LETTERS TO THE EDITORS

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Post-mortem Darkening of Plant Tissues and its Relation to Respiration

THE darkening of plant tissue following extensive mechanical damage is a frequently observed phenomenon and is due to irreversible oxidation of polyphenols. The effect is particularly well marked in the tea-leaf, where the tannin oxidation results in the development of the characteristic copper-red colour of 'fermented' tea-leaf.

It is possible to effect a full fermentation of tea-leaf, that is, a complete oxidation of all the tannins present, while still preserving the cellular nature of the tissue. After fermentation, following extensive mechanical damage, the cell walls are largely intact; and it is observed that the tannins are no longer localized in the vacuoles but are distributed throughout the whole tissue. It follows that rupture of the outer cell wall is not essential for irreversible oxidation of tannins to take place.

The necessary damage to the tea-leaf is best achieved by subjecting the wilted leaf to a shearing force such as is obtained by rubbing it between the finger and thumb or between the palms of the hands. While such treatment has but little effect on the integrity of the outer cell walls, it has been claimed by Phillis and Mason[1] that comparatively small shearing forces have a disruptive effect on the continuous phase of the cytoplasm.

The effect of such shearing forces on the respiratory activity of the leaf is marked. The capability of undergoing anaerobic fermentation may be almost completely inhibited[2] and oxygen uptake under aerobic conditions is also suppressed unless an oxidizable polyphenol is present[3].

Results to be presented shortly interpret this effect in tea-fermentation as due to coenzyme inactivation. The disruptive effect on the cytoplasm may be considered as affecting the orientation of molecules in protein-phosphatide monolayers where adsorption of coenzymes I and II is a necessary adjunct to the transfer of hydrogen from respiratory substrates to carriers of the cytochrome type. The effect of the destruction of the organization of such monolayers will be to inactivate the coenzymes by restricting their sites of activity, and hence to reduce respiratory activity to low levels. If the vacuole originally contained appreciable amounts of polyphenols, these latter substances may now penetrate into the cytoplasm where they undergo direct oxidation by the oxidase system to form deeply coloured pigments. Until this oxidation is complete, the tissue may consume oxygen at a rate higher than that when undamaged, but when the polyphenols are oxidized the uptake sinks to the same low levels found for tissues free from polyphenols. The respiratory quotient during the rapid oxygen uptake is low.

There are other means of inducing irreversible oxidation of polyphenols in vegetable tissues including treatment with anæsthetics such as chloroform, and subjecting the leaf to a temperature of about 50° C. In both these cases it is observed that the cytoplasm is rendered freely permeable to the vacuole contents. Further, the solvent effect of the chloroform on the phosphatides or the denaturing effect of the elevated temperature may be expected to result in a destruction of the organization of protein-phosphatide monolayers fully equal to that brought about by shearing forces.

The effects of anæsthetics, moderate heat and mechanical damage involving shearing forces on the respiratory activity of vegetable tissues are therefore to be considered as essentially similar. Enzyme inactivation is not responsible for the diminution in respiratory activity, and the effects are to be interpreted as due to the inability of the coenzymes to couple the oxidase system and dehydrogenases in the disorganized tissue. Polyphenols present in the vacuole may then penetrate into the cytoplasm to undergo oxidation catalysed by the oxidases. Some secondary oxidation of respiratory substrates by the *o*-quinones may be brought about through the coenzymes now in homogeneous solution, but the respiratory quotient values observed (0·2–0·5) for different plant tissues indicate that the greater part of these *o*-quinones undergo further irreversible changes, with pigment production, before being able to function as hydrogen acceptors in this way.

E. A. HOUGHTON ROBERTS.

Indian Tea Association,
Tocklai, Cinnamara P.O.,
Assam.
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[1] Phillis and Mason, NATURE, **140**, 370 (1937).
[2] Deb and Roberts, *Biochem. J.*, **34**, 1507 (1940).
[3] Roberts and Sarma, *Biochem. J.*, **34**, 1517 (1940).

Blackening of Potato Tubers on Boiling

IT might be of interest to state one or two facts which may have some relation to the hypothesis advanced by Miss Ursula M. Robison[1], that the blackening of potato tubers on boiling is caused by the black oxide of iron produced by oxidation from ferrous iron liberated from a loose complex, probably in association with proteins, as the result of hydrolysis on boiling.

From an examination of potato samples derived from about forty modern replicated fertilizer experiments, designed in association with Dr. E. M. Crowther of Rothamsted Experimental Station, I found that the typical grey to black discoloration which develops after boiling was confined to tubers grown on potash-deficient plots in association with a relatively high nitrogen level in the soil.

It has been shown by various workers that in potash-starved plants the amino acids increase relatively to the protein, and it has been suggested that this is due, at least partly, to the breakdown of protein in the prematurely ageing plants. These changes may cause an abnormal distribution of iron in potash-deficient plants and produce a greater concentration in potato tubers. Hoffer[2] has shown that