

chance, we must suspect that the key to their evolution is a dissipative mechanism not present in the solar system today. It might have involved a viscous medium in the early solar nebula or the influence of a passing star. A study of evolutionary models with such alternate mechanisms might provide useful clues about conditions in the young solar system.

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#### References and Notes

1. See, for example, P. Goldreich, *Mon. Notic. Roy. Astron. Soc.* **130**, 159 (1965).
2. A complete discussion is given by R. J. Greenberg, thesis, Massachusetts Institute of Technology (September 1972).
3. D. Brouwer and G. Clemence, *Methods of Celestial Mechanics* (Academic Press, New York, 1951), p. 492.
4. This qualitative aspect of the stability for relatively high values of  $e_2$  was described by Goldreich (1) and by C. J. Cohen and E. C. Hubbard [*Astron. J.* **70**, 10 (1965)].
5. The time scale for the evolution of the Titan-Hyperion resonance presents certain problems; these are discussed in (2).
6. R. R. Allan, in *Symposia Matematica* (Istituto Nazionale di Alta Matematica, Città Universitaria, Rome, 1970), vol. 3, p. 75. Here the conjunction longitude librates about the average longitude of the satellites' ascending nodes on Saturn's equatorial plane, so the resonance involves the inclinations of both satellites.

30 June 1972

range was not delimited. All samples were collected with oblique plankton tows by using the National Academy of Sciences (NAS) reference net (0.5 m in diameter at the mouth; mesh size 333  $\mu$ m) equipped with a flowmeter (3). The highest concentrations observed were in the Niantic Bay area with an average of about 1 spherule per cubic meter for 72 samples taken on six dates between February and May 1972. Concentrations up to 14  $m^{-3}$  were observed in this area. At other stations sampled in February to March 1972 the average concentrations were as follows: Long Island Sound (stations 22 to 27), 0.07  $m^{-3}$ ; east of Block Island (stations 13 to 18), 0.03  $m^{-3}$ ; Great Salt Pond on Block Island and west to Long Island Sound (stations 19 to 21), 0.02  $m^{-3}$ .

Bacteria and polychlorinated biphenyls (PCB's) are present on surfaces of the plastic particles. Freshly collected spherules from Niantic Bay were transferred through four washings of sterile seawater and plated onto A-C seawater medium (4), where rod-shaped gram-negative bacteria were observed after incubation. An extraction of the surface of the spherules from Niantic Bay with hexane showed that they contained PCB's (Aroclor 1254) in a concentration of 5 parts per million. Since PCB's are not added in the manufacture of polystyrene (2), it is probable that the source was ambient seawater.

## Polystyrene Spherules in Coastal Waters

**Abstract.** Polystyrene spherules averaging 0.5 millimeter in diameter (range 0.1 to 2 millimeters) are abundant in the coastal waters of southern New England. Two types are present, a crystalline (clear) form and a white, opaque form with pigmentation resulting from a diene rubber. The spherules have bacteria on their surfaces and contain polychlorinated biphenyls, apparently absorbed from ambient seawater, in a concentration of 5 parts per million. White, opaque spherules are selectively consumed by 8 species of fish out of 14 species examined, and a chaetognath. Ingestion of the plastic may lead to intestinal blockage in smaller fish.

Polystyrene spherules are widespread in the coastal waters of southern New England. We first observed spherical plastic particles in plankton tows in January 1971 while sampling to determine the effects of a nuclear power station on the ecology of Niantic Bay (northeastern Long Island Sound). The particles, although usually present in zooplankton samples throughout the year, were not investigated in detail until February 1972. The spherules are markedly different in size, shape, distribution, and chemical composition from the plastics on the Sargasso Sea surface (1).

Infrared spectrophotometry of the particles indicated that they were polystyrene plastic. Two types are present in seawater, in approximately equal proportions. One is a clear or crystalline polystyrene, and the other is a white, opaque form with pigmentation due to the presence of a diene rubber compound in the plastic, as indicated by infrared spectrophotometry and confirmed by a representative of the plastics industry (2). Both forms are virtually perfect spheres and average about 0.5 mm in diameter, ranging from 0.1 to 2 mm. They contain various sizes and numbers of gaseous voids. Thus, they are found at the sea surface, in the water column, and presumably in the

sediments since polystyrene is of a greater density than seawater.

The spherules are present in coastal waters from western Long Island Sound to Vineyard Sound (Table 1), and may be more widespread since their total

Table 1. Sample location, date, volume filtered, and concentration of plastic spherules in coastal water. Stations 1 to 12 were in an area of about 10 km<sup>2</sup>; the averages and ranges of the spherule concentrations at these 12 stations are presented.

Station	Location	Date (1972)	Volume filtered (m <sup>3</sup> )	Spherules per cubic meter	
				Avg.	Range
		<i>Niantic Bay</i>			
1-12	41°18'N, 72°10'W	1 February	475	0.75	0.39-1.94
1-12	41°18'N, 72°10'W	17 February	140	2.58	0.62-14.1
1-12	41°18'N, 72°10'W	16 March	513	0.79	0.00-2.52
1-12	41°18'N, 72°10'W	7 April	603	0.13	0.00-0.51
1-12	41°18'N, 72°10'W	25 May	387	0.61	0.03-2.44
		<i>Buzzards Bay</i>			
13	41°34'N, 70°43'W	9 March	59	0.03	
14	41°34'N, 70°43'W	9 March	50	0.02	
		<i>Vineyard Sound</i>			
15	41°30'N, 70°39'W	10 March	48	0.02	
16	41°30'N, 70°39'W	10 March	31	0.00	
		<i>Rhode Island Sound</i>			
17	41°20'N, 71°03'W	24 March	108	0.10	
18	41°13.5'N, 71°18'W	25 March	76	0.00	
		<i>Great Salt Pond</i>			
19	41°09'N, 71°33'W	25 March	94	0.04	
		<i>Block Island Sound</i>			
20	41°12'N, 71°44'W	25 March	191	0.01	
21	41°12'N, 72°00'W	25 March	104	0.01	
		<i>Long Island Sound</i>			
22	41°10'N, 72°20'W	25 March	280	0.10	
23	41°09'N, 72°36'W	25 March	122	0.05	
24	41°08'N, 72°52'W	25 March	48	0.10	
25	41°16'N, 72°01'W	23 March	109	0.05	
26	41°17'N, 72°03'W	23 March	125	0.07	
27	41°17'N, 71°59'W	23 March	151	0.04	

Spherules are consumed by fish in the Niantic Bay area; only the white, opaque form has been found ingested, which indicates selective feeding. Fourteen species of fish, totaling 270 individuals, were collected by oblique plankton tows with the NAS net in the bay or at the cooling water intake of the Millstone Point nuclear power station, also on Niantic Bay. Of these, eight species contained spherules in their gut contents. The species, common name, and occurrence of plastic, of fish with spherules for which at least five individuals were examined are: *Myoxocephalus aeneus*, grubby, 4.2 percent; *Pseudopleuronectes americanus*, winter flounder, 2.1 percent; *Roccus americanus*, white perch, 33 percent; and *Menidia menidia*, silversides, 33 percent (5). In addition, one chaetognath, *Sagitta elegans*, was collected on 12 July 1972; it was 20 mm long and had a spherule 0.6 mm in diameter in its intestine. The effects on fish of consuming the spherules themselves or the accompanying PCB's are unknown; however, it is likely that they can cause intestinal blockage in some of the smaller fish. Winter flounder and grubby larvae, 5 mm in length, contained spherules 0.5 mm in diameter. The percentage consumption of plastics by some species of fish may be greater than observed here if ingestion of the spherules directly or indirectly causes mortality through blockage, thereby preventing sampling of these fish.

The spherules appear identical to polystyrene plastic "suspension beads." These beads are not usually marketed commercially (6), but are molded into a pellet shape before being sold to plastic fabricators. Thus, the source of the spherules is probably a manufacturer and may be any of the many polystyrene producers in southern New England. Although the situation may be confined to this area, the bead suspension process is widely employed for the manufacture of polystyrene, and contamination of both marine and fresh waters and their sediments may occur in other areas as well.

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1. E. J. Carpenter and K. L. Smith, Jr., *Science* **175**, 1240 (1972).
2. K. Burgess, Dow Chemical Co., Midland, Michigan, personal communication.
3. *Recommended Procedures for Measuring the Productivity of Plankton Standing Stock and*

- Related Oceanic Properties* (National Academy of Sciences, Washington, D.C., 1969).
4. *Difco Manual* (Difco Laboratories, Detroit, ed. 9, 1953).
  5. The total number of fish examined, average total length in centimeters, and standard deviation of the total length for fish containing spherules are: *M. aeneus*, 47, 0.58, 0.12; *P. americanus*, 95, 0.46, 0.44; *R. americanus*, 12, 24.9, 3.98; *M. menidia*, 9, 1.61, 1.89; *Tautoglabrus adspersus*, 6, 9.16, 1.81. Two herring, *Clupea harengus*, each 4.2 cm; one pollack, *Pollachius virens*, 3 cm; and one sea robin, *Prionotis evolans*, 32.7 cm, were collected; each contained a spherule.
  6. R. Harding, Society of Plastics Industry, New

York, personal communication. Polystyrene beads also have some limited usage as absorbents for industrial water purification.

7. We thank R. Harding for his cooperation and for notifying all polystyrene manufacturers in the United States of the presence of plastic spherules in coastal waters. Gratitude is also expressed to F. Doohan and S. Young for allowing one of us (E.J.C.) to collect samples from the R.V. *Verrill*. We also appreciate the critical review of this manuscript by P. H. Wiebe, N. S. Fisher, J. H. Tuttle, V. Vreeland, and R. S. Scheltema. Woods Hole Oceanographic Institution Contribution 2920.

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## Rous Sarcoma Virus Nucleotide Sequences in Cellular DNA: Measurement by RNA-DNA Hybridization

**Abstract.** Kinetic analysis of the hybridization of 71S RNA from Prague strain of Rous sarcoma virus with an excess of DNA from virus induced sarcomas indicated the presence of the majority of the viral genome sequences in cellular DNA with a very low average frequency per cell. About one-third of the viral sequences were at least partially complementary to DNA sequences with a higher average frequency on the order of 50 to 100 per cell. Normal chick embryo DNA was distinctly different, but contained sequences at least partially homologous to some fraction of the viral RNA.

The proposal by Temin (1) that RNA tumor viruses replicate through a DNA intermediate has been supported by an increasing body of indirect evidence. The discovery of RNA-dependent DNA polymerase activity within tumor virus particles strengthened this "provirus" hypothesis. The direct detection of viral nucleotide sequences by annealing viral RNA with cellular DNA seems to be a reasonable approach for testing this idea. However, RNA-DNA hybridization studies have yielded conflicting results. Although most investigators have found viral complementary sequences in cellular DNA, some have found evidence for more complementary DNA in virus-transformed cells (2, 3), while others report no significant differences between normal and virus-infected tissue (4-8). Technical problems may underlie the varying conclusions since, in all cases, only a very small fraction of the viral RNA was detected in the RNA-DNA hybrids. Furthermore, the conditions under which the hybridization reactions were carried out would not be expected to reveal small numbers of complete viral genomes per cell genome, particularly if multiple copies of DNA in the cell complementary to a small fraction of the viral RNA were present. The rate of hybridization reported in these previous studies suggests the presence of such repetitive virus-related DNA (3). A method has been described for detecting full complements of RNA in cellular DNA and for obtaining rea-

sonable estimates of frequency even when very low (5-7). The method is based upon hybridization in the presence of an excess of DNA large enough for the kinetics of RNA-DNA interaction to be determined by the relative number of complementary sequences in the DNA. By this approach, it is possible to detect most, if not all, of the base sequences of Rous sarcoma virus in the DNA of virus-induced sarcomas, and to demonstrate major differences with DNA sequences complementary to viral RNA in normal embryos.

Prague strain of Rous sarcoma virus subgroup C (Pr RSV-C) was selected as the source of viral RNA because it is a helper-independent avian sarcoma virus that replicates efficiently in cell culture and produces rapidly growing sarcomas in newborn chicks (8). Chick embryo fibroblast cultures transformed by Pr RSV were incubated in medium 199 (prepared without unlabeled nucleosides and made 5 percent in fetal calf serum) with 100  $\mu$ C of [ $^3$ H]uridine, [ $^3$ H]cytidine, and [ $^3$ H]adenosine per milliliter (22, 26, and 9 c/mmole, respectively). Culture fluid was harvested after 24 hours, and labeled virus particles were purified from culture fluids by banding in sucrose gradients (20 to 60 percent). Viral RNA was extracted by the sodium dodecyl sulfate-phenol technique (9). The 71S viral genome, separated from low-molecular-weight nucleic acids by sedimentation in sucrose gradients (5 to 20 percent) in 0.01M tris buffer containing 0.001M