References and Notes

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Influence of Currents on Form of Sponges

Abstract. Small reconstituted *Microciona prolifera* produced central oscular chimneys, perpendicular to the surface of attachment, in standing water; in a slow, steady current, the oscular chimneys were eccentrically placed and directed obliquely downstream. Gravity and directional illumination did not affect the orientation of the chimney.

In 1923, Bidder (*1*) pointed out that sponges of the same species differ in shape, apparently in adaptation to the water currents in which they grow; those exposed to currents of constant direction bear most of their oscula on the downstream side, while those in still water or variable currents bear oscula opening upward. Since, so far as I am aware, no attempt has ever been made to verify this suggestion experimentally, I examined the influence of certain environmental factors, including water currents, on the growth of the oscular chimney in small *Microciona prolifera* obtained by the method of H. V. Wilson (*2*), that is, by allowing cells dissociated by squeezing the sponge through bolting silk to settle on glass slides in dishes of sea water.

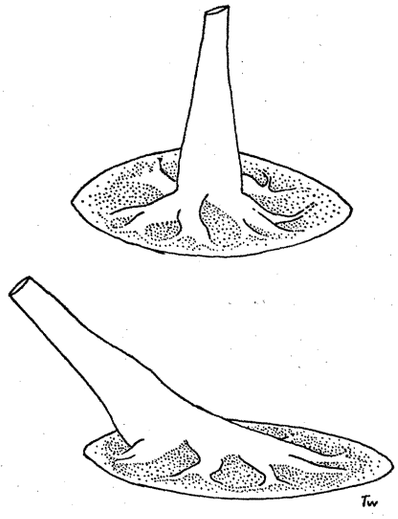

Fig. 1. Reconstituted sponges, *Microciona prolifera*, grown in standing water (top) and in slowly flowing water (bottom).

After 36 to 48 hours, before the reassociated masses of sponge cells had any detectable internal structure foreshadowing a canal system, they were well attached to the slides and could be transferred to other containers. Half the slides, with about 15 sponges, were placed in finger bowls of "standing" water—actually, kept gently turbulent by slowly dripping sea water. The other half of the slides were placed in a battery jar in which a constant, slow current was maintained by a stream of bubbles up one side.

Within a week each small sponge, 1 to 2 mm in diameter, had formed a system of excurrent canals radiating from the base of a tall oscular chimney. In standing water, every sponge produced a central chimney perpendicular to the slide on which it grew (Fig. 1, top). In flowing water, every sponge produced an eccentric chimney, pointing downstream at an angle of about 45° with the slide (Fig. 1, bottom). The same form was assumed whether the sponge grew on the upper surface of the slide, or on the lower surface, or on the vertical surface of a slide standing on edge, and whether illumination came from above, or from one side, or through the slide from below.

When a slide bearing such adapted sponges was transferred to the other kind of container, the sponges gradually transformed themselves to the form appropriate to the new conditions over a period of about 10 days. Except for a temporary shortening and constriction of the oscular chimney, the canal system seemed to remain functional throughout the transition.

These results agree well with Bidder's hypothesis that a growing sponge adopts a form which minimizes the quantity of exhaled water re-entering its incurrent pores (*3*).

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4 March 1960

Uterine Vascular Clamping: New Procedure for the Study of Congenital Malformations

Abstract. Clamping the uterine blood vessels of the rat on the 9th day of gestation for ½ to 3 hours resulted in fetal death, growth retardation, and severe congenital malformations. This procedure has broad applications in the fields of teratology and cancer chemotherapy, and in the investigation of fetal-maternal physiology.

Mechanical or operative techniques in teratology have never been widely adopted, because, in general, they are not potent teratogenic agents, or they have only minimal application to the general field of congenital malformations. The following operative procedure, developed in our laboratory, is a potent teratogenic agent and should find wide use in all phases of experimental teratology, since one animal provides both the control and experimental fetuses.

An inbred strain of pathogen-free rats with an extremely low rate of random malformations was used in the study. The rats were subjected to a 12-hour mating period, and vaginal smears were used to determine pregnancy. The first day of a positive smear was used as day zero in calculations of gestational age.

On the 9th day of pregnancy laparotomy was performed with pentobarbital anesthesia. The number, location, and condition of the implantation sites were recorded. One horn of the uterus was then clamped at its cervical and ovarian ends, the clamps extending across the uterus and its mesentery. By this means, one horn was completely isolated from the maternal circulation. The other horn served as a control. There were five rats in each of the six time periods of ½, 1, 1½, 2, 2½, and 3 hours. After the specified clamping interval, the hemostats were removed, and the abdomen was closed. The rats were killed on the 21st day.

Complete isolation of the clamped uterus from the maternal circulation was an essential condition of this experimental procedure. Intravenous injections of trypan blue and fluorescent dyes qualitatively confirmed uterine hemostasis, since no dye was noted on the clamped side. A more quantitative method was obtained by injecting 12.48 μc of radioiodinated albumin intravenously, after clamping a (nonpregnant) uterus. The two horns were then excised and divided into three segments. The counts per minute per milligram of tissue showed negligible radioactivity in the clamped segments (Fig. 1*a*).

Either of two reactions followed clamping: (i) a sudden arteriolar spasm