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The AAAS-Newcomb Cleveland Prize, which previously honored research papers presented at AAAS annual meetings, is now awarded annually to the author of an outstanding paper published from September through August in the Reports section of *Science*. The second competition year under the new rules starts with the 2 September 1977 issue of *Science* and ends with that of 25 August 1978. The value of the prize is $5000; the winner also receives a bronze medal.

To be eligible, a paper must be a first-time publication of the author's own research. Reference to pertinent earlier work by the author may be included to give perspective.

Throughout the year, readers are invited to nominate papers appearing in the Reports section. Nominations must be typed, and the following information provided: the title of the paper, issue in which it was published, author's name, and a brief statement of justification for nomination. Nominations should be submitted to AAAS-Newcomb Cleveland Prize, AAAS, 1515 Massachusetts Avenue, NW, Washington, D.C. 20005. Final selection will rest with a panel of distinguished scientists appointed by the Board of Directors.

The award will be presented at a session of the annual meeting at which the winner will be invited to present a paper reviewing the field related to the prize-winning research. The review paper will subsequently be published in *Science*. In cases of multiple authorship, the prize will be divided equally between or among the authors; the senior author will be invited to speak at the annual meeting.

Reports

Vibrio cholerae, *Vibrio parahaemolyticus*, and Other Vibrios: Occurrence and Distribution in Chesapeake Bay

Abstract. Vibrio cholerae *was isolated at several locations in Chesapeake Bay in fall 1976 and spring 1977. Strains induced fluid accumulation in rabbit ileal loops and positive activity in Y-1 adrenal cells.* Vibrio cholerae, Vibrio parahaemolyticus, *and related vibrios show a spatial and temporal distribution characteristic of* Vibrio *species in an estuary. The* Vibrio cholerae *strains isolated from Chesapeake Bay represent serotypes other than O-group I—that is, so-called nonagglutinable vibrios—and are not recognized as a serious epidemic threat, although they have caused cholera-like diarrhea sporadically.*

Vibrio cholerae, the causative agent of cholera, was one of the first bacterial agents to be implicated in a disease. Originally described by Koch in 1883 (*1*), *V. cholerae* still extracts a high toll of misery in certain parts of the world today. Outbreaks of cholera have been recorded in the United States since the settlement of the original colonies and were significant events in its history. However, modern sanitary facilities and treatment of water supplies have virtually eliminated cholera in the United States. Since 1911, only two cases of cholera have been documented that were not acquired in the laboratory—one in Texas in 1973 (*2*) and the other in Alabama in 1977 (*3*).

We report the isolation of *V. cholerae* from water samples collected in Chesapeake Bay (see Fig. 1). Isolations were made in late fall 1976 and early spring 1977, and we are continuing to isolate *V. cholerae*. Water samples were processed aboard ship immediately after collection or at the laboratory within 1 hour of collection. Details of sample collection are provided elsewhere (*4*). Samples incubated at 37°C in alkaline peptone enrichment broth for 18 to 24 hours were streaked onto thiosulfate citrate bile salts sucrose agar (Difco Laboratories, Detroit, Michigan) and inoculated plates were incubated for 18 to 24 hours at 37°C. *Vibrio cholerae* isolates were purified on tryptic soy agar (Difco), characterized by a series of biochemical tests, including the API 20E system (Analytab Products, Plainview, New York), and sent to the Center for Disease Control, Atlanta, Georgia, and to the Vibrio Reference Laboratory, Jefferson Medical College, Philadelphia, Pennsylvania, for serological confirmation.

To date, a total of 17 isolates has been obtained and confirmed as nonagglutinable (NAG) *V. cholerae*—that is, *V. cholerae* strains which do not agglutinate in antiserum against *V. cholerae* group I O-antigen. At the present time, only strains of *V. cholerae* which agglutinate in group I antiserum are recognized as an epidemic threat. However, many cases of choleralike diarrhea resulting from NAG vibrios have been documented (*5*). Furthermore, agglutinable strains of *V. cholerae* have been reported to lose the group I antigen entirely, and NAG vibrios have been reported to gain the ability to agglutinate in cholerae group I antiserum (*6*). Taxonomic data, including numerical taxonomy, the overall guanosine plus cytosine composition of DNA, and DNA homology (*7*), demonstrate the validity of including NAG vibrios within the taxospecies and genospecies of *V. cholerae*.

The *V. cholerae* isolates have been tested for ability to induce fluid accumulation in ligated ileal loops of rabbit intestine (*8*) (Table 1) and for toxin production by observations in Y-1 adrenal cells (*9*). Observations in Y-1 adrenal cells revealed positive activity with all strains except V35. Cell-free filtrates, when tested against CHO and Y-1 adrenal cell lines, also yielded positive results.

From the results of the detailed biochemical characterization, it is concluded that the Chesapeake Bay isolates are typical *V. cholerae*. In addition to conventional tests, such as gelatin hydrolysis and indole production, all strains hydrolyzed chitin. Several of the isolates were tested for the overall guanosine plus cytosine composition of DNA, which was found to be 48 to 49 percent, typical of *V. cholerae*.

As shown in Table 1, 11 isolates were recovered from Jones Falls in Baltimore

Harbor, where the water is shallow, brackish [salinity, 4 to 5 parts per thousand (ppt)], and polluted by sewage. Water collected from Jones Falls yielded high fecal coliform counts, ~ 2400 per 100 ml of water. A majority of the strains from Jones Falls produced fluid accumulation (Table 1). Three were typed. The serotypes of *V. cholerae* isolated at Jones Falls are of wide geographical occurrence: serotype 24 has been isolated from fresh water, diarrheal discharge, and animal feces in the Philippines, Bangladesh, and Thailand, and serotype 23 from shellfish in the Philippines and from a patient with diarrhea in Sudan. The most probable number for *V. cholerae* in water collected from Jones Falls was 3.3 per liter.

The Fort McHenry sampling site is approximately twice as deep as the Jones Falls site, and the water is slightly more brackish (salinity, 5 to 6 ppt), with fecal coliform counts of 100 to 2000 per 100 ml. The water at Fort McHenry is well mixed, more representative of Baltimore Harbor than that at Jones Falls, and not adjacent to known point sources of pollution. Serotype 14 was isolated from Fort McHenry water and has also been isolated by other investigators from a variety of sources, including fresh water, brackish water, diarrheal discharge, night soil, and animal feces in Bulgaria, Iraq, Czechoslovakia, Japan, and Bangladesh (*4*).

The Chester River station, located at the mouth of the Chester River, is representative of the upper Chesapeake Bay and has a salinity of 6 to 12 ppt and fecal coliform counts of approximately 0.5 per 100 ml, indicating little or no pollution with sewage. Strain V35 was found to be serotype 23, which was also isolated at Jones Falls, and strain V38 was serotype 17, which has been isolated in a dozen countries around the world from diarrheal discharge, sewage, fresh water, and nonenteric infections.

Water temperature appears to be a critical factor, since *V. cholerae* was isolated in the late fall and early spring, when the water temperature was 15° to 20°C. During the winter, when the water temperature was 0° to 10°C, *V. cholerae* strains were not isolated. No correlation between incidence of *V. cholerae* and fecal coliforms was observed, as was also the case for *V. parahaemolyticus* (*10*).

The discovery of *V. cholerae* in Chesapeake Bay raises many questions, some very disturbing, since *Vibrio* species are associated with shellfish and crustaceans in Chesapeake Bay. In 1968 a bacteriological analysis of oysters collected from the Marumsco Bar and

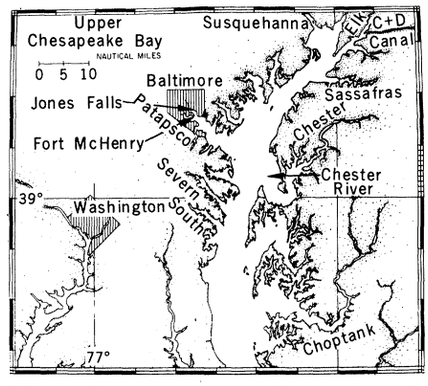

Fig. 1. Chesapeake Bay stations sampled in this study.

Eastern Bay areas of Chesapeake Bay showed a predominance of *Vibrio*, *Pseudomonas*, and *Achromobacter* spp., in that order, with a smaller percentage of other genera (*11*). The bacterial flora of blue crabs (*Callinectes sapidus*) from Chesapeake Bay comprises *Vibrio* spp., including *V. parahaemolyticus* (*12*). *Vibrio parahaemolyticus*, widely implicated in outbreaks of gastroenteritis related to the consumption of improperly processed seafood, was found in crabs collected between May and November 1968 (*13, 14*).

The incidence of *V. parahaemolyticus* in Chesapeake Bay has been shown to be related to water temperature (*15*), and the association with zooplankton of *V. parahaemolyticus* and related vibrios has also been established (*10, 15*). *Vibrio* spp. and related organisms comprise the total population associated with plankton in summer months (*10*). From zooplankton and crab studies, it was concluded that *Vibrio* spp., including *V. parahaemolyticus*, play an important ecological role in Chesapeake Bay because they are chitin digesters and mineralizers of organic matter.

Salinity influences the distribution of *V. parahaemolyticus* and related vibrios (*10*), as shown in a comprehensive bacteriological survey of the upper Chesapeake Bay (*16*). The salinity of full-strength seawater was demonstrated to be inimical to *V. parahaemolyticus* and, although *Vibrio* species were a major component of the bacterial flora of zooplankton in offshore waters where the salinity is high, these vibrios were different from *V. parahaemolyticus* (*17*). They were unable to grow at elevated temperatures, tolerated higher salinity, and had low nucleotide homology with *V. parahaemolyticus* (*18*). Other investigations have shown that *V. parahaemolyticus* can be readily isolated from estuaries and near-coastal water (*19, 20*).

From the data accumulated on the distribution of *V. parahaemolyticus*, *V. cholerae*, and related vibrios in Chesapeake Bay and environs, we offer the following hypothesis. *Vibrio* spp. are dominant bacterial species in brackish, estuarine, and coastal waters. Within the genus *Vibrio*, there are species that are restricted in their distribution by salinity and other factors. *Vibrio cholerae* and related vibrios are found in brackish waters, *V. parahaemolyticus* and related vibrios in estuarine water, and marine *Vibrio* species in coastal and offshore waters. Whether there are *Vibrio* species restricted to the deep ocean may be determined by examination of water samples collected from the Puerto Rican and Cayman trenches (*21, 22*). The association of *Vibrio* species with shellfish, crustaceans, and zooplankton is significant, if not dramatic. We hypothesize that these *Vibrio* species are important in the ecology of brackish and marine aquatic ecosystems because of their ability to mineralize organic matter and digest chitin, a major structural component of many aquatic invertebrates.

The pathogenicity demonstrated by *Vibrio* species is noteworthy since ingestion of water or seafood in areas of poor sanitation or bad food-handling practices is frequently involved in disease outbreaks recorded for *V. cholerae* and *V. parahaemolyticus*. The associa-

Table 1. Isolation of *Vibrio cholerae* from Chesapeake Bay.

Site of sample collection	Date of isolation	Strain	Serotype	Ileal loop activity
Jones Falls	September 1976	V2, V3	NT*	+
		V4	24	+
		V5	23	+
		V10	14	+
	October 1976	V11, V25, V26, V29	NT	−
		V15, V24	NT	+
Fort McHenry	April 1977	V31	NT	+
		V33	14	−
Chester River	May 1977	V35	23	+
		V36, V37	NT	ND†
		V38	17	ND

*NT, not typable. †ND, not determined.

tion of *Vibrio* species with crustaceans and other invertebrates suggests that further studies on the association of vibrios with their invertebrate hosts would provide a better understanding of the pathogenic mechanisms of these bacteria. Enterotoxin produced by *V. cholerae* and by some strains of *V. parahaemolyticus* (*23*) may impart a significant advantage to the vibrio in its association with invertebrates and zooplankton. Indeed, extracellular substances produced by vibrios may prove to be ectocrines, as defined by Lucas (*24*). The epidemiology and pathogenicity of *V. cholerae*, *V. parahaemolyticus*, *V. alginolyticus*, *V. anguillarum*, and other *Vibrio* species would be better understood if the pathogenic and nonpathogenic *Vibrio* species were considered in the perspective of microbial-host dynamics in aquatic ecosystems.

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Archean Microfossils Showing Cell Division from the Swaziland System of South Africa

Abstract. *A newly discovered population of organic walled microstructures from the Swaziland System, South Africa, is considered to be biological on the following grounds: (i) the structures are carbonaceous and occasionally have internal organic contents; (ii) the population has a narrow unimodal size frequency distribution (average diameter, 2.5 micrometers; range, 1 to 4 micrometers); (iii) the structures are not strictly spherical, but are commonly flattened and folded like younger microfossils; (iv) the sedimentary context is consistent with biogenic origins; and (v) various stages of binary division are clearly preserved.*

Discrete organic microstructures have been known from sediments of the Archean Swaziland System of South Africa for more than a decade (*1–6*), but the problem of their biogenicity has recently been questioned and is not satisfactorily resolved. Several investigators have voiced their skepticism of many of the reports of algalike fossils from these ancient rocks (*7, 8*), and with good reason. Most of the described spheroids are very large compared to extant prokaryotic cells and bona fide prokaryotic unicellular microfossils and, when subjected to elementary statistical tests, these Archean assemblages have often been found to have size frequency distributions quite unlike those of living and fossil unicell populations (*7, 9*). In addition, it should be noted that Swaziland assemblages have until now consisted almost solely of unelaborated individual spheroids (*10*), although Muir and Grant (*6*) have described what they interpret to represent paired cells in a poorly preserved assemblage from the Kromberg Formation. Primitive microbes are now and were assuredly in the past morphologically very simple; many extant prokaryotic taxa consist entirely of smooth coccoidal unicells. The only way one could hope to distinguish fossil populations of such primitive cells from abiotically formed spheroids would be to find assemblages some of whose members exhibit interpretable biological activity. In morphological terms, this criterion focuses on finding evidence of cell division. We here report the discovery of a well-preserved population of microstructures from the Swaziland System that contains not only isolated individuals and common paired cells, but also the intermediate stages of binary fission. We believe that the data described here constitute cogent evidence for the presence of discrete algalike biological remains in rocks exceeding 3 billion years in age (*11*).

The Swaziland System is an ancient Archean greenstone belt exposed in the Barberton mountain land of the eastern Transvaal, South Africa, and in adjacent Swaziland. Because of their great antiquity and remarkably low degree of metamorphic alteration, the carbonaceous sedimentary rocks of this sequence have been the focus of much scientific inquiry into the nature of early life on the earth. The general geology of the system has been elucidated in many publications (*12*) and need only be briefly outlined here. The Onverwacht and Fig Tree Groups of the Swaziland System include nearly 17,000 m of interbedded sedimentary rocks and volcanics. Conspicuous among the sediments are bedded cherts that are often rich in organic matter. The carbonaceous cherts in which we have found evidence of cell division come from an outcrop of the Swartkoppie Formation exposed in the valley of the Umsoli River 19 km southwest of the town of Barberton. Most geologists have considered the Swartkoppie to be the uppermost formation in the Onverwacht Group, but it is really transitional with the conformably overlying sediments of the Fig Tree Group. In fact, Reimer (*13*) now feels that this cherty horizon should definitely be regarded as Fig Tree in age. At our fossil locality, shallow-water to