Table 1. THE QUINIC, SUCCINIC AND MALIC ACID CONTENT OF THE PULP OF APPLES STORED UNDER VARIOUS ENVIRONMENTAL CONDITIONS

Treatment	Temp.	Days	Quinic (mgm./100 gm. fresh wt.)	Succinic (mgm./100 gm. fresh wt.)	Malic (mgm./100 gm. fresh wt.)	Condition of tissue
Air	37° F.	31	84·0	—	1,221·7	No damage
10 per cent CO_2	37° F.	31	87·5	6·35	1,228·5	No damage
20 per cent CO_2	37° F.	11	89·5	21·0	1,224·5	CO_2 damage
Air	50° F.	90	84·0	Trace	907·8	No damage
20 per cent CO_2	50° F.	90	96·3	1·10	879·5	No damage

that succinate is not a normal intermediate metabolite in the fruit; its turnover-rate may be so high as to prevent accumulation. It was noticed, however, that on paper chromatograms of the acid fractions from apples suffering from incipient carbon dioxide injury (a physiological disease of fruit stored in hypernormal concentrations of carbon dioxide, and resulting in the death of the affected tissue), there appeared a spot corresponding in position to that of succinic acid. A sample of the acid giving this spot was isolated from an extract of 1 kgm. of affected fruit and it proved to be a dibasic acid having an analysis of: C, 40·6; H, 5·3; O_2 (by difference) 54·1 (succinic acid = C, 40·7; H, 5·1). It melted at 185° C., and when mixed with authentic succinic acid produced no lowering of the melting point. The *p*-nitrobenzyl ester had a melting point of 87·5° C. A similar derivative from authentic succinic acid had a melting point of 88·0° C.

Determinations of the amount of the various organic acids present in fruit stored in air and in various concentrations of carbon dioxide at 3° C. and 10° C. (apples are more susceptible to carbon dioxide injury at lower, than at higher, temperatures) were made and the extent of carbon dioxide injury in affected fruit was recorded. The results are given in Table 1.

These results show that carbon dioxide injury is accompanied by an increase in succinic acid in the tissue. It appears that an abnormality of metabolism is induced in the tissue by hypernormal concentrations of carbon dioxide in the surrounding atmosphere which results in an accumulation of succinic acid. This, in turn, it is suggested, kills the tissue.

Further details of this work and its wider implications will be presented elsewhere.

A. C. HULME

Ditton Laboratory
(Food Investigation Organization,
Department of Scientific and Industrial Research),
Larkfield, Maidstone.
March 22.

[1] Hulme, A. C., and Neal, G. E. (in preparation).
[2] Turner, J. S., and Hanly, V. F., *New Phytol.*, **48**, 149 (1949).

Nitrogenous Reserves of Apple Trees

WHEN young apple trees assimilate nitrogen, reserve materials are generally laid down in the tissues of the stem and root[1]. Only part of these nitrogenous reserves can be extracted from the stem by exhaustive treatment with 70 per cent (v/v) aqueous ethanol, and a study has recently been made both of the nature of the reserves and of their extraction from the tissues by different solvents.

Amino-acids were separated chromatographically from the tissue extracts by a modification of the method of Moore and Stein[2,3], using ion-exchange resins 'Dowex 50' or 'Zeo-Karb 225'. In this way, arginine and asparagine were identified as the chief nitrogenous reserves; together, they represented 70–80 per cent of the total soluble nitrogen which could be extracted. Arginine alone accounted for more than 60 per cent of the soluble nitrogen in a typical analysis, and, although most of the amino-acids commonly found in plant tissues were present, none of them exceeded 1 per cent of the total soluble nitrogen.

It has also been shown that a large part of the arginine is bound in the tissue, so that it cannot be readily extracted with 70 per cent ethanol. Successive treatments of the finely divided tissue with hot or cold ethanol gave apparent completion of extraction, since the final extract yielded less than 1 per cent of the soluble nitrogen. Nevertheless, further treatment with hot water removed additional soluble nitrogen, amounting to 20–30 per cent of the total. The analyses, summarized in Table 1, indicated that large quantities of arginine were present in the water extract, despite the previous exhaustive treatment with ethanol.

Table 1. EXTRACTION OF APPLE STEM TISSUE WITH 70 PER CENT ETHANOL FOLLOWED BY WATER

	Total nitrogen in extract (per cent)	
	70 per cent ethanol	Water
Arginine	62·0	66·0
Asparagine	15·0	
Glutamic acid	1·0	
Serine	1·0	
Histidine	0·3	
γ-Amino-butyric acid	0·2	0·3–1·0
Alanine	0·2	
Valine	0·1	
Other α-amino-nitrogen	0·3	
Ammonia	0·8	0·6
Other soluble nitrogen	19·0	32·0

Although asparagine and some of the amino-acids were effectively extracted by aqueous ethanol, it is evident that this solvent should be used with caution for quantitative studies of the nitrogenous constituents of plants. In the present experiments, some direct evidence has been obtained that arginine, when added in ethanolic solution to apple stem tissue, is removed from solution, probably owing to reaction with acidic components. Hot water, or sodium chloride solution buffered at *p*H 7, as used by Danielsson[4], appears to be much more effective than aqueous ethanol for extracting the dialysable nitrogenous constituents, particularly when large reserves of arginine may be present, as in apple stems.

A full account of the investigation will be published elsewhere.

K. OLAND

Botanical Institute,
Agricultural College of Norway,
Vollebekk.

E. W. YEMM

Department of Botany,
University of Bristol.
March 27.

[1] Oland, K., *Phys. Plant.*, **7**, 463 (1954).
[2] Moore, S., and Stein, W. H., *J. Biol. Chem.*, **192**, 663 (1951).
[3] Yemm, E. W., and Cocking, E. C., *Analyst*, **80**, 209 (1955).
[4] Danielsson, C. E., *Acta Chem. Scand.*, **5**, 541 (1951).