The preinfection serum pool failed to prevent cytopathic changes, while a 1/10 dilution of the convalescent serum pool prevented such changes (50 percent serum titer log = 1.4).

The virus causes neither signs of illness nor pathological tissue changes in normal suckling or weaned Swiss white mice when it is inoculated by the intraperitoneal or intranasal routes. However, the virus causes illness and extensive myocardial necrosis in weaned mice when it is inoculated intraperitoneally after 2 to 3 daily intramuscular doses (2.5 mg) of cortisone acetate (7).

The etiological significance of this particular virus in the disease manifestations in cattle described has not been established. The disease-producing capacities of the virus in various animal species will be evaluated experimentally. The possible serologic relationships between this viral agent and other cytopathogenic viral agents isolated from the alimentary tract will also be investigated—for example, infectious bovine rhinotracheitis virus (8), poliomyelitis virus (6), Coxsackie virus (9), ECHO viruses (10), ECBO viruses (11), and adenoviruses (12) (13).

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Decomposition of Actinomycin by a Soil Organism

Several studies indicate the importance of microorganisms in the inactivation of antibiotics, although few comprehensive studies have been attempted. The most extensive reports have dealt with the capability of many bacterial species to inactivate penicillin by the hydrolytic action of an enzyme, penicillinase (1). In addition, Pramer and Starkey (2) have reported the isolation from soil of a Gram-negative bacterium capable of decomposing streptomycin. Smith *et al.* (3) have observed that growing and resting cells of *Escherichia coli, Bacillus mycoides, Bacillus subtilis,* and *Proteus vulgaris* can degrade chloramphenicol by hydrolysis, reduction, oxidation, and cleavage of the molecule. Waksman and Woodruff (4), as a result of an investigation of the stability of actinomycin in fresh soil, suggested that inactivation of actinomycin under these conditions may be the result, in part, of the activities of soil microorganisms. The present report describes the isolation of an actinomycin-decomposing organism from soil and presents observations concerning the nature of, and the optimum conditions for, this reaction (5). An enzyme system, tentatively designated *actinomycinase,* appears to be involved in the degradation process.

The medium employed, consisting of mineral salts, 0.1 percent yeast extract, and actinomycin D, was a modification of one used by Pramer and Starkey (2). Microbiological assays of actinomycin in culture fluids were performed by disk diffusion (6) and streak dilution methods (7). Isolation of an actinomycin-decomposing organism was accomplished by enrichment of a barnyard soil and subsequent cultivation of samples of this soil in media containing actinomycin D. On the basis of morphological, staining, cultural, and biochemical characteristics, it was established that the responsible bacterium belonged to the genus *Achromobacter.*

Growing cells of the *Achromobacter* sp. readily decomposed approximately 560 μg of actinomycin D per milliliter of medium in shaken (24 hours) and static (64 hours) cultures under aerobic conditions. The organism degraded actinomycin D (500 μg/ml) through a *p*H range of 6.0 to 8.0, *p*H 7.5 being optimum (48 hours), and at temperatures between 20° and 37°C, 28°C being the most favorable temperature. The extent of decomposition was found to vary within the range of 85 to 4010 μg of actinomycin per milliliter of medium. Up to 1000 μg/ml was destroyed in 48 hours; decomposition of 4010 μg/ml required 84 hours.

A direct relationship was found to exist between inoculum size and the decomposition process. For example, if 7.4×10^{10} cells were employed, decom-

Table 1. Influence of age of *Achromobacter* sp. on the decomposition of actinomycin D. *Achromobacter* sp. was cultivated in actinomycin medium; at various intervals, samples of the culture medium were harvested for cell counts, microbiological assays, and resting-cell studies. For the latter purpose, washed cells of the organism were suspended in 0.06*M* phosphate buffer, *p*H 7.5; the actinomycin-D concentration was 1000 μg/ml, temperature 28°C.

Age of culture (hr)	Cell count (No./ml)	Percentage of actinomycin (500 μg/ml) remaining in culture medium	Percentage of actinomycin (1000 μg/ml) remaining after 6 hr incubation with resting cells
0	1.5×10^7	100	100
8	7.9×10^7	100	100
16	2.0×10^9	100	100
20	2.1×10^9	70	90
24	1.9×10^9	52	50
28	1.8×10^9	24	33
32	1.2×10^9	0	0

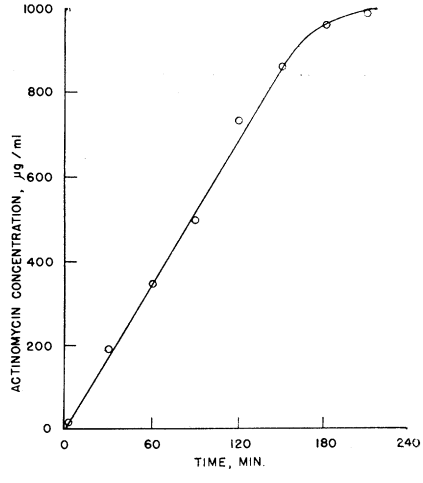

Fig. 1. The amount of Actinomysin D decomposed with time by a suspension of *Achromobacter* sp. cells. Cells were centrifuged out of actinomycin medium, washed twice with distilled water, and finally suspended in 0.06*M* phosphate buffer. One milliliter of this bacterial suspension was mixed with 1 ml of an actinomycin-D solution and 8 ml of 0.06*M* phosphate buffer, *p*H 7.5. Temperature of incubation was 28°C. Final concentration of actinomycin D was 1000 μg/ml.

position of 415 μg of actinomycin per milliliter occurred within 48 hours; if 7.4×10^6, within 72 hours; if 7.4×10^2, within 120 hours. In addition to actinomycin D, actinomycins B and C were also decomposed.

A study of the influence of various growth media on decomposition revealed that, as the growth medium became more complex in nature, the time required for actinomycin decomposition increased. The most rapid decomposition took place when actinomycin D was present as the sole organic compound. The decomposition of actinomycin in relation to the growth phase of the organism also was examined. Decomposition did not begin until quite late in the log or early in the stationary phase of growth, and complete decomposition did not take place until the cell population began to decline (Table 1). Further, it was observed that cells harvested during the lag or early log phase required 66 to 72 hours to degrade the drug in subculture, that cells obtained during the log phase required 48 to 54 hours, and that cells harvested late in the log or early in the stationary phase required 36 to 42 hours.

Resting cells of the organism, suspended in 0.06*M* phosphate buffer, rapidly (1 to 4 hours) decomposed actinomycin D over a wide concentration range (100 to 1400 μg/ml) (Fig. 1). The optimum *p*H for decomposition of actinomycin D was *p*H 7.5; the optimum temperature, 45°C. Destruction of actinomycin occurred with equal rapidity (2.5 hours) when cells were incubated under either static or shaken conditions. Whereas actinomycins B and D were completely decomposed under these conditions, only 60 percent of actinomycin C was destroyed within 6 hours. As in the case of growing cells, the age of the culture at time of harvest of the bacteria significantly affected decomposition by resting cells. Only cells harvested subsequent to the log phase of growth were able to decompose actinomycin (Table 1).

The data obtained thus far suggest that the destruction of actinomycin is enzymatic in nature. Nonspecific adsorption of the antibiotic by cells of the *Achromobacter* sp. does not appear to be involved, since only trace quantities of the drug can be detected after extraction of resting cells which have attacked actinomycin. Young cells of the organism, boiled cell preparations, growing cultures of *E. coli, Pr. vulgaris,* actinomycin-resistant *Micrococcus pyogenes* var. *aureus,* and *Penicillium notatum* all proved to be unable to adsorb or to attack the antibiotic. An acetone powder prepared from cells of the *Achromobacter* sp. readily decomposed actinomycin. Only the cells exhibit enzymatic activity; culture filtrates were found to be inactive.

Preliminary investigations have been carried out on the nature of the degradation products formed by the *Achromobacter* sp. from actinomycin D. Extraction of the decomposition products from culture fluids was accomplished through solvent extraction (acid-butanol). The residue obtained after distillation of the butanol in a vacuum was purified further through cellulose column chromatography; borate buffer (*p*H 8.2)—*n*-butanol and *n*-butyl ether—was employed as solvent system. The decomposition products separate on the column as two orange-red-colored bands, the slower-moving component representing 95 percent of the material. The colored bands were eluted separately from the column. After distillation, the residues were dissolved in warm ethyl acetate and precipitated with carbon disulfide.

Yields of the fast-moving component were low, but additional work was carried out with the slow-moving component. This component exhibited certain properties which differentiate it from the parent compound; for example, its behavior in circular paper chromatography, its visible and ultraviolet light absorption spectra, and its solubility in water and organic solvents differ from corresponding properties of actinomycin D. In contrast, qualitative amino acid analysis revealed the presence of the same amino acids in both the degradation product and actinomycin.

On the basis of these data, the degradation product does not appear to be similar to desaminoactinomycin or to actinomycin acid—compounds obtained by Brockmann and Franck (*8*) through chemical degradation of the actinomycin molecule. Further studies are in progress to purify and characterize the enzyme system responsible for actinomycin decomposition and to determine more completely the nature of the degradation products.

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Depths of Leaching in Glacial Deposits

The depth of leaching of carbonates in soils has been widely used for estimation or comparison of age of Pleistocene deposits in areas of temperate, humid climate. Leaching is influenced by many factors, such as time, climate, vegetation, surface topography, permeability and carbonate content of the material, and so forth. These factors have been discussed by several authors (*1*). C. S. Denny emphasized recently (*2*) that differences in depth of leaching may relate chiefly to the total amount of carbonates in the parent material of soil. However, he did not elaborate on this relationship in more detail.

The shallow depth of leaching in very calcareous or dolomitic materials may be explained by two principal factors: (i) A higher amount of carbonates requires a longer time of leaching, particularly if the carbonate fragments are coarse and if dolomite exceeds calcite in amount. (ii) The more carbonates that are present in the parent material, the thinner the resulting leached soil or profile of weathering will be, for the leached soil consists merely of the insoluble residues. A contrasting example may clarify this statement. If 10 ft of gravel, containing 10 percent carbonates, is leached, 10 percent of the volume will be lost by leaching, and the unleached residues will compact to 9 ft. If 10 ft of gravel with 90 percent carbonates is leached, the resulting thickness of the unleached residues will be merely 1 ft. Even if all other factors had been similar during the development of these two profiles of weathering, the great difference in the resulting measurable thickness (9 ft and 1 ft) does not mean that the 9-ft weathering profile is older than the one 1-ft deep. The opposite may be closer to a correct answer, since a longer time may be required for leaching of a 9 times larger volume of carbonates.

If the carbonate content varies only from 10 to 20 percent between individual places of measurement, the resulting differences in the depth of leaching may be in the usual range of variations which are observed even in one single exposure. Thus MacClintock (*3*) did not find any differences in depth of leaching in the region south of Utica, N.Y., where the percentage of carbonates ranged from 30 to 50 among pebbles.

Most authors do not report the amount of carbonates in the parent material at all. Therefore it is difficult to judge how much their measurements have been influenced by the compaction of the noncarbonate residues. Since the percentage of carbonates varies from zero to 95 in the glacial drifts of Ontario and probably to a considerable extent also in other areas, it is very difficult to com-