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EFFECTS OF HEXOSE ANALOGUES ON FUNGI: MECHANISMS OF INHIBITION AND OF RESISTANCE

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CONTENTS

I. SUMMARY

Although the effects of a number of sugar analogues are discussed in this review, most attention is devoted to 2-deoxy-D-glucose (deGlc) and L-sorbose. The growth of fungi is not uniformly inhibited by sugar analogues since some species or strains can metabolize sugars which severely inhibit others. Sugar analogues usually cause inhibitions by being used in metabolism in place of glucose. Both deGlc and sorbose are metabolized by several species but, in general, only a small fraction of the analogue is utilized.

The uptake and phosphorylation of readily metabolized sugars are inhibited by deGlc. Inhibitions by sugar analogues occur on media containing non-carbohydrate sources of carbon, so inhibition of sugar uptake is unlikely to be a major component of the growth inhibition which occurs on sugar-containing media. Hexokinase phosphorylates deGlc, and its 6-phosphate inhibits the activity of especially phosphohexose isomerase and glucose 6-phosphate dehydrogenase. Apart from an involvement in synthesis of more complex sugars, formation of deGlc-6P is the usual limit of metabolism of this sugar analogue. Accumulation of the phosphate ester leads to a considerable drain on phosphate pools and ATP levels decline drastically. Indeed, deGlc rapidly initiates degradation of purine nucleotides which can proceed at least to the level of hypoxanthine. In *Saccharomyces cerevisiae*, deGlc can be condensed to dideoxytrehalose and, in most fungi, polysaccharide synthesis is affected. Preformed wall material is eroded and

Abbreviations: ADP, adenosine diphosphate; AMP, adenosine monophosphate; ATP, adenosine triphosphate; cAMP, adenosine 3′,5′-cyclic monophosphate; deGlc, 2-deoxy-D-glucose; deGlc-6P, 2-deoxy-D-glucose 6-phosphate; NADP, nicotinamide adenine dinucleotide phosphate. All of the sugars and sugar derivatives referred to in this paper are the D-isomers with the exception of sorbose which should always be understood to mean L-sorbose. The unqualified term, 'yeast', is used to refer to *Saccharomyces cerevisiae*. Binomials of other yeasts are cited.

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synthesis of new wall components is prevented by sequestering of uridine and guanosine nucleotides through reaction with deGlc. Interference with the structure of the wall greatly reduces osmotic stability, and cell lysis is the major cause of inhibition of growth by deGlc in yeasts and filamentous fungi alike.

L-Sorbose is not phosphorylated, and the biochemical basis of the inhibitions caused by this analogue is obscure. Some enzymes involved in polysaccharide synthesis are sensitive to inhibition by sorbose, and a characteristic of growth on this sugar is formation of an abnormally thick cell wall. Filamentous fungi grown on solid medium containing sorbose assume an abnormal growth form in which cells are much shortened and branching frequency is increased. There are indications that sorbose, perhaps by interfering with cell wall structure, affects hyphal morphogenesis and that this effect may be magnified into a macroscopic change in the characteristics of the colony during growth on solid medium by secondary environmental effects.

Mutants, resistant to deGlc, have alterations in either transport, hexokinase or phosphatase functions, thus emphasizing the important of accumulation of deGlc-6P in the inhibitions caused by this analogue. Generalization is less easy about mutants resistant to sorbose. The majority are defective in transport, but others show defects in phosphoglucomutase and polysaccharide synthases and there are some correlations with morphological changes which underline the involvement of the sugar in the observed alterations in cell walls. Many aspects of early carbohydrate metabolism need investigation and, for such studies, the sugar analogues have much to offer. However, there is also particular scope for their use in studies of hyphal morphogenesis, especially in relation to the connection between wall construction and morphology. Counterparts of the abnormal hyphal growth forms caused by sugar analogues can be found in normal hyphal cells which contribute to the tissues of complex fungal structures such as the basidiomycete carpophore. Experimental induction of these cell forms in vegetative culture by the sugar analogues shows promise for study of events which contribute to the morphogenesis of these fungal structures.

II. INTRODUCTION

Metabolic inhibitors are often employed to interfere experimentally with normal processes in order to produce a recognizable abnormality so that study of the abnormal will lead to a better appreciation of the normal. To be successful, such an approach must employ inhibitors which cause the smallest disturbance possible at their primary site of action. Otherwise, the very magnitude of the initial effect and its confusion by secondary phenomena are likely to militate against determination of the nature and consequences of the application of the inhibitor. Such inhibitors can be analogues of normal metabolites. Frequently, though, they have an unexpectedly far-reaching impact because of the close integration of metabolism. Nevertheless, analogues have considerable attractions, not least of which is the chemical variety which is often available. Irrespective of the type of technique used to follow the effects of any inhibitor, its metabolic influence is very much a consequence of molecular interactions between the inhibitor and its target functional molecule. To understand better the molecular events involved in the inhibiting reactions, it is useful to have available a variety of chemically diverse analogues, as these potentially enable the significance of different aspects of the molecular structure of the inhibitor to be assessed.

The dual requirement of close similarity to a central metabolite and chemical variety is amply satisfied by the family of hexose sugars. The metabolic significance of hexoses, both as nutrients and as biosynthetic intermediates, is well documented and the molecular structure of hexoses provides a considerable variety of chemical modifications, though comparatively few sugar analogues have been examined in detail for their effect on fungal growth. Because of its ability to impose a restricted colonial growth habit on *Neurospora* (Tatum, Barratt and Cutter, 1949), L-sorbose

is probably the most widely used hexose analogue, but much work has been done with 2-deoxy-D-glucose and some with 3-O-methylglucose and a few others. Although only a few fungal species have been studied, a comprehensive range of inhibitory effects have been recognized and, among mutants selected for resistance to these inhibitions, a number of distinct responses occur.

The inhibitions caused by sugar analogues have not been reviewed since the treatises on enzyme and metabolic inhibitors of Hochster and Quastel (1963) and Webb (1966), and no review has ever attempted to emphasize the response of fungi to these agents. Not only do they have a great deal to offer as general tools to probe carbohydrate and energy metabolism, and polysaccharide synthesis and function but also, for filamentous fungi in particular, they hold promise for investigating the part played by polysaccharide components of the wall in determining hyphal morphogenesis. In this review, data dealing with inhibitions by sugar analogues and resistant mutants are brought together in the hope that the potential value of these agents in further work will be illustrated.

III. Hexose structure

Hexose analogues usually exert inhibitory effects because they are metabolized in place of glucose; their molecular structures are sufficiently similar to that of glucose for errors to occur. However, their differences from the structure of D-glucose subsequently cause metabolic lesions which inhibit growth. Appreciation of the molecular structure of hexose sugars is thus essential to an understanding of inhibitory and resistance mechanisms alike. Although the molecule of D-glucose can assume a number of shapes in solution, its most stable, and therefore most usual, structure is the chair form of the pyranose ring shown in Figure 1. This is the C1 conformation in the terminology introduced by Reeves (1949, 1951). Details of the stereochemistry of sugars have been dealt with by Pigman and Horton (1972), Pigman and Anet (1972) and Angyal (1972).

The structure shown in Figure 1 is a six-membered heterocyclic ring with an oxygen (hemiacetal) link between the first and fifth carbon atoms of the six-carbon 'chain'. Each carbon atom in the ring contributes two of its bonds to the ring structure. This is fairly flat, and the two bonds not involved in ring formation are

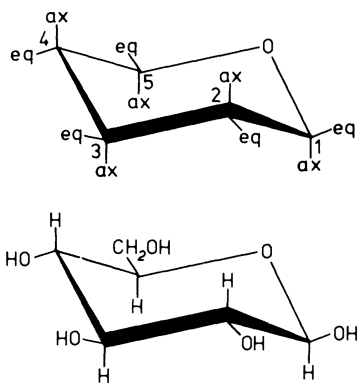

Fig. 1. Top: generalized structure of the C1 chair form of the pyranose ring. The five carbon atoms of the ring are indicated by number and the equatorial (eq) and axial (ax) bonds are identified. Bottom: the structure of β-D-glucopyranose.

directed one in the plane of the ring (described as equatorial) and one almost at right angles to the plane of the ring (axial). In β-D-glucopyranose, the form of the sugar which predominates in aqueous solution, all of the major substituents (hydroxyl groups on C-1, -2, -3 and -4, and a hydroxymethyl group on C-5) are arranged equatorially to the ring with the hydrogen atoms attached to the axial bonds (Fig. 1). Other aldose sugars can be related to this structure by simply indicating the manner by which they differ (Fig. 2 illustrates several). The hydroxyl group at C-2 is replaced by a hydrogen atom in deGlc, by an amino group in D-glucosamine and, in glucosone, by a ketonic group. In 3-O-methylglucose, the hydroxyl at C-3 is methylated. In D-galactose, the hydroxyl at C-4 is axial rather than equatorial while, in D-mannose, the C-2 hydroxyl is axial. For mannose, a secondary consequence is that the α-anomer predominates at equilibrium in aqueous solution as shown in Figure 2.

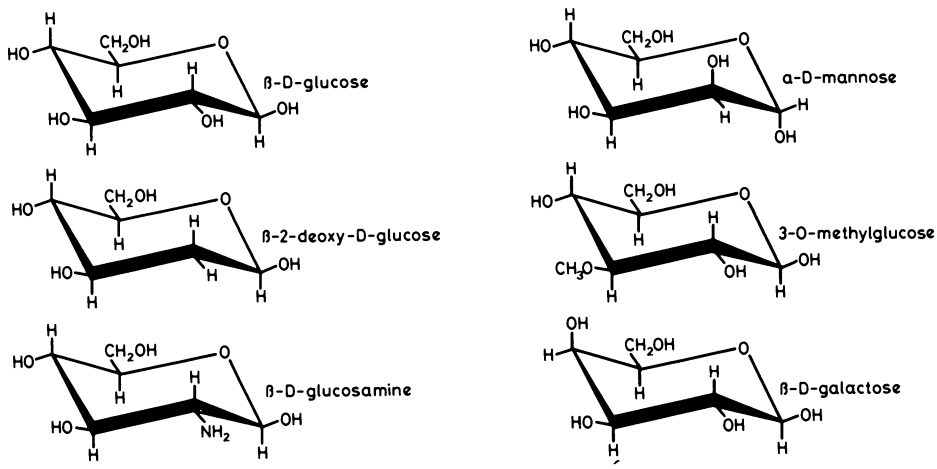

Fig. 2. Molecular structures of a selection of aldose sugars and sugar analogues.

It is not difficult to envisage the interaction between an analogue of an aldose and an enzyme which normally reacts with glucose. Such comparisons often emphasize the great specificity which can be displayed by enzymic and regulatory systems, Thus the different orientation of an hydroxyl group as in the glucose–galactose pair is a sufficient difference to enable discrimination between separate hexose transport systems in both *Aspergillus nidulans* (Mark and Romano, 1971) and *Saccharomyces cerevisiae* (Kotyk, 1967).

When ketose sugars like fructose and sorbose are considered, it is not immediately clear how their molecular conformations relate to enzymic and other proteins which normally react with glucose. Ketoses form pyranose rings, so again their structures can be compared directly with that of β-D-glucopyranose (Fig. 3). However, ring closure in ketopyranoses occurs between C-2 and C-6, so this ring structure can only be 'fitted' to the aldopyranose after inversion and rotation; i.e. the sixth carbon atom of the glucopyranose ring is equated, for the sake of diagrammatic comparison only, with C-1 of the ketopyranose, C-5 = C-2, etc. (Lardy, Wiebelhaus and Mann, 1950). The similarity between these diagrams is close and this ketopyranose conformation must play a part in any system involving fructose and sorbose in solution. However, such an interpretation makes it difficult to understand

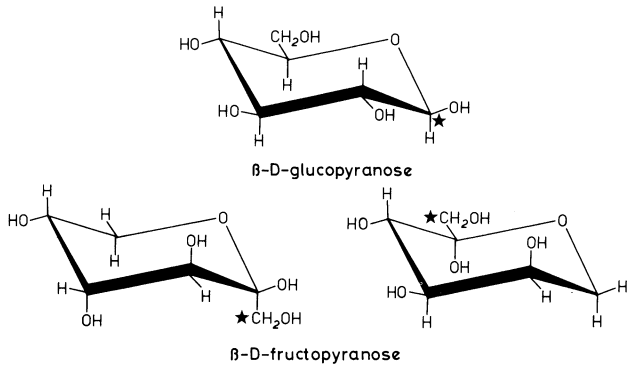

Fig. 3. Comparison of the pyranose ring forms of β-D-glucose and β-D-fructose. The fructopyranose ring is shown on the left in the conventional orientation and, in the right-hand diagram, the molecular structure has been inverted and rotated to present a view which allows a closer comparison with glucose. In each diagram, the C-1 carbon atom is indicated with a star.

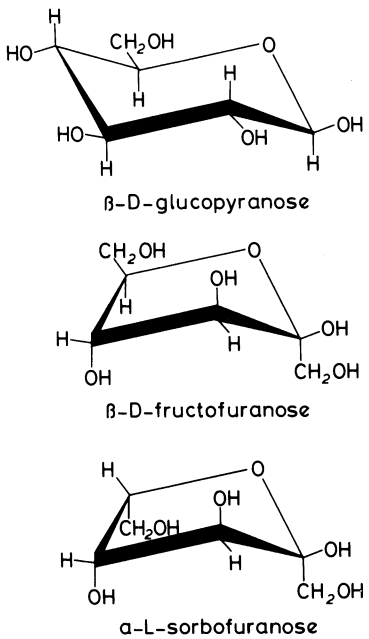

Fig. 4. Comparison of the pyranose ring form of glucose with the furanose forms of fructose and sorbose

some reactions involving specific carbon atoms, particularly the hexokinase reaction in which fructose 6-phosphate is formed. An alternative conformation of ketoses is the five-membered furanose ring. Indeed, it is in this form that fructose is always found when in combination in biological materials. Purich, Fromm and Rudolph (1973) accounted for the formation of fructose 6-phosphate by hexokinase by assuming that the enzyme acts on the furanose ring form of fructose. Figure 4 shows the comparison between β-D-glucopyranose and furanose ring forms of fructose and sorbose.

IV. INHIBITIONS BY SUGAR ANALOGUES

1. *Sensitivity to inhibition*

The overall effects of sugar analogues on fungal growth can be readily assessed by measurements of hyphal growth rates of filamentous fungi and by turbidimetric methods with yeasts. Sensitivities to inhibition observed for different organisms are very variable. The basidiomycete, *Coprinus cinereus* (often incorrectly called *C. lagopus* or *C. macrorhizus*), with which I have mainly worked is very sensitive to inhibition by hexose analogues and is almost restricted to using glucose as a carbon source (Moore, 1969a). It is intolerant of deviation from the β-D-glucopyranose structure. This leads to some contrasts between *Coprinus* and other fungi. For instance, while it is not surprising to find that its hyphal growth is inhibited by deGlc with an ED_{50} (concentration causing 50% inhibition of growth rate) of 0·05 mM and by sorbose (ED_{50} = 1·5 mM), it is unusual that hyphal growth is inhibited by galactose (ED_{50} = 10 mM) and glucosamine (ED_{50} = 0·2 mM). Only about 20% of the species surveyed by Lilly and Barnett (1953) were unable to use galactose, but it is generally a poorer carbon source than glucose. In some cases, good growth occurs in mixtures of galactose with other poor carbon sources (Steinberg, 1939). There are few reports of toxicity although Horr (1936) reported that, when used alone, galactose decreased rates of spore germination and mycelial development, and caused abnormal hyphal growth in *Aspergillus niger* and *Penicillium glaucum*. Similar effects have been reported for mixtures of galactose with fructose or glucose for *Podospora anserina* (Lysek and Esser, 1971). Glucosamine is similarly variable in effect, serving as sole source of carbon and nitrogen in *Aspergillus nidulans* yet being inhibitory to *Coprinus*. To some extent, such differences may be related to the ecology of the different organisms. The soil fungus *A. nidulans* grows in an environment in which 5 to 40% of the total nitrogen occurs in the form of polymers of aminosugars (Bremner, 1967), so it might be expected to make some use of these materials. On the other hand, *C. cinereus* grows in conditions where cellulose is by far the most important carbohydrate (Chang, 1967; Chang and Hudson, 1967). However, similar variations in sensitivity have also been observed with unnatural sugar analogues to which such ecological interpretations are unlikely to apply. Sorbose, for example, is rarely found in nature; it does not occur in the free form but is found in the pectin of *Passiflora edulis* (Martin and Reuter, 1949). Its occurrence in the fermented juice of *Sorbus* berries is a secondary feature resulting from bacterial metabolism (Schaffer, 1972). Although the growth of many fungi is inhibited by sorbose (Barnett and Lilly, 1951), some are able to utilize it either as their sole carbon source or synergistically in the presence of glucose or maltose (Lilly and Barnett, 1953). A similar spectrum of effects, complete inhibition in some cases contrasting with ability to utilize the analogue in other cases, has been observed for sorbose in studies of different genera of basidiomycetes (Oddoux, 1952), different species of *Phyllosticta* (Bilgrami, 1963) and different monosporidial isolates of *Ustilago maydis* (Matsushima and Klug, 1958). *Agaricus macrosporus* and *Neurospora* can metabolize sorbose, the latter apparently via D-glucitol (sorbitol), but only after a long period of adaptation (Bohus, 1967; Crocken and Tatum, 1968). *Pyrenochaeta terrestris* also grows slowly with sorbose as sole carbon source but the final yield of mycelium was only 20% less than that obtained with other hexoses and the mycelium was found to contain sorbitol in addition to the usually present mannitol (Wright and Le Tourneau, 1965). It is therefore impossible to generalize or confidently predict the likely response of any isolate to exposure to sorbose. Fewer organisms can utilize deGlc

but *Neurospora*, *Aspergillus spp.*, *Glomerella*, *Cunninghamella*, *Chaetomium*, *Neocosmospora* and *Xylaria* all have at least some ability to use this analogue as a sole source of carbon, though growth is generally poor and, in some cases, such high concentrations of analogue have been employed that cross-feeding through contamination of the analogue by readily metabolized sugars cannot be ruled out (Ruiz-Amil, 1959; Sols, Heredia and Ruiz-Amil, 1960; Atkin, Spencer and Wain, 1964; Moore, 1969b).

2. Mechanisms of inhibition: 2-deoxy-D-glucose and related sugars

Inhibitory sugar analogues seem to influence metabolism in two broad areas; in the initial stages of carbohydrate metabolism (energy-yielding metabolism) and in stages of polysaccharide synthesis. The potential sites involved in inhibition by deGlc are: (i) interference with sugar uptake, (ii) inhibition of hexokinase and (iii) inhibition of phosphoglucose isomerease by deGlc-6P. The latter compound is also likely to affect enzymes at other stages of glycolysis and in other pathways, and the fact that it is not further metabolized also has implications in terms of the depletion of pools of phosphate and ATP. Effects under these general headings occur in a wide range of cell types including yeasts and filamentous fungi. However, a great deal of work has been done with animal cells because early observations on the inhibition of metabolism in tumour cells in the rat (Woodward and Cramer, 1952) generated interest in deGlc as a potentially carcinostatic agent. Oddly, the similar observations for the effect of D-glucosamine on mouse tumours (Quastel and Cantero, 1953) have not been followed up so assiduously.

Interaction between utilizable sugars and their analogues at the transport step is indicated by the ability of the former to reverse inhibitions of growth caused by deGlc. In almost all cases in which the comparison has been made, inhibition is much more readily achieved on fructose-based media than on those containing glucose. Thus, in yeast, there was a two- to threefold difference in sensitivity (Heredia, de la Fuente and Sols, 1964) and about a tenfold difference among most of the filamentous fungi tested by Moore (1969b). *Coprinus cinereus* was exceptional in this latter study in having a 100-fold difference in sensitivity depending on whether the medium contained fructose or glucose. These effects are interpreted as being due to the relative abilities of glucose and fructose to inhibit uptake of the analogue into the cell. The differential sensitivities fall into patterns which reflect the affinities of transport mechanisms for the different sugars. In *Coprinus*, the transport carrier has a 100-fold greater affinity for glucose than for fructose (Moore and Devadatham, 1979) and, in yeast, there is a five- to tenfold difference in affinity (Kotyk, 1967; Cirillo, 1968). Clearly, in these organisms, fructose will be less able than glucose to inhibit uptake of inhibitory analogues. Conversely, the analogues must inhibit uptake of metabolizable sugars not only by their presence as competitors on the outside of the membrane but also through the effects of their intracellular accumulation on the regulation of sugar transport (Heredia, de la Fuente and Sols, 1964). Direct studies of mechanisms of transport, often employing sugar analogues for technical convenience, clearly demonstrate the inhibitions of uptake which can occur between different sugars and, in at least one case, the impact of metabolite transport on later stages of metabolism has also been explored (Höfer and Dahle, 1972). However, it is doubtful whether the inhibition of uptake contributes greatly to the overall inhibition of growth since, in most cases, the analogues cause considerable inhibitions of growth on media containing non-carbohydrate sources of carbon such as acetate or glycerol. An exception seems to be 6-deoxy-6-fluoro-D-glucose which appears to inhibit oxidation of glucose

in yeast prior to phosphorylation at a step suggested to be involved in the entry of sugar into the cell (Blakley and Boyer, 1955). The same conclusion was reached in studies involving animal tissues in which neither acetate nor lactate oxidation was sensitive to inhibition by this analogue (Serif and Wick, 1958). At high concentrations, inhibitions by 6-deoxy-6-fluoro-D-glucose reached maxima which differed from tissue to tissue but never reached 100%, suggesting that this analogue acts very specifically on only some of the routes by which sugar enters the cells (Serif *et al.*, 1958). If this is true, then it might be very useful for studying transport processes, particularly in those cases where multiple uptake systems differing in substrate specificity occur. Generally, though, interactions at the transport level are probably more important in relation to the ability of the normal metabolite to protect against the toxicity of the analogue. As already indicated, if the normal metabolite is a sugar it will protect in proportion to the affinity of the transport carrier and to its own ability to compete with the analogue for transport. An interesting exception occurs in *Aspergillus nidulans*, where acetyl coenzyme A exerts a regulatory influence on hexose transport (Romano and Kornberg, 1968, 1969; Desai and Modi, 1974, 1977). A consequence is that acetate protects against the toxicity of the sugar analogue such that, in acetate media, 50-fold higher concentrations of sorbose or deGlc are required to produce inhibitions equivalent to those produced with glycerol as carbon source (Elorza and Arst, 1971). This mechanism does not operate in *Coprinus*, and the greatest degrees of inhibition of growth of this organism are observed with acetate as carbon source (Moore and Stewart, 1972).

The hexokinase of yeast, *Neurospora* and *Aspergillus* readily phosphorylates deGlc (Sols *et al.*, 1958, 1960; Ruiz-Amil, 1959; Heredia *et al.*, 1964; Biely and Bauer, 1967). Yeast hexokinase does not phosphorylate either 4-deoxy-D-glucose or 6-deoxy-D-glucose (Kotyk *et al.*, 1975). Table 1 shows some data for the *Coprinus* enzyme. The similarities of affinity constants lead to considerable competitive inhibitions between the analogues and utilizable sugars for phosphorylation. It has been claimed that, for at least one strain of *Saccharomyces cerevisiae*, phosphorylation of deGlc is associated with its transport across the cell membrane (van Steveninck, 1968). There is considerable doubt about the generality of involvement of phosphorylation in sugar transport in yeast (Jennings, 1974; Kotyk and Janáček, 1975) and, even in this particular case, although van Steveninck (1968) showed deGlc-6P to be a precursor of intracellular free deGlc, intracellular

Table 1. *Kinetic constants of the hexokinase of* Coprinus cinereus *with different substrates*

Substrate	K_m (mM)	V_{max}	Phosphorylation coefficient
Glucose	0·107	264	1
2-Deoxy-D-glucose	0·410	263	0·26
Glucosamine	0·087	103	0·49
Fructose	2·858	181	0·03

The enzyme was partially purified by fractional precipitation with ammonium sulphate and assayed using a coupled system which detected formation of ADP. V_{max} is given in units of nmol product min^{-1} (mg protein)$^{-1}$. Phosphorylation coefficient calculated according to Sols & Crane (1954). Sorbose was completely inert as a substrate. Unpublished data of S. J. Taj-Aldeen.

hydrolysis of the phosphate was also demonstrated, so hexokinase activity remained of great importance for the accumulation of deGlc-6P. A related analogue, glucosone, inhibits fermentation and growth of yeast largely by its ability to inhibit activity of hexokinase (Mitchell and Bayne, 1952; Woodward, Cramer and Hudson, 1953; Hudson and Woodward, 1958). Sorbose is not phosphorylated, but some unphosphorylated sugar analogues show inhibitory activities even *in vitro;* for instance, 5-thio-D-glucose at concentrations of 0·25 mM and above completely inhibits activity of commercial preparations of mushroom tyrosinase (Prabhakaran, 1976).

However, it is clear from work with yeast and animal tissues that *in vivo* inhibition by deGlc is conditional on its phosphorylation (Heredia *et al.*, 1964). Phosphohexose isomerase and glucose 6-phosphate dehydrogenase are particularly sensitive to inhibition by deGlc-6P (Barban and Schulze, 1961). Glucosamine exerts a similar pattern of effects; it is readily phosphorylated by brain hexokinase (and see Table 1) and inhibits phosphorylation of glucose (Harpur and Quastel, 1949), while the glucose 6-phosphate dehydrogenase of yeast is competitively inhibited by glucosamine 6-phosphate (Glaser and Brown, 1955).

Formation of the 6-phosphate is probably the limit of analogue metabolism in most cases but there are other possibilities. 5-Thio-D-glucose and its 6-phosphate are, respectively, substrates for hexokinase and glucose 6-phosphate dehydrogenase of yeast, though the rate with the latter is only 5 % that with glucose 6-phosphate (Burton and Wells, 1977). The glucose oxidase of *Aspergillus niger* oxidizes deGlc at about 20 % of the rate observed with glucose; surprisingly, mannose was oxidized at only 1 % and galactose at only 0·5 % of the glucose rate (Pazur and Kleppe, 1964). Other glucose oxidases behave similarly (Sols and de la Fuente, 1957). Cell-free extracts of the polypore, *Trametes sanguinea*, oxidized sorbose although the sugar was not assimilated for growth. The purified enzyme was an L-sorbose oxidase which used substrate sorbose to form the products 5-keto-D-fructose and hydrogen peroxide. The metabolic function of the enzyme is unclear; fructose was inactive as a substrate although glucose, galactose, xylose and maltose were oxidized (Yamada *et al.*, 1966, 1967). There is evidence for sorbose metabolism in other fungi although, as will be discussed later, there is no evidence for the formation of sorbose phosphates. Galpin, Jennings and Thornton (1977) reported that mycelia of *Dendryphiella salina* exposed to randomly isotopically labelled sorbose for 48 h converted about 9 % of the available ^{14}C to $^{14}CO_2$. In *Neurospora*, Crocken and Tatum (1968) claim that sorbose is eventually converted to glucose with sorbitol as an intermediate, sorbose being utilized for growth when the medium also contains sucrose (Trinci and Collinge, 1973). Elorza and Arst (1971) have also demonstrated the formation of sorbitol from sorbose in *Aspergillus nidulans;* however, in this case, the evidence showed that ^{14}C introduced as sorbose eventually appeared as fructose. Conversion of sorbose to sorbitol is also evident in *Pyrenochaeta terrestris* (Wright and Le Tourneau, 1965). Although these reactions provide a means whereby sorbose can be detoxified, only a small fraction of the applied sorbose is usually metabolized in this way; the bulk of it remains unaltered.

An inducible coenzyme-dependent glucose dehydrogenase able to act on deGlc has been reported in *Aspergillus oryzae* (Ruiz-Amil, 1959) and two similar dehydrogenases have been found in *Pseudomonas aeruginosa* (Williams and Eagon, 1959). One of these latter enzymes could not be separated from glucose dehydrogenase while the other oxidized deGlc but not glucose and may have been an

expression of activity of a deoxyribose dehydrogenase. In neither case was the deoxygluconate formed metabolized further. In *Leuconostoc mesenteroides*, an enzyme able to oxidize deGlc-6P using NADP as coenzyme has been demonstrated. This enzyme activity was separable from glucose 6-phosphate dehydrogenase but 2-deoxy-D-gluconate 6-phosphate was not further metabolized (deMoss and Happel, 1955). No such activity could be found in *Neurospora* or *A. oryzae* (Sols *et al.*, 1960; Ruiz-Amil, 1959).

It seems that the most usual pathway for metabolism of deGlc leads, through hexokinase, to the formation of the 6-phosphate which, as it is not further metabolized, accumulates and inhibits early glycolytic enzymes. Among enzymes which deGlc-6P inhibits are phosphohexose isomerase, phosphofructokinase and aldolase (evidence mostly from animal cells; Webb, 1966). Both phosphoglucose isomerase and phosphomannose isomerase of yeast are sensitive to deGlc-6P, and inhibition of their activities *in vivo* causes drastic alteration to the balance of carbohydrate metabolism (Kuo and Lampen, 1972). Inhibition of enzymes by deGlc-6P is evidently an important aspect of the overall growth inhibition caused by this analogue but it must also be significant that formation of the 6-phosphate both depletes cellular supplies of ATP and impairs the metabolic ability to replenish those supplies. This aspect can have wide-ranging effects, since any part of metabolism which is regulated by the energy charge or is dependent on nucleotide pools will be influenced by changing levels of adenine nucleotides. Much of the evidence that such changes can be extremely dramatic again comes from work with animal tissues (Webb, 1966); one example is worth description. Within 12 min of first exposure to deGlc, Krebs ascites cells lose 65% of their adenine nucleotides; the loss proceeds through ATP, ADP and AMP, but the latter is then deaminated and the resulting inosine monophosphate dephosphorylated so that inosine appears within 1 min and, by the 12th min, inosine is the predominant 250 to 260 nm absorbing compound in the cell extracts (McComb and Yushok, 1964a, b). No fungal cell type has been examined in as great detail as have animal cells, but this drastic effect on purine nucleotide metabolism is probably a general one. Accumulation of deGlc-6P in *Saccharomyces cerevisiae* [and 2-deoxy-D-galactose 1-phosphate in *Kluyveromyces marxianus* (= *S. fragilis*)] was balanced quantitatively by simultaneous decreases in levels of ATP, orthophosphate and polyphosphate (van Steveninck, 1968; Jaspers and van Steveninck, 1976). In yeast exposed to 33 mM deGlc for 6 h, the concentration of inosine (not detectable in glucose-grown cells) increased until it represented about 60% of the total purines in the cell extract. There was a concomintant drop in the level of adenine nucleotides, ATP in particular being reduced to a level only 13% of that in cells exposed to glucose. Indications of accumulation of hypoxanthine in the yeast when incubation with deGlc was prolonged suggest that the cells embark upon full-scale purine degradation (Oppenheim and Avigad, 1965). Another study has illustrated the rapidity with which deGlc affects phosphate and purine nucleotide status (Maitra and Estabrook, 1962). Addition of ethanol to starved yeast leads to an immediate increase in ATP levels which is mirrored by reduction in amounts of both ADP and inorganic phosphate. Addition of 20 mM deGlc causes a rapid decline in both ATP and inorganic phosphate. Within 1 min of the addition of deGlc, the phosphate level is reduced to about 30% of its initial value and ATP to about 50% of its starting concentration. In the same period, ADP levels more than double. Under these conditions sufficient phosphate can be trapped in deGlc-6P to make respiration limited by phosphate availability even though ADP

levels remain high (Maitra and Estabrook, 1967). Six minutes after addition of deGlc, both inorganic phosphate and ATP declined to between 10 and 15% of the levels existing at the time the analogue was first administered, ADP levels also began to decline as, presumably, nucleotide degradation was initiated (Maitra and Estabrook, 1962). Anaerobic fermentation is stimulated in yeast. In dialysed extracts, a fermentation of fructose 1,6-bisphosphate which is independent of adenosine phosphates is stimulated by deGlc. In intact cells under anaerobic conditions, production of CO_2 was enhanced by deGlc by a factor of about four, endogenous glycogen stores being fermented to alcohol and CO_2. In the suggested pathway, hexose phosphates take the place of adenine nucleotides as phosphate acceptors (Scharff, Moffitt and Montgomery, 1965; Scharff and Montgomery, 1965, 1966). A transient stimulation of oxygen consumption occurs in both yeast and tumour cells very soon after first exposure to deGlc which is presumably a response to the rapid change in energy charge resulting from phosphorylation of the sugar (Feldheim, Augustin and Hofmann, 1966; Coe, 1968). Most metabolic processes, however, are inhibited. In ascites cells, contents of nucleotide triphosphates other than ATP also decline following treatment with deGlc (Letnansky, 1964) and, in consequence, rates of synthesis of both DNA and RNA can be greatly reduced (Klenow, 1963). Inhibitions of oxidation of Pyruvate, lactate, acetate and palmitate in yeast have been ascribed to depletion of ATP by deGlc (Feldheim *et al.*, 1966; Sauermann, 1968). On the other hand, inhibition of ethanol oxidation is thought to be due more to direct enzyme inhibition by deGlc-6P than to lack of ADP or phosphate (Feldheim *et al.*, 1966).

Recent work with baker's yeast has revealed what may be considered to be a detoxification reaction for deGlc-6P. After 60 min incubation with isotopically labelled deGlc, the bulk of the intracellular radioactivity is associated with 2,2′-dideoxy-α,α′-trehalose rather than with deGlc or its 6-phosphate (Farkaš, Bauer and Zemek, 1969; Meredith and Romano, 1977). A trehalose-like derivative has also been found within cells of *Kluyveromyces marxianus* exposed to 2-deoxy-D-galactose (Jaspers and van Steveninck, 1976). Large-scale synthesis of trehalose is a normal accompaniment of sugar uptake in yeast (Kotyk and Michaljaničová, 1974) so the synthesis of dideoxytrehalose on exposure to deGlc is another instance of the analogue being used metabolically in place of glucose; disaccharide synthesis in itself is not a pathological response to exposure to deGlc. Synthesis of disaccharide analogues is not restricted to yeast. β-D-Fructofuranosyl-2-deoxy-D-glucose has been isolated from leaves of several plant species following 2 days incubation with 50 mM deGlc (Barber, 1959). The same disaccharide was obtained *in vitro* when leaf homogenates of *Impatiens sultani* were incubated with deGlc and sucrose in conditions favouring invertase activity. In this disaccharide, the glycosidic bond occurred at either C-5 or C-6 of the deGlc moiety, so this was not a sucrose analogue (Barber, 1959). However, *in-vitro* synthesis of 'deoxysucrose' has been demonstrated with a sucrose synthetase preparation from pea seedlings (Farkaš, Biely and Bauer, 1968). The only report of deGlc in nature is in the form of a glycoside in *Erysimum* (Kowalewski, Schindler and Reichstein, 1960). The dideoxytrehalose in yeast represented the major portion of partially metabolized intracellular deGlc, but energy was still expended continuously to maintain an intracellular pool of deGlc-6P (Meredith and Romano, 1977). Diversion of deGlc into the disaccharide will provide some alleviation of phosphate sequestration but continued utilization of ATP will be damaging.

In their review of the effect of mannose and its analogues on green plants, Herold

and Lewis (1977) argue 'that the primary cause of almost all of the inhibitory effects of mannose, deoxyglucose and glucosamine in green plants is the synthesis and limited further metabolism of their phosphate esters'. The rapid effect exerted by deGlc on phosphate and purine nucleotide balances must make this a central feature of any growth inhibition caused by this sugar. However, it is unlikely that all of the inhibitory effects in fungi are due to this cause since, firstly, the inhibition of growth is generally much greater than any inhibition of respiration or glycolysis that can be measured and, secondly, there are some sugar analogues which are potent inhibitors of growth in filamentous fungi but which are not phosphorylated.

Fermentation in *Saccharomyces cerevisiae* is inhibited less than 50% by a 1:0·3 molar ratio of glucose:deGlc, whereas growth is inhibited more than 50% at a 1:0·05 ratio (Heredia *et al.*, 1964). These authors concluded that inhibition of growth by deGlc was not a direct consequence of the demonstrated inhibition of glycolysis, because quite high growth rates were obtained when disaccharide fermentation was considerably inhibited by other unfavourable conditions such as adverse pH values and concentrations of substrate. Similar results have been obtained with *Coprinus cinereus*. In this organism, mixtures of glucose + deGlc and fructose + deGlc which caused at least 50% inhibition of hyphal growth had very little effect on oxygen uptake. Even in media containing 5 mM deGlc as sole carbon + energy source, oxygen uptake was only 75% inhibited although linear growth did not occur (Moore, 1968). Clearly, factors other than inhibition of energy-yielding metabolism must contribute to inhibition of cell growth, and there is now convincing evidence that much of the inhibitory activity of deGlc concerns synthesis of polysaccharide.

It is well established that deGlc adversely affects cell wall synthesis in yeast. This is undoubtedly the basis of the fungicidal action that this compound has on yeasts, since sensitivity was proportional to the mean rate of extension of the individual yeast cells (Johnson and Rupert, 1967); only growing cells were killed (Heredia *et al.*, 1964) and fragments of cell wall were found in the treated suspensions (Megnet, 1965). Lysis occurred at points coinciding with the regions of growth of the glucan layers of the yeast cell wall (Johnson, 1968a). The major consequence to growth in deGlc is the osmotic fragility resulting from weakening of the cell walls. Erosion of preformed glucan seems to be a likely component of the weakening process. Johnson (1968b) showed that the amounts of glucan in cultures of *Schizosaccharomyces pombe* decreased after about 1 h exposure to deGlc. Svoboda and Smith (1972) distinguished early lysis, caused by degradation of wall material at the growing sites, from late lysis caused by deposition of aberrant material. However, the latter authors showed that deGlc was not incorporated into the walls of the fission yeast, although incorporation has been demonstrated into wall glucan of *Saccharomyces cerevisiae* (Biely *et al.*, 1971; Krátký, Biely and Bauer, 1975). Rather than being due to inclusion of the analogue, general weakening of the wall is thought to be largely the consequence of interference by phosphorylated metabolites of deGlc with metabolic processes involved in the incorporation of glucose and mannose into structural wall polysaccharides (Heredia *et al.*, 1964; Farkaš, Svoboda *et al.*, 1969; Biely *et al.*, 1971). The phosphorylated metabolites include both uridine and guanosine nucleotide derivatives (Biely and Bauer, 1966, 1968; Jaspers and van Steveninck, 1976). It is generally considered that, by trapping these nucleotides into a metabolically unusable form, deGlc (and 2-deoxy-D-galactose) is able to block synthesis of structural polysaccharides (Heredia *et al.*, 1964; Biely and Bauer, 1968). Much the same seems to be true

of 2-deoxy-2-fluoro-D-glucose, although this compound acts more specifically as an analogue of glucose (deGlc is an analogue of both glucose and mannose) and in cosequence glucan synthesis is the most sensitive process (Biely, Kovařík and Bauer, 1973). Although glucono-δ-lactone also inhibits glucanase and glucan formation in the yeast, *Pichia polymorpha*, as well as causing inhibitions of RNA and protein synthesis, there was only a small effect on the growth rate. After 12 h incubation, up to 20% of the cell population were aberrant – abnormally large, spherical and lacking buds (Villa *et al.*, 1976). The authors conclude that this agent seriously affects cell wall development.

Such detailed analyses have not been attempted with any filamentous fungus but it seems likely that similar biochemical events occur after their exposure to deGlc. It inhibited glucan formation in *Aspergillus nidulans* (Zonneveld, 1973) and lysis of hyphal tip cells occurred in actively growing hyphae of *Coprinus cinereus* exposed to deGlc (Moore and Stewart, 1972). Although glucosamine did not cause lysis, the tip cells of *Coprinus* hyphae swelled enormously, probably owing to interference with wall synthesis. A characteristic and structurally important component of the fungal wall is chitin, a (1-4)-β-homopolymer of *N*-acetylglucosamine. At low substrate concentrations, glucosamine is an allosteric activator of chitin synthase in *Coprinus* while glucosamine 6-phosphate is an inhibitor (de Rousset-Hall and Gooday, 1975). Wall growth is thought to depend on a balance between lysis of existing chitin fibrils by chitinase and synthesis of new chitin by the synthase (Gooday, 1975, 1977; Gooday, de Rousset-Hall and Hunsley, 1976; Gooday and Trinci, 1980) and it is possible that the swelling observed in *Coprinus* hyphae exposed to glucosamine results from disturbance of the lysis/synthesis balance. That this may well be the case is indicated by work with a temperature-sensitive mutant of *Aspergillus nidulans* in which the hyphal tips were osmotically unstable at 42 °C (Cohen, Katz and Rosenberger, 1969). The osmotic instability was caused by a lack of chitin in the cell wall, but this defect could be reversed by supplementing the growth medium with glucosamine (Katz and Rosenberger, 1970). Rather similar results have been reported for a mutant of *Saccharomyces cerevisiae* defective in glucosamine 6-phosphate ketol-isomerase. In this case, the lesion in chitin synthesis resulted in defective septation so that, in the absence of supplementation with glucosamine, vegetative daughter cells failed to separate. Ascospore walls were also abnormal, lacking a glucanase-resistant hydrophobic surface layer. Viability of vegetative cells decreased after 3 to 5 h incubation without glucosamine because of lysis in the highly disorganized septum region (Ballou *et al.*, 1977). Such observations emphasize the direct involvement of glucosamine in chitin synthesis and indicate the inability of other aspects of hyphal metabolism to compensate for imbalance in supply of the amino sugar. Similar work with an *A. nidulans* mutant in which the wall defect was relieved by growth on mannose (Valentine and Bainbridge, 1978) shows that synthesis of other wall polysaccharides is equally sensitive to the level of supply of their constituent sugars and again indicates that, in construction of the total wall, there is no ability to compensate for defects in any one of the wall components.

As far as non-wall polysaccharides are concerned, glucosamine and its *N*-acetyl derivative both inhibited conversion of glucose or fructose to glycogen by rat liver slices, probably via inhibition of glucose phosphorylation (Spiro, 1958). The reduced net accumulation of glycogen may result from acceleration of glycogen breakdown rather than a true inhibition of synthesis (Webb, 1966). Synthesis of glycogen in hepatoma cells is also inhibited by deGlc (Nigam, 1966) and it is

certainly the case that this analogue stimulates degradation of glycogen in yeast (Farkaš, Svoboda & Bauer, 1969). In this latter case, though, deGlc was not incorporated into glycogen *in vivo* despite the facts that an *in vitro* enzyme system can incorporate deGlc into some outer chains of the glycogen primer (Biely, Farkaš and Bauer, 1967) and incorporation into glycogen *in vivo* had been demonstrated in hepatoma cells (Nigam, 1967).

There is also firm evidence that deGlc inhibits the synthesis of exocellular enzymes, particularly invertase and acid phosphatase, in *Saccharomyces cerevisiae* and *Pichia polymorpha* (Krátký *et al.*, 1975; Kuo and Lampen, 1972; Villa *et al.*, 1975). There is a distinct difference of opinion about the basis of this inhibition in *S. cerevisiae*. Kuo and Lampen (1972) showed that the synthesis of invertase and acid phosphatase is inhibited by deGlc under conditions which cause only slight decreases in general protein synthesis. They suggest that deGlc-6P inhibits or restricts synthesis of the carbohydrate component of these glycoproteins and thereby reduces formation of the active extracellular enzyme. On the other hand, Krátký *et al.* (1975) could find no correlation between inhibitions of mannan synthesis and the appearance of invertase and acid phosphatase. They suggest that the effect of deGlc on the secretion of exoenzymes is complex but is related more to inhibition of synthesis of the protein component than to effects exerted on the carbohydrate moiety.

3. *Mechanisms of inhibition: sorbose and 3-O-methylglucose*

Sorbose causes what is probably the best known and most widely employed morphological change in the growth of susceptible filamentous fungi. The extension growth rate is reduced, cells are much shortened, branching frequency is increased with the enhanced occurrence of branched tips. Although sorbose has been much used to impose colonial growth morphology on rapidly growing filamentous fungi, very little is known about the enzymological aspects of these effects.

Hexokinase and aldolase of beef brain are inhibited by sorbose 1-phosphate but not by sorbose and its 6-phosphate (Lardy *et al.*, 1950). Sorbose 1-phosphate and the 1,6-bisphosphate behaved as competitive inhibitors of yeast aldolase, with K_i values of the same order of magnitude as the K_m for fructose 1,6-bisphosphate (Richards and Rutter, 1961) but sorbose 1,6-bisphosphate was not used as a substrate. However, no evidence has been obtained for phosphorylation of sorbose in fungi although formation of sorbose 1-phosphate by ketohexose kinases has been detected in mammalian systems (Hers, 1952; Cadenas and Sols, 1960).

In baker's yeast, sorbose is inert both as substrate and inhibitor of hexokinase (Sols *et al.*, 1958). The same is true for brain hexokinase under conditions able to detect phosphorylation rates less than 10^{-4} that of glucose (Sols and Crane, 1954). In *Aspergillus parasiticus*, the rate of phosphorylation of sorbose by hexokinase was no more than 2% that of glucose (Davidson, 1960). These studies leave open the possibility that ketokinases may exist. Indeed, as sorbose 1-phosphate formation has been observed *in vitro* by the action of crystalline rabbit muscle aldolase on hexose bisphosphate and L-glyceraldehyde (Lardy *et al.*, 1950), alternative modes of synthesis of sorbose phosphates may be real possibilities. It is particularly important, therefore, to note that several unsuccessful attempts have been made to extract sorbose phosphates from the cell contents. Most of the relevant work with *Neurospora* concerned sugar transport in that organism and, although there are many contradictions with regard to the sorts of transport systems

involved and their kinetic characters, there is unanimity that no phosphorylated forms of sorbose can be found in cell extracts (Klingmüller, 1967a, b; Crocken and Tatum, 1968; Scarborough, 1970). The same is true for *Aspergillus nidulans* (Elorza and Arst, 1971) and *Coprinus cinereus* (S. J. Taj-Aldeen, unpublished). In sharp contrast to deGlc, sorbose evidently exerts its inhibitory actions while chemically unchanged.

Although Crocken and Tatum (1967) claimed that sorbose was not accumulated against a concentration gradient in *Neurospora*, Klingmüller (1967a) reported its accumulation in this organism to more than 600 times the external concentration, and Scarborough (1970) showed that the internal concentration of sorbose reached 300 mM after 30 min exposure of germinated conidia of *Neurospora* to 10 mM sorbose. It may be that the strain used by Crocken and Tatum (1967) was in some way defective in its sugar transport apparatus, since the K_m, V_{max} and mode of transport reported by these authors differ considerably from any hexose transport process reported subsequently for *Neurospora* (Klingmüller and Huh, 1972). Sorbose is accumulated by an active uptake system in *Aspergillus nidulans* (Elorza and Arst, 1971) and *Coprinus cinereus* (Moore and Devadatham, 1979) and, in the latter organism, accumulation can proceed until the internal concentration is more than three orders of magnitude higher than the external. The problem remains, however, of identifying the point of action of this inhibitor. Sorbose does not inhibit phosphorylation of fructose in *Neurospora* (Klingmüller, 1967a) and has no effect on either *in vivo* or *in vitro* activities of hexokinase, glucosephosphate isomerase, phosphoglucomutase, glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase or NADP-linked isocitrate dehydrogenase (Mishra and Tatum, 1972). Although particular enzyme inhibitions have not been identified in primary metabolism, glucose is used less efficiently by *Neurospora* mycelia in the presence of sorbose. Mycelial dry wt yield is lessened, more CO_2 is produced and more O_2 is absorbed per unit glucose used. Because of the many similarities between the effects of sorbose and of compounds known to uncouple oxidative phosphorylation and respiration. Crocken and Tatum (1968) suggested that sorbose causes such uncoupling.

The morphological changes which affect *Neurospora* hyphae grown on agar media containing sorbose are accompanied by changes in the chemical composition of the hyphal walls. The ratio of glucosamine to glucose in wall hydrolysates more than doubles (de Terra and Tatum, 1961, 1963) and there is a marked decrease in the concentration of cell wall β-1,3-glucan in sorbose-grown mycelia (Mahadevan and Tatum, 1965). The obvious inference from these data is that the paramorphic change induced by sorbose results from a considerable alteration in the balance of important polymeric constituents (probably chitin and glucan) of the cell wall. A very similar glucosamine to glucose ratio was found in hydrolysates of cell walls from colonial mutants grown on sucrose. These are mutants which, on sucrose media, have a morphology very similar to the paramorphic growth of the wild type on sorbose + sucrose media (de Terra and Tatum, 1963). Sorbose inhibited the *in vitro* and *in vivo* activities of glycogen synthetases and β-1,3-glucan synthetases of *Neurospora* (Mishra and Tatum, 1972). The latter enzyme was found only in cell-wall preparations, a feature which might account for the apparent localization of the sorbose effect to the *Neurospora* hyphal surface for, although the *Neurospora* colony has a characteristic morphology imposed upon it by sorbose, not all of the hyphae are equally affected. Aerial hyphae do not show the abnormal growth form of submerged and surface hyphae (de Terra and Tatum, 1961).

Moreover, hyphae injected with sorbose did not develop the abnormal morphology despite the fact that the injected concentration was much greater than that usually found in paramorphic hyphae (Wilkins, unpublished, quoted in de Terra and Tatum, 1961). Thus it seems that the sorbose effect in *Neurospora* might be localized at the cell surface. If the enzyme system is located in the wall, then an explanation for the differential sensitivity is evident. Trinci and Collinge (1973) have provided an explanation for the differential effect of sorbose on hyphae in different parts of the *Neurospora* colony which does not depend on localization of enzymes in the hyphal wall. They demonstrated that sorbose had little or no effect on the morphology of *Neurospora* strains grown in liquid cultures. On solid media (using three different gelling agents), radial growth rates of colonies were greatly reduced although sorbose had little effect on the maximum specific growth rate. The same is true for *Dendryphiella salina* (Galpin *et al.*, 1977). Trinci and Collinge (1973) stress that the most significant effect of sorbose in solid media is its ability to induce profuse branching, because this radically alters the spatial distribution of hyphae, producing dense colonies in which adverse conditions (nutrient limitation, accumulation of staling products, pH change, etc.) are established much closer to the colony margin than is normal. In liquid culture, and for aerial hyphae, no such localization of adversity can occur. Trinci and Collinge (1973) thus interpret the characteristic macroscopic effects of sorbose as being secondary and dependent on growth on solid media. The primary effect of sorbose observed in cultures grown in liquid and on solid media seems to be an increase in wall thickness. This alteration in wall structure presumably so interferes with hyphal morphogenesis (Moore and Stewart, 1972) that branching patterns on solid media change and precipitate the secondary consequences referred to by Trinci and Collinge (1973). Apart from noting that there is no large-scale incorporation of sorbose into the hyphal wall (de Terra and Tatum, 1961) and the inhibitory effect of sorbose on glucan synthetase already referred to (Mishra and Tatum, 1972), the manner by which sorbose leads to an increase in wall thickness is unknown. Neither is there any clear understanding yet of the relation between wall structure and hyphal morphogenesis.

As well as accounting for differential sensitivity between different parts of the fungal colony, this interpretation of Trinci and Collinge (1973) can account for the reported variability in the response of *Neurospora* to sorbose, which depended on the nature and manner of preparation of the growth medium (de Serres, Kolmark and Brockman, 1962; Brockman and de Serres, 1963). It can also account for the absence of growth inhibition in yeasts (Woodward, Cramer and Hudson, 1953) for these have been tested in liquid culture; it would be interesting to investigate the effect of sorbose on yeast growth on solid media. A paramorphic effect of sorbose on *Saccharomyces* spp. has been reported. Lewis (1971) showed that sorbose restored normal morphology to inositol-requiring strains which otherwise grew as pseudomycelia in medium containing maltose or α-methyl glucoside.

The fact that no paramorphic changes occur in liquid medium shows that morphological changes induced by sorbose do not result from any osmotic imbalance. It is worth noting, though, that similar paramorphic changes can be induced by osmotic shocks (Robertson, 1958, 1959). Sorbose and 3-O-methylglucose cause extensive bursting of hyphal tips in the marine fungus *Dendryphiella salina* (Thornton, Galpin and Jennings, 1976) and sorbose causes apical disintegration in a spore colour mutant of *Neurospora* (Rizvi and Robertson,

1965). The glucose analogue, 3-O-methylglucose, is not phosphorylated by hexokinase (Sols and Crane, 1954; Sols *et al.*, 1958) but is readily accumulated by many cell types; accumulated material in *Neurospora* was completely extractable in unaltered form (Scarborough, 1970). In *D. salina*, 3-0-methylglucose promotes loss of mannitol into the medium and conversion of this hexitol to polysaccharide. The decline in mannitol level cannot be compensated because 3-O-methylglucose inhibits hydrolysis of glycogen. These events contribute to loss of osmotic control by the hyphae (McDermott and Jennings, 1976).

Galpin *et al.* (1977) have concluded that sorbose and 3-O-methylglucose exert their effects by influencing the level of intracelluar cAMP. Although there is no direct evidence, this conclusion was reached because of the similarity between the effect of the sugar analogues and of theophylline [an inhibitor of the cAMP-dependent phosphodiesterase in *Neurospora crassa* (Scott and Solomon, 1973)] on colony diameter and hyphal branching in *Dendryphiella*, and on the synergism existing between these agents. The interpretation is essentially that the glucose analogues so reduce carbohydrate metabolism that cAMP levels are affected and processes regulated by the nucleotide balance are consequently disturbed. Although speculative, this interpretation focuses attention on the purine nucleotides, the metabolism of which is also severely affected by deGlc and glucosamine (see above). However, simple comparison between morphological or other effects is a very weak foundation for any interpretation and it should be remembered that, on the same basis, Crocken and Tatum (1968) concluded that sorbose might act as an uncoupler of oxidative phosphorylation. Furthermore, although change in the concentration of cAMP is clearly implicated in a number of morphological alterations in *Neurospora*, the data are too contradictory for useful generalization. Thus Mishra (1976) showed that high concentrations of cAMP in the medium (up to 6 mg ml^{-1}) induced colonial growth in both wild-type *N. crassa* and the sorbose-resistant mutant *patch*. Conversely, the morphological abnormality of the *crisp*-1 mutant, which is correlated with a deficiency of intracellular cAMP, is at least partially repaired by growth in medium containing cAMP (10 to 30 mM) or some cAMP analogues (at 1 mM) (Rosenberg and Pall, 1979); however, the *frost* mutant, which lacks adenylate cyclase activity, does not respond to supplementation with cAMP. Drugs like theophylline, atropine and histamine, which adversely affect cAMP metabolism, induce wild-type *N. crassa* to grow with colonial or semi-colonial growth habit in liquid medium (Scott and Solomon, 1975). The morphological change is correlated with an approximate 75% *decrease* in endogenous cAMP concentration. On the other hand, drugs which uncouple oxidative phosphorylation cause 3- to 20-fold *increases* in endogenous cAMP levels in *N. crassa*, yeast and *Mucor racemosus* (Trevillyan and Pall, 1979) and, although transport fluxes of sugars (including deGlc and 3-O-methylglucose) cause transient increases in cAMP level in *N. crassa*, the same response is obtained when the cells are mechanically stressed by vigorous shaking (Pall, 1977).

It remains extremely difficult to understand the primary metabolic effect of sorbose. The absence of the characteristic 'sorbose growth' morphology in liquid cultures of *Neurospora* (Trinci and Collinge, 1973) is a key observation which needs to be verified for other fungi and which offers the promise of a technique which might distinguish primary from secondary effects.

V. SUGAR ANALOGUE-RESISTANT MUTANTS

Mutants resistant to the inhibitions of growth imposed by a variety of sugar analogues have been isolated from a number of organisms. Although mutational alterations in wall and cell structure have been reported, most of the mutants obtained have proved to have defects in early metabolic stages, specifically those which influence the accumulation of sugar analogue and/or analogue phosphate within the cell. Judging by the mutants which have been described, there are three main ways in which the last effect may be achieved; uptake of the analogue may be altered, a defect in phosphorylation may arise or the phosphorylated sugar may be hydrolysed by mutant phosphatases rather than accumulated.

In *Neurospora crassa*, mutations in any one of six chromosomal genes can give rise to resistance to sorbose (Klingmüller, 1967c, d, e). All of the resistant mutants were recessive to wild type, but only four of the genes were involved in transport. The phenotypes of the mutants have not yet been well characterized; the functions of the two genes not involved in transport have not been identified at all. However, from analysis of double mutants, it has been concluded that the different transport mutants contribute neither to a compound permease nor to components of a single uptake pathway. Instead, it is thought that the product of each gene catalyzes the same process so that, rather like isoenzymes, they constitute a set of isopermeases (Klingmüller, 1967f). Presumably, when mutation occurs in one of these genes, it causes a defect in sorbose uptake which cannot be compensated by the normal functioning of the remaining unmutated genes. Resistance conferred by the different genes is additive at least to the extent that double mutants are more resistant to sorbose than the original single mutants. *Neurospora* mutants selected for resistance to inhibition of growth by sorbose do not appear to have been tested for cross-resistance to other sugar analogues. However, in both *Coprinus cinereus* and *Aspergillus nidulans*, mutants selected for resistance to sorbose, and identified as having transport defects, are cross-resistant to deGlc. The situation in *C. cinereus* has been the most extensively analyzed. Here, mutants resistant to inhibitions of growth caused by deGlc, glucosamine and sorbose have been separately selected. All are cross-resistant to the two analogues not used in their selection. Furthermore, all of the mutants were later shown to be able to grow only poorly with fructose as sole carbon source, whereas the wild type grows equally well with either glucose or fructose (Moore and Stewart, 1971; Moore, 1973). In *N. crassa*, Klingmüller (1967d) isolated only 19 sorbose-resistant mutants yet found that they represented six different genes. In sharp contrast, over 450 analogue-resistant mutants have been reported in *C. cinereus*, but they all appear to be allelic mutations of a single genetic locus (Moore and Stewart, 1971; Moore, 1973). The definition of allelism has been based both on complementation tests in which the complementing dikaryon should be unable to grow on medium containing the inhibitor (Klingmüller and Kaudewitz, 1967; Moore and Stewart, 1971) and on studies of allelic recombination (Moore and Devadatham, 1975). These *Coprinus* mutants had apparently normal abilities to metabolize sugars but were impared in the accumulation of sugars from the medium. They were therefore assumed to be transport defective and, because fructose was the only utilizable sugar with which the transport defect was apparent, the gene was called the fructose-transport (*ftr*) cistron (Moore and Stewart, 1971). Analysis of transport of hexose in the *C. cinereus* wild type (Moore and Devadatham, 1979) showed that a single negatively co-operative uptake system was responsible for accumulation

of glucose, fructose and the sugar analogues. It is presumed that the *ftr* gene product is the carrier (or permease) component of this system. Utilization of glucose may not be seriously impaired in the mutants because the affinity of the carrier for glucose is so high in the wild type (K_m = 27 μM) that a mutational alteration which changes the affinity, even if it causes a drastic alteration, will still leave sufficient residual function to support good mycelial growth on normal media. As possible support for this view, the affinity of the carrier for fructose (K_m = 2·3 mM) is about 100 times less than that for glucose in the wild type, yet the mycelium grows equally well on these two sugars. However, the system for transport of glucose in the wild type does exhibit substrate-dependent modifications of its activity, and it may be that understanding of the behaviour of the mutated gene product is complicated by the dual role (modulating ligand and substrate) of the glucose molecule. A sorbose-resistant strain of *Coprinus fimetarius* [= *C. radiatus sensu* Guerdoux (1974)] has been reported (Prévost, 1958). The mutant, called *sor*^{r}, was isolated following u.v.-mutagenesis. It was markedly different from the *C. cinereus ftr* mutants in that *sor*^{r} was dominant and showed a pronounced dosage effect in homozygous dikaryons. All *ftr* alleles are recessive. The *sor*^{r} mutant was used to study nuclear migration but no attempt has been made to establish the biochemical basis of the resistant phenotype.

Elorza and Arst (1971) described sorbose-resistant mutants of *Aspergillus nidulans* which conferred cross-resistance to deGlc. The locus to which these recessive mutations belonged was designated *sorA*. The mutants were defective in accumulation of sorbose but utilization of fructose, glucose, glucosamine and other sugars as carbon sources was unaffected and uptake of glucose only slightly depressed. It was concluded that the *sorA* gene product contributed to an uptake system which accounted for the greater part of sorbose transport but was relatively unimportant in glucose transport (Elorza and Arst, 1971). If this interpretation is correct, it is difficult to understand how cross-resistance to deGlc can arise. The system for transporting glucose into *A. nidulans* has been characterized by use of deGlc as a non-metabolized analogue (Mark and Romano, 1971). Sorbose did not inhibit uptake of deGlc or fructose by the strain used in this latter study, suggesting that, in *Aspergillus*, the normal route of uptake of sorbose is independent of the separate permeases for glucose and fructose. At present, it is impossible to identify the normal function to which *sorA* corresponds. This will only be possible when the normal process of sorbose uptake in *A. nidulans* is properly described.

Interest in selection of mutants resistant to sugar analogues in other organisms has concentrated on selection for resistance to deGlc. The mutants obtained have proved to have either altered phosphorylation or phosphatase activities. Resistant mutants of *Schizosaccharomyces pombe* obtained by selection on media containing deGlc were unable to utilize glucose, fructose or mannose and were deficient in hexokinase (Megnet, 1965). Maitra (1970) isolated a mutant of *Saccharomyces cerevisiae* lacking hexokinase by screening cells for resistance in galactose medium containing increasing concentrations (1, 10 and 50 mM) of deGlc. *S. cerevisiae* has three enzymes able to phosphorylate glucose: hexokinase P1, hexokinase P2 and glucokinase (Lobo and Maitra, 1977a). Defects in each of these activities have been selected by exposing strains carrying only one of the three enzymes to selection for resistance to deGlc (Lobo and Maitra, 1977b).

Another type of resistance to deGlc in *S. cerevisiae* resulted from the occurence of an intracellular neutral phosphatase which was able to hydrolyse deGlc-6P fairly specifically (Heredia and Sols, 1964). This mutant was also obtained by serial

cultivation in (fructose) media containing increasing concentrations (0·1 %, 1 % and 2 %) of the inhibitor. The purified enzyme hydrolysed only deGlc-6P and fructose 1-phosphate. It was present in wild-type cells but only at about 5 to 10 % of the levels found in the mutant. The normal physiological function of the enzyme is unknown (Martin and Heredia, 1977). A somewhat similar situation occurs in HeLa human tumour cells where a resistant cell line accumulated deGlc-6P at a much slower rate than the sensitive parental line (Barban, 1962). The resistant strain had 4- to 9-fold greater activity of alkaline phosphatase than the original cells. Subsequently, Barban (1966) demonstrated a correlation between induced resistance to deGlc and alkaline phosphatase activity by treating cell populations with hormones known to cause increases in activity of this enzyme. A similar correlation was observed by Morrow and de Carli (1967). Unlike the yeast phosphatase mutant, the phosphatase variant of HeLa cells had an impaired glucose metabolism. The phosphatase acted as a net inhibitor of the hexokinase reaction, presumably by hydrolysing the product, glucose 6-phosphate, and the growth of the resistant line was much improved by addition of pyruvate to the medium (Barban, 1962). An interesting type of resistance has been observed in pig kidney cells (Bailey and Harris, 1968). Here, too, resistant mutants were isolated by serial cultivation in the presence of deGlc. The resistant cells accumulated less deGlc-6P, but rates of phosphorylation of hexose were higher in extracts of resistant mutants and alkaline phosphatase was not detectable in extracts of either sensitive or resistant cells. There was some increase in acid phosphatase levels in resistant cells but this enzyme was unable to hydrolyse deGlc-6P or glucose 6-phosphate *in vitro*. Resistant cells grew better than the sensitive ones at low glucose concentrations, and it was concluded that the basis of the resistance was that resistant cells were able to proliferate at glucose concentrations too low to support growth of sensitive cells (Bailey and Harris, 1968). This type of resistance has not been observed in fungi.

Analysis of glucosamine resistance in *Saccharomyces cerevisiae* has revealed some mutants with altered response to catabolite repression resulting perhaps from a change in membrane permeability (Elliott and Ball, 1973). When excess glucose is added to yeast growing aerobically with glycerol as carbon source, growth is first repressed but soon recovers and accelerates as fermentation is initiated. Respiration is also repressed and, because of catabolite repression, remains so. Glucosamine and deGlc act as gratuitous repressors and completely inhibit growth. Elliott and Ball (1973) isolated resistant mutants by selecting on medium containing 3 % glycerol and 0·05 % glucosamine. Among 34 mutants recovered, there were at least seven complementation groups. All but one of the mutants were recessive and three were cytoplasmic. Some, but not all, were cross-resistant to deGlc (Elliott and Ball, 1973; Ball, Wong and Elliott, 1976). Further analysis has concentrated on the cytoplasmic mutations, all of which showed altered colony and cell morphology. The resistant mutants produced rosettes of elongated pseudomycelial cells rather than the single budding cells of the wild type. This altered morphology was maintained in the presence and absence of glucosamine. One of the cytoplasmic mutations (designated *cry 1-r* for catabolite-repression-resistant yeast) was not cross-resistant to drugs which inhibit mitochondriogenesis and was assumed to be a mitochondrial mutation (Elliott and Ball, 1975). However, in a later study, these resistant mutations were crossed against strains carrying known mitochondrial markers and the results indicated that the glucosamine-resistant mutations were not located on mitochondrial DNA. Whether these mutations have identified a

distinct yeast plasmid is not yet known but their resistant phenotype apparently arises through some alteration in permeability to sugars (Kunz and Ball, 1977) and the π episome is thought to have some role in determining membrane architecture or function (Guerineau, Slonimski and Avner, 1974).

There remain a number of analogue-resistant mutations which are either less well understood or less well characterized. In the former category is the *sorB* mutation in *Aspergillus nidulans* (Elorza and Arst, 1971). Like *sorA*, this mutation was selected for resistant to sorbose but, unlike it, the *sorB* mutation did not confer cross-resistance to deGlc. However, colony morphology was altered, the mycelium growing with tightly compact morphology on virtually all carbon sources. Although levels of hexokinase, phosphoglucose isomerase, and glucose 6-phosphate dehydrogenase were normal, the *sorB* mutants had considerably reduced activity of phosphoglucomutase. It is obscure why reduction in this enzyme activity should result in acquisition of resistance to inhibition by sorbose. Elorza and Arst (1971) discuss the parallel between the morphological abnormality in the *A. nidulans sorB* mutation and morphological abnormalities which are associated with loss of phosphoglucomutase in *Neurospora* spp. (Brody and Tatum, 1967; Mishra and Tatum, 1970). They also show that the cell-wall composition of *sorB* mycelium grown on glucose medium differs drastically from that of the wild type. It seems that this strain avoids inhibition by sorbose because the mutation alters wall structure in such a way that it is no longer sensitive to sorbose-induced alteration in wall composition. The colonial morphological abnormality may result secondarily from this change in wall structure for, on solid medium, the *sorB* wall alteration could impose changes in branching patterns which precipitate the sorts of modification to the medium which Trinci and Collinge (1973) have suggested give rise to sorbose-induced paramorphic changes. A relevant comparison here is with the temperature-sensitive mutant of *A. nidulans* which grew normally only when mannose was supplied as sole carbon source (Valentine and Bainbridge, 1978). This mutant, designated *mnrA455*, produced an abnormally thermolabile phosphomannomutase. At restrictive temperatures in the absence of mannose, the mutant produced extensive areas of swollen hyphae and cell walls contained only about 30 % of the normal amount of mannose. The parallel with *sorB* is quite close, with the exception that the *sorB* morphological defect was evident during growth on normal carbon sources, whereas the *mnrA455* defect could be relieved by mannose supplementation. Nevertheless, these two mutations emphasize how dependent fungal morphology is on cell wall structure and how dependent the latter is on the ability to interconvert hexose sugars. A different example of a correlation between sorbose resistance and morphological abnormality is provided by the *Neurospora* mutant, *patch*. This mutant, isolated by its unusual cyclical growth pattern (Stadler, 1959), is resistant to inhibitions caused by sorbose, and the activities of its glycogen synthetase and glucan synthetase are not affected by sorbose either *in vivo* or *in vitro* (Mishra and Tatum, 1972). A transport-defective mutant arose as one of the progeny from a cross between *patch* and an acetate non-utilizer which showed a similar rhythmically altered colony morphology to the *patch* parental strain (Halaban, 1975). In contrast to the strictly circadian rhythm of *patch*, the new mutation had a period of about 50 h on medium containing 5 % glucose and the period decreased with decreasing glucose concentrations. The mutant lacks the low-affinity glucose transport system but, although 3-O-methylglucose was used as a transport substrate, sensitivity to inhibitions by glucose analogues was not assessed. However, as rates of sugar transport were

some eight times lower than in the wild type, it is highly likely that this strain would prove to be resistant to some degree at least. It is worth recalling that, in *Podospora anserina*, galactose is inhibitory to growth of the wild type, and that it so changes the branching habit of the hyphae that it induces the wild type to take on the morphology of a morphological mutant, *zonata*, which exhibits a rhythmic mycelial growth pattern on normal media (Lysek and Esser, 1971). These authors stress that their data indicate that *Podospora* strains with rhythmic growth are altered in carbohydrate metabolism such that sugar degradation is enhanced and substrates are wasted. Remembering that, in the presence of sorbose, *Neurospora* uses more than the normal amount of oxygen and produces more CO_2 per unit glucose used (Crocken and Tatum, 1968), it may be that the association of abnormality of rhythmic growth with enhanced degradation of sugar arises because, on solid medium, abnormally rapid carbon metabolism periodically gives rise to the type of adverse environmental condition induced by growth on sorbose (Trinci and Collinge, 1973).

Cell wall defects also occurred in mutants of *Saccharomyces cerevisiae* selected for resistance to 2-deoxy-2-fluoro-D-glucose (Biely *et al.*, 1973). In fluoroglucose-media, the resistant mutants grew in agglomerates consisting of large number of small cells. Cell division was defective in its final stages; in particular the septa were anomalously wide so that wide junctions occurred between mother cell and bud, rather than a bud neck. The biochemical basis of the resistant phenotype is unknown.

VI. CONCLUSIONS

I hope that this review will have indicated both the value of sugar analogues in dissecting metabolic function and the areas which remain to be explored. The fact that most of the resistant mutants which have been described somehow limit accumulation of either the sugar analogue itself or its phosphate emphasizes that the initial inhibitions do not depend on extensive metabolism. These compounds are therefore ideally suited to the study of the very earliest stages of carbohydrate metabolism, specifically transport and the production and processing of sugar phosphates. There is scope too for investigation of regulatory aspects both in terms of the direct effects of accumulation of sugar phosphates and of the consequential changes in nucleotide levels and phosphate availability. Transport mechanisms in *Neurospora* are inadequately understood to make a proper characterization of the resistant mutants already isolated, and much the same is true for *Aspergillus nidulans*, in which sorbose transport in particular must be analyzed before even the currently available mutants can be fully explained. Work with yeasts shows that mutants involving transport and kinase and phosphatase enzymes can be selected by exposure to sugar analogues, but in no filamentous fungus has any attempt been made to obtain a comprehensive set of resistant mutants. The relationships between phosphohexomutase mutations and hyphal morphology in the few instances discussed above suggest that several avenues exist for the investigation of the involvement of carbohydrate metabolism in hyphal morphogenesis. Sugar analogues induce morphological changes which are described as being abnormal. In the context of vegetative hyphal growth, the paramorphic changes observed certainly are abnormal but this is not to say that the paramorphic growth form is without relevance to other aspects of growth of the fungus. As illustration, consider the basidiomycete *Coprinus cinereus*. As in other organisms, sorbose induces compact colonial growth; instead of being well separated from one

another, the marginal hyphae are densely packed, have much shortened cells and branch profusely (Moore and Stewart, 1972). Although this is a totally abnormal growth pattern for the vegetative colony, it is precisely this sort of growth which is responsible for the formation of the gill hymenium during the earliest stages of morphogenesis of the carpophore (Moore, Elhiti and Butler, 1979). Further, treatment with inhibitory concentrations of glucosamine causes the tips of *Coprinus* hyphae to balloon into much expanded spheroids (Moore and Stewart, 1972). This behaviour also has its counterpart in normal development in that, during maturation of the carpophore, paraphyseal cells of the hymenium become enormously inflated though they remain connected to unchanged, hypha-like, subhymenial cells (Moore *et al.*, 1979). All fungal structures are ultimately derived from the hypha, the different cell types arising by modification of the basic hyphal compartment. Many different studies, using both inhibitory analogues to induce paramorphic changes and genetic alterations which impose morphological abnormalities, have contributed to the generalization that morphological change occurs when the balances between wall components, precursors or synthetic processes are disturbed. As originally formulated, the essential balance was seen as that existing between the rates of wall formation and of rigidification (Robertson, 1958, 1959). Biochemical knowledge about factors which might contribute to the latter process is only just beginning to emerge (Sietsma and Wessels, 1979; Gooday and Trinci, 1980) but there is now general agreement that growth of the polysaccharide components of the wall is itself a balance between lysis of the existing polymer and insertion of new sugar units. This concept has been particularly well developed recently for chitin synthesis (Gooday, 1977) but it was originally advanced as a description of the growth of glucan fibres in order to account for the effects of deGlc on yeast cells (Johnson, 1968a).

It is implicit in this concept that, during normal morphogenesis, change in cell shape results from controlled shifts in these balances which are directed by endogenous regulatory events. Study of morphogenesis involves attempting to understand these changes in wall synthesis and associated metabolism and the regulation underlying them. Sugar analogues offer one way of approaching this type of study. Even if they do not precipitate the same metabolic events which occur endogenously, their use can at least indicate ways in which the normally observed events could arise. Thus, the fact that glucosamine induces *Coprinus* hyphal tips to swell in a manner similar to that normally observed in maturation of the paraphyses of the gill hymenium does not necessarily imply that the same processes are operative in each case but, at least, the response to glucosamine provides a technique for the experimental study of cell inflation. The indication that paramorphic changes induced by sorbose arise largely through consequential environmental effects (Trinci and Collinge, 1973) opens the possibility of investigating the impact of environmental factors on morphogenetic plasticity. It should be possible to control mixing of the medium experimentally to assess the validity of the interpretation of Trinci and Collinge (1973). If, by this means, the characteristics of the phenomenon can be established, it may then be possible to devise ways in which developing tissues, like the gills of basidiomycete carpophores, in which similar morphological patterns are observed can be manipulated to see if their morphologies arise for similar reasons.

The hyphal morphologies which occur during the development of fungal tissues are closely similar to the paramorphologies induced by inhibitory sugar analogues and the opportunities offered by these compounds for investigation of morphogenesis are considerable.

VII. REFERENCES

ANGYAL, S. J. (1972). Conformation of sugars. In: *The Carbohydrates*, vol. IA (Ed. by W. Pigman & D. Horton), pp. 195–215. Academic Press, New York and London.

ATKIN, R. K., SPENCER, D. M. & WAIN, R. L. (1964). Investigations on fungicides. X. The antifungal activity of 2-deoxy-D-glucose. *Annals of Applied Biology*, **53**, 437–443.

BAILEY, P. J. & HARRIS, M. (1968). Patterns of resistance to 2-deoxy-D-glucose in pig kidney cells. *Journal of Cellular Physiology*, **71**, 23–32.

BALL, A. J. S., WONG, D. K. & ELLIOTT, J. J. (1976). Glucosamine resistance in yeast. I. A preliminary genetic analysis. *Genetics*, **84**, 311–317.

BALLOU, C. E., MAITRA, S. K., WALKER, J. W. & WHELAN, W. L. (1977). Developmental defects associated with glucosamine auxotrophy in *Saccharomyces cerevisiae*. *Proceedings of the National Academy of Sciences of the U.S.A.*, **74**, 4351–4355.

BARBAN, S. (1962). Studies on the mechanism of resistance to 2-deoxy-D-glucose in mammalian cell cultures. *Journal of Biological Chemistry*, **237**, 291–295.

BARBAN, S. (1966). Differential action of hormones on induced resistance to 2-deoxy-D-glucose in mammalian cell cultures. *Biochimica et Biophysica Acta*, **115**, 197–204.

BARBAN, S. & SCHULZE, H. O. (1961). The effects of 2-deoxyglucose on the growth and metabolism of cultured human cells. *Journal of Biological Chemistry*, **236**, 1887–1890.

BARBER, G. A. (1959). Characterization of a new disaccharide containing deoxy-D-glucose synthesised by higher plants. *Federation Proceedings*, **18**, 185 (*abstr.*).

BARNETT, H. L. & LILLY, V. G. (1951). The inhibitory effects of sorbose on fungi. *Science*, **114**, 439–440.

BIELY, P. & BAUER, Š. (1966). The formation of uridine diphosphate-2-deoxy-D-glucose in yeast. *Biochimica et Biophysica Acta*, **121**, 213–214.

BIELY, P. & BAUER, Š. (1967). Metabolism of 2-desoxy-D-glucose by baker's yeast. I. Isolation and identification of phosphorylated esters of 2-desoxy-D-glucose. *Collection of Czechoslovak Chemical Communications*, **32**, 1588–1594.

BIELY, P. & BAUER, Š. (1968). The formation of guanosine diphosphate-2-deoxy-D-glucose in yeast. *Biochimica et Biophysica Acta*, **156**, 432–434.

BIELY, P., FARKAŠ, V. & BAUER, Š. (1967). Incorporation of 2-deoxy-D-glucose into glycogen. *Biochimica et Biophysica Acta*, **158**, 487–488.

BIELY, P., KOVAŘÍK, J. & BAUER, Š. (1973). Lysis of *Saccharomyces cerevisiae* with 2-deoxy-2-fluoro-D-glucose, an inhibitor of the cell wall glucan synthesis. *Journal of Bacteriology*, **115**, 1108–1120.

BIELY, P., KRÁTKÝ, Z., KOVAŘÍK, J. & BAUER, Š. (1971). Effect of 2-deoxyglucose on cell wall formation in *Saccharomyces cerevisiae* and its relation to cell growth inhibition. *Journal of Bacteriology*, **107**, 121–129.

BILGRAMI, K. S. (1963). The utilization of L-sorbose by some pathogenic species of *Phyllosticta*. *Proceedings of the Indian Academy of Sciences* B, **57**, 230–234.

BLAKLEY, E. R. & BOYER, P. D. (1955). The effect of 6-deoxy-6-fluoro-D-glucose on yeast fermentation and on hexokinase. *Biochimica et Biophysica Acta*, **16**, 576–582.

BOHUS, G. (1967). The utilization of L-sorbose by *Agaricus macrosporus* (Meell. *et* J. Schaeff.) Pilat. *Acta Biologica* (*Hungarian Academy of Sciences*), **18**, 387–401.

BREMNER, J. M. (1967). Nitrogenous compounds. In: *Soil Biochemistry* (Ed. by A. D. McLaren & G. H. Peterson), pp. 19–66. Edward Arnold, London.

BROCKMAN, H. E. & DE SERRES, F. J. (1963). 'Sorbose toxicity' in *Neurospora*. *American Journal of Botany*, **50**, 709–714.

BRODY, S. & TATUM, E. L. (1967). Phosphoglucomutase mutants and morphological changes in *Neurospora crassa*. *Proceedings of the National Academy of Sciences of the U.S.A.*, **58**, 923–930.

BURTON, L. E. & WELLS, W. W. (1977). Studies on the effect of 5-thio-D-glucose and 2-deoxy-D-glucose on *myo*-inositol metabolism. *Archives of Biochemistry and Biophysics*, **181**, 384–392.

CADENAS, E. & SOLS, A. (1960). The ketokinase activity of the intestinal mucosa. *Biochimica et Biophysica Acta*, **42**, 490–498.

CHANG, Y. (1967). The fungi of wheat straw compost. II. Biochemical and physiological studies. *Transactions of the British Mycological Society*, **50**, 667–677.

CHANG, Y. & HUDSON, H. J. (1967). The fungi of wheat straw compost. I. Ecological studies. *Transactions of the British Mycological Society*, **50**, 649–666.

CIRILLO, V. P. (1968). Relationship between sugar structure and competition for the sugar transport system in baker's yeast. *Journal of Bacteriology*, **95**, 603–611.

COE, E. L. (1968). Stimulation of Ascites tumor cell respiration by low concentrations of 2-deoxyglucose. *Biochimica et Biophysica Acta*, **162**, 470–473.

COHEN, J., KATZ, D. & ROSENBERGER, R. F. (1969). Temperature sensitive mutant of *Aspergillus nidulans* lacking amino sugars in its cell wall. *Nature*, **224**, 713–715.

CROCKEN, B. & TATUM, E. L. (1967). Sorbose transport in *Neurospora crassa*. *Biochimica et Biophysica Acta*, **135**, 100–105.

CROCKEN, B. & TATUM, E. L. (1968). The effect of sorbose on metabolism and morphology of *Neurospora*. *Biochimica et Biophysica Acta*, **156**, 1–8.

DAVIDSON, E. A. (1960). Hexokinase from *Aspergillus parasiticus*. *Journal of Biological Chemistry*, **235**, 23–25.

DEMOSS, R. D. & HAPPEL, M. E. (1955). 2-desoxy-D-glucose metabolism in *Leuconostoc mesenteroides*. *Journal of Bacteriology*, **70**, 104–109.

DE ROUSSET-HALL, A. & GOODAY, G. W. (1975). A kinetic study of a solubilized chitin synthetase preparation from *Coprinus cinereus*. *Journal of General Microbiology*, **89**, 146–154.

DESAI, J. D. & MODI, V. V. (1974). Factors affecting glucose uptake in *Aspergillus nidulans*. *Indian Journal of Experimental Biology*, **12**, 438–440.

DESAI, J. D. & MODI, V. V. (1977). Regulation of glucose transport in *Aspergillus nidulans*. *Experientia*, **33**, 726–727.

DE SERRES, F. J., KOLMARK, H. G. & BROCKMAN, H. E. (1962). Factors influencing the survival of *Neurospora crassa* conidia in sorbose-sucrose media. *Nature*, **193**, 556–557.

DE TERRA, N. & TATUM, E. L. (1961). Colonial growth of *Neurospora crassa*. *Science*, **134**, 1066–1068.

DE TERRA, N. & TATUM, E. L. (1963). A relationship between cell wall structure and colonial growth in *Neurospora crassa*. *American Journal of Botany*, **50**, 669–677.

ELLIOTT, J. J. & BALL, A. J. S. (1973). Glucosamine resistance in the yeast *Saccharomyces cerevisiae*. *Genetics*, **74**, s71.

ELLIOTT, J. J. & BALL, A. J. S. (1975). A new mitochondrial mutation in yeast. *Biochemical and Biophysical Research Communications*, **64**, 277–281.

ELORZA, M. V. & ARST, H. N. (1971). Sorbose resistant mutants of *Aspergillus nidulans*. *Molecular and General Genetics*, **111**, 185–193.

FARKAŠ, V., BAUER, Š. & ZEMEK, J. (1969). Metabolism of 2-deoxy-D-glucose in baker's yeast. III. Formation of 2,2′-dideoxy-α,α′-trehalose. *Biochimica et Biophysica Acta*, **184**, 77–82.

FARKAŠ, V., BIELY, P. & BAUER, Š. (1968). Transglycosylic reactions of nucleotides of 2-deoxy-sugars. I. Biosynthesis of 2-deoxysucrose. *Biochimica et Biophysica Acta*, **165**, 63–67.

FARKAŠ, V., SVOBODA, A. & BAUER, Š. (1969). Inhibitory effect of 2-deoxy-D-glucose on the formation of the cell wall in yeast protoplasts. *Journal of Bacteriology*, **98**, 744–748.

FELDHEIM, M. E., AUGUSTIN, H. W. & HOFMANN, E. (1966). Uber die wirkung von 2-desoxy-D-glucose auf atmung und adenylsauresystem von hefezellen. *Biochemische Zeitschrift*, **344**, 238–255.

GALPIN, M. F. J., JENNINGS, D. H. & THORNTON, J. D. (1977). Hyphal branching in *Dendryphiella salina*: effect of various compounds and the further elucidation of the effect of sorbose and the role of cyclic AMP. *Transactions of the British Mycological Society*, **69**, 175–182.

GLASER, L. & BROWN, D. H. (1955). Purification and properties of D-glucose-6-phosphate dehydrogenase. *Journal of Biological Chemistry*, **216**, 67–79.

GOODAY, G. W. (1975). The control of differentiation in fruit bodies of *Coprinus cinereus*. *Reports of the Tottori Mycological Institute (Japan)*, **12**, 151–160.

GOODAY, G. W. (1977). Biosynthesis of the fungal wall – mechanisms and implications. *Journal of General Microbiology*, **99**, 1–11.

GOODAY, G. W., DE ROUSSET-HALL, A. & HUNSLEY, D. (1976). Effect of polyoxin D on chitin synthesis in *Coprinus cinereus*. *Transactions of the British Mycological Society*, **67**, 193–200.

GOODAY, G. W. & TRINCI, A. P. J. (1980). Wall structure and biosynthesis in fungi. In: *The Eukaryotic Microbial Cell* (Ed. by G. W. Gooday, D. Lloyd & A. P. J. Trinci), pp. 207–251. Society for General Microbiology Symposium 30. Cambridge University Press, Cambridge.

GUERDOUX, J. L. (1974). *Coprinus*. In: *Handbook of Genetics*, vol. I, *Bacteria, Bacteriophage and Fungi* (Ed. by R. C. King), pp. 627–636. Plenum Press, New York and London.

GUERINEAU, M., SLONIMSKI, P. P. & AVNER, P. R. (1974). Yeast episome: oligomycin resistance associated with a small covalently closed nonmitochondrial circular DNA. *Biochemical and Biophysical Research Communications*, **61**, 462–469.

HALABAN, R. (1975). Glucose transport-deficient mutant of *Neurospora crassa* with an unusual rhythmic growth pattern. *Journal of Bacteriology*, **121**, 1056–1063.

HARPUR, R. P. & QUASTEL, J. H. (1949). Phosphorylation of D-glucosamine by brain extracts. *Nature*, **164**, 693–694.

HEREDIA, C. F. & SOLS, A. (1964). Metabolic studies with 2-deoxyhexoses. II. Resistance to 2-deoxyglucose in a yeast mutant. *Biochimica et Biophysica Acta*, **86**, 224–228.

HEREDIA, C. F., DE LA FUENTE, G. & SOLS, A. (1964). Metabolic studies with 2-deoxyhexoses. I. Mechanisms of inhibition of growth and fermentation in baker's yeast. *Biochimica et Biophysica Acta*, **86**, 216–223.

HEROLD, A. & LEWIS, D. H. (1977). Mannose and green plants: occurrence, physiology and metabolism, and use as a tool to study the role of orthophosphate. *New Phytologist*, **79**, 1–40.

HERS, H. G. (1952). La fructokinase du foie. *Biochimica et Biophysica Acta*, **8**, 416–423.

HOCHSTER, R. M. & QUASTEL, J. H. (1963). *Metabolic Inhibitors. A Comprehensive Treatise*, vol. I. Academic Press, New York and London.

HÖFER, M. & DAHLE, P. (1972). Glucose repression of inducible enzyme synthesis in the yeast. *Rhodotorula gracilis*. Effect of the cell-membrane transport. *European Journal of Biochemistry*, **29**, 326–332.

HORR, W. H. (1936). Utilization of galactose by *Aspergillus niger* and *Penicillium glaucum*. *Plant Physiology*, **11**, 81–99.

HUDSON, M. T. & WOODWARD, G. E. (1958). Glucosone. II. Inhibition of yeast metabolism, yeast hexokinase activity and tissue glycolysis. *Biochimica et Biophysica Acta*, **28**, 127–133.

JASPERS, H. T. & VAN STEVENINCK, J. (1976). Transport of 2-deoxy-D-galactose in *Saccharomyces fragilis*. *Biochimica et Biophysica Acta*, **443**, 243–253.

JENNINGS, D. H. (1974). Sugar transport into fungi: an essay. *Transactions of the British Mycological Society*, **61**, 1–24.

JOHNSON, B. F. (1968a). Lysis of yeast cell walls induced by 2-deoxyglucose at their sites of glucan synthesis. *Journal of Bacteriology*, **95**, 1169–1172.

JOHNSON, B. F. (1968b) Dissolution of yeast glucan induced by 2-deoxyglucose. *Experimental Cell Research*, **50**, 692–694.

JOHNSON, B. F. & RUPERT, C. M. (1967). Cellular growth rates of the fission yeast, *Schizosaccharomyces pombe*, and variable sensitivity to 2-deoxyglucose. *Experimental Cell Research*, **48**, 618–620.

KATZ, D. & ROSENBERGER, R. F. (1970). A mutation in *Aspergillus nidulans* producing hyphal walls which lack chitin. *Biochimica et Biophysica Acta*, **208**, 452–460.

KLENOW, H. (1963). Inhibition by cordycepin and 2-deoxyglucose of the incorporation of ^{32}P orthophosphate into the nucleic acids of Ehrlich ascites-tumor cells in vitro. *Biochimica et Biophysica Acta*, **76**, 354–365.

KLINGMÜLLER, W. (1967a). Aktive Aufnahme von Zuckern durch Zellen von *Neurospora crassa* unter Beteiligung eines enzymatischen Systems mit Permeaseeigenschaften. *Zeitschrift für Naturforschung*, **22**b, 181–188.

KLINGMÜLLER, W. (1967b). Aktive Aufnahme von Zuckern durch Zellen von *Neurospora crassa* unter Beteiligung eines enzymatischen Systems mit Permeaseeigenschaften: II. *Zeitschrift für Naturforschung*, **22**b, 188–195.

KLINGMÜLLER, W. (1967c). Analyse der Sorbose-Resistenz von *Neurospora crassa* an Heterokaryen sorboseresistenter Mutanten; ein Beitrag zur Genetik des aktiven Transports: I. *Molecular and General Genetics*, **100**, 117–139.

KLINGMÜLLER, W. (1967d). Kreuzungs-analyse Sorbose-resistenter Mutanten von *Neurospora crassa*. *Molecular and General Genetics*, **100**, 109–116.

KLINGMÜLLER, W. (1967e). Die Aufnahme der Zucker Sorbose, Fructose und Glucose durch Sorbose-resistente Mutanten von *Neurospora crassa*. *Zeitschrift für Naturforschung*, **22**b, 327–335.

KLINGMÜLLER, W. (1967f). Analyse der Sorbose-Resistenz von *Neurospora crassa* an doppelmutanten; ein Beitrag zur Genetik des aktiven Transports: II. *Molecular and General Genetics*, **100**, 256–263.

KLINGMÜLLER, W. & HUH, H. (1972). Sugar transport in *Neurospora crassa*. *European Journal of Biochemistry*, **25**, 141–146.

KLINGMÜLLER, W. & KAUDEWITZ, F. (1967). 'No-growth' complementation in forced heterokaryons from sorbose resistant (transport defective) *Neurospora crassa* mutants. *Zeitschrift für Naturforschung*, **22**b, 1024–1027.

KOTYK, A. (1967). Properties of the sugar carrier in baker's yeast. II. Specificity of transport. *Folia Microbiologica*, **12**, 121–131.

KOTYK, A. & JANÁČEK, K. (1975). *Cell Membrane Transport: Principles and Techniques*, 2nd edn. Plenum Press, New York and London.

KOTYK, A. & MICHALJANIČOVÁ, D. (1974). Nature of the uptake of D-galactose, D-glucose and α-methyl-D-glucose by *Saccharomyces cerevisiae*. *Biochimica et Biophysica Acta*, **332**, 104–113.

KOTYK, A., MICHALJANIČOVÁ, D., VEREŠ, K. & SOUKUPOVÁ, V. (1975). Transport of 4-deoxy- and 6-deoxy-D-glucose in baker's yeast. *Folia Microbiologica*, **20**, 496–503.

KOWALEWSKI, Z., SCHINDLER, O. & REICHSTEIN, T. (1960). Die cardenolide von *Erysimum perofskianum* Fisch. *et.* mey. *Helvetica Chimica Acta*, **43**, 1280–1290.

KRÁTKÝ, Z., BIELY, P. & BAUER, Š. (1975). Mechanism of 2-deoxy-D-glucose inhibition of cell wall polysaccharide and glycoprotein biosynthesis in *Saccharomyces cerevisiae*. *European Journal of Biochemistry*, **54**, 459–467.

KUNZ, B. A. & BALL, A. J. S. (1977). Glucosamine resistance in yeast. II. Cytoplasmic determinants conferring resistance. *Molecular and General Genetics*, **153**, 169–177

KUO, S. C. & LAMPEN, J. O. (1972). Inhibition by 2-deoxy-D-glucose of synthesis of glycoprotein enzymes by protoplasts of *Saccharomyces*: relation to inhibition of sugar uptake and metabolism. *Journal of Bacteriology*, **111**, 419–429.

LARDY, H. A., WIEBELHAUS, V. D. & MANN, K. M. (1950). The mechanism by which glyceraldehyde inhibits glycolysis. *Journal of Biological Chemistry*, **187**, 325–337.

LETNANSKY, K. (1964). The influence of 2-deoxy-D-glucose on the nucleotide content of Ehrlich ascites carcinoma cells. *Biochimica et Biophysica Acta*, **87**, 1–8.

LEWIS, D. H. (1971). Metabolic aspects of the control of cell and colony morphology of *Saccharomyces* spp. by α-glucosides. *Abstracts, 1st International Mycological Congress*, p. 57.

LILLY, V. G. & BARNETT, H. L. (1953). The utilization of sugars by fungi. *Bulletin of the University of West Virginia Agricultural Experimental Station*, **362**T, 58 pp.

LOBO, Z. & MAITRA, P. K. (1977a). Genetics of yeast hexokinase. *Genetics*, **86**, 727–744.

LOBO, Z. & MAITRA, P. K. (1977b). Resistance to 2-deoxyglucose in yeast. Direct selection of mutants lacking glucose-phosphorylating enzymes. *Molecular and General Genetics*, **157**, 297–300.

LYSEK, G. & ESSER, K. (1971). Rhythmic mycelial growth in *Podospora anserina*. II. Evidence for a correlation with carbohydrate metabolism. *Archiv für Mikrobiologie*, **75**, 360–373.

MAHADEVAN, P. R. & TATUM, E. L. (1965). Relationship of the major constituents of the *Neurospora crassa* cell wall to wild-type and colonial morphology. *Journal of Bacteriology*, **90**, 1073–1081.

MAITRA, P. K. (1970). A glucokinase from *Saccharomyces cerevisiae*. *Journal of Biological Chemistry*, **245**, 2423–2431.

MAITRA, P. K. & ESTABROOK, R. W. (1962). The ADP and phosphate control of ethanol oxidation in baker's yeast. *Biochemical and Biophysical Research Communications*, **9**, 1–6.

MAITRA, P. K. & ESTABROOK, R. W. (1967). Studies of baker's yeast metabolism. II. The role of adenine nucleotides and inorganic phosphate in the control of respiration during alcohol oxidation. *Archives of Biochemistry and Biophysics*, **121**, 129–139.

MARK, C. G. & ROMANO, A. H. (1971). Properties of the hexose transport systems of *Aspergillus nidulans*. *Biochimica et Biophysica Acta*, **249**, 216–226.

MARTIN, C. M. & REUTER, F. H. (1949). Isolation of a pectic substance from Passion Fruit (*Passiflora edulis*). *Nature*, **164**, 407.

MARTIN, M. & HEREDIA, C. F. (1977). Characterization of a phosphatase specific for 2-deoxyglucose-6-phosphate in a yeast mutant. *Federation of European Biological Societies Letters*, **83**, 235–248.

MATSUSHIMA, T. & KLUG, R. J. (1958). Utilization of L-sorbose by mono-sporidial lines and mutants of *Ustilago maydis*. *American Journal of Botany*, **45**, 165–168.

MCCOMB, R. B. & YUSHOK, W. D. (1964a). Metabolism of ascites tumor cells. III. Effect of 2-deoxyglucose phosphorylation on phosphorus metabolism. *Cancer Research*, **24**, 193–197.

MCCOMB, R. B. & YUSHOK, W. D. (1964b). Metabolism of ascites tumor cells. IV. Enzymatic reactions involved in adenosinetriphosphate degradation induced by 2-deoxyglucose. *Cancer Research*, **24**, 198–209.

MCDERMOTT, J. C. B. & JENNINGS, D. H. (1976). The relationship between the uptake of glucose and 3-O-methylglucose and soluble carbohydrate and polysaccharide in the fungus *Dendryphiella salina*. *Journal of General Microbiology*, **97**, 193–209.

MEGNET, R. (1965). Effect of 2-deoxyglucose on *Schizosaccharomyces pombe*. *Journal of Bacteriology*, **90**, 1032–1035.

MEREDITH, S. A. & ROMANO, A. H. (1977). Uptake and phosphorylation of 2-deoxy-D-glucose by wild type and respiration-deficient baker's yeast. *Biochimica et Biophysica Acta*, **497**, 745–759.

MISHRA, M. C. (1976). The effect of cyclic adenosine monophosphate on the growth of *Neurospora crassa*. *Naturwissenschaften*, **63**, 485.

MISHRA, N. C. & TATUM, E. L. (1970). Phosphoglucomutase mutants of *Neurospora sitophila* and their relation to morphology. *Proceedings of the National Academy of Sciences of the U.S.A.*, **66**, 638–645.

MISHRA, N. C. & TATUM, E. L. (1972). Effect of L-sorbose on polysaccharide synthetases of *Neurospora crassa*. *Proceedings of the National Academy of Sciences of the U.S.A.*, **69**, 313–317.

MITCHELL, I. L. S. & BAYNE, S. (1952). The effect of D- and L-glucosone on yeast fermentation. *Biochemical Journal*, **50**, xxvii.

MOORE, D. (1968). The effect of 2-deoxy-D-glucose on the growth and respiration of *Coprinus lagopus*. *Journal of General Microbiology*, **52**, 433–439.

MOORE, D. (1969a). Sources of carbon and energy used by *Coprinus lagopus sensu* Buller. *Journal of General Microbiology*, **58**, 49–56.

MOORE, D. (1969b). Effect of 2-deoxy-D-glucose on mycelial growth of filamentous fungi. *Transactions of the British Mycological Society*, **53**, 139–141.

MOORE, D. (1973). Mutants of *Coprinus* selected for resistance to D-glucosamine and L-sorbose. *Genetical Research*, **22**, 205–209.

MOORE, D. & DEVADATHAM, M. S. (1975). Distribution of mutant sites in the *ftr* cistron depends upon the medium used for selection. *Molecular and General Genetics*, **138**, 81–84.

MOORE, D. & DEVADATHAM, M. S. (1979). Sugar transport in *Coprinus cinereus*. *Biochimica et Biophysica Acta*, **550**, 515–526.

MOORE, D., ELHITI, M. M. Y. & BUTLER, R. D. (1979). Morphogenesis of the carpophore of *Coprinus cinereus*. *New Phytologist*, **83**, 695–722.

MOORE, D. & STEWART, G. R. (1971). Mutants of *Coprinus lagopus* selected for resistance to 2-deoxy-D-glucose. *Genetical Research*, **18**, 341–352.

MOORE, D. & STEWART, G. R. (1972). Effects of 2-deoxy-D-glucose, D-glucosamine, and L-sorbose on the growth of *Coprinus lagopus* hyphae. *Journal of General Microbiology*, **71**, 333–342.

MORROW, J. & DE CARLI, L. (1967). The correlation of resistance to 2-deoxyglucose with alkaline phosphatase levels in a human cell line. *Experimental Cell Research*, **47**, 1–11.

NIGAM, V. N. (1966). A study of the Crabtree effect in Novikoff ascites-hepatoma cells. *Biochemical Journal*, **99**, 413–418.

NIGAM, V. N. (1967). Incorporation of 2-deoxyglucose into glycogen by intact Novikoff ascites-hepatoma. *Archives of Biochemistry and Biophysics*, **120**, 232–233.

ODDOUX, L. (1952). Utilization comparée du sorbose et du fructose naturels par une centaine d'Homobasidiomycètes. *Comptes rendus hebdomadaire des séances de l'Académie des Sciences*, **234**, 654–657.

OPPENHEIM, B. & AVIGAD, G. (1965). Accumulation of inosine in yeast treated with 2-deoxyglucose. *Biochemical and Biophysical Research Communications*, **20**, 475–481.

PALL, M. L. (1977). Cyclic AMP and the plasma membrane potential in *Neurospora crassa*. *Journal of Biological Chemistry*, **252**, 7146–7150.

PAZUR, J. H. & KLEPPE, K. (1964). The oxidation of glucose and related compounds by glucose oxidase from *Aspergillus niger*. *Biochemistry*, **3**, 578–583.

PIGMAN, W. & ANET, E. F. L. J. (1972). Mutarotations and actions of acids and bases. In: *The Carbohydrates*, vol. IA (Ed. by W. Pigman & D. Horton), pp. 165–194. Academic Press, New York and London.

PIGMAN, W. & HORTON, D. (1972). Structure and stereochemistry of the monosaccharides. In: *The Carbohydrates*, vol. IA (Ed. by W. Pigman & D. Horton), pp. 1–67. Academic Press, New York and London.

PRABHAKARAN, K. (1976). 5-thio-D-glucose: *o*-diphenoloxidase inhibition as its mechanism of action. *Experientia*, **32**, 152–153.

PRÉVOST, G. (1958). Sur le caractère 'résistance au sorbose' du mycelium de *Coprinus fimetarius* (Fr. *ex* L.). *Comptes rendus hebdomadaire des séances de l'Académie des Sciences*, **247**, 1897–1899.

PURICH, D. L., FROMM, H. J. & RUDOLPH, F. B. (1973). The hexokinases: kinetic, physical, and regulatory properties. *Advances in Enzymology*, **39**, 249–326.

QUASTEL, J. H. & CANTERO, A. (1953). Inhibition of tumour growth by D-glucosamine. *Nature*, **171**, 252–254.

REEVES, R. E. (1949). Cuprammonium-glycoside complexes. III. The conformation of the D-glucopyranoside ring in solution. *Journal of the American Chemical Society*, **71**, 215–217.

REEVES, R. E. (1951). Cuprammonium-glycoside complexes. *Advances in Carbohydrate Chemistry*, **6**, 107–134.

RICHARDS, O. C. & RUTTER, W. J. (1961). Comparative properties of yeast and muscle aldolase. *Journal of Biological Chemistry*, **236**, 3185–3192.

RIZVI, S. R. H. & ROBERTSON, N. F. (1965). Apical disintegration of hyphae of *Neurospora crassa* as a response to L-sorbose. *Transactions of the British Mycological Society*, **48**, 469–477.

ROBERTSON, N. F. (1958). Observations on the effect of water on the hyphal apices of *Fusarium oxysporum*. *Annals of Botany*, **22**, 159–173.

ROBERTSON, N. F. (1959). Experimental control of hyphal branching and branch form in hyphomycetous fungi. *Journal of the Linnaean Society*, **56**, 207–211.

ROMANO, A. H. & KORNBERG, H. L. (1968). Regulation of sugar utilization by *Aspergillus nidulans*. *Biochimica et Biophysica Acta*, **158**, 491–493.

ROMANO, A. H. & KORNBERG, H. L. (1969). Regulation of sugar uptake by *Aspergillus nidulans*. *Proceedings of the Royal Society* B, **173**, 475–490.

ROSENBERG, G. & PALL, M. L. (1979). Properties of two cyclic-nucleotide-deficient mutants of *Neurospora crassa*. *Journal of Bacteriology*, **137**, 1140–1144.

RUIZ-AMIL, M. (1959). Exploracion de las etapas enzimaticas iniciales en la utilizacion de azucares por el *Aspergillus oryzae* y de la influencia del molibdeno sobre las mismas. *Annales de Edafologia y Fisiologia Vegetal*, **18**, 701–721.

SAUERMANN, G. (1968). The inhibition of palmitate oxidation by 2-deoxy-D-glucose in ascites tumor cells. *Biochimica et Biophysica Acta*, **170**, 88–94.

SCARBOROUGH, G. A. (1970). Sugar transport in *Neurospora crassa*. *Journal of Biological Chemistry*, **245**, 1694–1698.

SCHAFFER, R. (1972). Occurrence, properties, and preparation of naturally occurring monosaccharides (including 6-deoxy sugars). In: *The Carbohydrates, vol.* IA (Ed. by W. Pigman & D. Horton), pp. 69–111. Academic Press, New York and London.

SCHARFF, T. G., MOFFITT, R. L. & MONTGOMERY, O. L. (1965). A tentative modified fermentation pathway in yeast extracts. *Life Sciences* **4**, 827–836.

SCHARFF, T. G. & MONTGOMERY, O. L. (1965). 2-deoxyglucose-stimulated fermentation in yeast cells; possible relationship to cyclic fermentation in extracts. *Life Sciences*, **4**, 1049–1053.

SCHARFF, T. G. & MONTGOMERY, O. L. (1966). Some characteristics of the stimulating effects of 2-deoxy-D-glucose on endogenous fermentation in yeast. *Proceedings of the Society for Experimental Biology and Medicine*, **121**, 475–478.

SCOTT, W. A. & SOLOMON, B. (1973). Cyclic 3′,5′-AMP phosphodiesterase of *Neurospora crassa*. *Biochemical and Biophysical Research Communications*, **53**, 1024–1030.

SCOTT, W. A. & SOLOMON, B. (1975). Adenosine 3′,5′-cyclic monophosphate and morphology in *Neurospora crassa*: drug induced alterations. *Journal of Bacteriology*, **122**, 454–463.

SERIF, G. S. & WICK, A. N. (1958). The effect of 6-deoxy-6-fluoroglucose on glucose utilization in kidney tissues. *Journal of Biological Chemistry*, **233**, 559–561.

SERIF, G. S., STEWART, C. J., NAKADA, H. I. & WICK, A. N. (1958). Influence of 6-deoxy-6-fluoroglucose on glucose utilization in rat epididymal adipose tissue and rat diaphragm. *Proceedings of the Society for Experimental Biology and Medicine*, **99**, 720–722.

SIETSMA, J. H. & WESSELS, J. G. H. (1979). Evidence for covalent linkages between chitin and β-glucan in a fungal wall. *Journal of General Microbiology*, **114**, 99–108.

SOLS, A. & CRANE, R. K. (1954). Substrate specificity of brain hexokinase. *Journal of Biological Chemistry*, **210**, 581–595.

SOLS, A. & DE LA FUENTE, G. (1957). On the substrate specificity of glucose oxidase. *Biochimica et Biophysica Acta*, **24**, 206–207.

SOLS, A., DE LA FUENTE, G., VILLAR-PALASI, C. & ARSENSIO, C. (1958). Substrate specificity and some other properties of baker's yeast hexokinase. *Biochimica et Biophysica Acta*, **30**, 92–101.

SOLS, A., HEREDIA, C. F. & RUIZ-AMIL, M. (1960). 2-deoxyglucose as metabolic substrate and inhibitor of glycolysis in fungi. *Biochemical and Biophysical Research Communications*, **2**, 126–129.

SPIRO, R. G. (1958). The effect of *N*-acetylglucosamine and glucosamine on carbohydrate metabolism in rat liver slices. *Journal of Biological Chemistry*, **233**, 546–550.

STADLER, D. R. (1959). Genetic control of a cyclic growth pattern in *Neurospora*. *Nature*, **184**, 170–171.

STEINBERG, R. A. (1939). Relation of carbon nutrition to trace-element and accessory requirements of *Aspergillus niger*. *Journal of Agricultural Research*, **59**, 749–763.

STEVENINCK, J. VAN (1968). Transport and transport-associated phosphorylation of 2-deoxy-D-glucose in yeast. *Biochimica et Biophysica Acta*, **163**, 386–394.

SVOBODA, A. & SMITH, D. G. (1972). Inhibitory effect of 2-deoxy-D-glucose on cell wall synthesis in cells and protoplasts of *Schizosaccharomyces pombe*. *Zeitschrift für allgemeine Mikrobiologie*, **12**, 685–699.

TATUM, E. L., BARRATT, R. W. & CUTTER, V. M. (1949). Chemical induction of colonial paramorphs in *Neurospora* and *Syncephalastrum*. *Science*, **109**, 509–511.

THORNTON, J. D., GALPIN, M. F. J. & JENNINGS, D. H. (1976). The bursting of the hyphal tips of *Dendryphiella salina* and the relevance of the phenomenon to repression of glucose transport. *Journal of General Microbiology*, **96**, 145–153.

TREVILLYAN, J. M. & PALL, M. L. (1979). Control of cyclic adenosine 3′,5′-monophosphate levels by depolarizing agents in fungi. *Journal of Bacteriology*, **138**, 397–403.

TRINCI, A. P. J. & COLLINGE, A. J. (1973). Influence of L-sorbose on the growth and morphology of *Neurospora crassa*. *Journal of General Microbiology*, **78**, 179–192.

VALENTINE, B. P. & BAINBRIDGE, B. W. (1978). The relevance of a study of a temperature-sensitive ballooning mutant of *Aspergillus nidulans* defective in mannose metabolism to our understanding of mannose as a wall component and carbon/energy source. *Journal of General Microbiology*, **109**, 155–168.

VILLA, T. G., NOTARIO, V. & VILLANEUVA, J. R. (1975). Synthesis of β-glucanase and other extracellular proteins by cells and protoplasts of the yeast *Pichia polymorpha*: effect of 2-deoxy-D-glucose. *Archives of Microbiology*, **105**, 335–337.

VILLA, T. G., NOTARIO, V., BENITEZ, T. & VILLANEUVA, J R. (1976). On the effect of glucono-δ-lactone on the yeast *Pichia polymorpha*. *Archives of Microbiology*, **109**, 157–161.

WEBB, J. L. (1966). *Enzyme and Metabolic Inhititors*, vol. II. Academic Press, New York and London.

WILLIAMS, A. K. & EAGON, R. G. (1959). Oxidation of 2-deoxy-D-glucose to 2-deoxy-D-gluconic acid by extracts of *Pseudomonas aeruginosa*. *Journal of Bacteriology*, **77**, 167–172.

WOODWARD, G. E. & CRAMER, F. B. (1952). 2-desoxy-D-glucose as an inhibitor of anaerobic glycolysis in tumor tissue. *Journal of the Franklin Institute*, **254**, 259–260.

WOODWARD, G. E., CRAMER, F. B. & HUDSON, M. T. (1953). Carbohydrate analogs as antagonists of glucose in carbohydrate metabolism of yeast. *Journal of the Franklin Institute*, **256**, 577–587.

WRIGHT, J. R. & LE TOURNEAU, D. (1965). Utilization and production of carbohydrates by *Pyrenochaeta terrestris*. *Physiologia Plantarum*, **18**, 1044–1053.

YAMADA, Y., IIZUKA, K., AIDA, K. & UEMURA, T. (1966). L-sorbose oxidase from *Trametes sanguinea*. *Agricultural and Biological Chemistry*, **30**, 97–98.

YAMADA, Y., IIZUKA, K., AIDA, K. & UEMURA, T. (1967). Enzymatic studies on the oxidation of sugar and sugar alcohol. III. Purification and properties of L-sorbose oxidase from *Trametes sanguinea*. *Journal of Biochemistry*, **62**, 223–229.

ZONNEVELD, B. J. M. (1973). Inhibitory effect of 2-deoxyglucose on cell wall α-1,3-glucan synthesis and cleistothecium development in *Aspergillus nidulans*. *Developmental Biology*, **34**, 1–8.