Mode of Action of Pesticin

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The mode of action of pesticin, a bacteriocin produced by many strains of *Pasturella pestis*, was studied. Pesticin action on macromolecular synthesis of a sensitive strain of *Escherichia coli*, strain ϕ, was found to have features similar to those of colicin E2-317 acting on the same strain. After exposure to pesticin, deoxyribonucleic acid synthesis was arrested and ribonucleic acid was degraded, but little effect was observed on protein synthesis. Pesticin, like colicin E2-317, induced lysogenic *E. coli* ϕ (P1), but, unlike the colicin, was active in the presence of dinitrophenol. Trypsin was found to reverse pesticin action up to 15 min after its addition at 40 C to *E. coli* ϕ. Pesticin action was studied on three sensitive bacterial strains, *P. pestis* 2C, *P. pseudotuberculosis*, and *E. coli* strain ϕ, which vary widely in their optimal growth temperature. *P. pestis* grows best at 29 C, *P. pseudotuberculosis* at 37 C, and *E. coli* ϕ at 40 C. It was found that pesticin action on all three strains was optimal at 40 C. Whereas the titer of pesticin was the same on all three strains when determined on agar, *E. coli* ϕ was the most sensitive to pesticin action in broth. No action of pesticin in broth on *P. pseudotuberculosis* was observed unless Ca ions were added. The effect was not immediate; that is, the cells had to be grown in a medium containing Ca^{++} before they displayed sensitivity to pesticin.

Pesticin, a bacteriocin produced by many strains of *Pasteurella pestis*, affects three different strains of bacteria, *P. pseudotuberculosis* C-134 (2), *P. pestis* 2C (8), and *Escherichia coli* strain ϕ (3). Very little is known concerning the mode of action of pesticin on these strains.

This paper describes experiments aimed at elucidating these effects. *E. coli* ϕ is sensitive to other bacteriocins as well as to pesticin. Therefore, it was possible to compare the mode of action of pesticin with that of known bacteriocins like colicins E2 and E1.

MATERIALS AND METHODS

Bacteria. *P. pseudotuberculosis* 134 C, *P. pestis* strain Kimberley, and a streptomycin-resistant non-pesticinogenic mutant of the Kimberley strain, strain 2C, have been described elsewhere (2, 8). Other strains used were as follows: *E. coli* strain ϕ (kindly provided by P. Fredericq); a lysogenic derivative of this strain, *E. coli* ϕ (P1) [P1 = P1*kc* of Lennox (10)]; a colicinogenic strain of *E. coli* K-12 producing colicin E2-317 (CL-137) and a colicinogenic strain of *E. coli* K-12 producing colicin E1-30 (kindly provided by B. A. D. Stocker); and a streptomycin-resistant strain of *Shigella*, sensitive to phage P1.

Media. Tryptose broth contained 2% tryptose broth powder (Difco) and 0.5% NaCl, in distilled water. Proteose peptone broth contained 2% Proteose Peptone No. 3 (Difco), 0.5% NaCl, 0.2% glucose, and 0.5% Na_2HPO_4, in distilled water. Tryptose-agar

and Proteose peptone-agar were prepared as described for liquid media with the addition of 2% agar (Difco).

Minimal medium for *E. coli* ϕ consisted of Na_2HPO_4, 0.7%; KH_2PO_4, 0.3%; NH_4Cl, 0.1%; NaCl, 0.05%; $CaCl_2$, 10^{-4} M; $MgSO_4$, 10^{-3} M; glucose, 0.4%; methionine, 0.005%; nicotinamide, 10 μg/ml; calcium pantothenate, 10 μg/ml; xanthine, 50 μg/ml; and distilled water.

Preparation of pesticin and colicins E1 and E2. Crude pesticin was prepared as follows. An overnight culture of *P. pestis* Kimberley growing at 29 C in a rich medium (tryptose broth or Proteose peptone broth) was irradiated with ultraviolet light [as previously described (2)] for 90 sec, and was incubated at 37 C. After 2.5 hr, a few drops of chloroform were added, to sterilize the suspension, and the cells were removed by centrifugation. The supernatant fluid, which contained pesticin, was stored at 4 C and its sterility was checked. The pesticin was titrated by spotting drops (0.01 ml) of a series of dilutions of the preparation to be titrated on the surface of a plate seeded with the indicator strain (*P. pseudotuberculosis*). The indicator plates were prepared some minutes before the titration by soaking filter paper in a suspension of the strain and leaving the paper on the plates for 5 min. The plates were then incubated at 37 C for 24 hr. In this way, a series of zones of decreasing inhibition was obtained, ranging from complete inhibition through partial inhibition to normal growth. The highest dilution that gave a visible inhibition zone was defined as containing 1 arbitrary unit/ml. The titer of pesticin obtained in this procedure was 10^3 to 10^4 arbitrary

units/ml. When a higher titer of pesticin was desired, bacteria were grown on solid agar in plates, which were irradiated in the same manner. Liquid medium was then added to the surface of the agar and, after 2.5 hr of incubation at 37 C, the suspension was sterilized with chloroform, the cells were removed by centrifugation, and the supernatant liquid was stored at 4 C. Solutions prepared in this way had a pesticin titer of 10^6 arbitrary units/ml, but contained also high concentrations of nucleic acids and proteins, which interfered with experiments conducted to determine the effect of pesticin on macromolecular synthesis of the sensitive cells. Partially purified pesticin was prepared by applying the crude pesticin preparation to a Sephadex G-75 column. The fractions containing pesticin were collected and lyophilized.

Colicin E2 was prepared as follows. A 2-day culture of *E. coli* C1-137 growing in liquid medium (Proteose peptone) at 37 C without shaking was centrifuged after the addition of chloroform. The supernatant fluid contained sterile colicin E2-317. Colicin E1 was prepared in the same manner, except that the chloroform treatment and the centrifugation were carried out on a 20-hr culture of strain C1-136 grown with shaking.

Viable count. Samples (0.2 ml) of diluted cells were plated on tryptose-agar plates. Colonies were counted after incubations of 16 hr at 40 C for *E. coli* φ, 24 hr at 37 C for *P. pseudotuberculosis*, and 48 hr at 29 C for *P. pestis*.

Induction of phage P1. Log-phase lysogenic cells of *E. coli* φ (P1), about 2×10^7 cells per ml, were incubated at 40 C with pesticin or the other bacteriocins. At various time intervals during incubation, samples were withdrawn and the free phage was titrated. This was done by dilution of the samples in 2.5 ml of top-layer soft agar (1) containing streptomycin and streptomycin-resistant indicator. The diluted samples were then poured onto tryptose-agar plates (containing $CaCl_2$, 2.5×10^{-3} M). These plates were incubated at 40 C, and the phage plaques were counted after 20 hr. The number of induced cells was determined in the same way as the number of free phage, except that the plates were incubated at 40 C for 2 hr and only then was streptomycin sprayed on top of the agar. The number of plaques on these plates represents the sum of the free phage in the solution present at the time of plating and the number of induced cells. By substracting the number of free phage, determined on plates containing streptomycin at zero time, the number of induced cells could be calculated.

Determination of protein and nucleic acids. Samples (9 ml) were taken, mixed with cold 50% trichloroacetic acid (1 ml), and left overnight at 4 C. The precipitates collected by centrifugation were resuspended in 1.5 ml of 5% trichloroacetic acid, and were extracted at 90 C for 30 min to obtain nucleic acid fractions. After centrifugation, the final precipitates were washed with 5 ml of 80% ethyl alcohol and were boiled with 2 ml of 1 N NaOH for 10 min; after centrifugation, protein was determined by the Folin-Ciocalteau reaction (11). Ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) were determined according to the orcinol (18) and diphenylamine methods (5).

Chemicals. The chemicals used were as follows: trypsin (Nutritional Biochemicals Corp., Cleveland, Ohio), 2,4-dinitrophenol (Fluka AG Chemische Fabrik, Switzerland), DNA, sodium salt highly polymerized A grade (Calbiochem, Los Angeles, Calif.), yeast RNA (Worthington Biochemical Corp., Freehold, N.J.), orcinol (Nutritional Biochemicals Corp.), and diphenylamine, reagent (Merck).

RESULTS

Antibacterial action of pesticin. The lethal action of pesticin at different concentrations was examined to determine appropriate activities for use in subsequent studies. Log cultures of *E. coli* φ were mixed with different concentrations of pesticin, and samples were plated at intervals to determine survivors. In the series of survival versus pesticin concentration curves (Fig. 1), the curve with the steepest slope represented the highest concentration of pesticin. The viable count decreased exponentially with time and was proportional to the pesticin concentration.

Reversibility of pesticin action by trypsin. It has been shown that in many cases the action of bacteriocins may be reversed when the sensitive cells are later treated with trypsin (15, 17). Trypsin has been reported to cause digestion of pesticin (2) as of other bacteriocins (6). In our experiments, it was found that the action of pesticin could be reversed if trypsin was applied up to 15 min after pesticin treatment (Fig. 2).

Action of dinitrophenol on pesticin-treated cells. Dinitrophenol, a known inhibitor of oxidative phosphorylation, prevents some post-adsorption step from taking place in the reaction between the bacterial cells and colicins E2 and E3 (13, 14). It was suggested that dinitrophenol prevents the transmission of the primary event caused by the colicins by interference with the energy supply. A direct interaction of dinitrophenol with some membrane component essential for transmission may also be postulated.

No effect of dinitrophenol (2×10^{-3} M) on pesticin action, as measured by its lethal activity, was found (Fig. 3). This was also true for colicin E1 action on the same indicator (*E. coli* φ), whereas the lethal action of colicin E2-317 was completely suppressed (Fig. 4). Trypsin reversed pesticin action in the presence of dinitrophenol (Fig. 3) for up to 15 min after the addition of pesticin.

That pesticin may act upon sensitive bacteria without energy supply was suggested also by an experiment carried out in a synthetic medium lacking nicotinamide. We found that *E. coli* φ requires, for growth in synthetic medium, the presence of the components xanthine, methionine, calcium pantothenate, and nicotinamide. The
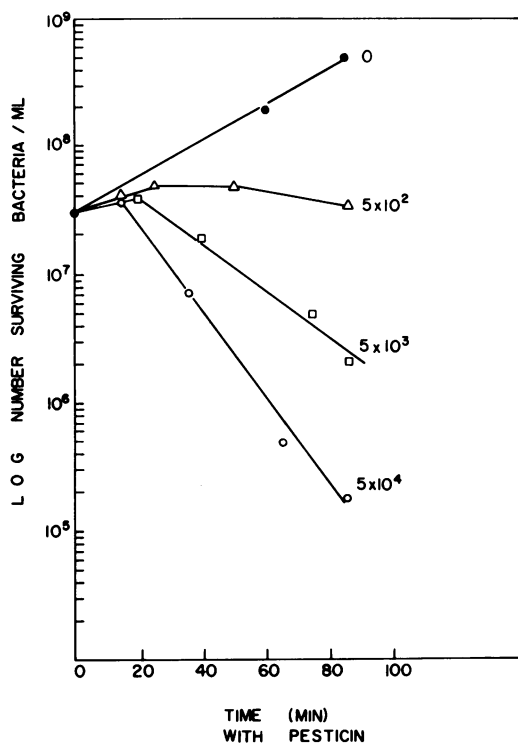
FIG. 1. *Survival of E. coli strain φ at different pesticin concentrations. A culture of E. coli strain φ growing in Proteose peptone broth at 40 C was treated with an equal volume of crude pesticin. Samples (0.5 ml) were removed at intervals and diluted in cold saline; the viable count was determined. Figures near the curves are the final pesticin concentrations in arbitrary units per milliliter.*

FIG. 2. *Reversibility of pesticin action by trypsin treatment. A culture of E. coli strain φ growing in Proteose peptone broth at 40 C was treated with an equal volume of crude pesticin. Samples were removed at intervals, and the number of viable bacteria was determined after immediate plating of suitable diluted samples on tryptose-agar plates (solid line) and after transfer of samples of cell suspensions into an incubation medium containing trypsin (5 mg/ml) and 2,4-dinitrophenol (2 × 10⁻³ m), for 20 min (dashed line). The latter sample was incubated at 40 C before plating.*

action of pesticin in a medium deficient in nicotinamide was investigated. *E. coli* φ was grown in the synthetic medium described in Materials and Methods, and was then diluted with the same medium lacking nicotinamide. The bacteria were thus starved for 30 hr before treatment with pesticin. Starvation for nicotinamide for 30 hr was found to stop cell growth. At intervals after the addition of pesticin, samples were withdrawn and treated with trypsin in the presence of 2,4-dinitrophenol (2×10^{-3} M) for 20 min; the number of survivors was determined by plating the samples on tryptose-agar plates (Fig. 5). Pesticin acted on cells starved for nicotinamide as well as on cells grown in complete minimal medium.

Effects of pesticin on macromolecular synthesis. The effects of pesticin on macromolecular synthesis of *E. coli* φ was tested in synthetic medium and in Proteose peptone broth. Crude or partially purified pesticin was added to a culture
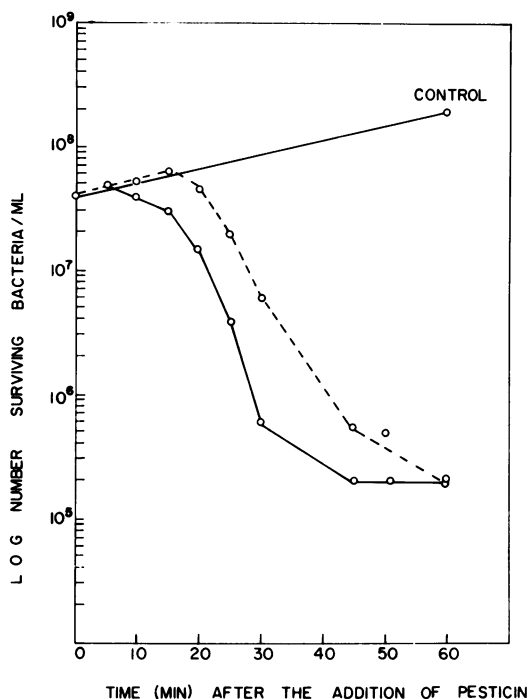
of strain φ growing exponentially at 40 C (with shaking) in Proteose peptone broth, or in synthetic medium to which 0.5% yeast extract had been added. Samples were removed at intervals, and viable count, protein, and nucleic acid content were determined as described in Materials and Methods.

The concentration of pesticin used was lethal for 90 to 92% after 60 to 75 min.

As can be seen from the results summarized in Fig. 6, DNA synthesis stopped immediately after the addition of pesticin to *E. coli* φ in synthetic medium. In Proteose peptone broth, DNA synthesis also stopped, but only some time after pesticin addition (Fig. 7a).

RNA synthesis continued for a period after the addition of pesticin to cells grown in Proteose peptone broth, at a lower rate than in the control, and then was degraded (Fig. 7b). Protein synthesis was less affected (Fig. 7c).
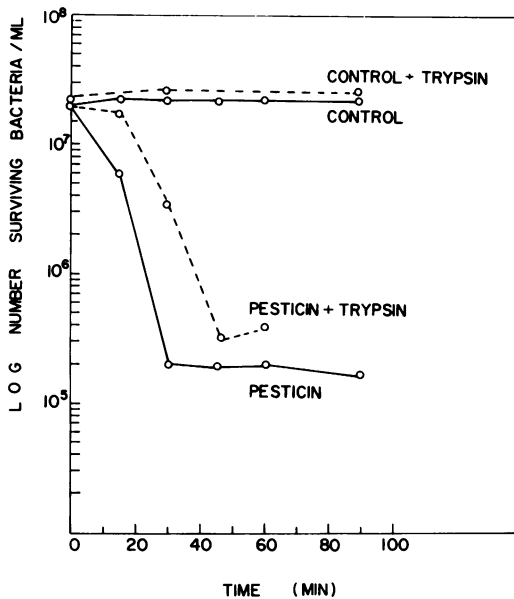
FIG. 3. *Effect of 2,4-dinitrophenol on pesticin action. Cultures of E. coli strain ϕ growing in Proteose peptone broth at 40 C were treated with 2,4-dinitrophenol (2 × 10⁻³ M) for 30 min before the addition of the same volume of pesticin and 2,4-dinitrophenol at the same concentration (time zero). At intervals, samples were withdrawn, and the viable count of bacteria was determined after immediate plating on tryptose-agar plates (solid line) and after transfer of samples of cell suspensions into medium containing trypsin (5 mg/ml) and 2,4-dinitrophenol (2 × 10⁻³ M) for 20 min (dashed line). The latter sample was incubated at 40 C before plating.*

For comparison, the effect of colicin E2-317 on the macromolecular synthesis of *E. coli* ϕ was determined under the same conditions used in the pesticin experiment. The general features of the effects of both bacteriocins on macromolecular synthesis in *E. coli* ϕ, growing in broth, were similar (Fig. 7a, b, c).

Induction of lysogenic bacteria by pesticin. A derivative of *E. coli* ϕ lysogenic for phage P1 was prepared as follows. A 0.1-ml amount of a log-phase culture of *E. coli* ϕ was plated in a soft tryptose-agar layer on a tryptose-agar plate with added $CaCl_2$ (2.5 × 10⁻³ M). Phage P1 (10⁹/ml) was spotted on the plate, and the plate was incubated overnight at 37 C. Bacteria from the lytic spot were streaked out, and the resulting isolated colonies were purified by re-streaking and tested for lysogenicity. One of the lysogenic strains obtained by the above method was treated with various bacteriocins, after which the titer of free phage was determined. Colicin
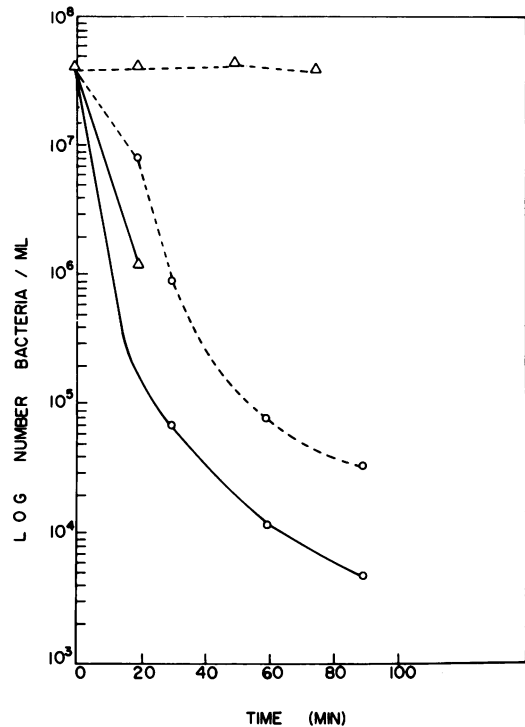

FIG. 4. *Effect of 2,4-dinitrophenol on colicin E1 and E2 action. Cultures of E. coli strain ϕ growing in Proteose peptone broth at 40 C were treated with 2,4-dinitrophenol (2 × 10⁻³ M) for 30 min before the addition of the same volume of the colicin and of dinitrophenol at the same concentration (time zero). At intervals, samples were withdrawn, and the viable count of bateria was determined. Solid lines: samples immediately plated on Tryptose agar plates after treatment with colicin E1 (O) or colicin E2 (△). Dashed lines: samples transferred into incubation medium containing trypsin (5 mg/ml) and 2,4-dinitrophenol (2 × 10⁻³ M) for 20 min after E1 treatment (O) and after E2 treatment (△). The latter samples were incubated at 40 C before plating.*

E2-317 and pesticin induced the prophage of lysogenic *E. coli* ϕ (P1) (Fig. 8), as evidenced by the increasing number of free phage after the addition of these bacteriocins to the culture. When colicin E1-30 was added to the lysogenic cells, the number of free phage decreased.

Similar results were obtained when, instead of free phage, the number of induced cells was tested. Pesticin and colicin E2 caused a 10-fold rise in the number of induced cells in the treated cultures, as compared with their number in the untreated one.

Sensitivity of P. pseudotuberculosis to pesticin in liquid and solid media. It was found that *P. pseudotuberculosis* was much more sensitive to
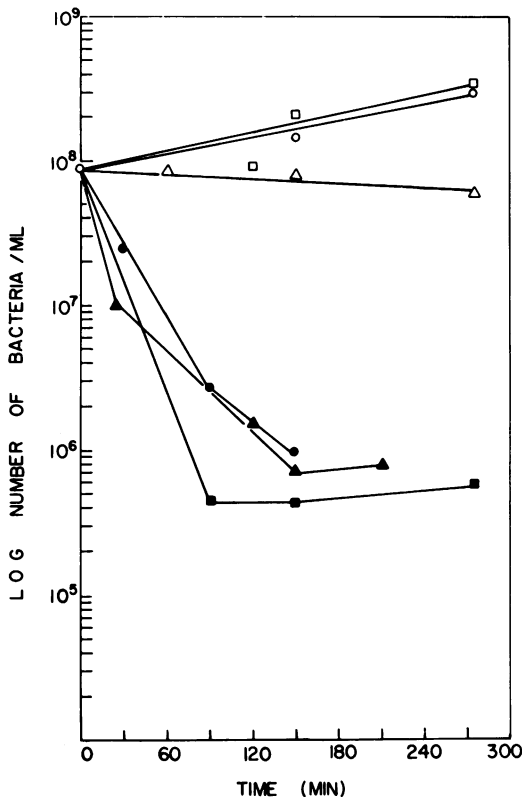
FIG. 5. *Effect of pesticin on bacteria starved for nicotinamide. E. coli strain φ was pregrown for 30 hr at 40 C with shaking in (a) complete synthetic medium and (b) synthetic medium lacking nicotinamide. At time zero, culture (b) was divided in two parts. To one (c), nicotinamide was added to its final concentration in the complete synthetic medium. These three cultures were further divided each into two parts, and partially purified pesticin was added to one set. At intervals, samples were withdrawn, diluted with trypsin in the presence of 2,4-dinitrophenol (2 × 10⁻³ m), and after 20 min were plated on tryptose-agar plates. (a) Pregrowth and growth in complete synthetic medium (O); (a) + pesticin at time zero (●). (b) Pregrowth and growth in synthetic medium lacking in nicotinamide (△); (b) + pesticin added at time zero (▲); (c) Pregrowth in synthetic medium lacking in nicotinamide and transfer at time zero into complete synthetic medium (□); (c) + pesticin added at time zero (■).*

FIG. 6. *Effect of pesticin on E. coli strain φ macromolecular synthesis in synthetic medium. An 80-ml amount of a growing culture of E. coli strain φ in synthetic medium was mixed with 80 ml of partially purified pesticin solution. Samples (9 ml) were removed at intervals, and viable count and protein and nucleic acid content were determined as described in Materials and Methods. DNA synthesis: O, control; ●, after addition of pesticin. RNA synthesis: △, control; ▲, after the addition of pesticin. Protein synthesis: □ control; ■, after addition of pesticin.*

pesticin action on agar plates than in liquid medium (2). Pesticin action in liquid medium was hardly apparent when *P. pseudotuberculosis* was used as indicator. Mayr-Harting and Shimeld obtained similar results with *E. coli* C6 cells, sensitive to E2 (12). However, they were able to increase the sensitivity of the cells in liquid medium by first growing the bacteria on agar plates. In our experiments, growing *P. pseudo-*
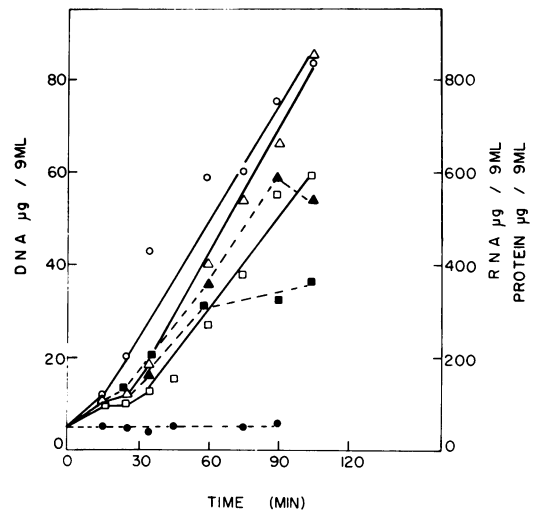
tuberculosis cells on agar plates before subjecting them to pesticin action in liquid medium still did not result in any apparent inhibition of the growth of these bacteria.

Brubaker and Surgalla found that by adding Ca ions they could enhance the effect of pesticin tested on cells growing on agar plates (3). We found that pesticin action in liquid medium became apparent only after CaCl₂ (0.05 m) was added to the mixture (Fig. 9). The number of sensitive cells (*P. pseudotuberculosis*) surviving started to decline only 3 hr after the pesticin was added. In other similar experiments, the effect of pesticin started after even longer periods of time. When the bacteria were grown in broth containing CaCl₂ before the addition of pesticin, they showed a much earlier sensitivity to pesticin when CaCl₂ was added with the pesticin (Fig. 10). Cells in the stationary phase were not sensitive to pesticin even when CaCl₂ was added to the mixture. However, they showed sensitivity if they had previously been grown with CaCl₂.

Absorption of pesticin. No decline in the titer of pesticin measured before and after its action on *P. pseudotuberculosis* could be found, even after concentrating the sensitive cells 100-fold. We could not show any decline in the titer of
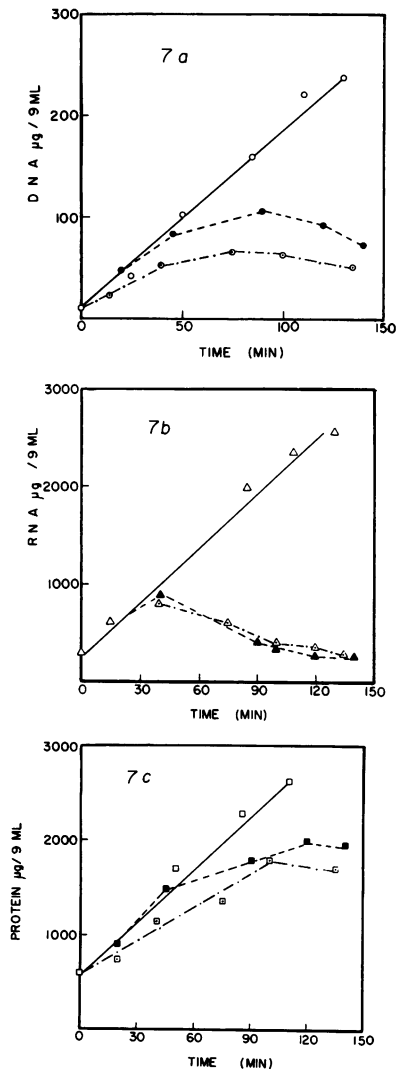
FIG. 7. Effects of pesticin and colicin E2 on macromolecular synthesis of E. coli strain φ in broth. An 80-ml amount of a growing culture in Proteose peptone broth was mixed with 80 ml of pesticin or colicin E2 solution. Nine samples were removed at intervals, and viable count and protein and nucleic acid content were determined as described in Materials and Methods. (7a) DNA synthesis: O, control; ●, after pesticin addition; ☉, after colicin E2 addition. (7b) RNA synthesis: △, control; ▲, after pesticin addition; △, after E2 addition. (7c) Protein synthesis: □, control; ■, after pesticin addition; ▣, after colicin E2 addition.

pesticin even after its dilution to levels that allowed survival of more than 50% of the sensitive bacteria. Similar results were obtained by Mayr-Harting (12) with colicin E2; however, he reported a decline in the titer of the colicin when
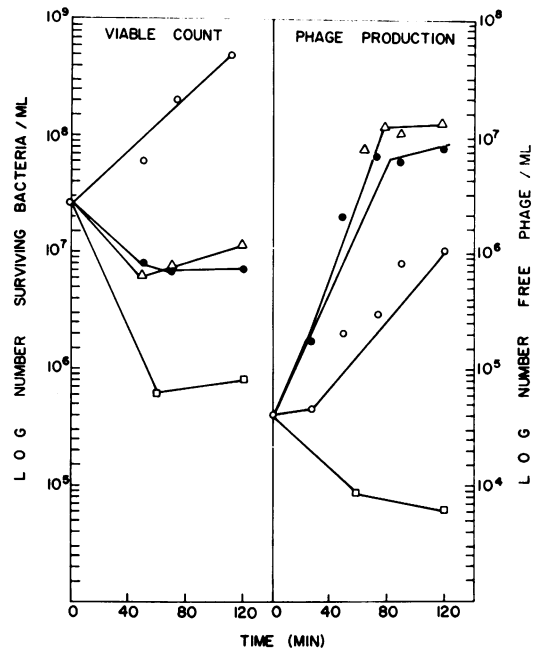

FIG. 8. Induction of lysogenic bacteria by pesticin and colicin E2. A growing culture of E. coli strain φ in Proteose peptone broth at 40 C was treated with equal volumes of pesticin, colicin E1, or colicin E2. At intervals, samples were withdrawn and diluted, and the viable count and free phage were determined, as described in Materials and Methods. Growing culture of E. coli φ (P1): O, control; ●, plus pesticin; △, plus colicin E2; □, plus colicin E1.

the sensitive cells were harvested before absorption from agar plates. No such decline in the titer of pesticin before and after its action on indicator cells grown on agar could be detected in our experiments.

Pesticin action at different temperatures. It has been reported previously (2) that pesticin acts better at 37 C than at 29 C on P. pseudotuberculosis grown on agar.

We repeated this test with three indicator strains: P. pestis 2C, P. pseudotuberculosis, and E. coli φ. Each of these three strains has a different optimal growth temperature: 29 C for P. pestis 2C, 37 C for P. pseudotuberculosis, and 40 C for E. coli φ.

The titration of pesticin was carried out as previously described (2). The plates were incubated at different temperatures. Incubation time was 24 hr, except for P. pestis 2C, which after 24 hr of incubation at 40 C was further incubated for 24 hr at 29 C. This procedure was necessary since the indicator (strain 2C) does not grow at 40 C.
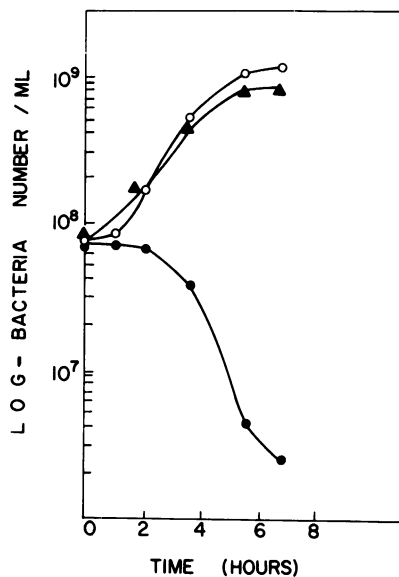
FIG. 9. *Effect of CaCl₂ on pesticin activity tested on P. pseudotuberculosis. A culture of P. pseudotuberculosis growing in tryptose broth at 37 C was divided at time zero into three parts. One part was mixed with equal volume of fresh tryptose broth containing 0.05 M CaCl₂ (final concentration). To another part was added an equal volume of pesticin (in tryptose broth) and CaCl₂ to 0.05 M final concentration. The third portion was treated with pesticin without addition of CaCl₂. At intervals, samples from the three mixtures were withdrawn, and the number of viable bacteria was determined by plating on tryptose plates. Control culture in tryptose broth ○, plus 0.05 M CaCl₂; ▲, plus pesticin and saline; ●, plus pesticin and 0.05 M CaCl₂.*

Figures 11, 12, and 13 indicate that pesticin acts more strongly on all three indicator strains at 40 C. When two other bacteriocins, colicins E1 and E2, to which *E. coli* φ is sensitive, were similarly tested, no differences were observed at the various temperatures.

When pesticin action was tested on the indicators *E. coli* φ and *P. pestis* 2C in broth, maximal effect was found with both indicators at 40 C; i.e., there is no correlation between optimal growth and sensitivity to pesticin.

DISCUSSION

The mode of action of bacteriocins has been studied by several groups of investigators (13, 17, 19). The apparent mode of action seems to be different, depending on the kind of bacteriocin studied.

The results presented in this paper show that the effect of pesticin on sensitive *E. coli* φ cells is in some ways similar to that of colicin E2. Both bacteriocins stop DNA synthesis and,
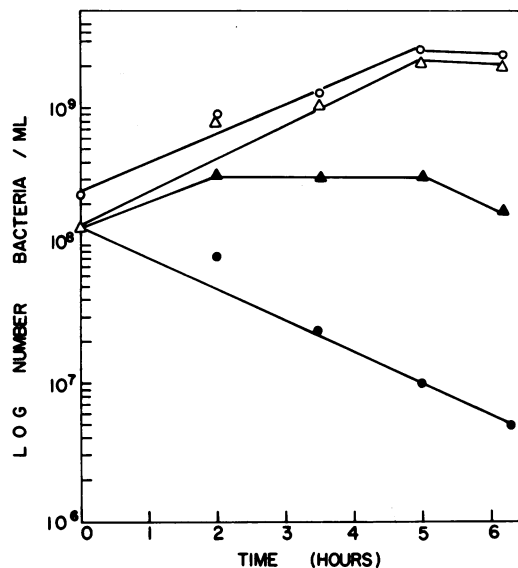

FIG. 10. *Effect of pregrowth with CaCl₂ on the sensitivity of P. pseudotuberculosis to pesticin. P. pseudotuberculosis was grown overnight at 37 C in either of two media: tryptose broth or tryptose broth with CaCl₂ (0.05 M). At time zero, CaCl₂ (0.05 M) was added to the culture growing in tryptose broth alone. Pesticin in tryptose broth was then added to the cultures. At intervals, samples were withdrawn, and the number of surviving bacteria was determined.* (○) *Bacteria growing in the presence of 0.05 M CaCl₂ (also pregrown for 16 hr with CaCl₂).* (△) *Bacteria growing in the presence of CaCl₂ (0.05 M) added at time zero.* (●) *Viable count of pesticin-treated bacteria, grown and pregrown with CaCl₂ (0.05 M).* (▲) *Viable count of pesticin-treated bacteria, to which CaCl₂ (0.05 M) was added at time zero.*

probably as a consequence, induce the lysogenic *E. coli* φ (P1). RNA synthesis is inhibited by both bacteriocins, whereas protein synthesis is the least affected.

An important difference in the mode of action of E2 as compared with that of pesticin is that E2 does not act in the presence of dinitrophenol whereas pesticin does. This observation suggests that the primary event between the bacterial cell and pesticin can occur also when the energy metabolism of the cells is impaired. This conclusion was strengthened by the observation that pesticin acts on bacteria that are unable to grow because they are starved for nicotinamide.

When pesticin action was studied in more detail, it was observed that, although DNA synthesis of cells growing in synthetic medium stopped immediately, the addition of pesticin to bacteria growing in a rich medium stopped only after a considerable time. Konisky and
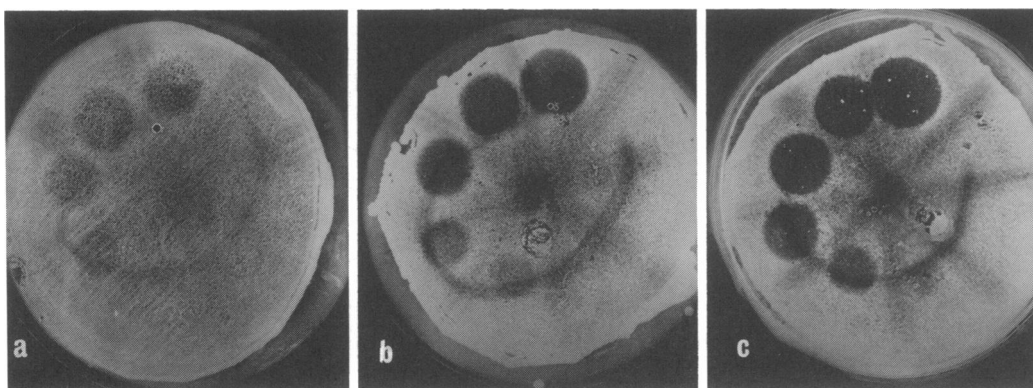
FIG. 11. *Titration of pesticin on E. coli strain φ at:* (a) 28 C, (b) 37 C, (c) 40 C.
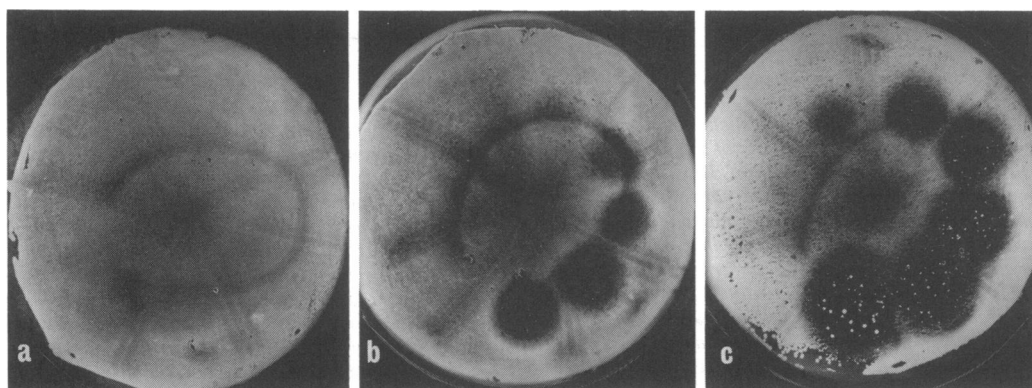

FIG. 12. *Titration of pesticin on P. pseudotuberculosis at:* (a) 28 C, (b) 37 C, (c) 40 C.
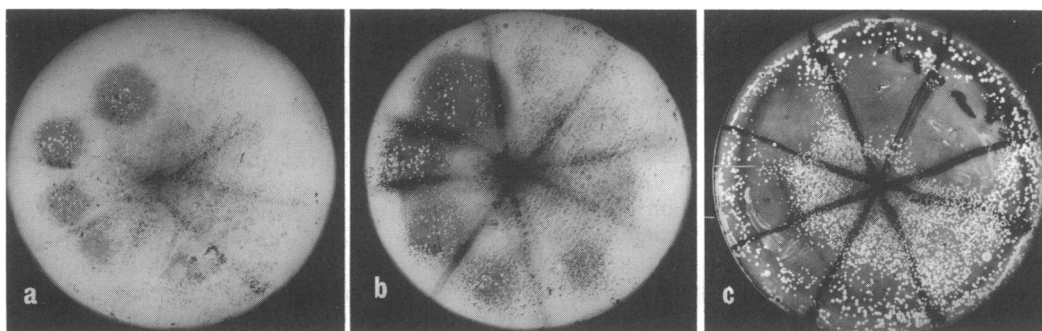

FIG. 13. *Titration of pesticin on P. pestis 2C at:* (a) 28 C, (b) 37 C, (c) 40 C.

Nomura (9), in a different system, also found that the composition of the growth medium changes the biochemical events following the treatment of sensitive cells with certain colicins. The reason for the difference in the apparent activity of a bacteriocin when tested in a synthetic medium as compared with that in broth is presently not understood. It may be due to the fact that the

observed effects of bacteriocins are only secondary effects. They may be influenced by the rate of growth or by control mechanisms operating differently in these two kinds of media, or by both.

In the present investigation, it was found that pesticin has an unusual temperature effect: the optimal temperature for pesticin action on the

three different bacteria tested was 40 C. This temperature was optimal for the growth of *E. coli* ϕ, whereas *P. pestis* and *P. pseudotuberculosis* grow best at 29 and 37 C, respectively. In this respect pesticin differs from colicins E2 and E1. The action of colicins E2 and E1 on *E. coli* ϕ grown on agar was not dependent on the incubation temperature.

A possible approach to the elucidation of the cause(s) of the temperature dependence in this system would be to follow pesticin action on *P. pestis* 2C at 40 C. At this temperature, these bacteria do not grow. Under these conditions of lowered metabolism, it would perhaps be easier to discern some earlier steps following pesticin adsorption, steps occurring before the observed effects on DNA or RNA synthesis.

Brubaker and Surgalla (3) reported that Ca ions enhanced pesticin action when tested on the sensitive cells of *P. pseudotuberculosis*. These authors suggested that various ions affect the action of an inhibitor of pesticin produced by many strains of bacteria. Studies carried out in our laboratory (Furman and Ben-Gurion, *unpublished data*) with *P. pestis* Kimberley demonstrated that this strain did not produce this inhibitor when tested according to the methods described by Brubaker and Surgalla (3, 4). Our results with *P. pseudotuberculosis* grown in liquid medium show that this strain is sensitive to pesticin only when grown in the presence of Ca ions prior to the addition of pesticin. A possible reason for the need for pregrowth in a medium containing Ca^{++} could be a better production of receptors to pesticin in the presence of Ca^{++}. This possibility was tested directly, but we failed to demonstrate adsorption of pesticin to the sensitive cells. The reason for this failure is not understood; we could not show adsorption under conditions under which practically all of the sensitive cells were killed. This was not due to an excess of pesticin in the mixture, as suggested by Reeves (16) for other bacteriocins, since dilution of pesticin to levels allowing survival of more than 50% of the sensitive bacterias still did not result in a decline in titer.

Similar difficulties in showing adsorption of other bacteriocins to sensitive cells have been encountered by other workers (7, 19). Attempts to demonstrate pesticin adsorption by the methods described by Mayr-Harting and Shimeld (12) for colicin E2-P9 were unsuccessful.

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