completely effective without some prior swelling of the particles. It should be noted that particles left in buffer suspension at 0° for periods of 5 h or more have been found to suffer loss of activity. The failure of ultrasonic treatment to produce full activity did not appear to be due to inactivation of the enzyme since a further 30-sec treatment did not lower the activity. The effect of increasing amounts of 'Triton X-100' is not surprising since the ferricyanide acceptor is not suitable when the particulate succinic dehydrogenase complex is disintegrated by various solubilizing treatments[1,2].

For some tissue preparations, a simple pre-treatment with calcium ions (0·75 mM) without freezing and thawing seems sufficient to obtain full activity using phenazine methosulphate as electron acceptor, but this was inadequate when applied to brain mitochondria[5]. The method described here has not been tested on a wide variety of tissues, but gives satisfactory results with liver and muscle fractions besides those from brain.

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[1] Blaschko, H., Hagen, J. M., and Hagen, P., *J. Physiol.*, **139**, 316 (1957).
[2] Whittaker, V. P., *Biochem. J.*, **72**, 694 (1959).
[3] Aldridge, W. N., and Johnson, M. K., *Biochem. J.*, **73**, 270 (1959).
[4] Johnson, M. K., *Biochem. J.*, **77**, 610 (1960).
[5] Singer, T. P., and Lusty, C. J., *Biochem. Biophys. Res. Comm.*, **2**, 276 (1960).
[6] Rose, S. P. R., *Biochem. J.*, **83**, 614 (1962).
[7] De Robertis, E., De Iraldi, A. P., Arnaiz, G. R. De L., and Salganicoff, L., *J. Neurochem.*, **9**, 23 (1962).
[8] Schwartz, A. (personal communication).
[9] Gray, E. G., and Whittaker, V. P., *J. Anat.*, **96**, 79 (1962).
[10] Johnson, M. K., and Whittaker, V. P. (to be published).
[11] Green, D. E., Mii, S., and Kohout, P. M., *J. Biol Chem.*, **217**, 551 (1955).
[12] Singer, T. P., and Kearney, E. B., *Methods Biochem. Anal.*, **4**, 307 (1957).

Iso-Butyric Acid in Silage

The presence of iso-butyric acid in silage has not been reported since Klinc[1], using a qualitative chemical method of detection, claimed to have found it in the material in the surface layers of a silo. Barnett[2] during comprehensive investigations into the formation of the volatile fatty acids in silage found no trace of it, but the results of Lessard, Briggs and Scaletti[3] suggest its presence in samples of alfalfa silage made in the laboratory. No quantitative determinations of the acid have been reported.

In the course of the determination of the volatile fatty acids present in 400 samples of silage, in this Department, iso-butyric acid has been found in measurable amounts in 38 of them. The silages were made from grass, or grass and clover mixtures, and were taken from farms in the south-east of England in the winter of 1961–62. The levels of the acid found were low with an average value of 0·35 mg/ml. in the silage juice, or 0·13 per cent when calculated to a dry matter basis. The iso-butyric acid occurred in the same range of silage *p*H as the *n*-butyric acid, but it made up only a very small proportion of the total butyric acid present. Table 1 shows the *p*H-range in which the iso-acid occurred, and also the substantial proportion of the total number of samples in the range which contained it.

These results suggest that iso-butyric acid occurs widely in small amounts probably as the result of secondary fermentation processes in the silage. Its distribution in samples, therefore, might be expected to be even more widespread in winters following poor silage-making seasons.

Table 1

	*p*H 4·0–4·5	4·6–5·0	5·1–5·5	5·5–6·0
No. of samples containing iso-butyric acid	6	6	23	3
Percentage of total samples containing iso-butyric acid	4·9	13·0	57·5	42·6

The method used for the separation and determination of the volatile fatty acids in this work was based on a gas-liquid chromatography technique. The acids were steam distilled from a sample of the silage juice, extracted by means of ether and injected directly on to the column. The column was 8½ ft. long with a stationary phase of silicone 550 containing 12 per cent behenic acid coated on to 100–120 mesh 'Celite 545'. Nitrogen was used as the carrier gas and the temperature of the column was 135° C. The acids were detected by automatic titration with N/100 sodium hydroxide.

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[1] Klinc, L., *Tierernahrung*, **7**, 281 (1935).
[2] Barnett, A. J. G., *Silage Fermentation* (Butterworths Scientific Publications, London, 1953).
[3] Lessard, J. R., Briggs, R. A., and Scaletti, J. V., *Canad. J. Plant Sci.*, **41**, 507 (1961).

Production of Humic Substances in Decomposing Peat and Compost Samples

During an investigation into the physical properties of humic acids, humic products were extracted from mixtures of papyrus and various additives such as different types of clays, which had been decomposing in natural tropical swamps for periods of up to 20 months, and from core samples collected from deep

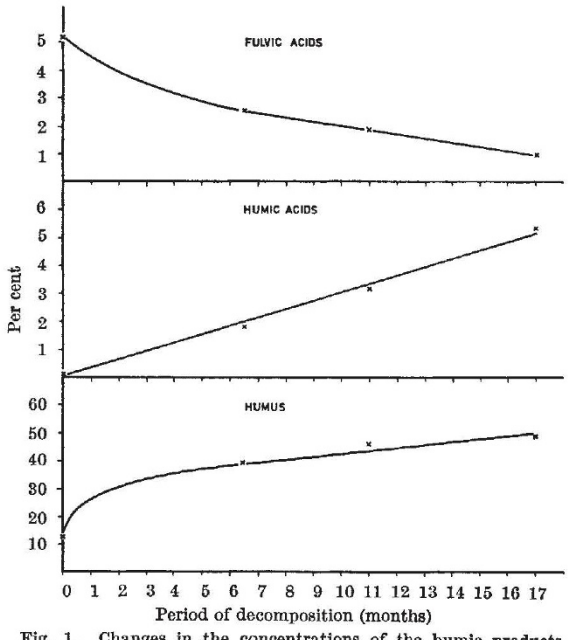

Fig. 1. Changes in the concentrations of the humic products in decomposing papyrus samples