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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.

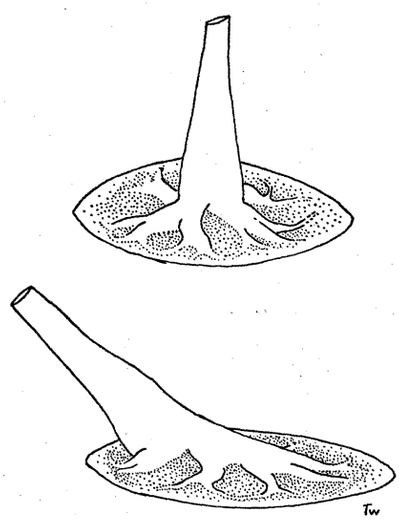

Fig. 1. Reconstituted sponges, *Microciona prolifera*, grown in standing water (top) and in slowly flowing water (bottom).

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

with blanching of the uterus, followed by deepening cyanosis during the next 15 minutes or (ii) gradual cyanosis, again reaching a peak between 10 and 15 minutes. The cyanosis persisted until the clamps were removed.

Uterine clamping affected the fetuses in several ways. Fetal death with resorption, or growth retardation or, sometimes, severe congenital malformations occured. Many fetuses were unaffected by the procedure, but most of these were in the shorter time intervals.

Occurrence of fetal resorption was directly related to the duration of clamping (Fig. 1*b*). Control mortality at ½ hour was 12 percent; it gradually rose, as the procedure was prolonged, to 33 percent in the 3-hour group. At ½ hour, 28 of 33 clamped embryos survived, a mortality rate of 15.1 percent. At 3 hours, 21 of 22 clamped embryos were resorbed; the one survivor was malformed.

The mean fetal weights in the surviving experimental fetuses clamped 1½ hours or less were not significantly different from those of the control groups (Fig. 1*c*). The mean fetal weights of the 2- and 2½-hour experimental groups were significantly less than the weights of the corresponding control groups.

One of the 116 surviving control animals was malformed, having a right-sided aortic arch. There were no malformations among the 49 survivors in the ½- and 1-hour experimental groups. In the groups clamped from 1½ to 3 hours, 8 of the 48 surviving fetuses (16.7 percent) were malformed. The 11 malformations consisted of anophthalmia (Fig. 1*e*), microphthalmia, renal aplasia (Fig. 1*g*), renal agenesis, uterine agenesis (Fig. 1*g*), pancake adrenal, anencephaly (Fig. 1*f*), omphalocele (Fig. 1*f*), absent external ear (Fig. 1*d*), hydrocephalus, and absent fourth aortic arch (infantile coarctation).

Similar experiments have been carried out at different gestational ages. Preliminary results of clamping on the 8th day show a higher mortality and more severe malformations.

The teratogenic action of vascular clamping may be due to either, or both, of two factors: (i) a deficiency of anabolic and metabolic products utilized by the embryonic tissues, or (ii) an excess of catabolic products produced in these tissues with no communication with the maternal circulation. Whether a single factor such as hypoxia, excessive carbon dioxide, or *p*H changes is the etiologic agent is unknown at this time, but it is clear that the cause lies in the alteration produced in the intrauterine environment. Low oxygen tension would appear to be the first mechanism to be investigated, since it has been shown to be teratogenic in mice (*1*). Experimental hypoxia in the rat has so far failed to produce congenital malformations (*2*), although excess carbon dioxide has been reported to be teratogenic (*3*). Certainly, valuable information can be obtained by measurement of the gas contents and chemical changes in the tissue and vascular compartments of the clamped uterus and embryos. More quantitative data on the possible roles of hypoxia and excessive carbon dioxide can be obtained by this procedure. Furthermore, attempts should be made to reverse the effects of clamping by hyperoxygenating the mother prior to clamping, exogenous oxygenation of the clamped segment, or alterations in maternal acid-base balance before clamping.

Since there is a period during the first hour of clamping, on the 9th day, in which there are no malformations and no weight reduction, the clamped side can be employed as a control for a wide variety of experiments using the unclamped horn as the experimental side. The determination of the length of action of a teratogenic drug is an example of one application of this technique. Thus, the presence of malformations on the clamped side after injection of a teratogenic chemical would characterize the drug as to its rapidity of action and degree of synergism with the clamping procedure. Chemotherapeutic agents could be similarly tested, since the embryo is a good test animal for antitumor drugs. The variations of the procedure are numerous, and spread into the field of fetal-maternal physiology. The broad applications of the procedure as a pharmacologic and physiologic technique are as worthy of investigation as is its mechanism of teratogenesis (*4*).

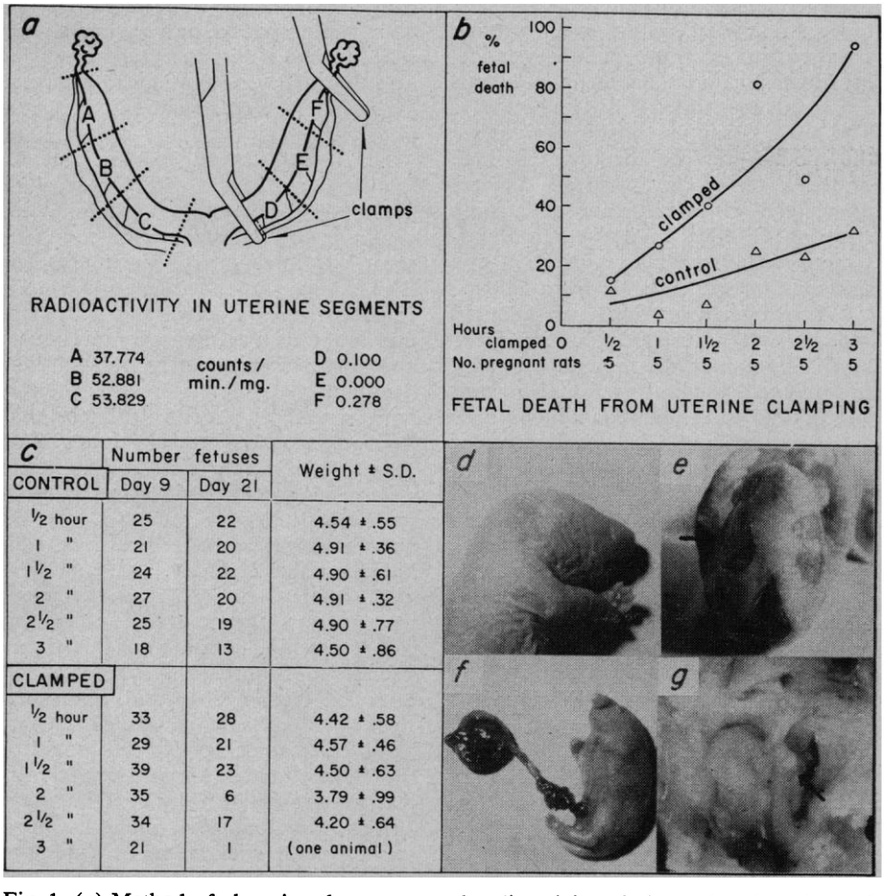

Fig. 1. (*a*) Method of clamping the uterus, and radioactivity of clamped and unclamped horns. (*b*) Percentage mortality among experimental and control fetuses after various periods of clamping. (*c*) Tabulation of the fetal weights of clamped and control animals. (*d*) Anophthalmia and absent pinna in animal from litter clamped 3 hours. (*e*) Anopthalmia on left (arrow) with normal eye on right in animal from 2-hour experimental group. Skin over orbit has been removed. (*f*) Anenecephaly and omphalocele in fetus from 2-hour experimental group. (*g*) Dissection of fetus from litter clamped 1½ hours, showing aplastic left kidney (arrow) and corresponding changes in left adrenal.

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8 April 1960

Increased Stress and Effectiveness of Placebos and "Active" Drugs

Abstract. Evidence is presented to indicate that placebos are far more effective in producing carefully defined relief of pathological pain than they are in the case of experimental pain. This is construed as further support for the view that placebos are more effective when stress is great than they are when stress is not so great. A similar situation holds for morphine. Certain drugs are effective in relieving visceral sensations only if an essential psychological state is present. This is, in effect, a new principle of drug action.

This report presents an experimental finding: placebos relieve pathological pain more effectively than they do experimental pain. Two general concepts grow out of this observation as working hypotheses: (i) the effectiveness of placebos increases with increased stress and (ii) the effectiveness of certain "active" drugs increases with increased stress.

In essence, this study is based upon the proposition that pain of pathological origin produces more anxiety, or stress, than does experimentally contrived pain. Like any axiom, this one may be unprovable in a tight mathematical sense, yet its truth is clearly evident. Even so, the material in this report is presented as evidence for, not proof of, the proposition, just as was the case in an earlier study (*1*), where material of another kind was presented. In that earlier paper it was found: (i) in terms of percentage of a given population relieved, placebos are significantly more effective when postoperative pain is severe than they are when the pain is less severe, and (ii) the work of Cleghorn, Graham, Campbell, Rublee, Elliott, and Saffran (*2*) demonstrates that firing of the adrenal glands (measured in objective terms) is far greater in response to a placebo in patients hospitalized for severe anxiety than in patients hospitalized for anxiety of less severe degree.

The new data are presented in Tables 1 and 2. The most important fact arising from these data is that the mean percentage effectiveness of placebos in relieving pathological pain is over ten times that found with experimental pain. It is realized that some of the studies compared are based on large samples and some on small, and that the *t*-test evaluation ignores this fact and gives all studies equal weight. For present purposes this is satisfactory since numerous studies are involved and the difference between the two conditions is great.

It is not my contention that placebo effectiveness is always low in experimental situations. This is demonstrably not the case. The matter is complex, and it may be in these other cases that stress of one kind or another operates when placebo effectiveness is high. It is important to deal, as here, with a limited area at a time; in the present instance attention is given solely to comparison of placebo effectiveness in relieving pain of (i) pathological and (ii) experimental origin.

Placebos, being "inert" agents, can affect only psychological processes. The assumption is that when the psychological component of a situation is important the placebo will have a correspondingly greater opportunity to produce an effect, and this seems to be the case. The primary purpose of this paper is, however, to present observations rather than speculations.

Effects similar to those of a placebo can be found with an "active" drug: morphine, even in large doses, does not dependably relieve pain of experimental origin in man, as indicated by some 15 different groups of investigators (*3*, pp. 123, 124). Morphine in comparable (or smaller) doses is highly effective in relieving pain of pathological origin. Pathology (stress, see below) provides the matrix on which the given drug (morphine), ineffective as it was in relieving experimentally contrived pain, becomes effective when the necessary component—apparently stress—is present (*3*, p. 164). Pathology alone (stimulation of pain endings in battle wounds) is often not enough to give rise to pain. The psychological significance to the subject determines the pain experienced (*3*).

The new principle is: certain drugs are effective in relieving visceral sensations only if an essential psychological state is present. It is not possible at present to define exactly the nature of this state. It appears to be related to the significance of the symptom, to anxiety, and to stress. (And apparently, the stronger the psychological state, the more effective the drugs.) Similarly, certain common symptoms, pain for example, appear to emerge only if an essential psychological state (anxiety, stress) is present (*3*). Physiological derangement (stimulation of pain end-

Table 1. Effectiveness of placebos in relieving pain of experimental origin. The numbers in parentheses in column 1 refer to studies cited in "References and Notes."

Study	Subjects (No.)	Average placebo effect (% relieved)	Comment
		Radiant heat	
(*4*)	16	0.8	Suprathreshold pain, untrained subjects
		0.4	Suprathreshold pain, semitrained subjects
		4.0	Suprathreshold pain, trained subjects
(*5*)	4	0	± 1.1% change from original value
(*6*)	3	0	Obstet. cases; radiant heat pain only
		Radiant heat to forehead	
(*7*)	1	19	1, no effect; four trials
	1		1, 20% rise; no effects 2nd and 3rd trials
	2		2, 28% rise
(*8*)	1	2	
(*9*)	29	0	No consistent trend
		Pressure on forehead	
(*10*)	4	0	Aching pain
		Pressure on forehead, cuff method	
(*11*)	63	0	Inconsistent, variable + and −
		Hydrostatic pressure in biliary system	
(*12*)	8	0	
		Electric shock	
(*13*)	30*	1.3†	Pain intensity comparison; no drug versus
		5.0‡	placebo
(*14*)	3	0	
		Tourniquet	
(*15*)	4	15.5	
(*16*)	4	0	
Totals (13 studies):			
	173	3.2 ± 1.8	Average percentage relieved

* Postaddicts; † $N = 16$; ‡ $N = 14$.