and crude trypsin (0.15 percent) (*6*) and then rapidly vibrating the embryos in the tube for 15 seconds (*7*). The cells were washed three times in culture medium and then placed in a drop of culture medium (approximately 0.005 ml) on a siliconed cover slip, which was inverted over a vaseline-ringed depression. The culture medium contained TC Yeastolate 200 mg/100 ml, but in other respects was essentially the same as that developed by Schneider for culture of imaginal discs (*8*). Fetal bovine serum was added routinely to the culture medium, at a final concentration of 10 percent. The cultures were maintained at 25°C for 5 to 7 days without changing the medium, during which time the cells formed aggregates which were fixed in Bouin's fluid or in a mixture of formalin, acetic acid, and alcohol. Successful cultures were established from embryos that were from 6½ to 16 hours old at the time of dissociation.

The dissociation method yielded a uniform suspension of single cells only when young embryos, up to about 11 hours old, were used. Sometime between the 11th and 14th hours of development, changes occurred in the embryo which rendered it progressively more difficult to release single cells. When embryos 17 hours old were dissociated, a few single cells were released, but most of the cells remained in fairly large, undissociated groups.

In suspensions of cells from both normal and mutant embryos aggregation begins very rapidly. By about 2 hours after the hanging drop was set up the cells were already noticeably more tightly grouped. The cells continued to move about and pile up to form a sphere or thick disc, sometimes with irregularly shaped projections. The process continued throughout the culture period, as evidenced by the constantly changing size and shape of the aggregate.

Soon after the aggregate formed it began to show pulsations resulting from contractions of muscle cells. This continued throughout the culture period, whether normal or mutant embryos were used. Some of the contractions were very strong, moving the whole aggregate; other contractions were weaker but more rapid, beating about 80 to 120 times per minute in both normal and mutant aggregates. Such contractions were first observed about 10 to 20 hours after the culture was set up; the older the embryos were at the time of dissociation, the sooner the contractions were observed in the culture.

Figures 1 to 3 show Azan-stained (*9*) sections through aggregates of normal and mutant embryos. The sections of aggregates were compared with sections of normal larvae fixed and stained in the same way so that as many cell types as possible could be identified in the aggregates. Oenocytes, muscle cells, and nerve cells, in addition to chitin, were easily distinguished in the sectioned aggregates, but several other cell types remain unidentified.

Within the aggregates the various cell types separate from one another, at least partially, and take up characteristic positions. As shown in Figs. 1 to 3, the very loosely adhering oenocytes were outermost. Internal to these were hypoderm cells, which in turn covered nervous tissue. Tracheal cells (*10*) and large muscle cells were innermost, though the latter often reached to the surface of the aggregate.

No differences were observed between the positions of the different cell types within aggregates of cells from *X2* mutants and the positions within aggregates from normal embryos. Aggregates from mutant embryos remained alive in tissue culture 6 days longer than intact mutant embryos live within the vitelline membrane and chorion.

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References and Notes

1. H. V. Wilson, *J. Exptl. Zool.* **5**, 245 (1907); J. Holtfreter, *Rev. Can. Biol.* **3**, 220 (1944); A. Moscona, *Exptl. Cell Res.* **3**, 535 (1952); J. P. Trinkaus and P. W. Groves, *Proc. Natl. Acad. Sci. U.S.* **41**, 787 (1955); P. Weiss and R. James, *Exptl. Cell Res.* suppl. 3, 381 (1955).
2. M. S. Steinberg, *Science* **141**, 401 (1963); ———, in *Cellular Membranes in Development*, M. Locke, Ed. (Academic Press, New York, 1964), p. 321.
3. This mutant is described by D. A. Ede, *Arch. Entwicklungsmech. Organ.* **148**, 437 (1956).
4. FM4 is an X-chromosome balancer of the Muller-5 type; see R. Mislove and E. B. Lewis, in *Drosophila Inform. Serv.* **28**, 77 (1954).
5. T. R. F. Wright, *J. Exptl. Zool.* **143**, 77 (1960).
6. Difco, 1:250.
7. R. Auerbach and C. Grobstein, *Exptl. Cell Res.* **15**, 384 (1958); R. Lesseps, *J. Exptl. Zool.* **153**, 171 (1963).
8. I. Schneider, *J. Exptl. Zool.* **156**, 91 (1964).
9. G. Humanson, *Animal Tissue Technique* (Freeman, San Francisco, 1962), pp. 149–151.
10. The identification of the tracheal cells is not definitive.
11. I thank Thomas Green and Roy Hemelt for valuable technical assistance during different stages of this work, which was supported by grants GB-307 and GB-2363 from the NSF. Part of the work was done in the biology laboratory of Loyola University, New Orleans, and part was done at Johns Hopkins University.

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Neoplasia in a Coral?

Abstract. *Anomalous growths of the colonial, deep-water coral* Madrepora kauaiensis *are interpreted as neoplasia. Since tissue was not preserved, evidence is derived solely from skeletal examination and consists of unusually rapid growth and progressively disordered growth of skeletal structures.*

Malformed corals are common, but in the great majority of such specimens that have been examined the malformations are directly attributable to the effects of predation or other physical injury. Pathologic morphologies other than those resulting from regeneration of injured tissues have generally been referred to under the term "abnormal" and not considered further.

The anomalous growths described here were found on the only known specimen of *Madrepora kauaiensis*. This oculinid coral was dredged in 1902 at "Albatross" Station 4136, off the Island of Kauai, Hawaiian Islands, from a depth of 529 to 635 m. Species of *Madrepora* are common deep- and cool-water colonial corals in the oceans of the world, usually occurring in depths below those in which the more widely known reef corals live. *Madrepora kauaiensis* was described by Vaughan (*1*), who noted the presence of at least one unusual corallite of the colony, interpreted it as an individual of another genus and superfamily of coral which had grown upon the *M. kauaiensis*, and suggested that this unusual portion was "*Mussa*? sp. young?": "a simple mussid coral was growing attached to *Madrepora kauaiensis* . . ." (*2*).

I believe Vaughan's interpretation of the specimen to be in error and suggest that the presumed mussid and two other corallites of the same degree of abnormality are the result of diseased growth of individuals of the colony. Because the specimen is unique and is a holotype, dissection of the skeleton is restricted.

Study of the soft tissues of the animal is not possible, for the specimen was dried after collection and the polyp tissue was destroyed. Information concerning the abnormal corallites is available from study of the skeleton, which tells much about the growth of the polyp, coral growth being accretionary, and developmental sequences are preserved in the structure of the skeleton.

The three individuals of *Madrepora*

kauaiensis considered to be pathologic differ from normal corallites in these important features: total size, arrangement of the septa in the corallites, and pattern of skeletal deposition.

Of the 239 normal corallites comprising the colony, 50 were randomly selected and measured. The mean diameter of the corallites is 2.7 mm; in contrast, the largest diameters of the three abnormal corallites are 23.7, 10.0+, and 5.6 mm. In a departure from normal symmetry, the largest individual has an outer edge which is scalloped, not round. The angle formed by the walls of the pathologic corallites varies from 78° to 86°, rather than the 10° to 15° of normal corallites. The smaller of the two living pathologic individuals (one pathologic corallite died during the life of the colony, presumably through burial by substratum) shows no real abnormalities in septal arrangement except for the greater development of septa of the third cycle. Septa of the larger corallite are inserted in an almost meaningless fashion. A total of 89 septa are present, which suggests that portions of a fifth cycle had been inserted. The union of higher-cycle septa to those of lower cycles is obvious, but the pattern lacks symmetry and is not systematic.

Madrepora characteristically has septa which are solid, quite thick, and have minutely dentate margins and sides. The pathologic corallites have septa which are highly fenestrate and lacerate. It was this condition which led Vaughan to suggest that the larger of the pathologic individuals was a young mussid coral, for its septa are like those of the mussid corals.

The following key points in the interpretation of the specimen have been made:

1) The three abnormal corallites of the colony were organically connected to the colony and are, therefore, the same species and were formed by polyps asexually produced by polyps lower on the branch.

2) The abnormal corallites were formed by polyps older than those budded from them, and younger than the polyps which gave rise to them. The corallites are thus a part of a time continuum of growth and reflect in their larger size growth rates that are significantly different from those of their "sibling" polyps.

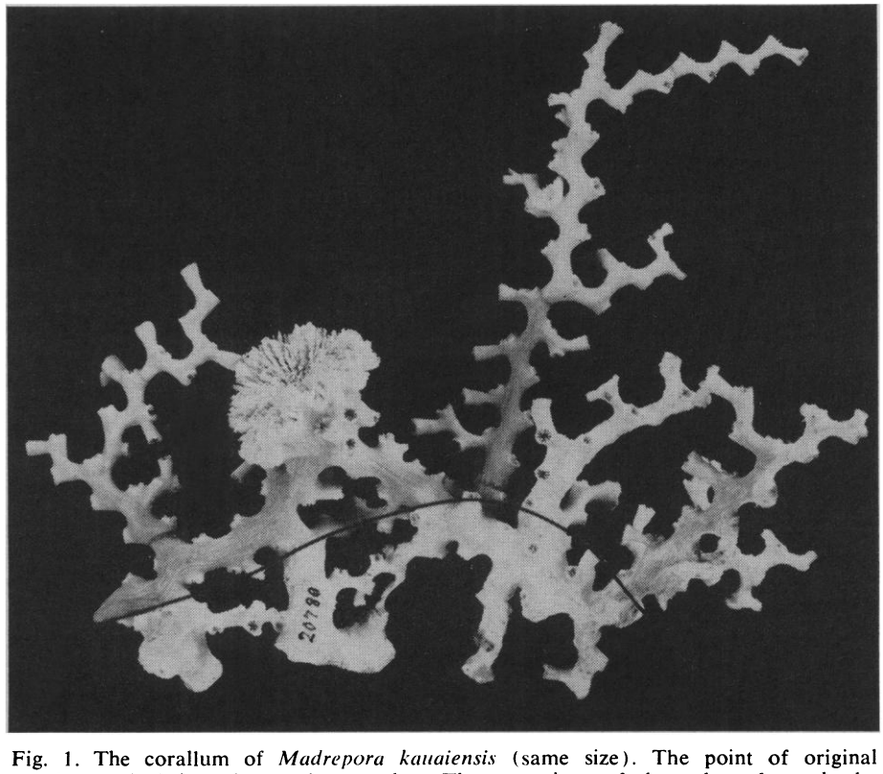

Fig. 1. The corallum of *Madrepora kauaiensis* (same size). The point of original attachment is below the catalog number. Those portions of the colony beneath the black line were dead, killed by burial in sediment. The largest polyp is apparent in the central left. The smaller live pathologic corallite is midway on the branch extending upwards and to the right. The third pathologic corallite is on the dead portion of the branch to the extreme right.

3) Structurally, the corallites show features indicative of rapid growth of both polyp and skeleton.

4) The arrangement of skeletal features suggests that the normal division and insertion of mesenteries was followed until a stage of growth beyond that of a normal polyp was reached, and then mesentery formation (and hence septal insertion) became disordered and chaotic.

Interpretation of these points leads to the conclusions that (i) in 3 of the 239 polyps represented in the skeleton of the colony, growth of unusual rate occurred; (ii) at its greatest development, this growth was chaotic and disordered with respect to the normal symmetry of the polyp; (iii) the corallites in question were descended from "normal" individuals and they in turn gave rise to "normal" individuals.

Whether injury, as opposed to disease, is a cause of the deformity may be considered. Deformation of the growth pattern as described here cannot be ascribed to unusual environmental factors, for the selectivity of the effect is too great, in that only three corallites were affected. The life span of such a coral colony as that described is not known, but would probably not be over 10 years. If a local change occurred, it would be expected to affect all the polyps alive at that time, or, at least, all those of a particular age. Polyps of similar age do not show similar effects.

Deep-water corals are preyed upon by fish and other carnivores which bite, suck, or digest the soft parts of the coral, with greater or lesser effects upon the skeletal materials. Because corals (and coelenterates in general) show great regenerative ability, many of the injuries sustained by predation are repaired during the life of the individual, if sufficient tissue remains for regeneration. Generally the disturbance to the growth of the coral in these instances is minor. Duplication of septa along the margins of an injury is not uncommon but is the result of duplication of a mesenterial pair. Increased skeletal porosity is more common in the regenerated tissue, as growth is speeded without a corresponding increase in the rate of calcium carbonate deposition. I do not think the present instance is the result of predation or accidental injury, largely because of the unusually large size of the individuals. Increased total size is not a characteristic of repaired or rejuvenated

coralla, for total size seems to be a genetically controlled feature in the coral. Again, the genetically controlled mesenterial pattern would not be chaotic as in the larger of the pathologic individuals were this a case of simple repair.

Within the realm of disease, nothing is known of the various afflictions of coral and the expression of their symptoms upon the growth of individuals. The requirements of the disease necessary to produce the observed pathology would be:

1) Removal of natural controls upon growth, thereby allowing unusual increase in size. The actual nature of the growth regulating mechanism in corals is not known, but is thought to be related to the attainment of sexual maturity, after which growth rates decrease sharply, while the metabolic activity of aragonite deposition continues little changed. Presumably, therefore, a disease affecting the sexual development of an individual within the colony would produce the observed results.

2) Malfunction of the controls and regulation of growth patterns so that ultimate complete breakdown of the symmetrical pattern of mesenterial and septal insertion occurs. The evidence for this breakdown primarily suggests that it results from a mechanism related to increasing rates of growth in time. Initially, at least, regular mesenterial formation kept pace with polyp growth; at some point, growth, as represented by increase in circumference of polyp, got ahead of mesenterial formation and aberrant local patterns of mesenterial insertion were consequently formed. Although polyploidy might be considered an explanation for increase in size and more rapid growth, it cannot explain disturbance of growth pattern and loss of symmetry.

3) No effect upon the rate at which aragonite was being deposited; that is, this segment of the metabolic activity of the individual was apparently not affected. Unusual porosity of skeletal structures is associated with temporarily increased growth rates, such as in areas of regeneration, not accompanied by apparent metabolic changes.

In total effect, the pathology observed derives from an alteration of the growth of the individual which was, at first, more rapid, and in the end became disordered to the point where normal regulation of development was lost.

The requirements listed suggest that the three anomalous growths of *Madrepora kauaiensis* resulted from processes similar to those of neoplastic change in higher animals. However, because no definition of neoplasia has universal acceptance among oncologists, a decision as to whether the anomalies under consideration are results of neoplastic growth cannot be made. Further complications arise because the evidence for neoplasia is indirect, owing to the absence of soft tissues, precluding cytologic and histologic studies, and the absence of analogous cases among other coelenterates (*3*, *4*). However, there is considerable literature and experience on neoplasms of bony skeletal origin in vertebrates in which retrospective and presumptive identifications have been based on the evidence of skeletal remains (*5*). Continued observation of pathologies in corals and an accumulation of gross macroscopic and biologic observations, coupled with experimental work on induced neoplasia among coelenterates (*4*), should offer better diagnosis of the abnormalities described. Although it is difficult to extrapolate from laboratory examples to the skeletal abnormalities of the coral, if neoplasia were to occur in corals it might be expected to express itself in a number of ways, as in the higher animals. The advantage of recognition of neoplasia in corals lies in the potential for recording its occurrence in other collections, and among fossil forms as well, in addition to the fact that the development of a neoplasm would be completely and consecutively recorded in the growth of the skeleton.

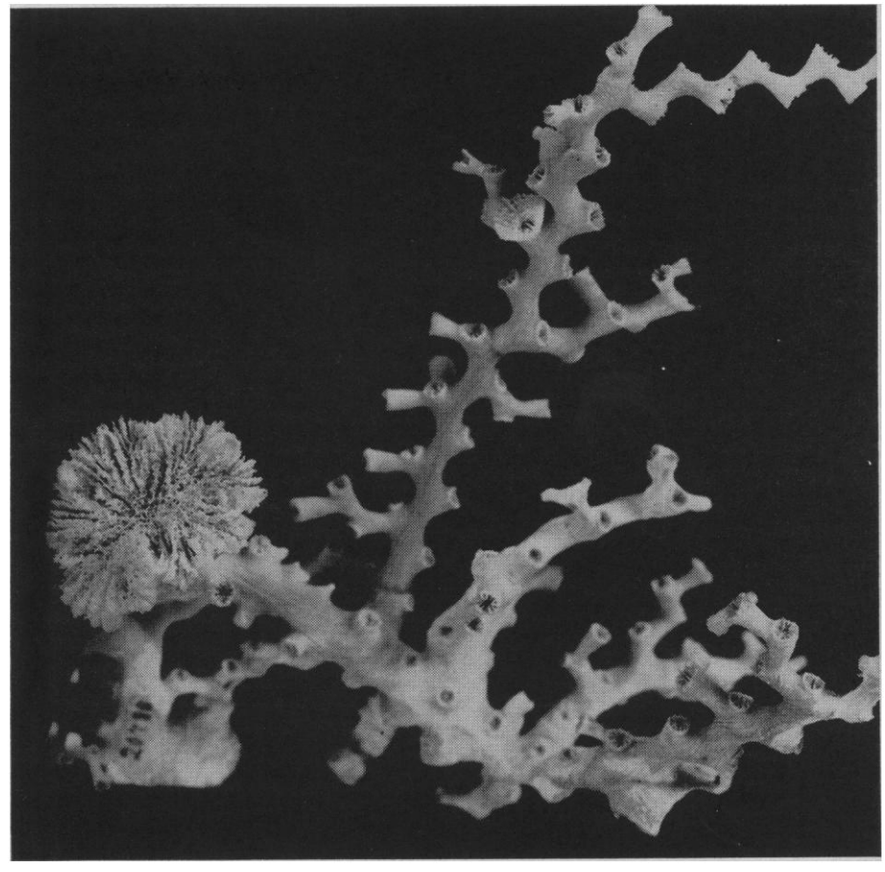

Fig. 2. The larger pathologic corallite (× 1½).

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References and Notes

1. T. W. Vaughan, *U.S. Natl. Museum Bull.* **59**, 81, and pl. 8, figs. 1–2a (1907).
2. ———, *ibid.*, p. 106, and pl. 8, figs. 3, 3a.
3. C. Kenton, "A bibliography of reports on neoplasia of invertebrates, 1950–1963," unpublished report available on request from Medical Library, National Institutes of Health, Bethesda, Md.; B. Scharrer and M. S. Lochhead, *Cancer Res.* **10**, 403 (1950).
4. H. D. Park and H. Chalkley, "Reaction of hydra to methylcholanthrene," unpublished report.
5. B. L. Coley, *Neoplasms of Bone and Related Conditions* (Hoeber, New York, 1949).
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