the sediment surface. On the basis of the n-C_{17}/pristane ratio, the oil below 2.5 cm is as fresh after 24 months as the oil at the surface was after 10 months (*3*). In the marshes penetration has been greater, extending to at least 60 cm, and bacterial degradation and dissolution are evident at that depth; this is probably the result of the greater permeability and aeration of the marsh sediments.

Some properties of the fuel oil have changed little, in spite of its gradual degradation. Initially the boiling range of the spilled oil extended from 170° to 370°C, and the normal alkanes ranged from decane to docosane. The boiling range of the fuel oil in the sediments is well preserved after 2 years; even at stations with low pollution level, C_{13} to C_{14} alkanes are still present. At station 31 and in the marshes dodecane can still be detected.

Adjacent members of the same or of closely related homologous series are affected by weathering to nearly the same degree; therefore, their concentration ratios change only slowly. Thus, the ratio of pristane to phytane at station 31 has remained 1.17 ± 0.09 during the 2-year period (Fig. 3A). This value differs characteristically from that encountered in unpolluted recent sediments (*6*). The pristane/phytane ratio and similar isomer ratios are distinguishing features that vary from one crude oil to another. That this ratio remains constant suggests that the identification of fossil fuel pollutants in the environment and their distinction from the natural hydrocarbon background may be possible over extended time periods.

Our continuing investigation will provide a framework for the consideration of the effect and fate of oil pollution in the coastal environment. Hydrocarbons in the boiling range of the oil spilled at West Falmouth are abundant in crude oil. Smith, in summarizing analyses of 6496 crude oils, showed that the "gas oil" content of the vast majority ranges from 20 to 35 percent, except for Tertiary oil, where higher values occur (*7*). Smith has defined gas oil as boiling between 200°C at 760 mm and 225°C at 40 mm (or 335°C at 760 mm). This range is comprised within that of this fuel oil (170° to 370°C, 760 mm). Thus, the "fuel oil" component, in the sense of the oil spilled at West Falmouth, constitutes one-quarter to one-third of the weight of a large majority of all crude oils. Therefore, we anticipate that the environmental effect and the persistence of a crude oil spill resembles that of a fuel oil spill qualitatively, and to a degree quantitatively. The presence in whole crude oils or residual oils of less rapidly degraded and less soluble hydrocarbons of higher molecular weight should lead to greater environmental persistence.

The preservation of hydrocarbons in marine sediments for geologically long time spans is one of the accepted key facts in the current thought on petroleum formation. Similarly, the uptake of dietary hydrocarbons in the food chain and their preservation in the lipids of organisms seem to be well documented. Our present findings do not contradict these background data.

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Development of Cellular Dependence on Infective Organisms: Micrurgical Studies in Amoebas

Abstract. *Nuclei and cytoplasm were transferred between a normal strain and a variant strain of* Amoeba discoides *heavily infected with bacteria. After 5 years of infection, the infective bacteria that were initially harmful to the host cells became harmless, and the nucleus of the host cell became dependent on the infective organisms for its normal functions.*

A strain of *Amoeba discoides* became spontaneously infected with a large number of rod-shaped bacteria (60,000 to 150,000 per cell) in 1966 (*1*) and has been carried in culture since then. After 5 years of infection, the infective organisms that affected the host cells adversely became harmless. Furthermore, the results of micrurgical experiments show that the nucleus of the host cell has become dependent on the infective organisms to the extent that it cannot function normally in the absence of the infective organisms. This is apparently the first recorded instance of intracellular parasitic organisms being converted to true symbionts within an observable period of time.

When the infection was first found (*1*), the bacteria were harmful to amoebas in that the infected amoebas (i) grew slower, with a mean generation time of 5 days at 18°C as compared to 2 days for the normal amoebas, (ii) had an extremely low percentage of clone formation when isolated singly, (iii) were more sensitive to starvation, (iv) were smaller in size, and (v) were much more fragile. Also, the bacteria could infect normal amoebas under experimental conditions, and killed most of the newly infected cells. On the basis of these observations, we regarded the bacteria as parasitic.

Attempts to infect amoebas with several different kinds of bacilli normally in the amoeba culture solutions failed, since amoebas usually digest foreign objects introduced into their cytoplasm (*2*). The infective bacteria would not grow outside the amoebas under any conditions tried, a result indicating that they were obligatory parasites. Within the past 5 years, the adverse effect of the infective bacteria has disappeared and at present the infected strain of amoebas grows normally while carrying the same number of bacteria within the cytoplasm as before. Examinations by light and electron microscopy indicate that the bacteria living in the amoebas are the same ones as those found originally.

In order to see if the infection during the last 5 years has caused any changes in cell characters of the infected strain, I examined the nucleocytoplasmic compatibility between the normal and infected strains. The in-

fected strain, called xD below, was derived from the D strain of *A. discoides*, which originated in Glasgow, Scotland (*3*). The amoebas were cultured in modified Chalkley's medium (*3*) with *Tetrahymena* as food organisms. The nuclei and cytoplasm were transferred on agar gel by the usual methods (*4*), and operated amoebas were kept singly in Syracuse watch glasses to observe their growth. Any amoeba that divided four times or more at regular intervals was regarded as forming a viable clone, in accord with previous experience.

The results of micrurgical experiments (Table 1) show that the combination of xD nuclei and D cytoplasm (xD_nD_c) is least viable. Thus only 26 percent of these cells divided and 7 percent formed viable clones, as compared to 98 and 91 percent, respectively, for cells with the reciprocal combination (D_nxD_c). It was apparent that the nucleus of the xD strain needed xD cytoplasm for normal functioning, while the D nucleus was perfectly viable in xD cytoplasm. Thus the xD cytoplasm containing the bacteria was not harmful to the D nucleus, and this was also shown by experimental infection of D amoebas by injection of xD cytoplasm ($xD_c \rightarrow D$). The injected D amoebas quickly became infected with large numbers of bacteria, but growth and clonability of infected amoebas were not altered. In earlier studies, such infection was fatal to the normal amoebas (*1*).

In order to assure that the low viability of xD_nD_c cells was due to the absence of the infective bacteria, a small volume (about 1/20 of cell volume) of xD cytoplasm containing the bacteria was transferred at the time of nuclear transplantation ($xD_c \rightarrow xD_nD_c$). A large proportion (91 percent) of the reconstituted cells grew and formed viable clones; this indicated that the presence of the bacteria was indeed necessary.

The dependence of the nucleus of the infected amoeba on the bacteria was further checked by introducing a small volume of xD cytoplasm into xD_nD_c amoebas after they had reached the stage where they showed symptoms of cell death (*5*), which occurred about 10 days after nuclear transplantation. During this period, half of them had divided once. Within a day or two after dying cells (xD_nD_c*) were injected with xD cytoplasm ($xD_c \rightarrow xD_nD_c$*), the xD_nD_c* cells recovered, entered mitosis after a delay of 2 to 3 days, and formed viable clones. Without exception, the viable clones resulting from the cells injected with xD_c contained the infective bacteria. Thus, while the D nucleus was viable in xD cytoplasm, the xD nucleus was not compatible with D cytoplasm.

Table 1. Summary of nuclear transplantation and cytoplasmic injection experiments between D and xD amoebas. A small proportion of amoebas that divided only once soon after an operation was not included, since such division does not seem to represent normal cell division. Nuclei that are ready to divide will do so irrespective of the state of cytoplasm. The asterisk denotes amoebas that would die if nothing further is done to them.

Experiment	Cell type	Cells studied (No.)	Cells dividing twice or more (No.)	Viable clones (No.)	Viability (%)	Presence of bacteria
		Nuclear transplantation				
Control	D_nD_c	52	48	48	92.3	No
	xD_nxD_c	38	32	31	81.6	Yes
Interstrain	D_nxD_c	43	42	39	90.7	Yes
	xD_nD_c	101	26	7	6.9	No
		Cytoplasmic injection				
Control	$D_c \rightarrow D$	48	46	46	95.8	No
Interstrain	$xD_c \rightarrow D$	46	44	44	95.6	Yes
	$xD_c \rightarrow xD_nD_c$	44	40	40	90.9	Yes
	$xD_c \rightarrow D_nD_c$*	42	34	30	71.4	Yes

The fact that a small proportion (7 percent) of xD nuclei placed in D cytoplasm is able to form viable clones in the absence of the infective bacteria indicates that the dependence of nuclei on the bacteria is not absolute, but complete dependence may develop after further culture. It is not known which particular aspect of the nuclear function of xD amoebas has become dependent on the presence of the infective organisms, or whether the xD cytoplasm retains its resuscitating ability after the bacteria have been removed, for example, by centrifugation.

The presence of intracellular infective organisms is known for many different types of protozoa, including other free-living amoebas (*6*). The DNA- and RNA-containing bodies found in several strains of mononucleate amoebas (*7*) are also considered to be endosymbionts. The possible role of these nucleic acid–containing bodies in cell heredity was studied (*8*), but the results were not definitive; the endosymbionts appeared to enhance the resistance of host amoebas to one drug, but were not related to the cellular response to other drugs.

Thus, in most of the known instances of intracellular infection, the precise host-symbiont relationship remains obscure. Even in the best-studied case of infection in *Paramecium*, it is not known whether the infective organisms are beneficial or harmful to the host cell (*9*). Intracellular symbionts are bound to have a profound effect on cellular divergence and variation, as has been suggested (*6, 10*). The results of my work add further evidence to support such a view, but more study is needed before we can generalize the hypothesis. My work also demonstrates that the host-symbiont relationship can be studied at the cellular level.

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