ON HABITAT PREDICTABILITY AND ALGAL PREADAPTATION TO NOVEL CHEMICALS

Fisher et al. (1973) reported that Sargasso Sea clones of three species of algae displayed greater growth inhibition by the organochlorine PCB than estuarine clones of the same species. Greater resistance of the estuarine clones was attributed to the unpredictability of their habitat compared with the Sargasso Sea.

Fisher (1977) also measured the relative toxicities to the clones of three chemicals which have never existed in the oceans. The estuarine clones were more resistant than their Sargasso Sea counterparts. He hypothesized that algae of unpredictable environments are generally preadapted to novel chemicals because adaptation to such habitats requires a "thick skin," i.e., a highly protective outer membrane. He demonstrated that growth of the green euryhaline flagellate, *Dunaliella tertiolecta*, reported to be resistant to 1,000 μg/L of PCB (Mosser et al. 1972), was inhibited at low salinity (10‰) by as little as 10 μg/L of PCB.

The mechanism proposed for this induced susceptibility was osmotic swelling, allowing PCB molecules to come into closer contact with sensitive sites of action because of the thinness of the distended cell membrane. In short, the hypothesis implies that physical thinning of the cell membrane makes the difference between sensitive and resistant cells. Inasmuch as Fisher et al. (1974), citing Eppley et al. (1969), reported that *D. tertiolecta* is an estuarine alga, Fisher's hypothesis states that unpredictable habitats like estuaries select for algae with thick skins. A close examination of this hypothesis in relation to data from this laboratory and elsewhere is warranted.

I exposed *D. tertiolecta*, WHOI clone "Dun" typical of tide pools and estuaries (Luard 1973; Menzel et al. 1970), to 100 times Fisher's PCB dose at varying salinity (fig. 1). Growth inhibition by PCB did not occur until salinity was reduced to 5‰ or below, salinities which in themselves inhibited *D. tertiolecta*. Even under these extreme conditions, PCB inhibited *D. tertiolecta* growth less than organochlorine-sensitive species exposed to much lower doses. For example, the diatom *Thalassiosira pseudonana* displayed growth inhibition by 15 μg/L of PCB, whereas *D. tertiolecta* was resistant to 2,000 μg/L. In contrast, Fisher's growth curves depicted differentials in PCB susceptibility of only a few percent between the estuarine and Sargasso Sea clones of the three species he compared, including *T. pseudonana*. Among the four species of algae, by far the largest and ecologically most important differences in susceptibility occurred between two species within the estuarine habitat.

Therefore, if adaptation to environmental agents is an important determinant of preadaptation to novel chemicals, the predominant selective agents must act within the estuary rather than between habitats. That algae whose susceptibilities to organochlorines are as disparate as *D. tertiolecta* and *T. pseudonana* can both be adapted to estuaries signifies that preadaptation to novel chemicals is not a necessary outcome of adaptation to an unpredictable habitat. Preadaptation to novel chemicals seems only loosely tied to those attributes which constitute a thick skin for algae living in unpredictable environments.

Even if osmotic swelling and distension of the *D. tertiolecta* cell membrane produces thinning of the membrane, other effects of low salinity, particularly

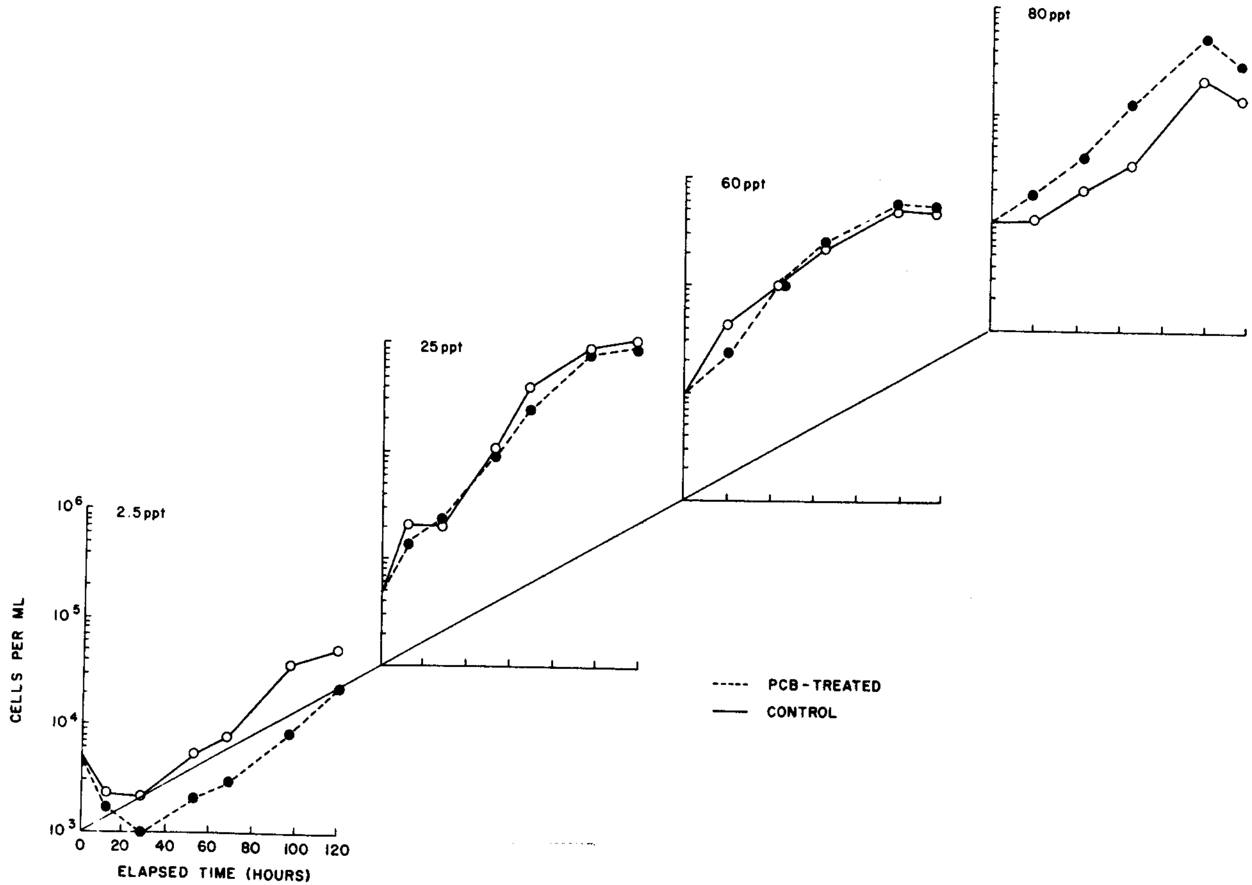

FIG. 1.—Effect of salinity on *Dunaliella tertiolecta* resistance to 1,000 μg/L of PCB. Points are means of two or three replicates. Depicted interaction of PCB resistance with salinity was verified statistically ($\alpha < 0.05$) by two-way analysis of variance of a larger data base than represented.

processes acting at the biochemical level, could have induced growth inhibition by PCB. In addition to osmotic effects of low salinity, nonosmotic effects could occur. These would involve altered concentrations of specific ions, most probably Na^+, K^+, Mg^{2+}, or Ca^{2+}, whose roles in animal membrane (especially nerve cell) physiology and organochlorine toxicity have been reported.

Suppose that *D. tertiolecta* resists the growth-inhibiting effects of PCB by carrying on a protective biochemical process at the cell surface characteristic of resistant species but not of susceptible species. Such a process is likely to possess a salinity optimum, and most certainly optimum values for a variety of relevant environmental parameters. Deviations in either direction from these optima would inhibit the protective process and enhance PCB-susceptibility at either end of the tolerance range of the alga. Such findings of optima for PCB resistance with respect to several environmental parameters would be highly convenient in that they would point to a unique surface process whose nature could be inferred from the response of the alga to PCB under varying environmental conditions. Organochlorine-sensitive species would be distinct in lacking this dependency of PCB toxicity on the environmental parameters relevant only to the resistant species.

To test this hypothesis, it is necessary to subject sensitive and resistant species of algae to PCB under varying culture conditions. Fisher (1977) reported absence of PCB inhibition of *D. tertiolecta* growth at 47‰ salinity. That salinity was not high relative to the tolerance range of the alga, and growth was not inhibited by the hypersaline medium in the non-PCB-treated controls as it had been in the low-salinity controls. In this laboratory, the toxicity of 1,000 μg/L of PCB was measured at the growth-inhibitory high salinities of 60‰ and 80‰. The results (fig. 1) show lack of PCB inhibition at 60‰ and stimulation at 80‰. If there is a protective surface process in *D. tertiolecta* it is uninhibited over a wider salinity range than growth of the species, displaying salinity-inhibition only at the extreme low tail of *D. tertiolecta*'s tolerance range.

To examine this further, *T. pseudonana* grown as previously described (Powers et al. 1975) was exposed to PCB at varying salinity to see whether salinity variation displayed the same relationship to PCB-induced inhibition of growth in this species as it had in *D. tertiolecta*. The results, to be presented in detail elsewhere, were opposite for *T. pseudonana*: Growth inhibition by PCB was reduced at low salinity, but increased at high salinity. These two differentially susceptible algae also behaved as opposites with respect to PCB toxicity at varying pH.

Two other observations from this investigation on PCB inhibition of *T. pseudonana* growth are worthy of mention here. First, when *T. pseudonana* was grown at low salinity (2.5‰) for several months, then transferred to a medium of normal salinity (25‰) and exposed to PCB, growth inhibition was less pronounced, just as if the cells were exposed to the PCB at low salinity. This indicates that the resistance acquired to PCB by adaptation to low salinity was a feature of the cells, and not of the dynamics of PCB in a medium of low salinity. It also indicates that within a species an altered regimen of salinity, rather than of habitat predictability, can account for differences in susceptibility to PCB.

Second, the reduced PCB toxicity to *T. pseudonana* at low salinity was produced by reduced concentrations of specific ions like Na^+, K^+, Mg^{2+}, and Cl^-, rather than by reduced osmolarity. To show this *T. pseudonana* was treated with PCB in medium of the same osmolarity as the normal culture medium but with the nonelectrolyte glycerol, in which PCB is insoluble (Hutzinger et al. 1974), substituted for electrolytes. Greater reduction of PCB toxicity occurred in this normal-osmolarity, low-ion medium than in the low-salinity medium. The fact that concentrations of specific ions rather than osmolarity governed the magnitude of PCB effect on *T. pseudonana* growth casts some doubt on Fisher's (1977) hypothesis that at low salinity PCB inhibited *D. tertiolecta* growth because lowered osmolarity caused membrane thinning.

Similarly, the finding that the estuarine blue-green alga, *Anacystis nidulans*, displayed reduced DDT tolerance at high salinity (NaCl concentration) was attributed to the presence of Na^+ rather than to high osmolarity (Batterton et al. 1972).

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