200-mg carbon filter was somewhat greater than that of the others.

Also shown in Table 1 are the amounts of ciliastatic materials in the condensable phase that the filters removed. Formaldehyde is included here because it has been reported to be ciliastatic, but its activity cannot be correlated by vapor phase chromatography (VPC) with that of the other compounds because it does not elute from the chromatographic column. The HCN (*9*, *10*) and NO, NO_2 (*11*) determinations were done by colorimetric methods described in the literature. Formaldehyde was determined by gas chromatographic analysis of its 2,4-dinitrophenylhydrazone (*12*). Acetaldehyde and acrolein were determined by a VPC method (*13*).

The effects of the five filters on ciliastasis are shown in Fig. 4. The data was analyzed statistically by the incomplete block method. There is no significant difference between points within the same circle. Points that fall in overlapping areas are not significantly different from points in either of the overlapping circles. At the 95-percent confidence level there was little significant difference in the early puffs. By the time the eighth puff was reached, however, differences could be seen. The smoke vapors through the basic filter were less ciliastatic than any of the others shown in the figure. The smoke through the plain cellulose acetate filters and the 100-mg carbon filters was less ciliastatic than the control, but no significant difference could be seen between the hydrazide and the control. The smoke through the 200-mg carbon filter was less ciliastatic than any of the others.

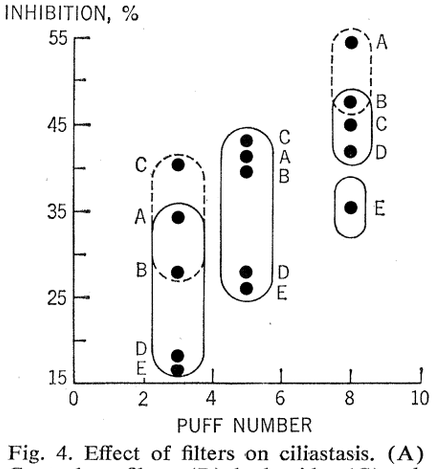

Fig. 4. Effect of filters on ciliastasis. (A) Control-nonfilter; (B) hydrazide; (C) cellulose acetate; (D) carbon; (E) base.

In conclusion, hydrogen cyanide appears to be an important contributor to the activity of smoke. This was shown partly by the chromatographic technique when the HCN strongly inhibited the cilia and partly by the sharp reduction in ciliastasis when most of the HCN was filtered from the smoke by the filter which contained base. Acetaldehyde seems to contribute little to the activity of smoke. Removal of most of it by the hydrazide filter showed no change in ciliastasis. This is not surprising, since it was noted in the chromatography of smoke that the cilia rapidly recovered from the effects of acetaldehyde. When the whole gas phase is smoked over the clam, the cilia would have time to recover from acetaldehyde before ciliastasis is recorded. And in fact some rapid recovery is seen immediately after a puff is taken.

Acrolein presents an interesting problem. The chromatographic method pointed to acrolein as a powerful ciliastat—at least as powerful as HCN; yet removal of most of the acrolein by the hydrazide filter had no effect on ciliastasis while removal of most of the HCN markedly reduced ciliastasis. One possible explanation is that while pure acrolein is a strong ciliastat, it is deactivated in the presence of smoke. Another explanation is that the acrolein passing across the specimen was more concentrated in the VPC method than in the puffing method, and the VPC method therefore did not give a true picture of its importance. Another possibility is that perhaps it is necessary to remove more than 60 to 70 percent of the acrolein to produce a reduction in ciliastasis; that is, the 25 μg acrolein in the filtered smoke may be about as effective as the 70 μg present in unfiltered smoke.

Table 1. Removal of gas phase components from cigarette smoke by filters.

Filter additive	Component removed (%)				
	NO, NO_2	HCHO	CH_3CHO	CH_2=CHCHO	HCN
None	< 10	16	0	0	0
Base	< 10	48	0	0	68
Hydrazide	< 10	61	82	66	0
Carbon, 100 mg	< 10	79	86	95	56
Carbon, 200 mg	< 10	92	> 95	> 95	80

TED R. WALKER
JOHN E. KIEFER
Research Laboratories, Tennessee Eastman Company, Division of Eastman Kodak Company, Kingsport, Tennessee

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1 August 1966

North Atlantic Deep-Sea Fertility

Abstract. *Observations have been made on two cruises in the North Atlantic in which large numbers of microscopic, unicellular flagellates have been found throughout the aphotic zone below 1000 meters. Preliminary measurements also indicate the uptake of dissolved organic substances, suggestive of an apparently viable, actively metabolizing community at these depths.*

The aphotic zone of the oceanic water column is generally considered to be sparsely populated with phytoplankton. Phytoplanktologists agree that organisms exist throughout the depths, but that both their biomass and rate of production are negligible relative to that in the euphotic layers. The view that aphotic microorganisms are insignificant can be attributed to three major reasons: first, those deep-sea expeditions concerned with this problem found few cells in abyssal samples (*1*); second, heterotrophy, so necessary for existence at these depths, has not been demonstrated to be a widespread nutritional mode in phytoplankton; third, the quantity of both dissolved and particulate organic ma-

terial in the sea is considered to be too low to support a population of any substantial size.

However, in recent years increasing evidence indicates that phytoplankton heterotrophy may be an important metabolic process in the oceans (*2, 3*). Much of the evidence supporting oceanic heterotrophy, however, rests on implicit rather than empirical information. The strongest evidence is provided by Bernard's observations in the Mediterranean and Eastern Atlantic (*4*). In these areas Bernard has found large numbers of coccolithophores throughout the aphotic zone, quite often exceeding those observed near the surface. Because of their viable appearance when collected, he has assumed that they are existing on organic material in the dark and are possibly autotrophic or heterotrophic or both when at the surface. However, based on the evidence presented, Bernard's contention of heterotrophic survival in the abyss is not well supported. It is quite possible that these organisms may exist on reserve material previously synthesized in the euphotic zone, should they have sunk from that layer.

In addition to Bernard's work, Lewin and Lewin (*5*) have demonstrated experimentally that various diatoms, heretofore considered to be strict autotrophs, can grow on an organic medium in the dark. Thus, heterotrophy has now been demonstrated in all algal classes (*6*). This knowledge of potential heterotrophism in many organisms, coupled with our present information on the organic chemistry of sea water (*7*), suggests the widespread occurrence of phytoplankters existing heterotrophically in the aphotic zone. Furthermore, observations of Vinogradov (*8*) and Wolff (*9*) that copepods occur at great depths throughout the Pacific imply an indigenous food source for these animals. Various schemes have been devised to answer the obvious question of the nature of abyssal zooplankton sustenance. Certainly, the presence of large numbers of coccolithophores or even smaller flagellates would help to explain the nutrition of these deep herbivores.

An attempt to investigate this question of the importance of a deep-sea flora was made on two cruises aboard *R. V. Trident*, research vessel of the University of Rhode Island. Ten stations were made during the third leg of cruise No. 23 ("Sweat"), 8 May to 8 June, 1965, between Rio de Janeiro and the island of Barbados (Fig. 1). On cruise No. 28 ("Casbah"), 11 October to 30 November 1965, 24 stations were occupied along a transect extending from the coast of Rhode Island to the Algerian coast in the Mediterranean Sea (Fig. 1). The Casbah stations were planned in this manner to examine the possible criticism that any aphotic phytoplankters found in abundance, such as in the Mediterranean Sea (*4*) or tropical Atlantic, were the product of anomalous situations. Also, any information obtained on such a transect would be more applicable to the North Atlantic as a whole than if the study were conducted off the Rhode Island coast alone.

The sampling and data-processing procedure were similar on both cruises, as follows. At each station water was collected by using nonsterile Niskin 5-liter samplers at prearranged depths. Sterile Cobet samplers were also used occasionally. Samples were collected at 50 m, 1000 m, and thereafter at 1000-m intervals to the bottom. This water was utilized in many ways, including: routine hydrographic observations, dissolved and particulate organic carbon determinations, chlorophyll determinations, estimates of cell numbers and, on the Casbah cruise, incubation of known volumes with carbon-tagged dissolved organics (*2*). Only the last two will be mentioned in this report, since the relationship between all these parameters is not yet clear. Cell counts were made by filtering from 300 to 1000 ml of water through an HA Millipore filter (0.45 μ pore size), washing with 10-percent formalin, desiccating, and then clearing and mounting (Permount) on a slide for microscopical examination, usually at 1500×.

The Sweat cruise demonstrated the presence of flagellated microorganisms throughout the aphotic zone. Figure 2 shows the organism which predominated at every depth of every station sampled from 1000 m and below. It is biflagellated (although the majority do not display the flagella when observed on a membrane filter), apparently spherical, and ranges in diameter from 3 to 5 μ. On the membrane filter the organisms are distinctly yellow-green in color and appear to contain numerous opaque inclusions which are barely visible in Fig. 2. A preliminary attempt at classification indicates a possible affiliation with the class Chrysophyta.

Flagella were frequently absent in the

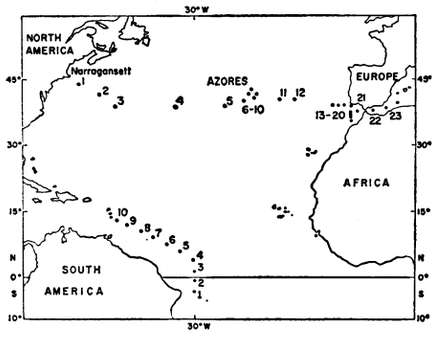

Fig. 1. "Sweat" cruise, 10 stations along coast of South America. "Casbah" cruise, 24 stations from Narragansett to Algerian coast.

fixed specimens. It is unknown whether this is an artifact of preservation or is a natural condition. The former is suggested from observations on organisms obtained on the Casbah cruise and now growing in culture. These organisms, collected with a nonsterile sampler, are morphologically similar to the preserved specimens, except for the presence of flagella. They have survived eight subcultures on an organic medium, living in the dark at 3°C.

At least a dozen other organisms have also been found in these samples, including *Coccolithus fragilis*, next in abundance, and also large numbers of what appear to be rhizopods of the genus *Limax*. A full quantitative and qualitative description of the organisms found at these deep stations will be presented in succeeding reports.

Some interesting trends exist in the flagellate (Fig. 2) cell numbers obtained from the Sweat cruise (Fig. 3). The average value for 37 separate observations from 1000 to 5000 m is 66,000 cells per liter, with a range of 14,200 to 220,000 per liter. Although the

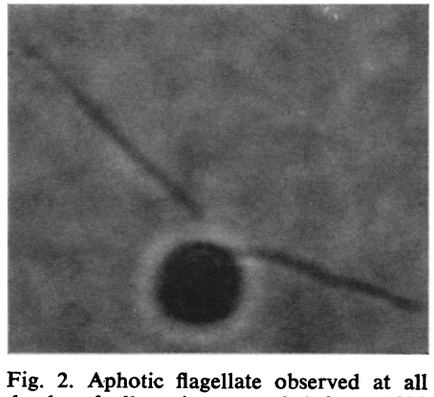

Fig. 2. Aphotic flagellate observed at all depths of all stations sampled from 1000 to 5000 m. Magnification is 1800×; mottled background is due to Millipore filter.

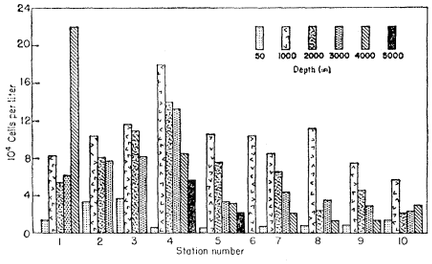

Fig. 3. Abundance of flagellates in tens of thousands of cells per liter observed on "Sweat" cruise.

Casbah cell counts are not complete yet, those completed are of a magnitude similar to those of Sweat. Although these data were collected with nonsterile samplers, their order of magnitude has been corroborated by the use of the previously mentioned Cobet samplers. The vertical distribution (Fig. 3) indicates a relative paucity in the euphotic zone (50 m averaged 15,000 cells/liter), followed by maximum numbers in almost every case at 1000 m (average, 107,000 cells/liter). Below 1000 m, a gradual decline occurs, yielding numbers at 5000 m (average, 39,000 cells/liter) almost three times those found near the surface. It is interesting to note that the maximum number (220,000 cells/liter) observed for Sweat occurred at 4000 m at station No. 1. A horizontal variability also appears to exist, indicating either a decline with approach to land, that is, at both ends of the transect, or possibly an increase in numbers with approach to that area most influenced by the Amazon discharge. It is hoped that the completed Casbah data will help to clear up some of the reasons for this variability.

Rates of glucose and acetate uptake determined during the Casbah cruise, although quite variable, suggest that this deep community may utilize dissolved organic substances. Although substantial numbers of organisms were present in each case, and shipboard techniques were sterile, some of the observed uptake is open to question because of the lack of sterility in the samplers. These experiments will soon be reproduced again at sea and in the laboratory with axenic cultures.

It would appear that these unicellular, motile, microorganisms are ideally suited for this aphotic environment where diffusion of organics in low concentration is so essential. Their small size results in a high area-to-volume ratio for increased efficiency in uptake, while their motility allows them to maintain a high internal/external gradient across external membranes. It appears that these results support Bernard's contention of a heterotrophic existence for his Mediterranean coccolithophores, since the conditions for existence of both are quite similar.

Thus, these results suggest that a fairly abundant phytoplankton population, possibly capable of heterotrophic existence, exists at a depth between 1000 and 5000 m, between latitudes 40°N and 3°S in the Atlantic Ocean.

The reason these organisms have seldom been observed seems to rest with the method of sample treatment and observation, namely, either centrifugation prior to examination, or sedimentation with the inverted microscope. The former method was used by the expeditions (*1*) mentioned earlier, but has since been shown to be quite inefficient with certain organisms (*10*). The inverted microscope is also inadequate for these small flagellates, although Bernard has used it successfully in the enumeration of coccolithophores (*4*, *10*). A comparative study made with identical Sweat samples demonstrated that after 48 hours most of the flagellate cells (Fig. 2) could still be recovered by filtering the supernatant from the settling chambers. This means that after fixation these cells either require a very long time to settle out or do not settle out at all. Therefore, it appears that both settling and centrifugation are methods which are inadequate to detect these organisms.

The implications of a community, whose presence has been generally unknown, which is both abundant and apparently autochthonous throughout the aphotic zone of the North Atlantic would appear to be far-reaching; for the presence of an actively metabolizing flora with cell numbers 100 times greater than has been previously reported (*1*) has general oceanographic importance. To mention several areas possibly influenced by the knowledge of the presence of these organisms, we should include: present estimates of recycling rates of organic matter in the sea; the nutrition of herbivorous deep zooplankton communities; and the question of the apparent paucity of bacteria in the deep sea where organic material is available and other organisms are abundant. In any case, the presence of these organisms presents an important area of investigation which biological oceanographers have long overlooked.

ROBERT O. FOURNIER
Graduate School of Oceanography, University of Rhode Island, Kingston

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Susceptibility of Human Diploid Fibroblast Strains to Transformation by SV40 Virus

Abstract. *A quantitative system has been developed for the study of transformation of human diploid fibroblasts in culture by two oncogenic viruses, SV40 and the E46 strain of adeno 7-SV40 "hybrid" virus. Seven of the eleven cell strains derived from human skin biopsies when infected with SV40 (10^9 tissue culture infective doses per milliliter) gave rise to transformed colonies with approximately the same frequency (0.03 percent). Two strains derived from patients with Fanconi's anemia, an autosomal recessive disease associated with a high incidence of chromosome abnormalities and spontaneous neoplasms, gave values more than ten times higher. Two strains from persons heterozygous for this gene were also considerably more susceptible to viral transformation.*

Several quantitative systems for studying the in vitro transformation of cells in tissue culture by oncogenic viruses have been described; these have employed both diploid fibroblast strains