

advantages. When the problem involved is that of estimating low concentrations of AER cells (for example, 0.01 percent) in a predominantly aer population, the advantages of the selective lactate agar technique over TTC overlay become apparent (6).

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6. This work was supported in part by grant N17D from the American Cancer Society.

11 February 1957

Catabolism of Hexuronic Acids by *Erwinia* and *Aerobacter*

Only casual information is presently available on the metabolism of hexuronic acids by microorganisms (1); somewhat greater development of this field is evident with plant (2) and animal (3) materials. Our interest in this subject stems from an earlier study of the pectinolytic action of *Erwinia* soft-rot bacteria in which it was shown that pectin is broken down to galacturonic acid (4) and that the uronate is further catabolized to a mixture of end-products (5). For the past few years we have sought the initial step in the breakdown of galacturonate. We wish now to report that our investigations (6) indicate that the first detectable step in the catabolism of D-galacturonate and D-glucuronate by cell-free extracts of *Erwinia carotovora* and *Aerobacter cloacae* is a reduction, with either reduced triphosphopyridine nucleotide (TPNH) or reduced diphosphopyridine nucleotide (DPNH), to the corresponding hexonic acid, namely, L-galactonate and L-gulonate.

These reductions are carried out by substrate-induced enzymes. Glucose-grown intact cells oxidize glucose at once in manometric experiments, but act on galacturonate and glucuronate only after an induction period. On the other hand, galacturonate-grown intact cells of *Erwinia carotovora* respire galacturonate at once or after a brief induction phase; glucuronate is used very slowly. These galacturonate-grown cells are adapted at the same time to the oxidation of L-galactonate, but not to D-galactonate, D- or L-galactose, mucate, D- or L-lyxose, D-xylitol, L-arabitol, L-ascorbate, L-arabinose, or D-fucose. This sequential in-

duction pattern suggests L-galactonate as a possible intermediate and reduces the likelihood that some often-suggested schemes (1) for galacturonic acid catabolism are operative in *E. carotovora*.

Glucuronate-grown intact cells of *Erwinia carotovora* oxidize glucuronate and galacturonate after a short induction phase; galacturonate- and glucuronate-grown cells of *Aerobacter cloacae* oxidize both uronic acids rapidly without a lag period; the last mentioned cells are sequentially induced to oxidize L-gulonate.

Cell-free extracts were prepared from both *Erwinia* and *Aerobacter* that had been grown on either galacturonate or glucuronate, by sonic oscillation and centrifugation. The supernatant was dialyzed overnight at 4°C against 0.05M tris-HCl buffer of pH 7.2 and centrifuged at 100,000 g for 90 minutes; the clear solution was used. These preparations contain an enzyme that catalyzes the reduction of both galacturonate and glucuronate by TPNH or DPNH. No reduction of D-mannuronate has ever been observed. The enzyme is provisionally named uronic reductase. When a preparation from galacturonate-grown *Erwinia* is used, DPNH reacts slightly faster with galacturonate than it does with glucuronate; when TPNH is the reductant, the reaction with glucuronate is about 5 times faster than it is with galacturonate under the conditions of our experiments. This difference is less distinct with preparations from cells of *Erwinia* grown on glucuronate, or *Aerobacter* grown on either uronate. The activity of the uronic reductase is very weak in extracts from glucose-grown cells of either species.

The end-products of the reduction of the uronates by both bacterial species, with either DPNH or TPNH, are non-reducing, nonlactonized acids: L-galactonate from galacturonate, and L-gulonate from glucuronate. By boiling for 5 minutes with 1N HCl, the acids are converted into the corresponding lactones which, on paper chromatograms, behave in every respect like L-galactono-γ-lactone after galacturonate reduction, or L-gulonono-γ-lactone after glucuronate reduction. It is highly unlikely that the reaction proceeds by lactonization of the uronic acid followed by reduction to the corresponding L-hexonolactone; this opinion is based on (i) the failure of extracts of *Aerobacter cloacae* to reduce D-glucuronono-γ-lactone with DPNH or TPNH, and (ii) the actual accumulation of the free L-hexonic acids taken together with the observed lack of de-lactonizing enzyme activity in the extracts for either of the L-hexono-γ-lactones.

The recent report by Payne (1) regarding the ability of dried cells of *Ser-*

ratia marcescens to form from glucuronic acid a substance tentatively identified as a "1,6-ester linked dihexuronic acid," prompted an examination by us of uronic reductase activity in that microorganism: an enzyme preparation from glucuronate-grown *Serratia* reduces glucuronate with TPNH or, somewhat more slowly, with DPNH. The relationship of the aforementioned reactions to the system described by Isherwood *et al.* (2) in pea mitochondria (which reduces the methyl ester of galacturonate, but not galacturonate, to L-galactono-γ-lactone) is under investigation, as are extensions of this general reaction to some of the less common uronic acids.

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6. This work was supported in part by research grant RG4544 from the National Institutes of Health, U.S. Public Health Service.
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30 January 1957

Effects of Pentobarbital on Intermittently Reinforced Behavior

The behavioral effects of drugs have recently (1-5) been measured in terms of changes in the stable temporal patterns of responding that occur when behavior is reinforced intermittently. It has been shown that drugs may influence both the average rate and the pattern of emission of responses (4). The latter effects are of particular interest, since these patterns probably represent the operation of more subtle behavioral processes than are reflected in the average rate of response. In this report, a new measure of the temporal pattern of responding is used in order to compare the effects of a drug on temporal patterning with the effects on rate of response.

One form of intermittent reinforcement is designated as a fixed-interval