taken into account when nitrite-balance studies in smoked meats are carried out.

M. E. KNOWLES
J. GILBERT
D. J. MCWEENY

Food Science Division, Ministry of Agriculture, Fisheries and Food, Colney Lane, Norwich, NOR 70F, UK

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1 Lijinsky, W., and Epstein, S. S., *Nature*, 225, 21–23 (1970).
2 Knowles, M. E., McWeeny, D. J., Couchman, L., and Thorogood, M., *Nature*, 247, 288 (1974).
3 Mirna, A., and Hofmann, K., *Fleischwirtschaft*, 49, 1361–1366 (1969).
4 Archer, M. C., Clarke, S. D., Thilby, J. E., and Tannenbaum, S. R., *Science*, 174, 1341–1343 (1971).
5 Knowles, M. E., Gilbert, J., and McWeeny, D. J., *The Potential Formation of C-Nitroso Compounds in Smoked, Cured Meats*, (3rd Meeting on the Analysis and Formation of N-Nitroso Compounds, Lyon, 1973).
6 Challis, B. C., *Nature*, 244, 466 (1973).
7 Issenberg, P., Kornreich, M. R., and Lustre, A. O., *J. Agric. Fd Chem.*, 36, 107–109 (1971).

Inhibition of inter-microbial predation by chlorinated hydrocarbons

TIDAL or estuarine regions have traditionally served as sinks for municipal sewage. Some microbial predators in the sea feed on the enteric bacteria contained in such sewage[1]. Recently, the concentration of chlorinated hydrocarbons in the sea, especially in estuarine regions, has increased although it remains considerably below the concentration which directly affects the viability of predators or intestional pathogens. But, estuarine chlorinated hydrocarbon concentrations are within a factor of 20 of those which we have observed to inhibit the chemotactic response of marine bacteria[2]. Because bacterial predators are chemotactic specifically to exudates of their prey[3], inhibition of chemotaxis by chlorinated hydrocarbons may directly affect estuarine self-purification rates. We report here the impact of two chlorinated hydrocarbons, at concentrations observed to inhibit chemotaxis[4], on enteric bacteria predation.

We examined the rate of kill of *Escherichia coli* by marine predators in the presence of 2,4-dichlorophenoxyacetate (2,4-D) and *o,o*-dichlorobiphenyl. The predators were isolated from seawater by repeated subculture in artificial seawater[5] using *E. coli* as the sole carbon source. The resulting predators displayed positive chemotaxis to *E. coli*. The level of kill of *E. coli* in 48 h by the predators in 50 ml portions of artificial seawater was determined by serial dilution and plating in triplicate on EMB agar (Difco) containing lactose. The die-off experiments were run in duplicate with two flasks placed on a rotary shaker (approximately 60 r.p.m.) and two on the laboratory bench at room temperature. Initial predator populations were estimated by five tube most-probable-number counts using the clearing of a tube containing 10 ml of a 10^{6} ml^{-1} solution of *E. coli* in artificial seawater as a positive result.

We made several attempts to isolate a single predator species from the mixed predator culture. Methods used included: colony isolation on a broad spectrum of enriched artificial seawater-agar or gelatin solid media; isolation by serial dilution; or isolation by filtration through a series of Millipore filters. In every case a mixed culture resulted when the isolates were reinoculated into artificial seawater with *E. coli* as the sole carbon source. The predator culture consisted of at least two distinct *Spirillum* species as well as one motile pseudomonad. The dominant predator species (90% by visual count in liquid culture) was a *Spirillum* (approximately 1 μm diameter; two spiral turns). The presence of this predator mixture of visable organisms was necessary for *E. coli* lysis. The predator culture filtrate, when passed through a 0.45 μm Millipore filter, did not contain these organisms and also did not produce lysis of *E. coli*. There was no evidence of Bdellovibrios or bacteriophages in the filtrate.

The mechanism of *E. coli* lysis by the dominant *Spirillum* does not seem to be direct contact. The lysis mechanism seems to be based on the maintenance of a critical concentration of lytic enzymes although we were unable to obtain culture extracts which would lyse *E. coli* cultures in the absence of living predator cells. The predacious *Spirilla* utilised materials available in cell walls in that they could be subcultured in artificial seawater containing washed *E. coli* cell walls as the sole carbon source.

Inhibition of kill of *E. coli* by marine predators in the presence of chlorinated hydrocarbons is shown in Table 1. A decrease in the rate of kill was generally observed in the shaken flasks containing the chlorinated hydrocarbons and the percentage kill was no longer directly related to the initial population of the predators. In the standing flasks, the percentage kill generally decreased in the presence of the lower concentration of chlorinated hydrocarbons. In the presence of higher concentrations of the chlorinated hydrocarbons, the percentage kill of *E. coli* in the standing flasks was generally increased in those flasks in which the initial predator population was less than 10^{7} ml^{-1}. The viability of the predators and of *E. coli* was not affected by either the low or the high concentrations of the chlorinated hydrocarbons. This was determined by predator cell counts in control flasks lacking *E. coli* and by *E. coli* counts in control flasks lacking the predators. The activity of the predators also did not seem to be affected by the chlorinated hydrocarbons as shown by a glucostat determination[6] in which the rate of consumption of glucose did not decrease even in the presence of the higher concentration of hydrocarbons.

Table 1 Percentage kill of *E. coli* in 48 h by motile marine predators in the presence of chlorinated hydrocarbons

Initial predator population no. per ml.	No chlorinated hydrocarbons	2, 4-D 10^{-4} M	2, 4-D 7×10^{-3} M	*o, o*-Dichlorobiphenyl 10^{-5} M	*o, o*-Dichlorobiphenyl 10^{-3} M
	% Kill of *E. coli*				
Standing					
10	0	7	31	0	31
10^{3}	6	3	32	17	35
10^{5}	30	10	29	13	39
10^{7}	72	26	41	41	47
Fully mixed					
10	20	23	25	18	27
10^{3}	54	29	41	31	44
10^{5}	61	32	43	30	43
10^{7}	71	77	44	55	51

The initial *E. coli* population was 10^{7} ml^{-1}. The data are corrected for kill observed in the absence of the predators.

The lower concentrations of chlorinated hydrocarbons, which significantly decreased the percentage kill of *E. coli* by marine predators in shaken culture, are approximately a factor of 20 greater than those observed in natural ecosystems. Waste effluents, which contain intestinal pathogens of which *E. coli* is an indicator, may contain chlorinated hydrocarbons at concentrations ten to a hundred times greater than seawater residual levels. Such chlorinated hydrocarbons, acting either singly or in combination, may decrease the level of predator–prey interactions responsible for self-purification in natural ecosystems in the vicinity of outfalls.

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The lower chlorinated hydrocarbon concentrations, which decreased the percentage kill of *E. coli*, are similar to those we previously reported to inhibit chemotaxis in motile marine bacteria[1]. The higher hydrocarbon concentrations, which increased kill rates in the standing culture flasks for lower predator populations, are those which we observed to increase chemotaxis. This correlation suggests that the observed changes in *E. coli* kill rates are the result of inhibition or excitation of the chemotactic response in the marine predators of *E. coli*.

F. Walsh
R. Mitchell

Laboratory of Applied Microbiology,
Division of Engineering and Applied Physics,
Harvard University,
Cambridge, Massachusetts 02138

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1 Mitchell, R., *Nature*, 230, 257 (1971).
2 Walsh, F., and Mitchell, R., in *The microbial degradation of oil pollutants* (edit. by Meyers, S. P.) (Center for Wetland Resources, LSU-SG-73-01, 1973).
3 Chet, I., Fogel, S., and Mitchell, R., *J. Bact.*, 106, 863 (1971).
4 Walsh, F., and Mitchell, R., *Bact. Proc.*, G244 (1972).
5 Lyman, J., and Fleming, R. H., *J. mar Res.*, 3, 134 (1940).
6 Washko, M. E., and Rice, E. W., *Clin. Chem.*, 7, 542 (1961).

Formation of methylmercury in a terrestrial environment

Wood *et al.*[1] demonstrated that the mercury from inorganic chemicals can be methylated by aquatic organisms and also, enzymatically, by extracts of methanogenic bacteria. Since the organic forms of mercury are reported to be more biologically available, these methylation processes in aquatic or sedimentary environments, together with the tendency of methylated mercury to accumulate in biota, are a major reason for bioconcentration of mercury in fish and other organisms found in lakes, streams and bodies of seawater that are contaminated with mercury. As methanogenic bacteria also occur in terrestrial environments, we have investigated possible organomercury compound formation in soils contaminated with an inorganic mercury compound.

In a preliminary laboratory study, several 100 g soil samples were spiked with ^{203}Hg as mercuric nitrate, and incubated. Extraction of these samples with hydrochloric acid, followed by benzene extraction of the aqueous phase resulted in increased activity in the benzene fraction compared with reagent blanks. This indicated some conversion of the mercuric nitrate to a chemical form more soluble in benzene, presumably some organic form.

These results prompted us to spike small agricultural plots, at the Environmental Protection Agency's experimental farm at the Nevada Test Site, with radioactively labelled mercuric nitrate. Approximately 1 μCi ^{203}Hg m^{-2} (specific activity 2-20m Ci g^{-1} according to the supplier's specification) was applied in the form of an aqueous solution to the bare sandy loam soil (soil *p*H about 8.5). Plots were watered daily, and starting at the time of application, soil samples were collected periodically and extracted with 2.2 N HCl; organic mercury compounds contained in the filtered aqueous solutions were extracted into benzene. The benzene extracts were concentrated by column distillation; the concentrate was washed with 2.2 N HCl, and the mercury compounds extracted into 1% cysteine acetate solution as described by G. Westöö[2]. The mercury compounds in the cysteine acetate solution were then extracted into a small volume of a benzene solution of dithizone (0.4% w/v). Excess dithizone was removed by treatment with H_2SO_4 and NH_4OH (ref. 3); no attempt was made to achieve a quantitative recovery.

Thin-layer chromatography (TLC) of a portion of the dithizonate solution was performed on Gelman silica gel instant TLC sheets in a Gelman chromatography chamber using a carbon tetrachloride:benzene mixture (75:25 v/v) as the mobile phase. Isolated spots consisting of a mixture of stable mercuric dithizonate, phenylmercuric dithizonate and methylmercuric dithizonate were applied on each side and in the centre of the same TLC sheet and developed together with the radioactive dithizonate extract, to serve as indicators. In control experiments carried out under identical conditions the R_f values for mercuric dithizonate, phenylmercuric

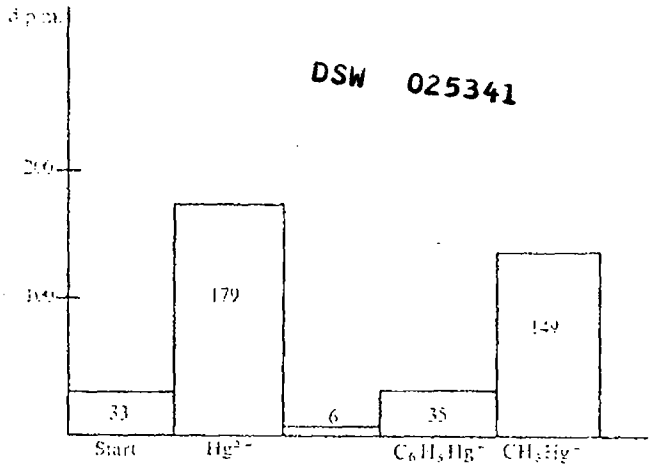


Fig. 1 Distribution of ^{203}Hg on thin-layer chromatography sheet.

dithizonate and methylmercuric dithizonate were determined as 0.19, 0.52 and 0.63, respectively.

The developed, air-dried TLC sheets were cut immediately into horizontal segments using the coloured indicator spots of the above-mentioned stable mercury compounds as reference points, and the individual segments were analysed for radioactivity content by gamma-counting. Figure 1 shows that more than one-third of the total activity of the dithizonates was concentrated in the range of the methylmercuric dithizonate.

It is not known whether the activity found in the range of the phenylmercuric dithizonate was due to phenylmercuric dithizonate originating from mercuration of benzene during extraction, tailing of the methylmercuric dithizonate, or to some as yet unidentified mercury compound with an R_f similar to that of phenylmercuric dithizonate. A control experiment using a heat-sterilised soil sample which was spiked with $^{203}Hg(NO_3)_2$ solution and analysed as described above showed no activity in the methylmercuric dithizonate range but some activity (about 2%) in the phenylmercuric dithizonate range of the TLC sheet. A portion (2-10%) of the total ^{203}Hg contained in the soil samples was not soluble in 2.2 N HCl after repeated extractions. It could, however, be extracted readily into cold 2.2 N HNO_3, indicating some conversion to elemental mercury. This conversion is not surprising as certain bacteria can convert virtually all mercury compounds into elemental mercury[4-6].

These results show that mercuric salts can, under agricultural conditions, be transformed into methylmercury. We could not differentiate between methyl and dimethylmercury because during extraction, dimethylmercury would have been converted to the monomethyl compound, according to $(CH_3)_2Hg + HCl \rightarrow CH_4 + CH_3HgCl$. The conversion in a terrestrial environment of inorganic mercury compounds to methylmercury implies that recycling of mercury from soil to air and back to soil or water could occur faster than presently assumed on the basis of the volatility of metallic mercury and inorganic mercury compounds alone. Due to the formation of methylmercury compounds and their high volatility,

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