ages than in Holocene sediments. Siliceous microfossils are less abundant and more corroded in glacial sediments than in interglacial sediments off California, Oregon, and Washington (*19*), and in the northeastern Atlantic (*20*). Siliceous microfossils may be better preserved in some hemipelagic sediments deposited during ice ages, especially where greater siliceous microfossil productivity due to intensified upwelling during glacial periods outweighed the effects of increased terrigenous sedimentation caused by lowered sea level. Such appears to be the case off Cape Barbas, northwest Africa, where the abundance and preservation of siliceous microfossils are enhanced in sediments of glacial age (*21*).

In conclusion, the preservation of siliceous microfossils in eastern tropical Pacific sediments is most strongly influenced by the relative sedimentation rates of biogenic opal and certain detrital silicate minerals, especially minerals depleted in silica by intense chemical weathering in tropical regions. If the ratio of the sedimentation rate of biogenic opal to the sedimentation rate of detrital silicates is low, a large sink for dissolved interstitial silica is created by silicate reconstitution reactions, perhaps of the type suggested by Sillén and others (*22*). Biogenic opal present in these sediments dissolves to replace the dissolved silica taken up by these reactions and, if the silica sink is sufficiently large, all of the opal ultimately disappears. If, on the other hand, the ratio is high, the silicate conversion reactions that can take place fairly rapidly occur before a significant proportion of the siliceous microfossils is lost to dissolution. The kinetics of subsequent reactions for the uptake of dissolved silica may be significantly slower, allowing interstitial silica concentrations to increase to a level that considerably reduces the dissolution rate of biogenic opal.

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23. This research was conducted at Scripps Institution of Oceanography in La Jolla, California, and constituted part of my doctoral dissertation. I thank W. H. Berger, W. R. Riedel, M. Kastner, and K. Bjorklund for helpful discussions. This work was supported by the Oceanography Section of the National Science Foundation, grants GA 36697 and DES 75-04335 to the Scripps Institution of Oceanography.

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Gametogenesis in Planktonic Foraminifera

Abstract. *Gametogenesis in* Globigerinella aequilateralis *and* Globigerinoides sacculifer *in culture is preceded by sinking of the organism and loss of its spines. Hundreds of thousands of flagellated gametes, about 5 micrometers in diameter, are produced within the parent shell and released within a period of 13 hours.*

Life cycles of several species of benthic Foraminifera are known (*1*), but no such knowledge exists for those foraminiferal species having a planktonic existence. The delicate nature of the latter group frustrated previous attempts to study them in culture. Since June 1975, we have succeeded in maintaining planktonic Foraminifera at the Bermuda Biological Station; and recently gametogenesis was observed for the first time in *Globigerinella aequilateralis* and *Globigerinoides sacculifer*. They are among six spinose species of planktonic Foraminifera, which are routinely hand-collected by scuba diving off Bermuda. This collecting method does not damage the fragile cytoplasm and shells which bear long, extended spines and pseudopodia.

Gamete formation was first observed on 6 September 1975 in an adult specimen of *G. aequilateralis* having a shell length of about 1000 μm collected on 15 August 1975. The previous day it appeared in normal health, was floating, had long extended spines, and accepted food organisms. At 1630 on 6 September, the specimen was on the bottom of the culture vessel and had lost most of its spines. Although short pseudopodial strands radiated outward from the shell and protoplasmic streaming was evident, our previous experience indicated that it was in poor condition because loss of spines and sinking usually precedes the death of the organism. At 2130 a cloud of protoplasm was observed around the shell (Fig. 1a), containing myriads of individually moving cells, which later were shown by electron microscopy to be gametes. The gametes and large dense clumps of parental protoplasm were distributed near the aperture and appeared to have emerged from it. Individual gametes moved away from these dense clumps; but each remained connected by a fine thread of protoplasm until, after further vigorous movement, they broke loose. The gametes had a granular appearance under the light microscope and had variable shapes and sizes (Fig. 1b). The cell surface seemed rough and the perimeter seemed angular and irregular. The smallest are nearly spherical with a diameter of 2 to 5 μm.

It is difficult to observe the details of cellular division by light microscopy, but it was clear that individual gametes eventually were released since they became free-swimming. Prior to the free-swimming stage the majority of gametes in the clumps outside the shell rotated rapidly and moved in an oscillating manner. Occasionally, individual gametes seemed to move toward one another and stick together in chains, rods, or irregular clumps. It was not possible to determine whether any gametes fused.

At 0100 on 7 September, the gametes had moved farther from the shell and appeared to be sluggish and homogeneous in size. The number of gametes had also increased, while the large clumps had diminished in size and number. The

gametes appeared to move and "dance" in pairs along the fringe region.

At 0740, only a little residual protoplasm remained inside the shell, which was partly translucent. The number of gametes outside the shell had greatly decreased, and their movement slowed. At 1100, the gametes had disappeared and apparently degenerated. Only residual protoplasm and granulated masses remained. We estimate that gametogenesis was completed within 13 hours.

On 17 September 1975, we observed gametogenesis in a second species, *Globigerinoides sacculifer* (Fig. 2a), which was collected on 13 September. Its shell measured 413 μm in length and 280 μm in width. At 2045 on 16 September, it was photographed feeding on brine shrimp (*Artemia*); its shell had short spines and was covered by short pseudopodial strands.

At 1040 on 17 September, the specimen lay at the bottom of the culture vessel and all its spines were broken off. Two bulging masses of protoplasm were emerging, one from the primary and the other from the secondary aperture. Gametes from the larger mass (Fig. 2a, right) were being dispersed along outwardly radiating pseudopodial strands. Most gametes, although actively wiggling, appeared to be passively transported by the pseudopodia. Some were free-swimming or moved in small clumps.

The fate of *G. sacculifer*'s symbiotic dinoflagellates remains an enigma. These symbionts, which have been described (*2*) differ from the gametes in their slightly larger size (5 by 8 μm), golden-brown color, and immotility. Electron microscopy showed no symbionts within the shell (Fig. 2b). It is possible that the symbionts had either vacated the shell before the main exodus of the gametes or were digested in the cytoplasm. The number of symbionts scattered outside the shell was relatively small and certainly did not approach their normal density.

At 1225 on 17 September, the finger-like extensions of the pseudopodia had retracted, while the two protoplasmic masses emerging from the apertures had expanded and joined to cover most of the shell. It was clear that the gametes were being released from the parent shell en masse. Vigorous movement of tens of thousands of minute gametes within these masses gave a scintillating effect. The specimen was fixed in glutaraldehyde at 1238 on 17 September for transmission electron microscopic study.

An ultrathin section through a chamber is presented in Fig. 2c. The organic layers within the decalcified shell mark the perimeter of the chamber. This corresponds to a segment of the largest chamber presented in Fig. 2b. Numerous flagellated gametes and occasional membranous masses of residual parent cell cytoplasm fill the chamber. The mean diameter of the nuclei is 3 μm and of the gametes is 5 μm. These flagellated cells are not zooxanthellae (symbionts) since they do not contain puffy, coiled chromosomes characteristic of mesokaryotic nuclei in zooxanthellae nor do they possess plastids.

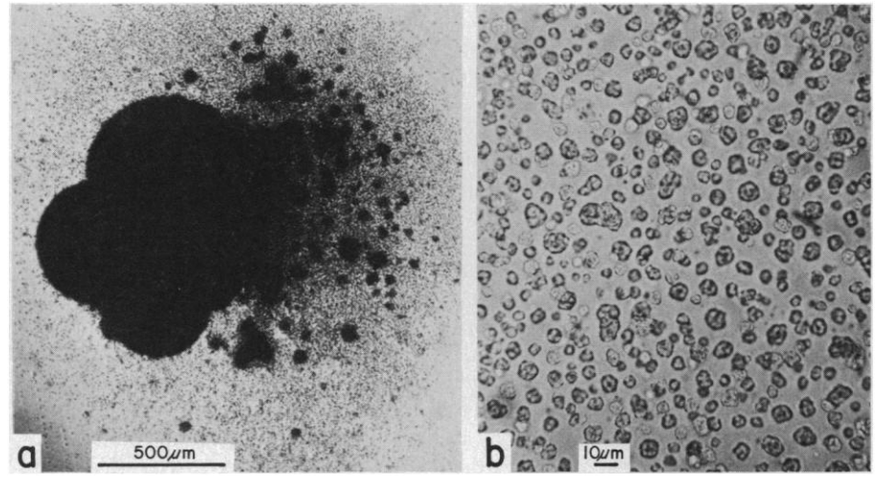

Fig. 1. (a) Gametogenesis in *Globigerinella aequilateralis*. Tens of thousands of gametes as well as clumps of parent cytoplasm form a cloud around the parent shell. (b) Light micrograph of individual gametes from (a).

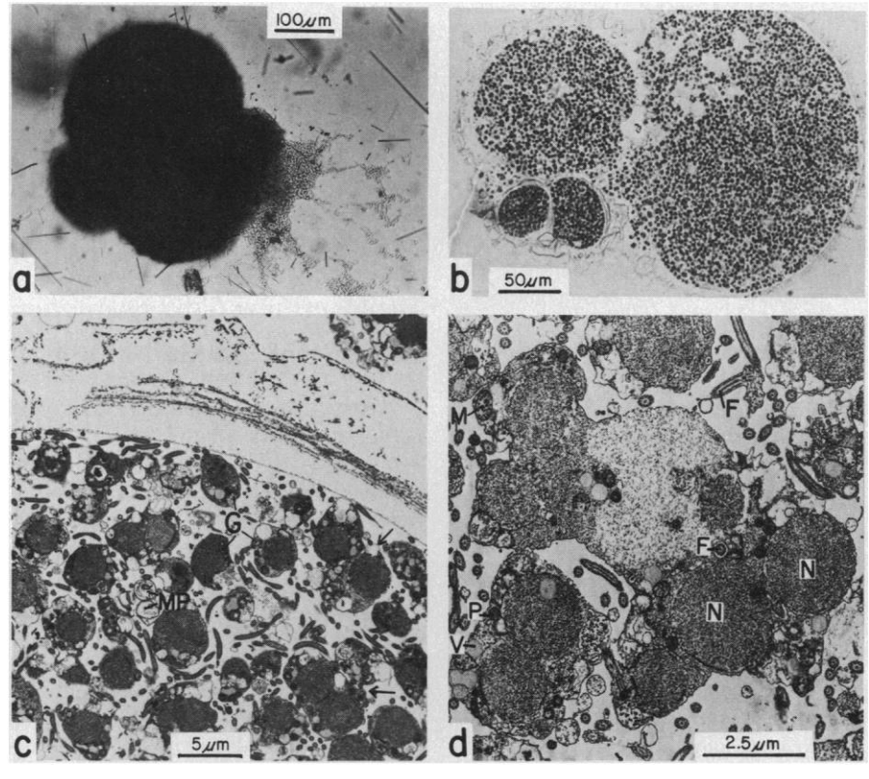

Fig. 2. Gametogenesis in *Globigerinoides sacculifer* (all four photos are of same specimen). (a) Two masses of protoplasm protruding from the shell's apertures. At right, gametes are being dispersed in a pseudopodial network. (b) This longitudinal section of *G. sacculifer* reveals a total of 4295 gametes. Little remains of the parent protoplasm and no symbiotic dinoflagellates remain within the shell. (c) Numerous gametes (*G*), clumps of parent cytoplasm (arrows) containing nuclei, and residual membranous material (*MP*) from the parent cell fill the chamber in this ultrathin section. Part of the chamber wall is visible at the top. (d) A mass of parent cytoplasm shows several nuclei (*N*), mitochondria (*M*), flagella (*F*), single-membrane bodies (*P*) that resemble peroxisomes, and small vacuoles (*V*) and contain a fibrous material typically produced by parent cytoplasm. The nuclei are segregated from the cytoplasm and appear to be undergoing cytokinesis to produce isolated gametes.

Some of the gametes are incompletely separated (Fig. 2c, arrows) and are organized into multilobed masses of foraminiferal cytoplasm containing two or more nuclei. These probably correspond to the clumps of cytoplasm seen with the light microscope. Higher magnifications (Fig. 2d) show that the incipient, undivided gametes are derived from parent cytoplasm. The small vacuoles filled with translucent masses of fibrillar material are characteristic of parent cytoplasm (*2*). Within the cytoplasmic clumps (Fig. 2d) the nuclei are being separated from the cytoplasm and possess double membranes.

The gametes are evidently in their terminal stage of development. Sequential sections were taken through the entire specimen, but no residual parent nucleus was observed. We suspect that the single large nucleus of the parent cell (*2*) had already undergone repeated division to yield the numerous nuclei producing the gametes. This is consistent with many observations in benthic Foraminifera (*3*), in which the gamete-producing (gamont) cell contains a single large nucleus, whereas the agamont stage is multinucleate.

Flagella are present in various stages of development. We have observed no more than two flagella in cross sections of individual gametes. The cytoplasm of the gametes contains tubular mitochondria and single-membrane organelles with a granular matrix and a dense inclusion body. These organelles resemble peroxisomes. Golgi bodies and occasional lipid droplets were observed. Biflagellated gametes have been reported in several species of benthic Foraminifera (*4–6*). The cytological events during gametogenesis in planktonic Foraminifera are similar to those seen in some species of benthic Foraminifera—*Myxotheca* sp. (*4*), *Boderia turneri* (*5*), and *Nemogullmia* sp. (*6*).

We have estimated the total number of gametes within the shell by counting the number in a single section of the specimen. There were 4295 gametes in a cross-sectional area of 5×10^4 μm^2 (Fig. 2b). The section's volume was determined by assigning a thickness equivalent to the mean diameter of the gametes (5 μm). This volume was divided by the number of gametes to yield a volume-to-gamete ratio of 60 μm^3 per gamete. The volume of the entire shell was 17×10^6 μm^3, which, when divided by the foregoing ratio of volume to gamete, yielded the total number of gametes in the shell (2.8×10^5 gametes). The total number of gametes produced exceeds this estimate as a sizable proportion of them had already been released.

The combined evidence from light microscopy and electron microscopy confirms that sexual reproduction (gametogenesis) occurs in the life cycle of these planktonic Foraminifera. The large number of gametes released from the shell suggests that these species are gametogamous (fusion outside the shells of parent organisms) and not gamontogamous (fusion within the paired shells of parent organisms) (*3*). Hence we know that one part of their life cycle is a sexual phase, but we have not yet observed fusion of gametes. We do not have evidence as yet that there is asexual reproduction, although multiple nuclei have been reported in *Globigerina bulloides*, which was presumed to be an agamont stage (*7*). Further observations of reproduction in isolated cells and in cultures of two or more gamonts of the same species are required to determine whether planktonic Foraminifera are monoecious or dioecious. Such studies may allow us to deduce their complete life cycle.

The prodigious number of gametes in planktonic Foraminifera is frequently observed in lower organisms that release their gametes into the environment. Such large numbers would be expected if the foraminiferal species were dioecious. Although these organisms are abundant in the ocean, they are so widely spaced that only the production of myriads of gametes would ensure fusion of sufficient numbers to yield enough offspring to maintain the population. If sexual reproduction occurs in planktonic Foraminifera, as indicated by our evidence, this would allow genetic diversification and may help to explain their great abundance in widely diversified habitats.

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Hepatocellular Transplantation for Metabolic Deficiencies: Decrease of Plasma Bilirubin in Gunn Rats

Abstract. *A sustained decrease of plasma bilirubin concentrations occurred in homozygous recessive Gunn rats lacking the enzyme uridine diphosphate glucuronyltransferase following infusion into the portal vein of hepatocytes from heterozygous nonjaundiced Gunn rats possessing the enzyme. Transplantation of cells capable of continuous enzyme production could be an effective mode of therapy for congenital enzyme deficiency diseases.*

There is currently no effective treatment for many congenital enzyme deficiency diseases. Enzyme infusion therapy has only a temporary effect (*1*), and whole organ transplantation is associated with considerable morbidity and mortality (*2*). An excellent model of congenital enzyme deficiency disease is provided by the Gunn rat, a noninbred mutant Wistar rat. The homozygous recessive Gunn rat has a deficiency of the liver enzyme uridine diphosphate glucuronyltransferase (UDPGT), which is necessary for the conjugation of bilirubin to bilirubin diglucuronide. Affected animals are jaundiced at birth, and all bilirubin is unconjugated. Heterozygous animals have decreased enzyme levels but are unable to maintain a normal concentration of plasma bilirubin unless they are challenged with a bilirubin load.

Previous reports have described the lowering of serum bilirubin in jaundiced Gunn rats by subcutaneous implantation of rat hepatoma cells (*3*) and by direct implantation of pieces of Wistar rat liver into punch biopsy sites in the livers of Gunn rats (*4*). Results of these studies stimulated us to develop a technique of hepatocellular transplantation that would be easily applicable to human congenital enzyme deficiency disease. Two