

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 0.06M phosphate buffer, rapidly (1 to 4 hours) decomposed actinomycin D over a wide concentration range (100 to 1400 $\mu\text{g}/\text{ml}$) (Fig. 1). The optimum pH for decomposition of actinomycin D was pH 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 (pH 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 non-carbonate 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-

Table 1. Depth of leaching in selected areas. The italic numbers in parentheses in columns 1 and 3 indicate sources listed under "References and Notes." The italic numbers without parentheses in columns 3 and 4 indicate the most abundant thicknesses or depths.

Location and description of material	Carbonates (%)	Measured depth of leaching (ft)	Calculated original thickness of leached portion (ft)
<i>I. Gravelly soils that began to form during Cary time</i>			
A Fanshawe, near London, Ont., pebbly gravel, probably of Lake Maumee time and thus about 14,000 yr old (5)	80	0.5-1-1.5-3	2.5-5-7.5-15
B Eastern North America (6)	50-60	3.5-5 and 7 (7)	7.5-11 and 16
C Eastern North America (6)	30-40	4-5-6-7 (7)	6-7-9-11
D Eastern North America (6)	10	5-8 (7)	5.5-9
<i>II. Gravelly soils formed since the end of Cary time or since Mankato and Valders time</i>			
E Ridgetown, Ont., pebbly gravel, submerged Lake Whittlesey bar, younger than 12,660 ± 440 yr (8)	45	1-1.3-2-3.5	2-2.5-3.5-6.5
F Union, Ont., Lake Warren beach barrier (9); 9600 to 12,000 yr old (5)	50	1.3-2	2.5-4
G Orillia, Ont., Lake Algonquin beach (10); 8000 to 9000 yr old (5)	70	1-1.3-2.5	3.5-4.5-8.5
<i>III. Soils in clayey till; began to form during Cary time</i>			
H Between London, Ont., and Mitchell moraine	50-60	Mostly 1.7	4
I Mitchell moraine at Elginfield, Ont., very stony till with 30 percent clay; 90 percent carbonates among pebbles	65	1	3
J Northern Ohio, late Cary, according to G. W. White (11)	14	Mostly 2.7 (11)	3.2

pare the measurements of leaching without introducing a correction factor. Such a correction factor for calculation of the original thickness of weathering profiles in calcareous and dolomitic weathering profiles is suggested in this report (4).

Some actual examples with several measurements in each area are selected from Ontario and other neighboring regions where the soil formation began during the Cary subage or slightly later. They are arranged in Table 1 in groups of similar age, texture of the material, and topography, in order to avoid the influence of variables other than the carbonate content.

The original thickness of the leached layer is calculated by using the following formula:

$$h = \frac{100d}{n}$$

where h is the original thickness of the leached layer, d is the measured thickness of the leached weathering profile, and n is the percentage of noncarbonates before leaching in the parent material.

Materials of groups I and II (see Table 1) have a similar texture (per-

meable gravel), and the original thickness of the leached weathering profile agrees well with their age differences (average above 5 ft in I and 4.5 ft or less in II). This difference is not conspicuous from actual measurements—for instance, in gravels A and E—because of the higher percentage of carbonates in the older soils at Fanshawe (A).

The measured depths of leaching vary in each of the groups I and III to such an extent that it would be difficult to assume that each group represents a unit of similar age and texture. These differences level out if the leached thickness of carbonates is added to the measured depth (see Table 1, last column).

The exceptionally shallow depth of leaching (12 in.) in the very stony till at Elginfield (I) indicates that leaching of large fragments has required a longer time, and therefore the depth is slightly less than in H, though both tills are clayey and of a similar age.

The relatively deeper leaching at Orillia (G) than at Ridgetown (E) may be explained by presence of dolomite in the gravel E, while G contains only limestone pebbles (all other Ontario

samples of Table 1 contain dolomite, but less dolomite than limestone). Although differences in particle size of carbonates and calcite/dolomite ratios also affect the depth of leaching, the total amount of carbonates seems to be of a greater importance.

In order to facilitate comparison of depth of leaching while deciphering the Pleistocene stratigraphy, I suggest that the percentage of carbonates also be published with the other characteristics of the drift. If gravels are sandy, the percentage of carbonates is usually higher among pebbles than it is in the entire sample. Therefore both figures would be required. If the percentage of carbonates is higher than 20 or the measured depth of leaching is several feet, or both, the original thickness of the leached weathering profile should also be calculated, for this thickness indicates the age of soil better than the measured depth of leaching. For materials low in carbonate, this calculation may not be necessary.

If leaching has affected several layers of drift, the amount of carbonates may have been different in each of them—for example, in silt overlying gravel or till. In such cases a recalculation of the original thickness of the leached weathering profile is more difficult, requiring determination of the carbonate content of each layer.

The speed of leaching and the resulting depth of the leached profile is certainly influenced also by the particle size of carbonate fragments, their porosity, and whether they consist of calcite or dolomite. An insufficient number of contrasting cases does not permit me to introduce any correction factors for these variables at present, but studies of them are in progress.

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