containing the "lactic acid" group but were unable to translocate those that did not contain this group. If incorporated into the structure of some other plant regulators or pesticides, the "lactic acid" group may also enhance their translocatibility.

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30 March 1954.

Enzymatic Oxidation of DPNH by Diketosuccinate and Dihydroxyfumarate

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In the course of an investigation (*1*) of pyridine nucleotide dehydrogenases in higher plants, we have observed that various plant extracts catalyze an oxidation of DPNH (*2*) by diketosuccinate. Preliminary experiments indicate that such an enzyme is widely distributed in the roots, seeds, and leaves of higher plants and in animal tissues as well. The more detailed experiments described here have been done mainly with preparations from pea roots.

Because diketosuccinate is decarboxylated rapidly in aqueous solution (*3*), it was necessary to show that the enzymatic oxidation of DPNH is due to the diketosuccinate itself and not to its decarboxylation product. On standing at room temperature, solutions of diketosuccinic acid lost their ability to oxidize DPNH enzymatically within 20 to 40 min. The rate of loss of enzymatic activity paralleled the rate of decarboxylation. This showed that the enzymatic oxidation is not due to the decarboxylation product.

The enzyme has tentatively been called diketosuccinate reductase. The product of the reduction of diketosuccinate has not yet been unequivocally identified. However, a substance that reduces 2,6-dichlorophenol indophenol (*4*) accumulates when the reduction of diketosuccinate is coupled with the oxidation

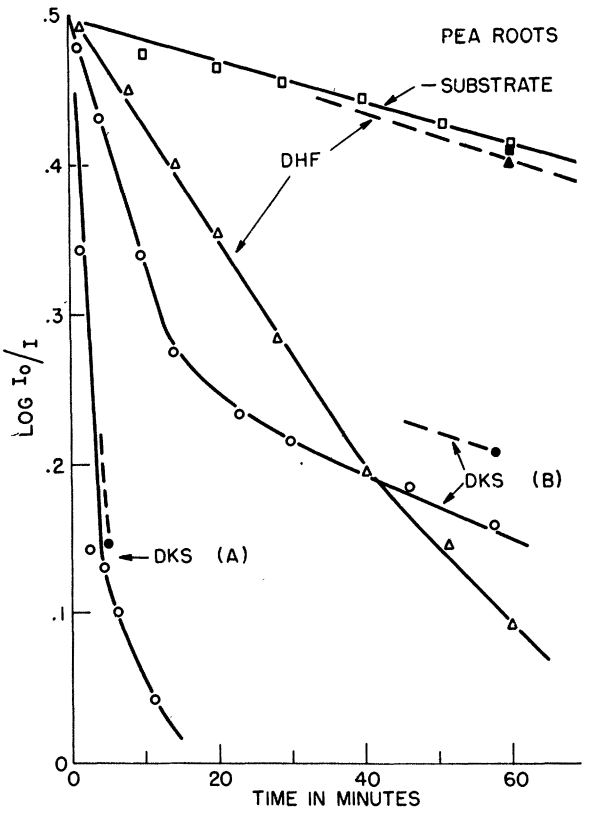

Fig. 1. The oxidation of DPNH by diketosuccinate (DKS) and dihydroxyfumarate (DHF). The enzyme was a dialized extract of an acetone powder prepared from pea roots. Each reaction mixture contained 0.1 ml of enzyme, 0.2 ml of 0.5*M* phosphate buffer of *p*H 6.5, DPNH as shown, and substrate, made up to a total volume of 3.0 ml. The open circles with solid lines indicate aerobic conditions; the solid circles with dashed lines indicate anaerobic conditions (N_2 freed from O_2 with yellow P). The dihydroxyfumarate was prepared according to Hartree (*9*), and the diketosuccinate according to Fenton (*3*). Both substrates were added as solids at the beginning of the experiment to avoid preliminary decomposition.

of ethanol in the presence of diketosuccinate reductase, alcohol dehydrogenase, and DPN^+. This may be regarded as partial evidence that the reduction product is either α-keto-β-hydroxy succinate or an enediol form of this compound. These substances are interconvertible in solution. The reaction may be formulated provisionally according to either Eq. 1 or Eq. 2.

$$COO^- \cdot CO \cdot CO \cdot COO^- + DPNH + H^+ \rightarrow COO^- \cdot CO \cdot CHOH \cdot COO^- + DPN^+ \quad (1)$$

$$COO^- \cdot CO \cdot CO \cdot COO^- + DPNH + H^+ \rightarrow COO^- \cdot COH{:}\,COH \cdot COO^- + DPN^+ \quad (2)$$

Equation 1 would be preferred from analogy with other pyridine nucleotide dehydrogenase reactions.

The enediol of α-keto-β-hydroxy succinic acid is available as a solid; Hartree (*5*) and Gupta (*6*) have shown that this solid is dihydroxyfumaric acid and

not dihydroxymaleic acid, as Fenton (*7, 8*) originally thought. The term *dihydroxyfumaric acid* is used in this paper to designate both the keto form and its enediols.

Attempts to demonstrate the enzyme reaction in the reverse direction—that is, reduction of DPN^+ by dihydroxyfumarate—have not been successful. Dye methods are unsatisfactory because of the high reducing power of dihydroxyfumarate in the absence of enzyme. Direct observation of a reduction of DPN^+ by spectrophotometric measurement at 340 mμ is made difficult by the fact that the concentration of dihydroxyfumarate must be kept small in order to avoid appreciable changes in light absorption due to the substrate alone (*5*). Furthermore, such measurements must be made under anaerobic conditions, since dihydroxyfumarate causes an enzyme-dependent oxidation of DPNH when it is added to a reaction mixture in a Beckman cuvette under aerobic conditions. This reaction is dependent on the presence of oxygen and is, thereby, sharply differentiated from the enzymatic oxidation of DPNH by diketosuccinate. The latter reaction proceeds at equal rates under aerobic and anaerobic conditions.

The results of a typical set of experiments illustrating these facts are shown in Fig. 1. The experiments were done with a pea root preparation buffered with phosphate and supplemented with DPNH. The light absorption at 340 mμ due to DPNH is plotted against time. The solid lines show the change that occurred when no precautions were taken to exclude oxygen. A precise duplicate of each reaction mixture was prepared under anaerobic conditions, opened at an appropriate time, and transferred immediately to a cuvette for spectrophotometric reading. The solid points represent the values thus observed. The upper curve shows the slow decline in DPNH in the absence of added substrate. This change is the same anaerobically and aerobically. An anaerobic incubation mixture with added dihydroxyfumarate (0.2 mg) contained as much DPNH after 60 min as the samples without added substrate, whereas the DPNH was almost completely oxidized when the same amount of dihydroxyfumarate was added aerobically. When diketosuccinate was added, on the other hand, the anaerobic and aerobic changes were equal within the limits of experimental error. Two sets of experiments are shown on the graph. Curve *A* was obtained with about 2 mg of diketosuccinate. Curve *B* was obtained with 0.2 mg of diketosuccinate and shows the marked decline in the reaction rate at about 20 min. This decline is believed to be due to the disappearance of the substrate by decarboxylation. The diketosuccinate does not oxidize DPNH in the absence of enzyme and TPNH cannot be substituted for DPNH in the enzyme reaction.

The results obtained with dihydroxyfumarate can be explained by the rapid autoxidation of the compound to diketosuccinate. The autoxidation occurs spontaneously and is also accelerated by an oxidase widely distributed in plants (*10–14*). The diketosuccinate so formed must then be reduced by the DPNH and diketosuccinate reductase. The reductase thus provides a link between any DPN-reducing system and dihydroxyfumaric acid oxidase. The possibility that a system of this nature may function in plant respiration has been suggested by Szent-Györgyi and his collaborators (*11, 12*). Diketosuccinate reductase furnishes the hitherto missing reducing system (*15*) [See (*16*), however].

A full evaluation of the results obtained with dihydroxyfumarate must take into account not only the autoxidations but also the decarboxylation of this substance (*17, 18*). In some plant preparations, for example, the anaerobic decarboxylation product of dihydroxyfumarate oxidizes DPNH enzymatically, and the dependence of the dihydroxyfumarate-DPNH reaction on oxygen is thereby partially obscured. A more detailed description of the nonenzymatic and enzymatic reactions that diketosuccinate and dihydroxyfumarate undergo will be presented elsewhere.

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15 March 1954.

Stability and Absorption Spectrum of Malononitrile

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The investigation of Hydén and Hartelius (*1*) into the effects of malononitrile on neuronal metabolism and mental disease aroused much interest in its mechanism of action and possible therapeutic value. They stated (*1*):

> It is possible by means of a chemical agent—in this case malononitrile—to stimulate the large nerve cells of the central nervous system to an increased production of nucleic acid and protein substances.