was seen repeatedly, exhibited by many individuals, obviously being a regular part of daily life. For example, on 19 Oct., in one period of 2.5 hr spent watching a small stream along 7 m of its length, five frogs were seen to defend their territories in the manner just described. On 22 Oct. at the same place, on the other hand, it was 2.5 hr before the full aggressive pattern was seen, although challenging behavior had occurred earlier.

A specific example of territoriality may be cited in the actions on 4 Oct. of a female, which had been watched in her territory on previous days as well. A subadult frog landed about 4 cm from her at the end of a jump. For 3 or 4 min the adult sat facing the young frog, with head elevated and anterior body raised high on straightened forelegs, slowly pulsating her orange-yellow throat through an amplitude of several millimeters. The smaller frog remained quiet. Then the resident jumped on the intruder, which made a short leap, dislodging the adult. Two more jumping attacks followed in rapid succession before the intruder hid beneath a rock. In a short time, the young frog came out and was quickly attacked again. This time its retreat carried it outside the territory of the resident female, and no further attack was seen.

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Oxidation of Indoleacetic Acid by an Extracellular Enzyme from *Polyporus versicolor* and a Similar Oxidation Catalyzed by Nitrite

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During an investigation in this laboratory concerning the action of the fungus *Polyporus versicolor* on lignin and various related phenolic compounds, it was noted that the culture filtrate was able to oxidize rapidly indole-3-acetic acid (IAA). Further work (*1*) was therefore done to characterize this enzyme and its reaction. The enzyme preparation was obtained by growing the organism for a 10-day period at 28°C in flasks of Dion's medium (*2*) on a rotary shaker and then dialyzing the culture filtrate for 9 days against daily changes of distilled water at 4°C. One-milliliter portions of this dialyzed filtrate, containing 1.20 mg of dry solids, were generally employed in the various experiments.

Measurements of gas exchanges in the Warburg apparatus at 37°C showed that 1 mole O_2 was used and 1 mole CO_2 was formed per mole of IAA during the reaction. This corresponds with the data reported for the IAA oxidase of pea seedlings (*3, 4*), bean roots (*4*), and pineapples (*5*). The enzyme from *P. versicolor* showed optimal activity at about *p*H 4.5, compared with *p*H 6.5 for the enzymes from peas and beans and *p*H 3.5 for the enzyme from pineapples. Attempts were made to concentrate this fungus enzyme by methods used for other IAA oxidases, but precipitation by addition of 40 vol of acetone (*6*) inactivated and dialysis (*3*) failed to precipitate the enzyme. Heating for 10 min at 100°C effected complete inactivation. The reaction had a Q_{10} of 2.0 from 27° to 37°C. Riboflavin at a concentration of 10 μg/ml or higher produced no oxidation of IAA under the usual lighting conditions. The Q_{O_2} on various indole derivatives at a level of 10 μM was as follows: IAA, 153; indole-3-butyric acid, 68; indole-3-propionic acid, 28; and indole-3-acetaldehyde, 29 (see Fig. 1 for typical curves and conditions used). Phenylacetic acid, DL-tryptophone, DL-β-phenylalanine, indole, skatole, and indole-3-acetamide were not oxidized.

Hydrogen peroxide does not seem to be involved in the reaction, as is shown by the failure of either puri-

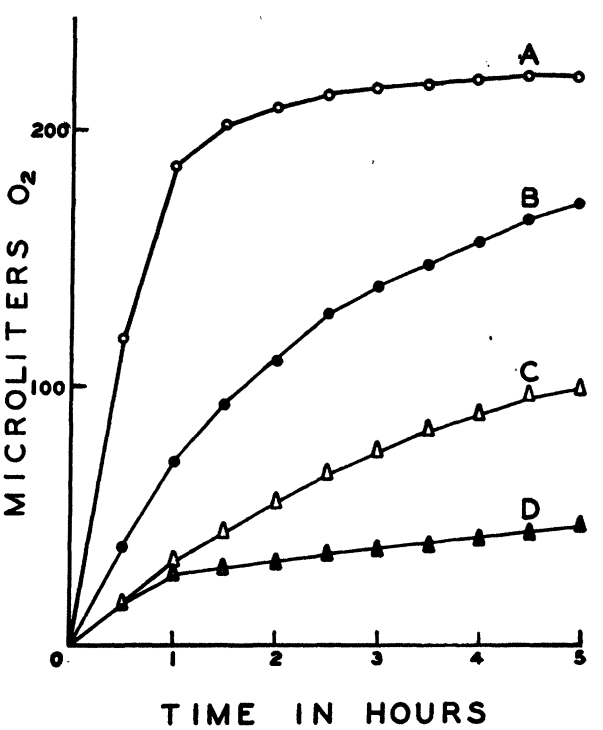

Fig. 1. Oxidation of (*A*) indole-3-acetic, (*B*) indole-3-butyric, and (*C*) indole-3-propionic acids present as the sodium salts, and of (*D*) indole-3-acetaldehyde by 1 ml of dialyzed culture filtrate of *P. versicolor*. Warburg vessels contained 10 μM substrate, 0.5 ml of M/5 sodium acetate buffer at *p*H 4.73, distilled water to 3.0 ml, and 0.1 ml of 25 percent KOH in center wells. Temperature, 37°C; gas phase, air.

fied catalase (*Kat. f.* 5400) or pyruvic acid to reduce the apparent uptake of O_2 by destruction of H_2O_2. No peroxidase activity could be detected in the filtrate by measurement of purpurogallin formed from pyrogallol in the presence and absence of H_2O_2, and a purified horseradish peroxidase (*P.Z.* 270) did not significantly alter the course of oxidation of IAA. The failure of the filtrate to oxidize cadaverine or putrescine indicated that no true diamine oxidase was present. An oxidation of *p*-phenylenediamine did not produce H_2O_2 and probably proceeded by means of hydrolytic deamination.

Up to 48-percent stimulation of the maximum oxidative rate on 10 μM IAA occurred with 0.001*M* $MnCl_2$, and various compounds produced the following percentage inhibitions: 0.01*M* azide, 100; 0.01*M* cyanide, 100; 0.1*M* semicarbazide, 100; 0.01*M* phenylhydrazine, 95; 0.01*M* fluoride, 87; 0.001*M* methylene blue, 86; 0.005*M* 2,4-dinitrophenol, 65; 0.01*M* 8-hydroxyquinoline, 47; 0.00015*M* $CuSO_4$, 46; 0.001*M* bisulfite, 40; 0.00005*M* 2,4-dichlorophenol, 36; and 0.005*M* iodoacetate, 31. This enzyme is similar to the enzyme from peas but not that from pineapples in inhibition by 8-hydroxyquinoline, and differs from both in being inhibited by 2,4-dichlorophenol instead of either no effect (*5*) or stimulation (*7*), respectively. It resembles the enzyme from pineapples (*5*) but not that from peas (*8*) in lack of stimulation by 0.0018*M* maleic hydrazide. Activation by Mn^{++} is common to all the IAA oxidases, although Mn^{++} does not appear to be an absolute requirement for the enzyme from *P. versicolor*. A large number of other compounds tested were without significant effect.

The end-product of the reaction has not been identified but is a tan-colored precipitate of carbonyl nature and may be a polymerized form of indole-3-aldehyde on the basis of theoretical considerations and various tests for higher aldehydes. The presence of bisulfite prevents the formation of this precipitate.

Sodium nitrite at 0.001*M* concentration greatly accelerated the oxidation of IAA enzymatically. It was soon found that nitrite alone, but not nitrate, could produce this oxidation. This reaction was similar to one of Salkowski's (*9*) color tests for IAA; however, HNO_3 was not employed in our situation. A straight-line response in rate to increasing concentrations of nitrite up to 0.002*M* and an uptake of 1 mole O_2 regardless of nitrite level suggests that this salt acts as a homogeneous catalyst in solution. The activity of 0.001*M* $NaNO_2$ was about equal to that of 1 ml of culture filtrate. Optimal oxidation occurred at the same *p*H as with enzyme. The R.Q. of the system was also 1. The influence of inhibitors was generally similar to that mentioned for the enzymatic reaction, except for failure of NaF to produce any appreciable inhibition. Heating did not inactivate nitrite alone in solution or added to filtrate. No nitrite could be detected in the filtrate by means of the sulfanilic acid-α-naphthylamine test (*10*), which was found to be sensitive to $1.65 \times 10^{-5}M$ NO_2, a level far too low to induce observable oxidation of IAA. The use of up to 80 μM of ammonium sulfamate to destroy NO_2 in the Warburg vessels had no effect on enzymatic oxidation but inhibited the catalytic oxidation by nitrite nearly 40 percent. No dehydrogenase activity of the enzyme could be demonstrated, but it was observed that IAA alone could slowly reduce methylene blue and that this reduction took place rapidly in the presence of nitrite. A cyclic shift from

$$HNO_2 \rightarrow HNO + H_2O \rightarrow H_3NO_2 \rightarrow HNO_2$$

may be responsible for such catalytic action.

Additional and more detailed data regarding the IAA oxidase from this new source will be published elsewhere.

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Adsorbability of Bacteriophage T_1 after Ultraviolet Irradiation in the Dry State

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It has been reported previously that phage T_1, after ultraviolet irradiation in the dry state, cannot be photoreactivated (*1*). Since photoreactivation requires that the phage be adsorbed to its host, *Escherichia coli* strain B, it is possible that the simultaneous combination of dryness and ultraviolet impairs the adsorbability of the phage (*2*). Therefore, this property was investigated (*3*).

A stock of T_1 labeled with P^{32} was prepared (*4*) in the following manner: *E. coli* strain B from an overnight slant culture and phage from a broth stock were added to peptone-glucose medium (*5*) in final concentrations of 1×10^8 bacteria and 1.5×10^4 phages/ml; P^{32} was added so that the final concentration was 144 μc/ml, and the suspension was aerated at 37°C. After preliminary centrifugation at 3200 rev/min, to eliminate bacterial debris, two ultracentrifugations were carried out. The first pellet was resuspended in 2-percent ammonium acetate and the second in broth. Radioactive assays were made of (i) the original P^{32} solution, (ii) the acetate suspension after the first ultracentrifugation, (iii) the acetate supernatant, and (iv) the final broth suspension. This was done by drying 1-ml samples in planchettes and counting with an end-window type of Geiger counter.