would appear to afford an argument against concepts of localization in the brain. On the other hand, experiments that use restitutive ablation may produce particularly persuasive evidence of localization should the restitution be found to depend on the area ablated.

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Podocoryne carnea, a Reliable Differentiating System

Abstract. The number of nutritive hydranths in a slide-grown colony of this marine hydroid increases exponentially until sexual forms appear in the colony.

Recent studies have emphasized the importance of population factors to cellular differentiation. The size of the cell mass (*1*), its geography, and the effect of metabolic products in controlling subsequent activities (*2*) all play a role in the differentiation of units in a biological mass. It is difficult to evaluate the effects of these factors in embryos because of (i) the size of the units involved—cells, (ii) the difficulty of defining operationally useful end points of differentiation, (iii) the large spectrum of differentiated tissues, and (iv) the fact that cells in an embryo are not end products but on their way to becoming something else.

A reasonable approach would be to find a system that increases in size, originates from a single known unit, and reaches a point where a second distinctly defined type appears. The units of variation in this system should be conveniently visible and analyzable and the gross structure of the system simple enough so that the effect of spatial orientation could be evaluated. In theory it would not be important what the size or complexity of the units in the mass were; they might be as small as cells or as large as multitissue zooids in a colonial population.

The marine hydroid *Podocoryne carnea* (*3*) satisfies these criteria for a reliable system. Colonies can be initiated by placing a single hydranth on a microscope slide in standing sea water. Stolons grow out along the surface of the slide from the base of the hydranth; new hydranths form and increase in size; finally a new type of zooid, which bears medusae, appears. The units, hydranths, are easily visible at × 10 magnification and the medusa-bearing zooids are recognizable from the time of their first appearance (Fig. 1). The anastomoses of stolons are simple enough up to the time of sexual zooid appearance to allow stolon length to be measured and for the pattern and distribution of hydranths on the stolons to be recorded. Generalizations about differentiation in this simple model should be applicable to more complex embryonic systems.

Important observations have already been made along these lines by Loomis and Lenhoff (*4*), who suggested this approach with the use of hydra as a model system. They showed that sexual differentiation is nonobligatory and is alternative to exponential growth and the nonsexual condition.

However, the hydra as an experimental animal presents certain drawbacks. Its social differentiation ("social" to distinguish this process from the differentiation of cells in a single animal) is not analogous to that of most other animals in which the end product of differentiation is a spectrum of differentiated types. Only two alternatives exist in hydra; the range of differential expression is limited to numerical increase of nonsexual animals on one hand and to gamete formation on the other. Biochemical studies involving nutrient additions or inhibitors can be carried out only with difficulty (*5*), because additives to the medium of this fresh water animal are not taken up by the cells. Although the concept of the unity of an aquarium of hydra is a valid one, the animals are in fact spatially separated and motile, and questions pertaining to the spread of pattern in a differentiating system cannot be asked. Since both sexual and asexual zooids can exist simultaneously in a *P. carnea* colony, it approaches the embryonic situation more realistically than

Fig. 1. Slide-grown *P. carnea* colony after the appearance of sexual zooids. The medusa-bearing zooids can be easily distinguished from the nutritive hydranths.

the hydra does. The marine animal will take up small organic molecules from sea water, so that labeling and biochemical studies are easier than with hydras (*6*). Being a sessile animal, *Podocoryne* allows investigations regarding the spread of pattern and spatial distribution of hydranths that are not possible with the fluid hydra system.

An analysis of the factors affecting sexuality in another hydroid, *Hydractinia echinata,* has been published by Hauenschild (*7*). His findings are generally in agreement with those reported here, despite his having had to work with material that, because of the density of the stolon system, could not be studied quantitatively.

The technique for laboratory culture of colonies of *P. carnea* is simple and allows relatively unlimited production

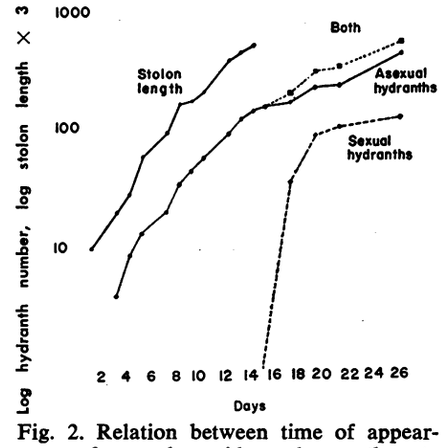

Fig. 2. Relation between time of appearance of sexual zooids and growth rate of asexual zooids in a growing colony of *P. carnea.* Stolon length is measured in arbitrary units.

of replicate colonies (*8*). A single hydranth is removed from a hermit crab shell, where it occurs naturally, and is placed on a microscope slide in standing sea water. In 2 or 3 days the hydranth attaches to the slide, which can then be transferred to a glass slide holder in an aquarium. After 1 month at 23°C, the colony reaches a size that permits using it as a source for about 75 replicate colonies.

To feed the 20 colonies that fit into one glass slide tray, it is only necessary to immerse the tray in a finger bowl of hatched eggs of *Artemia salina* for 10 minutes, after which the hydranths have ingested as many brine shrimp as they can. Each day the *P. carnea* colonies were fed for 10 minutes and the filtered sea water was changed. The water in a 15-liter aquarium is constantly aerated and is stirred by means of a glass propeller (*9*).

To determine the time course of the appearance of sexual zooids, each of nine similar colonies was photographed almost every day for a month. (The daily growth of colony 11, clone W, is typical, Fig. 2.) Stolon lengths and hydranth numbers were calculated from enlargements. Initial growth of stolons and increase in hydranth numbers were exponential. After the second week density made it no longer possible to calculate stolon lengths from the photographs. During the third week, the rate of increase of asexual hydranths fell off sharply; simultaneously, small medusa-bearing zooids began to appear in the central part of the colony.

Thus, *P. carnea*, although continuing to propagate nutritive hydranths, initiates its second hydranth differentiation at a time when the rate of increase in number of the first form declines. In the hydra, too, the onset of sexuality coincides with the cessation of logarithmic growth. Another similar situation is that described by Braun (*10*) for *Brucella abortis*, a bacterium that is sensitive to one of its own metabolic products, D-alanine. After a period of logarithmic growth, the rate of increase of the bacterium declines; at this time selection pressure encourages the reproduction of a mutant form less sensitive to the inhibiting substance.

Experiments are in progress to determine the mechanism of asexual hydranth inhibition and sexual hydranth inception and to determine what relations, if any, exist between the two. Detailed reports on the determination of hydranth-to-hydranth distance, the pattern of growth in single hydranths, the effect of CO_2 on sexuality and the mechanism of this effect, and descriptions of the five clones being maintained in this laboratory (*11*) are in preparation (*12*).

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8. The method for culturing is essentially that described for *Campanularia* by S. Crowell, *Physiol. Zool.* **26**, 319 (1953). Dr. Crowell and his assistants were kind enough to demonstrate the details of his culture method for marine hydroids.
9. That sea water—an unknown factor—is the medium is an objection that has been raised by C. Fulton, *Science* **132**, 473 (1961), but which can hardly be considered seriously regarding any experimental setup that requires the feeding of live food of unknown chemical composition. What is important in such studies is that one variable at a time can be changed and controlled. The totality of a defined milieu is no more precisely defined than its least known quantity.
10. W. Braun, *Am. Naturalist* **86**, 355 (1952).
11. The Stazione Zoologica, Naples, Italy, has agreed to maintain cultures of five clones of *P. carnea* and to make them available to anyone wishing to use the animal either in Naples or elsewhere. Since the clones differ considerably in rate of growth, rate of sexuality, and stolon-hydranth ratio, it would be advantageous for investigators wishing to work on this species to use the same clones, rather than to isolate new ones.
12. This research was carried out while I was a NATO post-doctoral fellow at the Stazione Zoologica, Naples. Thanks are due to Dr. Peter Dohrn and the staff of the Stazione Zoologica for their cooperation during the course of these experiments. The use of *P. carnea* was suggested by Dr. Howard Schneiderman while I was a student in the invertebrate course at the Marine Biological Laboratory, Woods Hole, Mass.

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Iron Chelates in Soybean Exudate

Abstract. Soybean exudates contain iron compounds which can be separated electrophoretically into anodic bands and eluted with water. Chromatography of the water extracts separated iron from chelating agents which were identified as malic acid and malonic acid. Hence organic acids seem to function in the translocation of iron in plants.

Although much has been done to characterize metal chelates of organic acids in simple systems (*1*), very little is known about these compounds in the aqueous systems of plants. However, a considerable amount of information concerning metal binding and translocation is being accumulated from studies with expressed fluids and exudates from plants. Schmid and Gerloff (*2*) have reported a negatively charged iron compound in tobacco stem exudate, but unfortunately did not identify the chelating agent involved. Jones (*3*) has recently shown that organic acids (especially malic acid) may be responsible for the absorption and translocation of aluminum.

Several studies in this laboratory (*4–6*) have provided information leading to the identification of iron compounds in plant exudates. The data may be summarized as follows: (i) large amounts of iron absorbed from synthetic chelate were found in exudate (*4, 5*); (ii) exudate from controls of several species bound iron in vitro and held it soluble against heat and filtering at high *p*H [the solubility of iron was attributed to natural chelators (*4*)]; (iii) iron compounds in the exudate migrated electrophoretically as anions and had mobilities different from those of the chelates that supplied iron to the roots (*5*); and (iv) tests for iron protein (*6*) by dialysis, centrifugation, heat, and high salt treatment, combined with electrophoresis, failed to reveal iron protein but indicated, rather, that the iron carriers were small, stable, anodic molecules. The study reported here shows that the iron-binding agents were organic acids.

The methods used, discussed in detail elsewhere (*5*), are briefly as follows: Hawkeye seedlings, 6 days old, were bound in groups and transferred to a standard nutrient (*5*) containing $10^{-6}M$ ferric ethylenediamine di(*o*-hydroxyphenylacetate) (FeEDDHA). This level of iron produced green plants and prevented a build-up of iron in the roots. On the 16th day from germination each plant group was transferred to standard nutrient (1 liter) labeled with $10^{-6}M$ Fe^{59}EDDHA (526 count/sec ml). The tops of the plants were cut off, and exudate from the stems was delivered by plastic tubing into vials in vacuum jars that were packed with ice.

Electrophoresis of the iron compounds was accomplished by means of 1,3-iminodiproprionitrile-acetic acid-formamide buffer (*p*H 5.9) (*7*) and a Misco ionophoresis apparatus (*8*). By running the negatively charged dye amaranth (*7*), 9 cm from the origin,