tion and thus were moderately stressed. Some additional stress may also have resulted from caging the animals singly. Hence, the difference between experimental and control groups might be more striking under ideal conditions.

Even though the physiological pathways of action are still poorly defined, it is clear that chronic, avoidance-learning stress induces a prolongation of homograft survival times to a small but significant degree in genetically homogeneous as well as in heterogeneous lines of mice (*10*).

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Growth Pattern in the Green Hydra, Chlorohydra viridissima

Abstract. A method was employed whereby it was possible to determine the growth pattern of hydra without using the traditional techniques of grafting and vital staining. It has been shown that hydra possess a growth region just below the hypostome where constant cell proliferation renews the tissues of the animal completely every few weeks.

In 1949 Brien and Reniers-Decoen (*1*) published an account of the growth pattern exhibited by the common brown hydra, *Pelmatohydra oligactis* (Pallas). These investigators grafted upper portions (mouth, tentacles, hypostome, and the region just below the hypostome) of animals which had been stained vitally with methylene blue, neutral red, and Nile blue sulfate to the stalk or peduncular region of unstained animals. During the next few weeks the stained material migrated towards the basal disk, where, after a period of from 3 to 5 weeks, the stained material disappeared entirely. These workers concluded from the experiments that hydra possess a growth region just under the hypostome where rapid cell division occurs in both epidermis and gastrodermis. As cells divide they force nondividing cells proximally to the basal disk and distally to the tentacle tips, where these migrating cells die and are sloughed off. Thus, it appears that the hydra is constantly renewing its tissues every 3 to 5 weeks.

This discovery of Brien and Reniers-Decoen, if correct, will influence to a great extent future interpretations of experiments dealing with growth and cell differentiation in hydra, because it was formerly believed that the hydra's "immortality" was dependent only upon a constant tissue replacement by interstitial cells in all areas of the body.

The present study was an attempt to confirm the existence of a growth region in hydra and to determine whether a growth region exists in species of hydra other than *Pelmatohydra oligactis*. Also, it was necessary to devise a method of staining which did not involve vital dyes, since there is a possibility that these dyes are capable of diffusing from cell to cell, thus mimicking cell movement. Furthermore, it was desirable to eliminate the grafting techniques applied to experimental animals, because it appeared that these procedures were not altogther satisfactory for studying the normal growth process of an animal. In any grafting precedure applied to hydra a wound must be made in each of the pieces to be joined. Once the pieces are united, a certain amount of regeneration is necessary to repair the wounded tissue. New nervous and muscular tissue must be made, for example. It is never possible to cut sections which exactly complement each other from two different animals. In a given hydra there is a certain distance which separates the hypostome from the budding region, the distance depending upon the dominance exerted by the mouth in the immediate vicinity. This distance is further dependent upon the size and physiological state of the animal. Thus, when a proximal portion of one animal is grafted to the distal portion of another, a process of regulation must occur while the body regions of the newly formed animal are being adjusted. During this period of regeneration and regulation it is possible that cells are called to service from distant body areas to aid in repair processes and so on. Also it appeared possible that active cell proliferation might have begun at the site of the wound, thus establishing a temporary growth region.

A relatively simple procedure was found for marking the cells of the green hydra, *Chlorohydra viridissima*. Whitney (*2*) found that a green hydra when placed in a 0.5-percent glycerin solution for a few days voided its algal bodies. Several "white" animals resulting from this treatment were cut through the center of their budding zones into two pieces. The piece containing the head and tentacles was then grafted to the base, stalk, and lower half of the budding region of a normal green animal. This results in an animal whose budding region is half green and half white. The buds produced from such animals are also sometimes half green and half white, but usually the buds are white with irregular green blotches. These irregularly colored forms were then allowed to reproduce, and after several hundred of their offspring and of the offspring of succeeding generations had been examined, it was possible to find about two dozen animals with only a single patch or two of algal-laden digestive cells in their bodies. Thus, we had essentially a stained animal which was not directly involved in grafting procedures. Also, it may be assumed that no diffusion of algal cells from one cell to another in hydra will occur, since an individual algal cell is as large as the digestive cell nucleus. All that was necessary at this point was to observe the fate of algal-laden digestive cells in the growth region over a period of several days.

In 15 animals examined there was a distal and proximal migration of colored cells from the growth region. In many cases the colored material moved down only one side of the body column, showing that diffusion was not taking place. It usually took from 2 to 3 weeks for cells to be forced from the growth region to the basal disk and less than a week (average, 5 days) for cells to be forced from the growth region to the tips of the tentacles.

It may be concluded that a growth region does exist in *Chlorohydra viridissima* similar to that found in *Pelmatohydra oligactis*. Furthermore, these experiments indicate that the cell migration witnessed after staining *P. oligactis* with vital dyes was not due to regenerative and regulative processes involved in grafting procedures.

It is hoped that this simple marking procedure will be employed more in the future for the study of such processes as budding, wound healing, and reorganization of animals from bits of tissue.

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26 August 1959

Hollow Crystals from Buffer Solutions of Sodium Diethyl Barbiturate

Abstract. Buffer solutions of sodium 5,5′-diethyl barbiturate, after standing at 4° to 8°C for 7 weeks, were observed to contain large tubular crystals. The crystals appear to have the composition of 5,5′-diethyl barbituric acid. They can be grown readily from fresh seeded solutions.

Large, hollow, tubular crystals in buffer of sodium 5,5′-diethyl barbiturate were unexpectedly observed in this laboratory. Chrysotile asbestos (*1*), halloysite (*1*), pyromorphite (*2*), nitroguanidine (*3*), and vanadinite (*4*) have been previously reported as cavernous, tubular, or hollow crystals. The first two are microscopic; nitroguanidine crystals are about 2 mm long, the vanadinite ones about 10 mm. Various crystal forms of 5,5′-diethyl barbituric acid, of which none were hollow, have been described (*5*). Recently, tubular hexamethylene-tetramine-triphenol was reported (*6*). Because of their unusual form, dimensions, and pyrimidine nature, the observed crystals are described here.

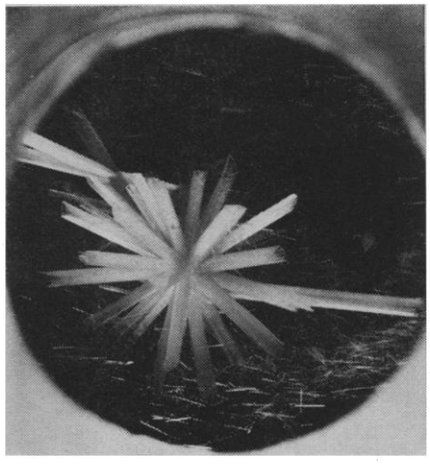

Fig. 1. Appearance of rosette of crystals in mother liquor (about × ½).

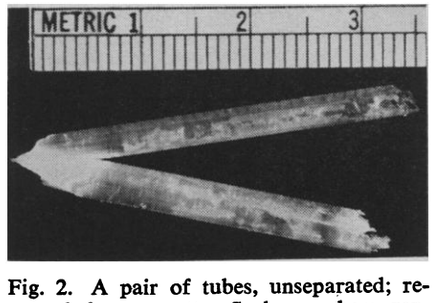

Fig. 2. A pair of tubes, unseparated; removed from rosette. Scale numbers represent centimeters.

16.0 gm of NaOH (reagent grade, Merck) and 88.4 gm of 5,5′-diethyl barbituric acid (N.F. Barbital Merck) were dissolved in water at a total volume of 4 liters. The solution was inadvertently left unstoppered at 4° to 8°C for about 7 weeks. During that period, crystal growth occurred to produce the large rosette shown in Fig. 1. The rosette consists of 30 hollow tubes, each with parallel outer edges in the long axis, all radiating from a common area at a closed end of each tube. Each tube was open at the other end and was about 30 to 40 mm long. The tubes could be broken away in smaller clusters, in pairs (Fig. 2), or as single crystals. Each central end was pointed and solid, not hollow. The two crystals shown in Fig. 2 formed an angle of 17 ± 0.5 deg with each other. The open shaft in one crystal (Fig. 2) was found to end 3 mm from the pointed, closed tip, and in all crystals the open shaft extended to a similar distance from the tip. The tubes were 2 to 4 mm in outside diameter, colorless, moderately translucent, brittle, and smooth to the touch. The outer edges of four crystals, measured in photographs, were found to be parallel (± 0.2 mm over a length of 20 mm).

For analysis, crystals were removed from the rosettes while in the mother liquor by means of a slight wedging motion of a spatula. After the fluid contents from the interior of each had been aspirated with a fine capillary, and the crystals had been washed inside and out quickly with cold water, the separated tubes were dried in a desiccator at 12 mm-Hg over P_2O_5 for 18 hours. After being dried they were sectioned transversely with a razor blade; some fracturing occurred. The open ends could be gently ground until somewhat flat. The interior and contours of the walls were somewhat hexagonal (see Fig. 3).

Analysis showed mp 181° to 184°C (uncorrected) with simultaneously measured mp on acid barbital (N.F., Merck) of 184.5°C and mixed mp 184.5°C, [reported 176°, 183°, and 190°C for various crystal forms (*5*)]. Sodium barbital (USP, Merck) decomposed well over 200°C. Sodium content (flame, Beckman) of supernatant removed from a solution of the dried crystals that had been allowed to re-

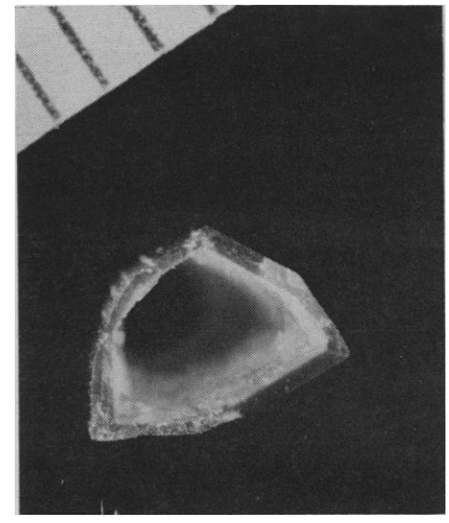

Fig. 3. Cross section of hollow crystal near open end. One scale division equals 1 mm.

crystallize (needles) overnight at room temperature showed a value of 0.3 percent of that expected for a saturated solution of sodium barbital at 25°C. This supernatant had a *p*H of 5.7 ± 0.05 and after evaporation was found to contain 0.77 ± 0.05 gm of solute per 100 ml (solubility of 5,5′-diethyl barbituric acid = 0.69 gm/100 ml at 25°C; of sodium 5,5′-diethyl barbiturate = 20.0 gm/100 ml at 25°C). It is quite possible, therefore, that the hollow crystals are simply monomeric 5,5′-diethyl barbituric acid.

Crystals were grown once before in a similar buffer solution at *p*H 8.6 ± 0.05. Also, four times they have been grown in a matter of days by seeding with very small crystal fragments (*7*).

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