Delayed Lethal Effect of 2, 4-Dichlorophenoxyacetic Acid on Bacteria

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Colmer (1) and more recently Dean and Law (2) noted that the tolerance of bacteria to 2, 4-dichlorophenoxyacetic acid (2, 4-D) was increased by continued culturing in the presence of sublethal concentrations of the herbicide. The latter authors reported that sublethal quantities of 2, 4-D did not effect cell size or the total cellular yield of Aerobacter aerogenes. They also presented convincing evidence that magnesium deficiency in A. aerogenes did not mimic the action of sublethal quantities of 2, 4-D.

Hart and Larson (3) reported on the effect of 2, 4-D on different metabolic types of bacteria and had observed that certain bacteria, particularly the Gram-negative facultatively anaerobic bacteria, showed a graded response to increasing concentrations of the herbicide. In further studies we have noted a delayed lethal effect and an effect on length of cells by 2, 4-D at concentrations which do not inhibit growth completely. The present report is concerned with these effects.

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Bulletin of Environmental Contamination & Toxicology,
Vol. 4, No. 2, 1969, published by Springer-Verlag New York Inc.

Materials

The bacterial cultures were obtained from the departmental culture collection. Unless indicated otherwise the medium employed was composed of 1% Bacto peptone (Difco) and 1% glucose. Incubations in the growth experiments and for growing cells for respirometry studies were done at 25°C on a rotary shaker. Optical densities (OD) were determined in a Bausch and Lomb Spectronic 20 spectrophotometer at a wavelength of 600 mu. Dimensions of cells were measured with a Bausch and Lomb filar micrometer which was standardized with a stage micrometer. The cellular dimensions reported are the results of averaging the measurements of 20 cells. Viable counts were obtained using standard procedures and an agar medium containing 1% peptone and 1% glucose. Total cell counts were obtained with a Petroff-Hauser counting chamber. Cells employed in microrespirometry studies were grown in the presence or absence of 2, 4-D. Standard manometric procedures (4) were performed at 30°C. 2, 4-D was furnished by Dow Chemical Co., Midland, Michigan.

Results

Experiments with different bacteria grown in the presence of 2, 4-D showed that 2, 4-D affected the respiratory capacity of cells. Escherichia coli grown in a medium containing 2, 4-D (10 mM) completely lacked respiratory capacity on glucose whereas cells grown in the absence of 2, 4-D

showed good oxygen utilization (Fig. 1). Similar results were obtained with the following bacteria grown in the presence of 2, 4-D at the concentrations indicated: Pseudomonas fluorescens (15 mM), Aerobacter aerogenes (10 mM), Staphylococcus aureus (1.5 mM), and Micrococcus lysodeikticus (10 mM). These results suggested that either the respiratory system of cells grown in a medium containing 2, 4-D was impaired or that the cells were non-viable.

To determine the viability of the cells viable counts, total counts, and optical densities of E. coli cultures growing in the presence and absence of 2, 4-D were compared. From the results obtained (Fig. 2) it is apparent that the viable count, total count and optical densities of E. coli grown in the absence of 2, 4-D are well-correlated over a long period of incubation. However, by comparison of the same parameters of E. coli grown in a 2, 4-D containing medium (Fig. 3) the OD, total counts, and viable counts showed good correlations until the stationary phase was reached after which the viable count dropped sharply. During the stationary phase the OD and total count remained constant but after 52 hours only about 10% of the cells were viable.

In the medium containing glucose the pH decreased from an initial value of 7.0 to 4.5 during growth. It seemed possible that the delayed bactericidal activity of 2, 4-D could be correlated with the change in pH. However, a similar delayed bactericidal effect was obtained in

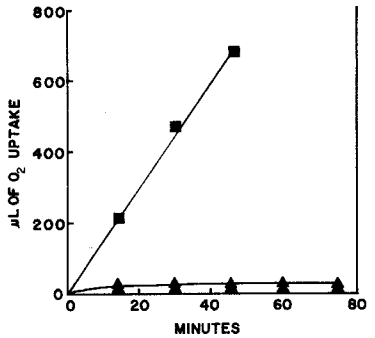

Fig. 1. Glucose oxidation by resting cells of E. coli. The vessels contained phosphate buffer, pH 7.2, 5 mg dry weight of cells, KOH in the center well, 25 μmoles of glucose and water to 3.0 ml. Vessels for endogenous activity were identical except the glucose was omitted. Gas phase, air; temp. 30 C. Symbols: ■ =cells grown in glucose-peptone medium; ▲= cells grown in glucose-peptone medium containing 10 mM 2,4-D; ● = endogenous activities.

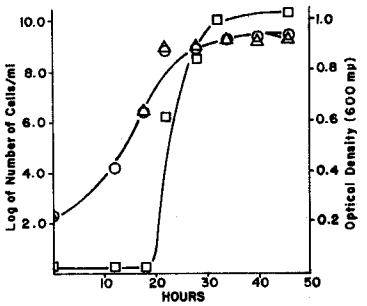

Fig. 2. Growth of E. coli in glucose-peptone medium. Symbols: ○ =viable count; △ = total count; □ = optical densities.

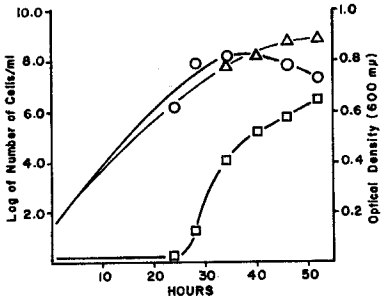

Fig. 3. Growth of E. coli in glucose-peptone medium containing 10 mM 2,4-D. Symbols: ○ = viable count; △ =total count; □ = optical densities.

experiments employing a 1% peptone medium in which the pH increased during growth from 7.0 to 7.4.

Cells of E. coli grown in the presence of 2,4-D appeared long when observed in the counting chamber. Therefore, the effect of 2,4-D on cell length of E. coli was investigated. As shown in Table 1, cells grown in the absence of 2,4-D were long during the early log phase of growth (low OD) but in the late log phase (high OD) the cells became shorter. Cells grown in the presence of 2,4-D were also long during the early log phase (low OD) but these cells did not shorten during growth. In the late log and stationary phases of growth cells grown in the presence of 2,4-D were two to three times as long as cells grown in the control medium. A similar response was noted with Aerobacter aerogenes and Clostridium tetanomorphum although the effect was not as pronounced.

Table 1

The Effect of 2,4-D on the Length of E. coli Cells

Glucose-peptone medium		Glucose-peptone medium with 10 mM 2,4-D	
OD (600 mu)	length (u)	OD (600 mu)	length (u)
.14	3.1	.08	2.4
.35	2.6	.14	3.2
.43	2.0	.25	3.6
.55	1.4	.46	3.6
.75	1.2	.46	3.5

Dean and Law (2) using a glucose-salts medium in which the concentration of magnesium was the growth-limiting ingredient, found that 2,4-D had little effect on the lag, growth-rate and total population of Aerobacter aerogenes. Similar experiments carried out in our laboratory with E. coli provided comparable results.

Discussion

The initial observation that cells grown in the presence of 2,4-D possessed no oxidative ability can be explained on the basis of non-viability. The cells employed for microrespirometry studies were harvested from cultures in the stationary phase of growth. As shown by comparison of total and viable cell counts of cultures grown in the presence of 2,4-D the stationary phase of growth is characterized by a rapid cellular death.

As far as we are aware the delayed bactericidal effect of 2,4-D on bacteria which we have observed has not been reported previously. The exact mechanism of the action of 2,4-D has not been ascertained; however, from the data presented in this report two possibilities exist. From the measurements made on cell length it seems that cross wall formation and cell division may be affected. In the absence of cell division the cells synthesize protoplasm, but eventually die. Secondly, because cellular death does not occur until the stationary phase of growth is reached it seems that the 2,4-D may effect endogenous

activity more severely than metabolism involved in cellular growth. Therefore, cellular death occurs in the stationary growth phase. Recent evidence (5) has implicated fumarase as the enzyme most sensitive to 2,4-D which may result in cessation of endogenous activity with resultant cellular death.

The delayed lethal effect of 2,4-D noted in the present research has ecological implications for soil in that serious effects on the microflora would not be noted unless exogenous carbon sources were absent. It is possible that 2,4-D could have serious effects on the soil microflora under conditions of carbon deprivation. The length of time required for the microflora to return to normal is not known.

Acknowledgements

C. A. Lamartiniere and L. T. Hart are trainees under Public Health Service Grant NIH, 5TI-GM692.

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