UNSATURATED FAT OXIDASE: DISTRIBUTION, FUNCTION AND HISTOCHEMICAL IDENTIFICATION IN PLANT TISSUES

Sir:

Tauber[1] and Strain[2] reported that the enzyme associated with unsaturated fats in soy meal is an "unsaturated fat oxidase." "Carotene oxidase"[3] has been renamed "unsaturated fat oxidase" by these authors because the oxidation of carotene in soy meal is a result of the enzymatic oxidation of unsaturated fat, and it is further apparent that the "lipoxidase" demonstrated earlier[4,5] is also the same as unsaturated fat oxidase.

By means of some new methods of observation recorded here it is possible to confirm the work of Tauber and Strain and to reveal at the same time the function and intimate association of unsaturated fat oxidase with specific tissues in numerous crop plants. The presence of unsaturated fats in microscopic preparations may be detected by means of Nile blue sulfate, osmium tetroxide, Sudan III and IV and Sudan black B. The oxidation and oxidase system of these fats is best studied by using the leuco form of methylene blue, leuco indigo carmine, guaiacol, and "Nadi" reagent. By means of the above indicators the location and function of the unsaturated fat oxidase may be demonstrated *in vivo*.[6,7]

Recent preliminary observations indicate that the unsaturated fat oxidases are activated in regions of the plant that are alkaline or neutral, where water loss is taking place, beneath wound surfaces, and where inhibitols or antioxidants are absent or inactivated. Dilute solutions (0.0012–0.00028%) of sodium selenite, an antioxidant to many respiration systems, were applied to sustaining sections and it was found that the general oxidase reaction is halted and that the oxidation of indicators is confined to those tissues (epidermis, hypodermis, etc.) which have an accumulation of unsaturated fats. Sodium selenite, as an alkaline salt, depressed oxidase activity in tissues (cortex and xylem) that are not fatty in nature and where the oxidases are activated only at a *p*H of 4.8–5.8. Sodium selenite and alkaline buffer systems activated the oxidation of unsaturated fats by apparently freeing the lipoids and the associated oxidase system from naturally occurring alkali-labile inhibitols and loose addition compounds introduced (iodine, carbonic acid, etc.) into sustaining sections. Root and stem tissues from six species of crop plants grown in buffered media at a *p*H of 7.2–7.6 had a high oxidase activity in fatty tissues at the seedling stage, but at the end of five weeks the unsaturated fat supply was very low and the fatty tissues were characterized by a low oxidase activity and the presence of fatty degradational products. The reverse of this condition was obtained in the case of plants grown in buffered media at a *p*H of 4.8–5.6. Plants grown in relatively acid media had a higher fatty accumulation and a low oxidase activity until treated in section with alkaline buffered solutions. It is thus possible to visually isolate and activate oxidase systems in sustaining sections and in growing plants by means of alkaline salts which activate unsaturated fat oxidase systems but depress the oxidases associated with non-fatty tissues.

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(1) H. Tauber, THIS JOURNAL, **62**, 2251 (1940).
(2) H. H. Strain, *ibid.*, **63**, 3542 (1941).
(3) J. B. Sumner and R. J. Sumner, *J. Biol. Chem.*, **134**, 531–533 (1940).
(4) E. Andre and K. Hon, *Compt. rend.*, **195**, 172–174 (1932).
(5) C. H. Lea, *Rep. Food Invest. Bd., Lond.*, 55–56 (1937).
(6) D. S. Van Fleet, *Am. J. Bot.*, **29**, 1–15 (1942).
(7) D. S. Van Fleet, *ibid.*, **29**, 747–755 (1942).

THE MUTAROTATION OF β-D-ALTROSE

Sir:

When D-altrose was first isolated in crystalline form[1] we reported that mutarotation was not observed, the first reading being taken 3.8 minutes after solution of the sugar in water at 20°. Austin and Humoller[2] had reported a small mutarotation, $[\alpha]^{20-25}D$ $-28.75 \rightarrow -32.30°$ in water, for the antipodal L-altrose, and accordingly had designated it as the β-modification.

With a larger amount of D-altrose now available from the deacetylation of its α-pentaacetate, which was obtained by the acetolysis of 4,6-benzylidene-α-methyl-D-altroside,[3] we have found that this sugar exhibits a complex mutarotation. Thus, 2.000 g. of D-altrose in 50 ml. of water showed $[\alpha]^{20}D$ +11.7, 19.3, 25.1, 32.1 and 33.1° (final) at the end of 2.83, 4.10, 6.00, 17.8 and 31 minutes, respectively. From calculations of the velocity coefficients, derived from these and other intermediate measurements, it would appear that the mutarotation consists of a very rapid interconversion of the furanose and pyranose modi-

(1) Richtmyer and Hudson, THIS JOURNAL, **57**, 1720 (1935).
(2) Austin and Humoller, *ibid.*, **56**, 1154 (1934).
(3) Richtmyer and Hudson, *ibid.*, **63**, 1730 (1941).