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4 March 1957

Inability of Nitrate to Serve as a Terminal Oxidant for Hydrocarbons

Denitrifying or nitrate-reducing bacteria are capable of growing aerobically in the absence of nitrate. All compounds which serve aerobically as energy sources for these organisms will serve in the same capacity anaerobically if nitrate is present as the electron acceptor. The only known exception to this rule is the inability of denitrifying bacteria to utilize aromatic acids anaerobically in the presence of nitrate (*1*).

During a study of the mechanisms of microbial hydrocarbon oxidation, attempts were made to isolate nitrate-reducing bacteria by the technique of van Iterson (*2*), with various straight chain, aliphatic hydrocarbons as the oxidizable substrate (*3*). Such isolation procedures were uniformly unsuccessful. The same technique, on the other hand, when citrate and ethanol were used as sources of energy, yielded large numbers of denitrifiers from soil samples. Numerous cultures conforming to the description of *Pseudomonas stutzeri* (*4*) were isolated in pure culture.

To ascertain whether cultures isolated in this manner would oxidize hydrocarbons anaerobically in the presence of nitrate, the technique of Allen and van Niel was employed (*5*). Essentially, the technique measures substrate oxidation by a conventional manometric determination of the nitrate reduction product (nitrogen). Hydrocarbons were supplied to washed, resting cells of organisms grown anaerobically in yeast extract-peptone-nitrate broth as a 1-percent (volume by volume) emulsion in water. Homologous oxygen-containing derivatives were similarly dispersed and tested. Commercially available hydrocarbons appeared to be oxidized to a small but significant extent, but, when highly purified hydrocarbons (*6*) from the American Petroleum Institute were used, no oxidation was detected under anaerobic conditions with nitrate present (Fig. 1). All the homologous oxygen-containing compounds tested were readily oxidized anaerobically. No particular significance should be attached to the rates of oxidation, since they probably depend, in part, on degree of dispersion and surface area of substrate available.

The data present another exception to the general rule concerning oxidative abilities of nitrate-reducing bacteria. A possible explanation for the failure of aromatic acids to function as oxidizable substrates for denitrifying organisms anaerobically is found in the suggestion of Stanier (*7*) and Parr *et al.* (*8*) that the primary attack on the aromatic nucleus by bacterial enzymes involves the simultaneous introduction of two hydroxyl groups—that is, a peroxidation. Peroxidations rarely occur in the absence of oxygen, since molecular oxygen is generally implicated directly in the formation of the peroxide involved. Alternatively, the primary enzymatic attack on the paraffin molecule may involve the direct participation of oxygen in the reaction, as is the case in pyrocatechase (*9*). Hayaishi and coworkers (*10*) have recently shown that two types of these "oxygenases" exist; one type (phenolytic oxidases) adds both atoms of an oxygen molecule to the substrate, while the second type splits the oxygen molecule, reducing one to water and adding the other to the substrate. *P. stutzeri* will grow at the expense of benzoic acid aerobically but not in the absence of oxygen, even if nitrate is supplied (*5*).

A parallel situation exists with the hydrocarbons. Strains of *P. stutzeri* that are unable to oxidize hydrocarbons anaerobically readily oxidize paraffins and olefins aerobically (Fig. 1, right). Aerobically grown cells did not oxidize hydrocarbons anaerobically. It is interesting to note that the "aerobic" oxidation of both paraffins and olefins among those tested (C_{12}, C_{14}, and C_{16}) occurred without detectable induction period, even though hydrocarbon was absent from the growth medium.

These observations lend some credence to the suggestion (*11*) that peroxidation or some other oxygen-requiring mechanism is the first step in the biological oxidation of hydrocarbons and, together with the findings of Updegraff and Wren (*12*), suggests that there are probably very few anaerobic oxidations of paraffins (with the possible exception of methane) by either nitrate-reducing or sulfate-reducing bacteria. Once the hydrocarbon structure has been breached by introduction of oxygen, by means of peroxidation or oxygenase action, oxidation at the expense of nitrate reduction and, presumably, sulfate reduction (by *Desulfvibrio* species) occurs readily.

It is not suggested here that the oxygen-containing compounds tested are the immediate intermediates in the oxidation of paraffins. Intermediates isolated during the course of paraffin oxidation are being characterized and will be described later.

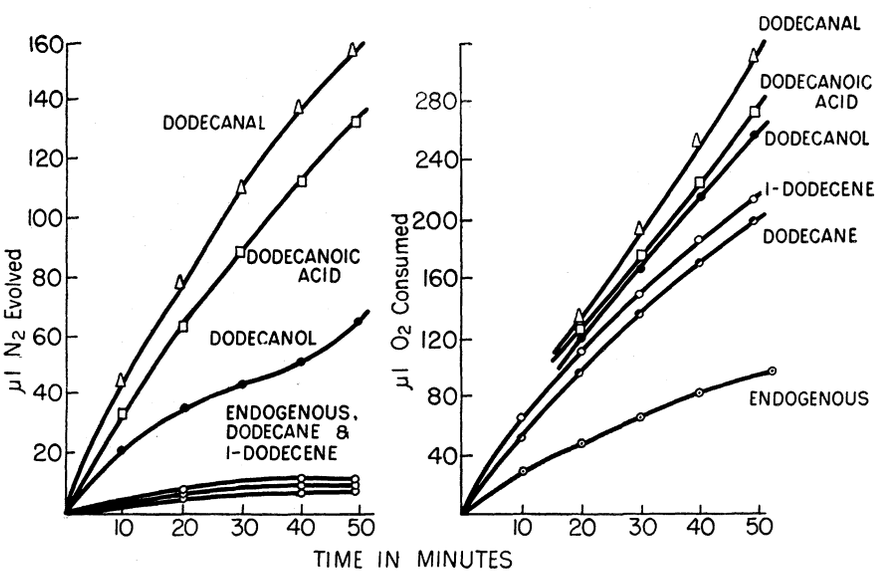

Fig. 1. Oxidations of dodecane, 1-dodecene, and corresponding oxygen-containing derivatives by resting cells of *P. stutzeri*, grown in yeast extract-peptone-nitrate medium. Each vessel contained 8 mg (dry weight) of cells, 0.5 ml of 1 percent (volume by volume) suspension of substrate, and 0.8 ml of 0.02*M* phosphate buffer (*p*H 7.2). Each flask contained in addition, 20 μmole of nitrate. Incubation was at 30°C. N_2 evolution was measured under anaerobiosis; both sets of data were derived from the same cell suspension.

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25 March 1957

Relationship between Auxin and Membrane-Integrity in Tissue Senescence and Abscission

Previous investigations have shown that the physiological aging and abscission of fruits and foliar organs attend a drop in the auxin level of these determinant organs (*1*). This report presents some experimental evidence of the probable role of auxin in such phenomena (*2*).

Segments of commercially grown Kentucky Wonder pole beans were prepared as described by Bonner and English (*3*). From 10 to 20 segments were placed, with all, part, or none of the exocarp removed, in petri dishes on filter papers, which were kept moist with distilled water or distilled water plux auxin [indole-3-acetic acid (IAA) or naphthalene acetic acid (NAA)] at concentrations of 4 to 50 ppm. The experiments were conducted both with and without asepsis. The dishes were stored in the dark at 25°C.

At the end of 2½ to 3 days, under aseptic conditions, the segments treated with 4 ppm auxin were plump and rigid, while the controls (water-treated) were soft and flaccid. Rigidity, however, was not directly related to the amount of water absorbed, for under some conditions the auxin-treated segments took up less water than did the control segments.

Hand sections made after 2½ to 3 days revealed that, in the segments treated with auxin, the intercellular spaces were filled with air (Fig. 1); this is a normal situation, as is shown by comparison with sections of fresh, whole beans. In contrast, sections of the controls showed that their intercellular spaces were filled with liquids (Fig. 2). Thus, it appears that the role of auxin in maintaining rigidity of the bean tissue is the result of an effect on membrane permeability; the auxin functions to maintain the selective permeability of the membranes, thereby preventing the exosmosis of cellular substances into the intercellular spaces. The latter process may be visualized as causal of disturbances in equilibria, which accelerate senescence.

Subsequent to liquid-logging of the intercellular spaces, there occurred a dissociation of cells, manifested by their separating at the middle lamella and rounding-up. The possibility that pectinases may be among the cellular substances liberated into the spaces is being investigated.

With concentrations of 4, 25, and 50 ppm auxin, membrane-integrity in the bean segments was maintained for 7, 11, and 17 days, respectively. In experiments performed without asepsis, the first sign of contamination appeared after the loss of membrane-integrity. Rapid bacterial decomposition followed as a result of the favorable substratum provided by the loss of cellular substances.

Auxin was most effective in maintenance of membrane-integrity when it was applied immediately. After 24 hours of water treatment it gave partial effects, and after 48 hours, no effects. This indicates that membrane-alteration begins during 24 hours, and is irreversible after 48 hours, of water treatment.

Sections of limp, whole beans showed that the effect of auxin on membrane-integrity is unrelated to the phenomenon of water loss that occurs as beans wilt, for the intercellular spaces were filled with air, although much water had been lost.

There was a differential response of tissues of the bean segments to loss of membrane-integrity. If such differential developments were to take place in selected tissues in other material, such as the abscission zone, the following hypothesis is suggested. The cells of the abscission zone are especially sensitive to a drop in auxin level. Below a critical level, the cells in this zone lose the integrity of their membranes; this permits the displacement of cellular fluids which affect the middle lamella, causing dissociation of cells, and the leaf abscises. Following are the results of some experiments on the effects of auxin on the abscission zone in *Coleus*.

A profusely branched stock of *Coleus* was used for the experimental work (*4*). Longitudinal hand sections show that the cells of the petiole are arranged in longitudinal rows, with conspicuous and continuous intercellular spaces oriented longitudinally and extending through the abscission zone. These spaces are normally filled with air, as is evidenced by their interference with transmission of light owing to reflection phenomena (Fig. 3).

Coleus shoots were spirally debladed from nodes 1 to 6, inclusive (*5*). Within 3 days the debladed petiolar stumps at nodes 3 to 6 had abscised, while petioles at the uppermost nodes 1 and 2 were intact. Hand sections of debladed petioles from node 1 showed that the air in the intercellular spaces had been displaced by cellular fluids only in the cell layers of the abscission zone (Fig. 4). Various stages in the liquid-logging of the spaces were observed by means of fresh sections of many debladed petioles from nodes 1 and 2. At each node the opposite, nondebladed petiole served as a control. The controls always showed intact air columns through the abscission zone.

Subsequent to the loss of selective permeability of the cell membranes in the abscission zone (evidenced by liquid-logging of the spaces) there was apparent a dissociation of cells, manifested by

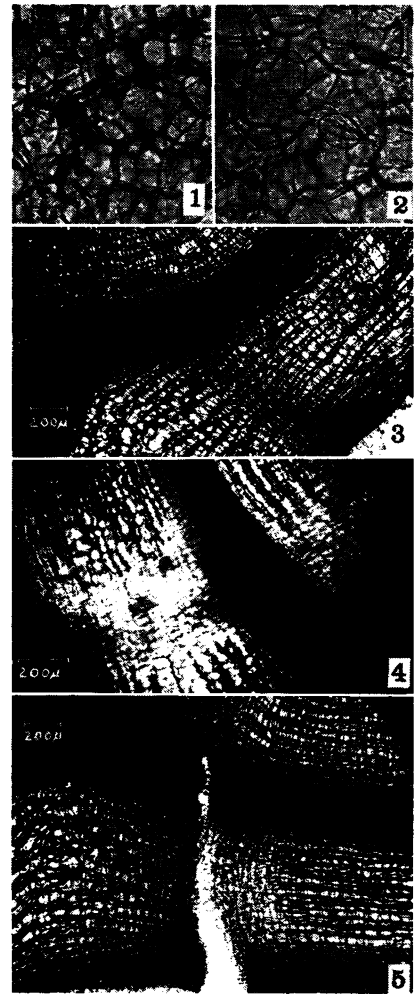

Fig. 1. Fresh section of bean segment after 3 + days of auxin treatment, showing air-filled intercellular spaces (they appear black, owing to reflection phenomena). Fig. 2. Fresh section of bean segment after 3 days of water treatment. Intercellular spaces are filled with liquid as a result of loss of membrane-integrity. Fig. 3. Longisection of control petiole (with blade intact) of *Coleus*. Note the air-filled intercellular spaces traversing abscission zone. Fig. 4. Longisection of petiolar stump (node 1), 3 days after debladding. Intercellular spaces in abscission zone are filled with liquid, owing to loss of membrane-integrity. Fig. 5. Longisection of petiolar stump (node 2), 3 days after debladding. Note centripetal progression of abscission, owing to dissolution of middle lamella.