties. To date, the most important determinant for maintenance of both cell viability and contractility appears to be the composition of the medium in which the cells are resuspended after dissociation. Preliminary studies have indicated that this type of preparation can be useful in investigating metabolic pathways and control mechanisms in cardiac tissue.

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Asexual Reproduction in a Sipunculan Worm

Abstract. *The sipunculan worm* Aspidosiphon brocki *reproduces asexually by transverse fision into two unequal parts, the smaller part comprising the posterior fifth of the animal. Prior to fission each part regenerates the structures essential to the formation of a new individual. The smaller posterior part (daughter) regenerates an anterior body, including introvert, anterior gut, retractor muscles, and nephridia, whereas the larger anterior part (parent) regenerates only the posterior body wall.*

Asexual reproduction was unknown in sipunculan worms until Rajulu and Krishnan (*1*) reported "budding" in four specimens of *Sipunculus robustus* after they had maintained the animals in stale seawater in the laboratory. I now report that a small species of *Aspidosiphon* from the Caribbean Sea and the Straits of Florida undergoes asexual reproduction both in the field and in the laboratory by constriction and subsequent detachment of the posterior end to form a new individual. The constriction occurs at a distance approximately one-fifth of the total length of the trunk from the posterior extremity. The internal organs which are severed by the constriction and thereby segregated into the posterior body are incorporated along with a regenerated anterior end in the formation of the "daughter" individual. When the narrow neck of the constriction closes, the posterior portion of the "parent" animal drops off and is at once replaced by the eversion of a new posterior end that is formed during constriction. At the time of its detachment or within 1 to 3 days the separated posterior entity or daughter individual everts the regenerated anterior end, formed prior to detachment, thus completing the formation of the "juvenile" (*2*).

The specimens observed in this study, tentatively identified as *Aspidosiphon brocki* Augener 1903 (*3*), were collected in Jamaica, Puerto Rico, and the Florida Keys from intertidal coralline limestone where they inhabit burrows, presumably of their own formation (*4*). Adult specimens range from 3 to 10 mm long with introvert retracted. Characteristic of the genus, horny cuticular shields are present on the anterior and posterior ends of the trunk, although in this species the posterior shield is weakly developed. The introvert, when extended, is nearly as long as the trunk. A single pair of retractor muscles that function in the withdrawal of the introvert originates from the body wall in the posterior quarter of the trunk.

Twenty-three specimens were collected in which posterior constrictions were present. Sixteen were preserved or fixed for histological study. The remaining 7 were maintained alive in the laboratory until detachment of the posterior end occurred (5 to 14 days after collection) and the juvenile was fully formed. At one station in the Florida Keys, where all specimens were counted and carefully examined for asexually reproducing individuals, 15 percent (8 of 53) had posterior constrictions. Moreover, in two different observations, a juvenile, apparently recently detached, and a detached posterior end were found, each occupying the posterior portion of a burrow of an adult worm.

In the constricted animal, the area of the constriction is encircled externally by a blackened band, presumably of cuticular origin (Fig. 1a). Internally, the stricture is traversed by a noncellular sheet of unknown composition (Fig. 2). The constriction occurs immediately caudad to the origin of the re-

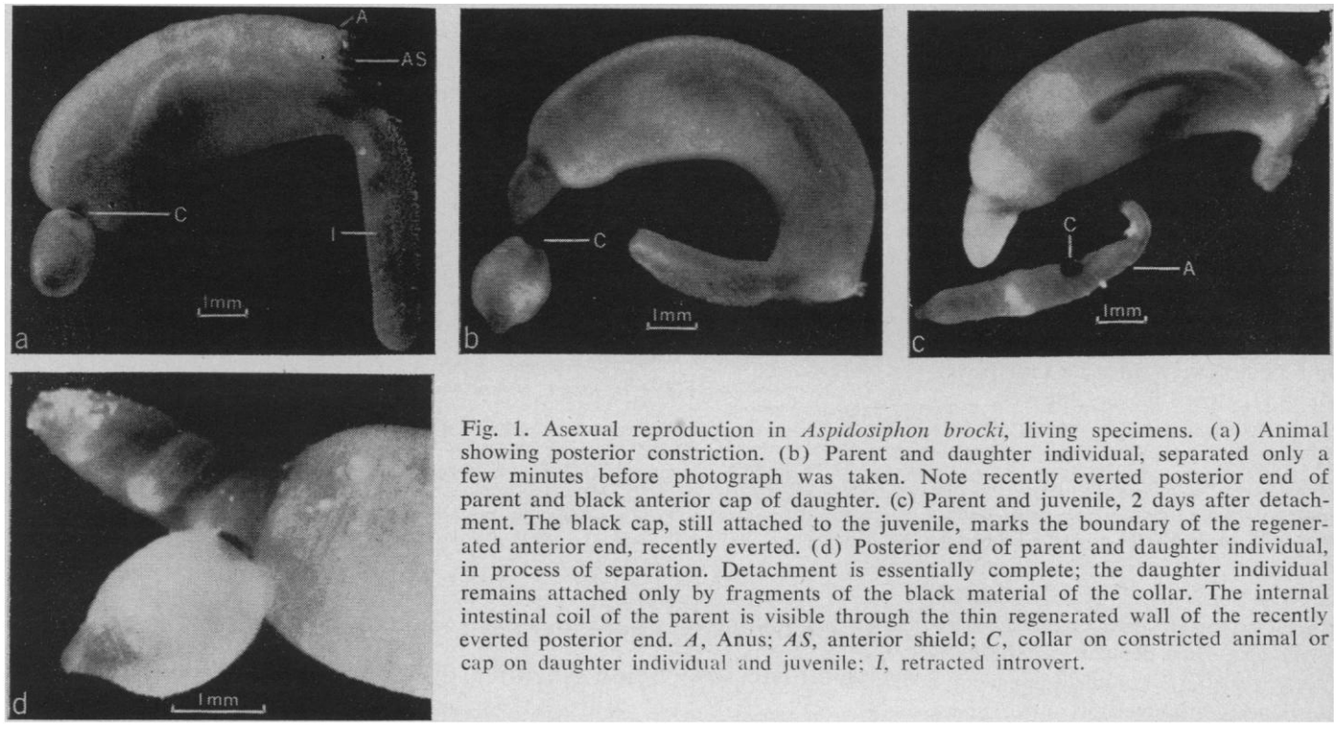

Fig. 1. Asexual reproduction in *Aspidosiphon brocki*, living specimens. (a) Animal showing posterior constriction. (b) Parent and daughter individual, separated only a few minutes before photograph was taken. Note recently everted posterior end of parent and black anterior cap of daughter. (c) Parent and juvenile, 2 days after detachment. The black cap, still attached to the juvenile, marks the boundary of the regenerated anterior end, recently everted. (d) Posterior end of parent and daughter individual, in process of separation. Detachment is essentially complete; the daughter individual remains attached only by fragments of the black material of the collar. The internal intestinal coil of the parent is visible through the thin regenerated wall of the recently everted posterior end. *A*, Anus; *AS*, anterior shield; *C*, collar on constricted animal or cap on daughter individual and juvenile; *I*, retracted introvert.

tractor muscles, separating the posterior portions of the intestinal spiral, spindle muscle, and ventral nerve cord from the anterior portions. On either side of the stricture is an invaginated epidermal proliferation with thin cuticular covering (Fig. 2).

Anterior to the constriction, in the parent organism, the epidermal invagination is attached to the thickened spindle muscle which emerges from the posterior end of the intestinal coil. The ascending and descending portions of the intestinal spiral are joined terminally, at the point of their rupture from the posterior gut. At the time of detachment of the posterior end, the anterior epidermal invagination with cuticular covering is everted to form a new posterior end which is characterized by its narrow, pointed shape and its thin body wall, through which the internal organs are visible (Fig. 1d). Cuticular thickenings of the posterior shield can be recognized within 12 days after detachment. Occasionally, after eversion of the new posterior end of the parent, the daughter individual remains loosely attached to the parent by fragments of the cuticular collar; complete separation is effected within a few hours by body movements of both the parent and daughter individuals.

In the posterior portion of the constricted animal prior to detachment (daughter organism), the epidermal proliferation is invaginated to a distance about one-third of the length of the organism and from this is formed the epidermis of the anterior trunk and introvert of the juvenile (Fig. 2). Slender retractor muscles pass forward from either side of the posterior invagination to attach to the body wall. A thin esophageal tube extends posteriorly from the invaginated epidermis to attach to the descending loop of the severed intestinal coil. Joining the end of the ascending intestinal loop, the newly formed rectum of the daughter organism unites dorsally with the epidermal invagination about halfway along its length (Fig. 2). Additionally, in a ventrolateral position on either side of the rectal attachment, a thin-walled nephridial sac is present. The spindle muscle, fastened to the posterior end of the body cavity, courses anteriorly through the center of the intestinal coil and runs along with the rectum to join the epidermal inpocketing. At the base of the retractor muscles a few primordial germ cells mark the rudimentary gonad. The entire body cavity of the daughter organism is densely packed with coelomocytes.

When the daughter individual drops off, the pigmented material of the constricted collar adheres to it as a rounded black anterior cap (Fig. 1b). Within 1 to 3 days, the newly formed anterior end is everted and the black cap is pushed aside. With the eversion of the anterior end, the animal resembles the

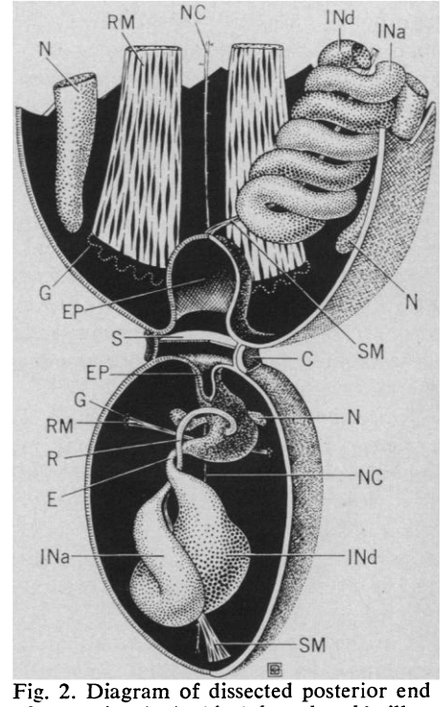

Fig. 2. Diagram of dissected posterior end of constricted *Aspidosiphon brocki*, illustrating internal structures of daughter organism and posterior portion of parent. *C*, Collar; *E*, esophagus; *EP*, epidermal invagination; *G*, gonad; *INa*, ascending intestine; *INd*, descending intestine; *N*, nephridium; *NC*, nerve cord; *R*, rectum; *RM*, retractor muscle; *S*, internal noncellular sheet traversing the stricture; *SM*, spindle muscle. Regenerated structures in the daughter organism are the esophagus, rectum, retractor muscles, gonad, nephridia, and epidermal invagination which gives rise to the anterior end of the juvenile, including the introvert.

adult and is referred to as a juvenile. The cap may adhere to the juvenile for several days, marking the site of detachment and the boundary between that portion of the juvenile contributed by the parent and the newly formed portion (Fig. 1c). The body wall posterior to the boundary is notably thicker and the apex is marked externally by the cuticular thickenings of the posterior shield. Anterior to the boundary, the body wall of the newly formed anterior trunk and introvert is exceedingly thin. Immediately after its initial eversion the activity of the introvert commences; it is continually retracted to a position just above the anus and extended to reveal the terminal tentacular anlage, consisting of two long dorsal lobes and two shorter ventral lobes. As in the adult, the introvert is covered by brownish horny spines and encircled anteriorly by several rows of hooks. Within 12 days after detachment the early traces of the anterior shield appear in a dorsal position above the anus and the introvert is displaced slightly in a ventral direction.

Although asexual reproduction in sipunculans has been discovered only recently, their regenerative capabilities have been recognized for some time. Regeneration of the posterior end has been described (*5*). Experimentally induced regeneration of the introvert with the formation of anterior gut, retractor muscles, and brain, has been reported (*6*). According to these reports, regenerative cells migrate from the nerve cord to form ectodermal elements and the coelomocytes may give rise to mesodermal elements.

A comparison of the morphological aspects of asexual reproduction in *Sipunculus robustus* as related in the brief description by Rajulu and Krishnan (*1*) and in *Aspidosiphon brocki* shows that differences occur in the sequence of events relative to the separation of individuals. Whereas in *S. robustus* the introvert is formed only after separation by an elongation and subsequent inversion of the anterior portion of the new individual, in *A. brocki* the introvert is formed prior to separation by an epidermal ingrowth and the organs of the newly formed anterior end are clearly recognizable in the constricted individual before detachment.

Prior to Rajulu and Krishnan's report (*1*), sipunculans were known to reproduce only sexually (*7*). Whether sexual phases alternate with asexual phases in *Sipunculus robustus* and *Aspidosiphon brocki*, as described for some planarians and annelids (*8*), is unknown. However, the presence of gonadal tissue at the base of the retractor muscles in both the adult and the juvenile of *A. brocki* suggests that this species is capable of reproducing sexually, even though no mature gametes have been detected free in the coelom.

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3 December 1969

Enhancement of Photoalteration of Cyclodiene Insecticide Chemical Residues by Rotenone

Abstract. *When applied at low concentrations to plant foliage, rotenone catalyzes the photoisomerization of dieldrin and other cyclodiene insecticide chemical residues. This finding of a new type of interaction between pesticide chemicals suggests the possibility of controlling the persistence of residues on plants by use of certain pesticide-photosensitizer combinations and application sequences.*

Pesticide chemicals should ideally persist, on the surface to which they are applied, only for the period required for pest control. Their residues should then rapidly dissipate in order to minimize or, hopefully, obviate contamination of the agricultural commodities and the adjacent environment. It might be possible to achieve pest control for the desired period without leaving significant residues at the end of this time by the simultaneous or subsequent use of a second chemical that will regulate, as desired, the rate of dissipation of the pesticide chemical applied. For example, a photosensitizing chemical might be applied to a surface treated with a persistent pesticide to accelerate the dissipation of residue associated with the pesticide. Our finding that the presence of rotenone residues on plant foliage catalyzes the photoisomerization of dieldrin and similar insecticide chemicals indicates that the suggested idea for regulation of pesticide chemical residues is indeed possible (*1*). The structures of dieldrin and its "half birdcage" photoisomer, photodieldrin, are shown in Fig. 1.

In screening possible photosensitizers, [^{14}C]dieldrin and the candidate chemical, each at a concentration of 10 parts per million based on the weight of the fresh leaf, were separately dissolved in 50 μl of methanol and applied to the surface of growing bean leaves; the treated plants were subsequently placed in sunlight for 1 hour. The amount of [^{14}C]dieldrin, [^{14}C]photodieldrin, and other compounds containing ^{14}C that persisted on the foliage after exposure to sunlight was determined by rinsing the treated leaves with ether and analyzing the rinses by thin-layer chromatography (TLC), radioautography, and liquid scintillation counting. We used a similar procedure with certain other pesticide chemicals labeled with radioactive components, including [^{14}C]aldrin and [^{14}C]endrin, sometimes varying the time intervals, concentrations, or sequences of applying the pesticides and photosensitizers. In addition, each chlorinated hydrocarbon insecticide chemical tested was analyzed by gas-liquid chromatography (GLC) with electron capture detection, and by TLC of unlabeled material with detection by suitable chromogenic procedures (*1, 2*).

On the basis of a survey of 16 known chemical photosensitizers and related compounds (*3*), and 29 pesticide chemicals and analogs having structural similarities to known photosensitizers, rotenone, of the compounds