Effect of Carbon Dioxide on the Manganese Absorption of Red Beet Tissue

In an earlier publication[1] the discovery was reported of a substance extractable by water from red beetroot and capable of inhibiting the active accumulation of manganese by slices of storage tissue. It was suspected at the time that the substance might be produced by the dark fixation of respiratory carbon dioxide which, as Burton[2] showed for potatoes, may reach concentrations of about 7 per cent in bulky storage organs. I have now shown that the presence of carbon dioxide in solutions from which beetroot tissue slices are absorbing manganese reduces the rate of absorption. The reduction in rate is proportional to the concentration of carbon dioxide up to about 40 per cent, at which the rate appears to be minimal.

When slices of beetroot tissue are freed from natural inhibitor by prolonged washing in running water, inhibition of salt absorption capacity can again be produced by exposing the tissues to carbon dioxide in gaseous form before placing in the manganese solutions. The degree and duration of this condition depend on the concentration and duration of exposure to carbon dioxide. These facts support the hypothesis that a naturally occurring inhibitor of salt absorption may be a carbon dioxide fixation product. Further support is provided by the fact that a greater degree of inhibition results when beetroots are stored at temperatures of about 25° C. instead of the more usual 10° C., and when they are stored in closed containers so that respiratory carbon dioxide accumulates.

The exposure of inhibitor-free beetroot tissue to carbon dioxide concentrations of about 3 per cent (or short exposures to concentrations up to 10 per cent) when oxygen was the complementary gas produced a subsequent stimulation of manganese absorption on placing in solutions free from carbon dioxide, although higher concentrations or longer exposures produced the usual inhibition. This suggests the possibility of the formation of another fixation product or the oxidation of the inhibitor to form a metabolic stimulant. It is hoped that full results will be reported elsewhere.

Dale and Sutcliffe[3] question the significance of a water-extractable inhibiting substance in connexion with the dormancy of stored beetroot or the development of a capacity for salt absorption in slices of cut tissue, and they claim that the observations of Skelding and Rees were due to the presence of relatively indiffusible anions such as malate and citrate in the extracts. This may well have been the case, although Dale and Sutcliffe quote no figures for the organic acid content of their tissue extracts to support it. The results reported above are, however, consistent with the formation in beetroot tissue of an inhibiting substance, and the formation of acids by carbon dioxide fixation in plant tissues is well known. It could be the case that the presence of organic acids in the cytoplasm in certain concentrations is inhibitory; but if so the explanation would more probably lie in a specific action of the acid on absorption centres rather than in the presence of an ion of low mobility in the external solution. The effect of externally applied malate might also be on absorption centres. If the hypothesis put forward here as to the reason for the undoubted low level of salt absorption capacity in newly cut storage tissue is correct, an inhibiting substance may well be of significance in the life of tuber-forming plants.

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March 20.

[1] Skelding, A. D., and Rees, W. J., *Ann. Bot.*, N.S., **16**, 513 (1952).
[2] Burton, W. G., *New Phyt.*, **50**, 287 (1951).
[3] Dale, J. E., and Sutcliffe, J. F., *Nature*, **177**, 192 (1956).

A Selective Medium for the Isolation of Basidiomycetes

In the course of a programme of work involving the isolation of wood-rotting fungi from infected Scandinavian wood-pulp, considerable trouble was experienced with rapidly spreading mould contaminants, particularly *Trichoderma viride*[1]. In many cases, attempts to isolate Basidiomycetes from stains known to be caused by these fungi failed, due not only to the growth of *Trichoderma* which in a short time covered the surface of the malt agar medium, but doubtless also to secretion of the antibiotics viridin and gliotoxin by this mould, which are active against many fungi[2]. Experiments have shown that *Trichoderma* isolated from wood-pulp is able to retard, and in some cases prevent, the growth in artificial culture of various Basidiomycetes.

This difficulty has now largely been overcome in these laboratories by the development of a selective medium containing 0·006 per cent *o*-phenyl phenol, which has given good results both when used for the isolation of wood-rotting fungi from wood-pulp and also, in conjunction with an air-sampling apparatus, from the air in a pulp mill. The latter work was done in an attempt to demonstrate the presence of Basidiomycete diaspores in the aerial microflora of a Norwegian pulp mill, and was very successful. Using the selective medium, *Fomes annosus* was isolated from several locations together with four other (at present unidentified) Basidiomycetes. None was obtained by exposing plates of normal malt agar.

The selective medium has the following composition:

'Oxoid' desiccated malt extract	3 gm.
'Oxoid' mycological peptone	0·5 gm.
Agar-agar	2·5 gm.
o-Phenyl phenol	0·006 gm.
Distilled water	100 ml.

The medium is autoclaved at 10 lb./in.^{2} for 10 min. The required amount of *o*-phenyl phenol is added from a stock solution prepared by dissolving 1 gm. in 50 ml. industrial alcohol. This solution is diluted to 100 ml. with distilled water and is quite stable.

Extensive tests have been carried out with this medium. We have found that several Basidiomycetes isolated from wood pulp will grow, albeit slowly, in media containing up to 0·01 per cent w/v of *o*-phenyl phenol. The growth of bacteria can at the same time be prevented by the normal method of lowering the *p*H of the medium to about 3·5 with sterile lactic acid, without affecting the selective action of the *o*-phenyl phenol towards Basidiomycetes.

At a concentration of 0·006 per cent *o*-phenyl phenol, certain moulds (for example, *Cladosporium* sp., *Alternaria* sp., *Penicillium* spp.) will produce restricted colonies after about a fortnight on the medium. Also, from one pulp sample, a strain of