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Aldrin: Removal from Lake Water by Flocculent Bacteria

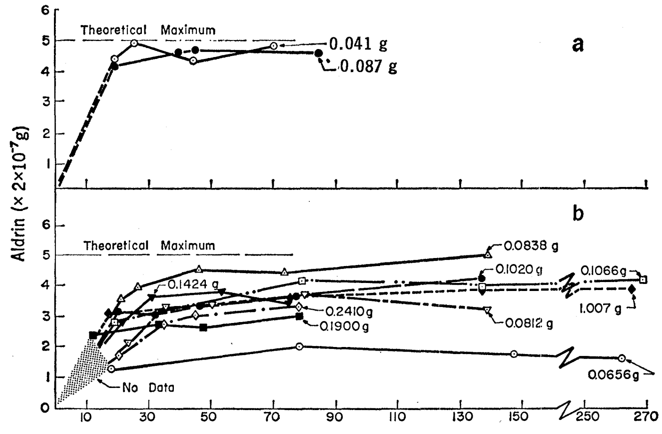
Abstract. *Floc-forming bacteria isolated from Lake Erie adsorb and concentrate aldrin from colloidal dispersion so that the settling of the bacterial flocs removes aldrin from the water phase. Contemporary sediments forming in Lake Erie contain aldrin and could adsorb more. The sediments consist of a conglomerate floc of bacteria, diatoms, and inorganic and detrital particles. Flocculent bacteria also adsorb microparticulates, and this adsorption capacity represents a mechanism for sediment formation and for the removal of suspended particles including aldrin from the water column.*

Many chlorinated hydrocarbon insecticides have been isolated from surface waters, usually in concentrations of less than 1 μg/liter or 1 part per billion (ppb). The deleterious effects of pesticide in water have been established (*1*). Our interest is in the fate of these chemicals in a water column and particularly in their adsorption to silt- and floc-forming bacteria which form contemporary sediment in lakes. Bacterial floc is an aggregation of cells which results in a macroscopic bacterial clump that settles from the liquid, thus leaving that medium less turbid. This type of growth appears to result from physical, chemical, and biological interactions when extracellular fibrillar polymers are synthesized by organisms (see *2*).

Our study of aerobic bacteria isolated from Lake Erie revealed that of 33 isolates tested in six different growth media 19 formed flocs in at least one medium, whereas ten formed flocs in two or more of the media. We report here a study of the ability of two of the floc-forming isolates to concentrate and accumulate the pesticide aldrin (*3*) from solution. One bacterium was an orange-red pigmented Gram-negative rod, tentatively identified as either a *Flavobacterium* or *Protaminobacter*. The other was a Gram-positive species of *Bacillus*.

Our experimental procedure was as follows: The test organisms were grown in a shake flask at ambient temperature (22° ± 2°C) in nutrient broth (8 g/liter, Difco), harvested by centrifugation, washed twice with distilled water, and resuspended in 25 ml of distilled water. Erlenmeyer flasks containing 50-ml suspensions of bacterial floc were then placed on a rotary shaker and 1 ml of aldrin dissolved in acetone was added to give a final aldrin concentration of 1×10^{-6} g/ml or 1 part per million (ppm). After being shaken at 120 rev/min for the desired time period, the flasks were removed from the shaker and the floc was separated from the supernatant by centrifugation. The flocs were washed twice with distilled water and the washings were added to the original supernatant. The pesticide exposure time was calculated as that period between the addition of aldrin to the solution and the separation of the second washing from the bacterial floc. The floc and supernatant fractions were extracted separately with a mixture of heptane and acetone (3 : 1, by volume). The organic solvent fractions containing the aldrin were concentrated by evaporation and adjusted to a volume of 4 ml. Samples (2 μl each) were injected into a gas chromatograph (Aerograph model 200) fitted with an electron capture detector (*4*).

The total amount of aldrin adsorbed to bacterial floc as a function of time is plotted in Fig. 1. The theoretical maximum for aldrin adsorption calculated from a standard curve is 1 ppm. The recovery values for aldrin varied in individual experiments between 70 and 130 percent (0.7 to 1.3 ppm), with the variation possibly due either to adsorption on glassware (*5*) or to the varying sensitivity of the electron capture detector. Almost all of the aldrin ad-



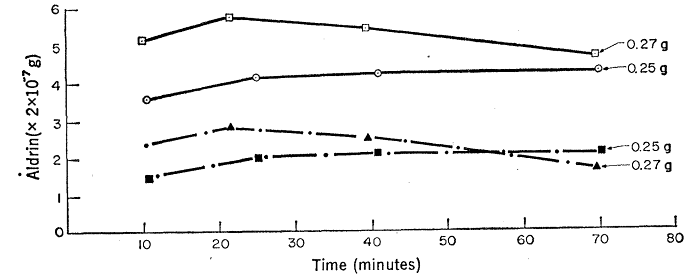


Fig. 1 (left). Curves showing adsorption of aldrin by both (a) Gram-positive bacterial floc and (b) Gram-negative bacterial floc as a function of time (solid lines). Numerals on each curve indicate the dry weight of bacterial floc used in each experiment. Broken and dashed lines indicate extrapolation from the first experimental point to zero time. Fig. 2 (right). Curves showing the inital amount of aldrin found in two different samples of contemporary sediment (silt) (broken lines) and the additional aldrin adsorbed during the experiment by the two samples as a function of time (solid lines).

sorption to floc took place within the first 20 minutes of contact. That the amount of aldrin adsorbed in most cases remained nearly constant after 20 minutes was verified statistically by single variable linear regression at both the 1 and the 5 percent levels of significance when the amounts of aldrin adsorbed as a function of time were compared. All of the aldrin added to the Gram-positive bacteria was recovered from the floc; none was recovered from the supernatant. The recovery value averaged 88 percent. The slope of the line is linear and $\beta = 0$ (*6*) at both the 1 and 5 percent significance levels when floc weight as a function of pesticide adsorbed was evaluated. This linearity is due to the high rate of aldrin adsorption by floc which resulted in maximum adsorption within the minimum time period required to obtain the first adsorption value (that is, 12 to 15 minutes). The fact that there was no significant difference in the amount of aldrin adsorbed by 0.041 g of floc as compared to 0.087 g of floc indicates that 0.41 g was sufficient to adsorb all of the available aldrin.

Adsorption curves shown in Fig. 1 indicate a rapid uptake of aldrin during the first 20 minutes until maximum theoretical adsorption is reached. As aldrin is adsorbed, less is available in solution to be adsorbed. Therefore, the flocs effectively adsorb from more dilute solutions than anticipated by the initial test concentration. This would essentially represent adsorption from lower, more realistic, pesticide concentrations found in Lake Erie.

Data for the Gram-negative organism show that the amount of aldrin adsorbed by an equal weight of cell flocs remained the same or increased only slightly with time beyond 20 minutes and either decreased or remained unchanged in the supernatant. The adsorption curve was linear at the 1 and 5 percent significance levels, but only if the 0.0656 g value was omitted from the calculations. If this value was included, the data did not represent a linear relation at either level. However, in neither case was $\beta = 0$, an indication of a relationship between adsorption and floc weight in this case. Deviation from $\beta = 0$ results from a slower adsorption by the Gram-negative bacteria as compared to the Gram-positive bacteria.

The concentrating effect of these bacteria is considerable. For example, when 0.041 g of Gram-positive floc adsorbed pesticide from 25 g of water, the concentration factor was about 625 to 1 within 20 minutes. A similar but slightly smaller amount of adsorption occurred with the Gram-negative organism. Analogous findings have been reported for algae (*7*).

Samples of natural sediment that were in the process of settling and accumulating in Lake Erie were collected by specially designed sediment collectors placed on reefs (*8*). This sediment consisted primarily of inorganic matter. We analyzed the sediment in a manner identical to that described for bacterial floc, and we also examined the sediment on a microcoulometer (Dohrmann Instrument Company model 200A) (*9*).

The presence of both aldrin and dieldrin (*3*) in contemporary sediment was detected by both gas chromatography and microcoulometry. Additional aldrin added experimentally was absorbed and, as shown in Fig. 2, the concentrations after 10 and 70 minutes were almost equal. No aldrin was detected in the supernatant. The data were linear at both the 1 and 5 percent levels of significance and β was equal to zero.

Contemporary lake sediments appear to accumulate pesticide from suspension in a manner similar to that shown for bacterial floc. Floc-forming bacteria are common in the lake environment and experimentally have a rapid and high adsorption capacity for aldrin. Organic "floc-like" bottom sediments from the eastern basin of Lake Erie have been reported (*10*), and electron microscopic examination of contemporary sediments from Lake Erie shows that these sediments consist of a conglomerate of bacteria, diatoms, and inorganic and detrital particulates.

Counts of 10^5 aerobic and 10^6 anaerobic heterotrophic bacteria have been obtained per gram (wet weight) of contemporary lake sediment. In this regard, clay particles are known to adsorb pesticide and lake sediments are known to adsorb lindane (*3, 11*). Pfister *et al.* (*12*) reported that chlorinated hydrocarbons both behave as suspended microparticulates and are associated with other microparticulates including detritus in the water column. Other researchers (*13*) have established that DDT (*3*) is taken up from organic detritus by fiddler crabs. Significant concentrations of chlorinated pesticides have been detected in algae and lake bottom mud (*14*). It is known that actinomycetes, fungi, and other bacteria adsorb and concentrate pesticides from solution (*15*) and that microparticulates associate with microorganisms (*16*). Particulate organic material has been considered a potentially important source of food for filter-feeding marine organisms (*17*). The suggestion has been made that most of the particulate organic carbon at depths shallower than 175 m in the Atlantic Ocean off South America consists of living organisms and decomposable organic matter (*18*).

We conclude that floc-forming microorganisms act as adsorbants for other suspended microparticles including chlorinated hydrocarbons and that this adsorption represents a natural process for the removal of microparticles from the water column. Once the microparticles have settled from suspension, the fate of the pesticides is in question, but they may be degraded under anaerobic conditions (*19*). It is likely that pesticides concentrated in bottom sediments for even short periods of time would exert an insecticidal effect on the bottom insects and other susceptible fauna. Jensen and Gaufin (*20*) and Carlson (*21*) have shown that different species of stone fly and mayfly naiads have varying susceptibilities to the same pesticide. This may explain the disappearance of certain insects from Lake Erie such as mayflies, and the persistence or increase of others. The same may hold true for other organisms in the lake.

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4. Operating conditions were as follows: 250 mc of titanium tritritide, Ti^3H_3; column temperature, 185°C; detector temperature, 200°C; injector port temperature, 225°C; glass column 152 cm long and 0.32 cm in internal diameter packed with Chromosorb W 60/80 mesh, coated with 5 percent Dow silicone SE-30; high-purity nitrogen carrier gas; flow rate, 60 ml/min.
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Intromission Pattern and Species Vaginal Code in Relation to Induction of Pseudopregnancy

Abstract. *Mechanical stimulation was used to mimic normal vaginal stimuli during copulation in the mouse; the number and rate of intromissions were crucial influences on reproductive success. The best combinations for the mechanical induction of pseudopregnancy were comparable to those male behaviors normally seen during mating. The behavior of the male coincides with a species-related vaginal code, increasing chances for reproductive success between cospecifics.*

Observation of the copulatory behavior of many species, rodents in particular, reveals a definite series of intromissions interspersed with other sexual or nonsexual behaviors. With the report (*1*) indicating that the induction of pregnancy and pseudopregnancy in the hamster is related to the number and rate of intromissions, the hypothesis was formulated that the proper combination of the two would be crucial for successful reproduction in other species. Appreciation of such interacting factors would amplify the data for the rat. For the rat, Ball indicated that the number of intromissions per se is inconsequential for pregnancy as long as at least one or two ejaculations occur (evidenced by the finding of sperm plugs) (*2*). More recently, four or more intromissions, rather than three or fewer, are reported to significantly increase chances of pregnancy (*3*).

I have studied the combined effect of number and frequency of intromissions (insertions and interval between insertions) by means of mechanical induction of pseudopregnancy in the mouse. Heretofore, the induction of pseudopregnancy in this species has been impossible or rare without the use of an ejaculating vasectomized male (*4–6*).

Virgin DBA/2J strain mice obtained from the Jackson Laboratories (Bar Harbor, Maine) or bred in our laboratory were used. This strain was chosen because its copulatory behavior has been extensively studied (*7*).

All females were given freshly prepared ovulation-inducing gonadotropins. Pregnant mares' serum (PMS) [Equinex (Ayerst), serum gonadotropin; 2 international units] was administered at 4:30 to 5:30 p.m. followed 48 hours later by 3 units of human chorionic gonadotropin (HCG) ["A.P.L." (Ayerst), chorionic gonadotropin]. Between 9:00 and 11:00 a.m. on the day after administration of HCG, the females were vaginally stimulated so as to simulate intromission. Stimulation was with a mechanical vibrator (Vibro-Graver, Burgess Vibro Crafters, Inc., Chicago) fitted with a polished brass "penis" 3.5 mm in diameter. The penis rapidly moved back and forth (7200 strokes per minute) and was inserted up to the cervix. The day after stimulation was considered day 1. Until stimulation, the test females were kept in the colony room. After stimulation, the females were kept in a separate room without males to forestall pheromonal effects.

Various aspects of stimulation were tested (Table 1). The number (I) of insertions used was 3, 5, 10, or 15; the intervals between insertions (III) varied from 30 to 270 seconds. The median duration of intromission for males of this strain varies from 17 to 20 seconds; therefore, duration of each insertion (II) was standardized to last 20 seconds. The selection of these values was predicated on the extensive behavioral studies by McGill and his colleagues (*5, 7*). The comparative efficiency of single prolonged stimuli and several other aspects of stimulation were also tested.

The effectiveness of stimuli in the induction of pseudopregnancy was evaluated in every case by a modification of the uterine decidual reaction technique (*8*). One uterine horn was traumatized by being extensively cut along the antimesometrial wall 3 days after stimulation (day 3); it was checked for a decidual reaction 3 days later (day 6). A decidual reaction was said to have occurred if the traumatized uterine horn weighed at least 50 percent more than the untraumatized horn and at least 90.0 mg (mean weight of the untraumatized control horn was 47.3 mg). Occasionally, ovaries were examined histologically for the prolonged maintenance of corpora lutea necessary for pregnancy and pseudopregnancy.

Throughout the experiment the mice were grouped one to four per clear plastic container (approximately 28 by 18 by 12 cm) with wood-chip bedding. The light cycle was maintained with darkness occurring from 6 p.m. to 6 a.m. Food and water were available as desired. The room was air-conditioned; temperatures varied from 65° to 75°F (18.3° to 24°C).

Mice can be mechanically induced to pseudopregnancy (Table 1). Consistent with our findings for the hamster (*1*), neither many insertions nor a single prolonged stimulation alone will insure the induction of pseudopregnancy. The successful stimulatory patterns appear to represent a key to a vaginal code which, within certain limits, is relatively specific for each species. Patterns of stimulation optimum for the induction of pseudopregnancy in the hamster (*1*) (I = 30, II = 5, III = 5) or capable of inducing 100 percent pseudopregnancy in the rat (*9*) (I = 1, II = 20) are relatively ineffective in the mouse. The pattern of stimulation sufficient to induce pseudopregnancy in the rat had been reported ineffective in the hamster and mouse (*4*). For the mouse, as for the hamster, the combination of factors most successful are those which resemble the normal mating pattern. McGill and colleagues (*5, 7*) have reported that the median number of intromissions for this strain of mice varied in