

Further evidence that the high confining pressure allowed the mineral grains to be pressed into the limestone or to make deep tracks and then be half-buried in the limestone is shown in Fig. 1j; two indentations have concentric inner walls, indicating plastic deformation produced first by a large grain, then by a smaller grain.

So far the most common mineral grains found to have produced the pressure striae are quartz. Other mineral grains identified are K-feldspar, pyrite, and aggregates of calcite.

Interpretation. The origin of these striae on limestone fragments and the conditions under which they were formed are relatively easy to determine. The remnant mineral grains in the terminal positions of striae give proof that the striae were produced by scouring by mineral grains. The polish was produced by movement of fragment-poor clay matrix against the surfaces. The observation that relatively deep striae in porous substrates and shallower striae in smooth polished surfaces are juxtaposed (Fig. 1, e and g) clearly indicates that the mineral grains that formed the striae were embedded in clay; thus, the striae and polish were produced simultaneously by the relative movement between the limestone fragment and the adjacent clay matrix with its variable content of mineral grains. The accompanying local plastic deformation (Fig. 1, h and j) indicates that these striae must have been produced at least locally under extremely high confining pressure. The fact that such pressure striae and polish are found on angular fragments shows that the pressure striae and clay polish were produced within the time span of the cratering event after the fragment was broken up by the impact, that is, within a time span measured in terms of seconds.

It is evident that the development of the mineral-produced striae and polish on surfaces freshly fractured during the impact event depended on the nature of the clay matrix and the mineral grains it contained. In field studies in the summer of 1976 I found that striated and polished fragments are more common in parts of the varicolored ejecta with a clayey matrix of purple Keuper and gray Dogger shale, abundant quartz grains, and Malm limestone fragments. Striated and polished fragments are generally not found in large blocks of Dogger or Lias gray shale free of sand-size grains (where plastically deformed concretions are found) or in Tertiary sandy clay. The shale blocks do not tend to mix intimately with Malm limestone or quartz

grains from the Mesozoic sandstones or the basement crystalline rocks.

The order of magnitude of the high confining pressure can be only roughly estimated from the nature of plastic deformation in micaceous ferruginous sandstones in the Dogger gray shales found within meters of these striated limestone fragments. The deformational features detectable in these concretions are ruptures and weak kink bands in mica, suggesting that the deformation is low and is of the slow-strain-rate type. The differential pressure required to produce such deformation under confining pressures is probably in excess of about 2 kbar and may be as much as 4 kbar, appreciably greater than the normal compressive strength of these concretions. Nevertheless, the confining pressure exerted by the enclosing clay matrix must have been very great (many times the differential pressures) since the entire process of deformation lasted only a few seconds. Such confining pressures must have been present to produce the plastic flow locally and essentially on the surficial layer of the limestone fragments. It would be difficult if not impossible to explain the observations shown in Fig. 1, h and j, if the limestone did not behave plastically locally.

If these pressure striae and clay polish can be reproduced experimentally in the laboratory, then they may be used not only for estimating the velocity of the nonballistic ejecta transport under confining pressures but also the duration of the cratering event.

Implications. Sand-size fragments from every varicolored sedimentary ejecta mass at the Ries can be examined for the presence of high-pressure mineral-produced striae and clay polish. With this key criterion, the distribution and abundance of nonballistically and ballistically transported ejecta can be delineated, leading to a better understanding of the detailed development of the Ries crater.

There are no limestones on the moon or other planetary surfaces. But such striae may occur on other rock types, where they may be more difficult to identify. Similar striae may occur on 14053, a basalt, and 14047, a breccia, two unshocked lunar samples returned from Fra Mauro by the Apollo 14 mission.

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Hydra hymanae: Regulation of the Life Cycle by Time and Temperature

Abstract. *Hydra hymanae*, a hermaphroditic freshwater coelenterate, reproduces asexually at 24°C and sexually at 15°C. The appearance of gonads begins 12 days after transfer from 24° to 15°C and is complete 35 days after the temperature transition. Testes appear before eggs. Fifty percent of the mature embryos maintained at 15°C hatch by day 61, but they have a low level of survival. Fifty percent of the mature embryos pretreated for from 5 to 25 days at 4°C hatch by about day 45, and these have a high level of survival. Embryos maintained at 4°C for longer periods (55 to 85 days) accumulate in a prehatching state and hatch with a high degree of synchrony approximately 7.5 days after return to 15°C. Populations derived from newly hatched polyps are refractory to sex induction for approximately 120 days. The system is well adapted to ensure a regular alternation of reproductive modes in the natural environment.

Both environmental and intrinsic factors have been implicated in the control of reproduction in various species of *Hydra* (1, 2). I present here evidence that the life cycle of *H. hymanae* is regulated by the interaction of the environmental

temperature with an endogenous component related to the age of the population.

The stock used was derived from a few asexual polyps collected from Winooski Pond near the campus of the University

Table 1. Summary of quantitative data derived from Fig. 3.

Days at 4°C	Total days to 50% hatching	Days at 15°C to 50% hatching
0	61.3	61.3
5	45.8	40.8
15	47.3	32.3
20	43.0	23.0
25	42.7	17.7
38	53.3	15.3
55	62.5	7.5
64	72.0	8.0
75	82.5	7.5
85	92.8	7.8

of Vermont in the fall of 1973. The sizes and shapes of the nematocysts, the relative length and mode of origin of the tentacles, the shape of the embryonic theca (shell), and the hermaphroditic nature of the sexual state all fit the description of *H. hymanae* (3).

As in earlier studies in this laboratory, the culture medium was tap water (from Lake Champlain) drawn from the main input into the Marsh Life Science Building (4). Animals were fed each day with an excess of newly hatched *Artemia* larvae (rinsed in tap water), after which the medium was changed. Polyps were maintained in groups of from 20 to 75 individuals in 100 ml of water contained in stacking dishes 100 mm in diameter. An individual was defined as a polyp regardless of whether the polyp had buds or not. Unless noted otherwise, the photoperiod was fixed at LD 12:12 (about 550 lu/m², "cool white" fluorescent bulb).

At 24°C *H. hymanae* reproduced strictly by budding. After transfer to 15°C a large fraction of the population de-

veloped gonads, in agreement with field observations (2) that this species is sexual in the late fall. On return to 24°C sexual polyps resorbed their gonads and resumed budding within 9 days. Figure 1 depicts a sexual polyp with distal conical testes and a single basal embryo. The embryos typically detach from the parent polyp very early, sometimes even before cell division, and become firmly attached to the substrate within 5 days after fertilization.

Figure 2 illustrates a typical time course for sex induction. Twenty-five polyps without buds were transferred from 24° to 15°C on day 0. Each day for the next 50 days the total number of polyps, the total number of polyps with gonads, and the total number of embryos released were recorded. The embryos were removed daily. During the first 10 days the original stem animals produced buds which began detaching on day 13. On day 12 testes began to appear. The appearance of developing eggs lagged behind that of developing testes by about 3 days, and the first embryos were released on day 21. By day 35 nearly all the polyps were sexual, but a few animals escaped sex induction and continued to bud. The sexual state was maintained at 15°C for at least 3 months during which time the polyps gradually decreased in size and reproductive vigor.

In order to examine the kinetics of hatching, additional sexual populations were produced by transfer from 24° to 15°C. Embryos were removed daily and transferred to stacking dishes containing 100 ml of water. Each dish received embryos collected over a 10-day period. The dishes were then maintained at 15°C for an additional 10-day period to ensure maturation of all the embryos. At the end of the second 10-day period the medium was changed and the date arbitrarily was designated as day 0 for experiments testing the effects of low temperature on subsequent hatching. I simulated a winter regime by transferring dishes to 4°C (in the dark); a control dish was also placed in the dark but remained at 15°C. At various times dishes were returned to 15°C. All dishes remained in the dark except for brief daily periods when hatched animals (if any) were removed and recorded. Each experimental group consisted of from 150 to 300 embryos with the precise number unknown until the termination of the hatching period. Figure 3 depicts the effects of exposure to low temperature on subsequent hatching. The control population, marked 0, exhibited the least synchronization of hatching with 61.3 days required for 50 percent of

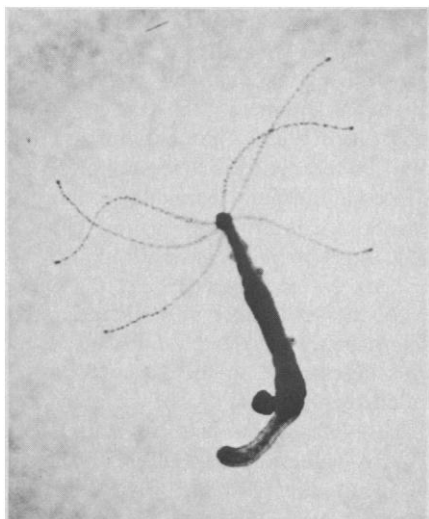


Fig. 1. A sexual polyp of *Hydra hymanae* with distal conical testes and a single basal embryo. The body column is about 4 mm long.

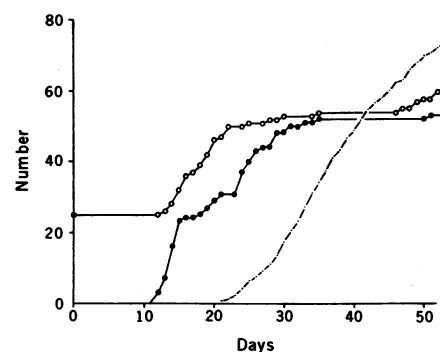


Fig. 2. The time course of sex induction after the transfer from 24° to 15°C; (○) total number of polyps; (●) total number of polyps with gonads; (---) total number of embryos produced. The numbers on the ordinate should be multiplied by 10 for the embryos only.

the population to hatch. It is not evident from Fig. 3 that a high percentage of the control animals were abnormal, many undergoing cell dissociation as they hatched and others failing to feed. In contrast with the control population, all the experimental populations, which were exposed to 4°C, hatched with a high degree of synchrony to yield normal vigorous polyps.

The pertinent quantitative data derived from Fig. 3 are summarized in Table 1. Exposure of the embryos to 4°C for from 5 to 25 days results in the hatching (50 percent of the population) of normal polyps between days 42.7 and 47.3. For these times of cold exposure, the total time to the hatching of 50 percent of the population is approximately constant, suggesting a physical process such as diffusion controlling the events leading to hatching. The cold-initiated process does not, however, extend into the terminal 7 to 8 days prior to hatching, since for long periods of cold exposure

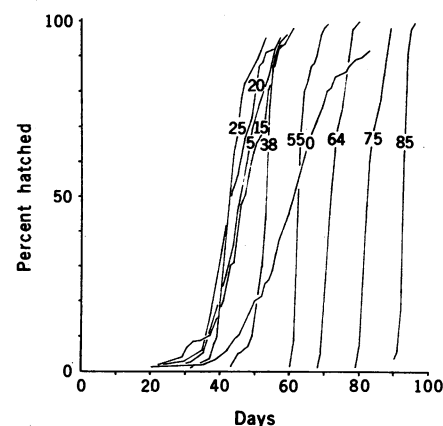


Fig. 3. Kinetics of hatching of the embryos. The abscissa includes both the time at 4°C and the time at 15°C. The number associated with each trace indicates the number of days spent at 4°C prior to return to 15°C.

(55 to 85 days) hatching does not commence until a few days after return to 15°C.

Newly hatched polyps rapidly grow to full size and bud vigorously whether they are maintained at 15° or 24°C. The derived populations are refractory to sex induction for approximately 120 days during which period they continue to bud after transfer from 24° to 15°C. Thus, although 15°C is required for the realization of the sexual state, a prior and relatively long period of asexual activity is also mandatory for the induction of the sexual phase of the life-cycle. The original material was collected in the fall and can be assumed to have spent the preceding summer months in the asexual state.

Time plays a critical role in two phases of the life-cycle. First, exposure of the embryos to 4°C initiates the processes leading to normal and synchronous hatching, but no hatching occurs until after return to 15°C, an indication that embryos produced in the fall will not hatch

until the next spring. Second, newly hatched polyps are refractory to sex induction for a long period roughly equivalent to the duration of the summer months. Such a regulatory mechanism is well adapted to produce a regular alternation of reproductive modes, especially in north temperate regions where seasonal variation in environmental temperature is the rule.

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High Concentration of GABA and High Glutamate Decarboxylase Activity in Rat Pancreatic Islets and Human Insulinoma

Abstract. *The concentration of γ -aminobutyric acid (GABA) and the activity of glutamate decarboxylase (GAD) in rat and human pancreas were measured by sensitive assay methods. The GABA concentration in rat pancreas was 2.51 millimoles per kilogram (dry weight) and GAD activity was 2.58 mmole per kilogram per hour. The GABA concentration and GAD activity in rat Langerhans' islets were 18.9 mmole kg^{-1} and 66.7 mmole $\text{kg}^{-1} \text{ hour}^{-1}$, whereas those in the exocrine acini were 1.97 mmole kg^{-1} and 4.67 mmole $\text{kg}^{-1} \text{ hour}^{-1}$, respectively. In an insulinoma region of human pancreas the GABA concentration was 25.5 mmole kg^{-1} and the GAD activity was 138.2 mmole $\text{kg}^{-1} \text{ hour}^{-1}$, but in the surrounding nontumor region these values were only 2.81 mmole kg^{-1} and 2.01 mmole $\text{kg}^{-1} \text{ hour}^{-1}$, respectively, similar to the values in normal rat pancreas.*

Since the discovery of γ -aminobutyric acid (GABA) in mammalian brain in 1950 many studies of its function and metabolism have been carried out (1). While there is now good evidence that GABA is an inhibitory neurotransmitter in the mammalian brain as well as in the invertebrate central nervous system (CNS) (2, 3), there is also evidence that GABA as well as glutamate decarboxylase (GAD) occur in nonneuronal tissues such as kidney, liver, and adrenal gland (4, 5), although at much lower concentrations than in nerve tissue. Tallan *et al.* (6) and Drummond *et al.* (7) also found a very small amount of GABA in pancreas, although their assay method seemed not to be sensitive enough to measure the exact amount of the compound in the tissue. We found previously a high concentration of GABA in Langer-

hans' islets of rat pancreas (8). In the studies reported here, GAD activity and GABA concentration were determined in rat pancreas, rat pancreatic endocrine and exocrine glands, and in insulinoma of human pancreas by means of a sensitive assay method (9).

Albino rats weighing 85 to 100 g were anesthetized with pentobarbitone sodium (50 mg kg^{-1}), the abdominal cavity was opened, and the pancreas together with exocrine and endocrine glands was carefully removed. The tissue was immediately immersed in Freon-12 and chilled to its freezing point (-150°C) in liquid nitrogen. The tissue block was cut with a microtome in a cryostat (-20°C), and serial sections (15 to 20 μm) were freeze-dried overnight at -30°C in a vacuum. For the determination of GABA and GAD in whole pancreas (exocrine plus

endocrine glands), two or three sections of freeze-dried tissue (100 to 200 μg) were used. For the assay of GABA and GAD separately in the endocrine and exocrine glands, the Langerhans' islets and acini were isolated with a fine dissecting knife under a stereomicroscope. The islets of Langerhans were well differentiated from the exocrine gland. Each isolated islet and exocrine sample was weighed with a fish-pole quartz fiber balance. The weight of a single islet ranged from 40 to 60 ng and that of the exocrine area from 40 to 200 ng.

For the assay of GABA (direct method) in whole, freeze-dried sections of pancreas, each sample was transferred to a 2-ml fluorometer tube, and 20 μl of 0.01N HCl was added; the sample was then heated at 60°C for 10 minutes to destroy enzyme activity and endogenous reduced nicotinamide adenine dinucleotide phosphate (NADPH). The freeze-dried sample was easily dissolved by this treatment. After the addition of 80 μl of GABA assay reagent, the samples were incubated at 38°C for 30 minutes. The GABA assay reagent consisted of 0.3M tris-HCl buffer (pH 8.9), 10 mM α -ketoglutarate, 1 mM NADP, 0.01 percent mercaptoethanol, and 60 μg of protein per milliliter of bacterial-derived enzymes: γ -aminobutyrate aminotransferase (E.C. 2.6.1.19) and succinate semialdehyde dehydrogenase (E.C. 1.2.1.16). After incubation, the reaction mixture was diluted to 1.0 ml with 0.1M tris-HCl buffer (pH 8.0) to stop the reaction. The fluorescence of the NADPH was determined by means of a Farrand fluorometer.

For the GAD assay (direct method) the tissue sample was transferred to 30 μl of GAD assay reagent in a fluorometer tube and incubated at 38°C for 60 minutes. The GAD assay reagent consisted of 0.1M phosphate buffer (pH 6.8), 50 mM glutamate, 250 μM pyridoxal phosphate, and 0.2 percent mercaptoethanol. After incubation, the samples were heated at 100°C for 2 minutes to stop the reaction. The mixture was incubated at 38°C for 30 minutes after adding 80 μl of GABA assay reagent. Then 0.8 ml of 0.1M tris-HCl buffer was added and the fluorescence of the NADPH produced was determined in the fluorometer. To calculate the GAD activity, endogenous GABA was subtracted from the total GABA value obtained after 60 minutes of incubation with the GAD assay reagent.

For the microassay of GABA and GAD from an isolated single islet and small area of acinus, an oil-well tech-