Noting a parallelism between the thermal inactivation of glucose 6-phosphatase and that of mitochondrial NADH dehydrogenase (Awasthi, Ruzicka & Crane, 1970), we began testing the effects on glucose 6-phosphatase of adding fatty acids and lysolecithin (lysophosphatidylcholine) to untreated microsomal fraction. Observing temperature-dependent inactivation (Cater & Hallinan, 1971), we set out to identify the origin of acylhydrolase products on phospholipase C treatment. The phospholipase C preparation showed no detectable acylhydrolase activity, and significant endogenous phospholipase A action seemed unlikely because phospholipase C treatment and preincubation were both at pH 6.8. This pH was chosen to be as far as feasible from the optimum pH (about 9) of the Ca^{2+} -dependent microsomal phospholipase A (Bjornstad, 1966). Therefore lipids were extracted from phospholipase C-treated and post-incubated microsomal-fraction and analysed by t.l.c. on silica gel. Significant amounts of monoglyceride and fatty acid were formed on phospholipase C treatment at 5°C, but after addition of EGTA and post-incubation at 37°C the microsomal-fraction accumulated much more fatty acid at the expense of the diglyceride and monoglyceride fractions. More than a tenfold increase in fatty acid over controls was demonstrated by a quantitative assay (Duncombe, 1963). Microsomal fraction treated at 5°C with Ca^{2+} but without phospholipase C and then post-incubated at 37°C after EGTA addition, and microsomal fraction heated for 10 min at 100°C before phospholipase C treatment and post-incubation, each showed no significant fatty acid accumulation. Hence fatty acids must be formed by endogenous acylhydrolases acting on the diglyceride products of phospholipase C action.

If it proves a general phenomenon, fatty acid release from diglycerides will need to be seriously considered during interpretation of the results of studies on biomembranes involving the use of phospholipase C.

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Water Regime as a Factor in Determining Flavour Strength in Vegetables

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In studies of environmental factors that influence the flavour components of vegetables, the importance of sulphur nutrition in determining the flavour strength of onion (Freeman & Mossadeghi, 1970a,b), garlic and *Allium vineale* (Freeman & Mossadeghi, 1971) and its effect on certain aspects of the flavour of Cruciferae (G. G. Freeman & N. Mossadeghi, unpublished work) has been established. Another factor, independent of sulphur nutrition, that has a marked influence on flavour is the water content of the soil in which the plants are grown. Growers have appreciated for some time that vegetables that have received an abundant water supply tend to be large and lush but relatively lacking in flavour, whereas those grown with restricted amounts of water are smaller and often less attractive in appearance but richer in the characteristic flavours. These qualitative observations have been confirmed by taste panel tests and g.l.c. analysis. The results of glasshouse experiments on watercress (*Rorippa nasturtium-aquaticum*) and a field experiment on cabbage (*Brassica oleracea capitata*) will be presented.

The mechanism of the influence of water regime on vegetable flavour has not hitherto been established, but some possible explanations will be discussed. The observed flavour differences were too great to be explicable in terms of a dilution effect in the samples that received adequate water. There is much evidence in the literature that plants subjected to stress conditions, e.g. nutrient deficiencies and water shortage, accumulate abnormally large amounts of certain metabolites such as organic acids (Coic & Lesaint, 1960; Freeman, 1967), sugars (Gauch & Eaton, 1942; Henrici, 1952) and amino acids (Steinberg, Bowling & McMurtrey, 1950; Stewart, 1967), which are rapidly transformed into macromolecular end products (e.g. cellulose and proteins) during growth in the normal plant. Similar changes have been found to occur in starch concentrations in certain plants (Henrici, 1952). A further example was established by Richards & Coleman (1952), who showed that K^+ deficiency in barley led to a striking increase of leaf putrescine content.

By analogy with these increases, the accumulation of flavour precursors during water stress is a plausible explanation of the experimental observations reported above. The effects of stress conditions on amino acid and sugar concentration respectively are of particular relevance because the

flavour precursors in Liliaceae are predominantly substituted cysteines and those of some Cruciferae include glucose derivatives (glucosinolates).

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Deoxyribonucleic Acid Synthesis in Cells Infected with Type 1 or Type 2 Herpes Simplex Virus

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Two types of herpes simplex virus with different antigenic and biological properties are recognized: type 1 is associated primarily with non-genital lesions, whereas type 2 is isolated primarily from genital sites (Pauls & Dowdle, 1967).

To investigate genetic differences between the two types, DNA synthesis was examined in infected BHK21/C13 cells.* DNA was extracted by the method of Marmur (1961) up to the first spooling stage from cells infected with either type 1 or type 2 virus. Viral DNA and cellular DNA were then separated by centrifugation to equilibrium in caesium chloride.

Infection of cells with strains of either type results in an immediate and progressive decrease in cellular DNA synthesis, although even by 11–13h after infection, when maximal virus yields have been attained, a small amount of cellular DNA is still synthesized. At the same time viral DNA synthesis increases, reaching its maximal rate at 5–7h after infection.

The viral DNA densities were determined in the Spinco model E ultracentrifuge and gave values of 1.7254 ± 0.00018 and 1.7275 ± 0.00006 , which con-

* Abbreviation: BHK cells, baby-hamster kidney cells.

form with G+C contents of 66.7 and 68.9% for type 1 and type 2 respectively, in agreement with the findings of Goodheart, Plummer & Waner (1968) with different strains of herpes viruses. Nearest-neighbour analysis on the DNA of type 1 and type 2 herpes simplex viruses disclosed no obvious differences in doublet pattern or general design (Subak-Sharpe, 1969).

Mixed samples of the two DNA types run on the Spinco model E ultracentrifuge gave one peak, the density difference of 0.002 apparently being too small to permit resolution. However, when type 1 DNA labelled with [³H]thymidine was co-centrifuged in a Spinco model L ultracentrifuge with type 2 DNA labelled with [¹⁴C]thymidine, the labelled viral DNA peaks were clearly separated. Three strains of each type were examined, the type 2 DNA always being found to be denser than the type 1, reversed-label experiments and homogeneous mixes being run as controls.

In addition, it appears that type 1 strains more efficiently incorporate viral DNA into completed virions than do type 2 strains, even though type 2 strains synthesize at least as much DNA as do type 1 strains.

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Studies on Nuclear Proteins in Cells Infected with Type 1 or Type 2 Herpes Simplex Virus

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Herpes simplex viruses have been classified into two types on the basis of site of infection and different antigenic, biological and biochemical characteristics (Pauls & Dowdle, 1967; Goodheart, Plummer & Waner, 1968; Figueroa & Rawls, 1969).

After infection of BHK21/C13 cells* with virus of either type there is a decrease in host-cell DNA synthesis that is accompanied by a gradual decrease in histone synthesis. However, whereas host-cell DNA synthesis can still be detected 13h after infection (Halliburton, Hill & Russell, 1971), arrest of histone synthesis is complete by 6h after infection. In addition to the histones, a number of virus-induced proteins appear in acid extracts of

* Abbreviation: BHK cells, baby-hamster kidney cells.