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Mutants of *Coprinus lagopus* selected for resistance to 2-Deoxy-D-glucose

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SUMMARY

The glucose analogue 2-deoxy-D-glucose seriously inhibits the growth of *Coprinus lagopus*. Following treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine 388 resistant mutants were isolated. It is shown that the mutants isolated are probably allelic; they were phenotypically similar and no complementation was observed. The mutants were pleiotropic in the sense that although they were initially selected only for resistance to 2-deoxy-D-glucose they were found to be cross-resistant to both of the related analogues, sorbose and glucosamine. Furthermore, the mutants were unable to utilize fructose as a sole carbon source. It is demonstrated that the inability to utilize fructose results from a defect in sugar transport. The gene symbol *ftr* is proposed for this cistron and it is shown that though the gene is quite closely linked to its own centromere, it is unlinked to centromere markers of the six known linkage groups.

1. INTRODUCTION

The glucose analogue 2-deoxy-D-glucose (deGlc) causes growth inhibition in a wide range of cell types (Webb, 1966). Mutants resistant to such inhibition have been isolated in *Saccharomyces cerevisiae* (Heredia & Sols, 1964; Maitra, 1970), *Schizosaccharomyces pombe* (Megnet, 1965) and HeLa cells (Barban, 1962, 1963). In all cases the resistant mutants were able to grow in media which completely prevented growth of the wild-type (parent) strain.

In *Saccharomyces cerevisiae* the resistance was shown to result from the occurrence of an intracellular neutral phosphatase in the resistant cells. This enzyme was able to hydrolyse deGlc-6-phosphate fairly specifically. Since the inhibitory effects of deGlc are conditional on its phosphorylation the reduced internal concentration of deGlc-6-phosphate consequent to the possession of the phosphatase thus accounted well for the resistance of the cells. A somewhat similar situation was found to obtain in HeLa cells; the resistant cell line being shown to accumulate deGlc-6-phosphate at a much slower rate than the sensitive parental line. The resistant line also possessed a higher alkaline phosphatase activity than the parental line. However, it was also demonstrated that resistants phosphorylated deGlc and other hexoses more slowly than did extracts of sensitive cells and that the growth of resistant cells on deGlc-containing media was much improved by the addition of pyruvate. In *Schizosaccharomyces pombe* (Megnet, 1965) and in another study

involving *S. cerevisiae* (Maitra, 1970) resistance was shown to result from a complete or partial defect in hexokinase.

It has been shown that deGlc is a potent inhibitor of hyphal extension growth, spore germination and spore respiration in the Basidiomycete fungus *Coprinus lagopus* (Moore, 1968; Moore & Stewart, 1971). Moreover it was also demonstrated that two related hexose analogues, D-glucosamine (2-amino, 2-deoxy-D-glucose) and L-sorbose, cause broadly similar effects and that some competitive interaction occurred between the three inhibitors (Moore & Stewart, 1971). It was found that deGlc was about four times more inhibitory than glucosamine while the latter was about five times more inhibitory than sorbose; the inhibitions varying only slightly between different wild type strains of the organism. The normal carbon sources, acetate, fructose, mannose and glucose, differed greatly in their abilities to reverse the inhibitions. Acetate and fructose were virtually ineffective; glucose was the most effective in reversing inhibitions, being about 10 times more effective than mannose and about 100 times more effective than fructose or acetate. All three analogues caused their greatest inhibtions on media which either lacked any normal carbon source or which contained acetate as the sole available carbon source. This fact, together with the results of experiments in which the effects of the analogues on sugar uptake were directly measured, led to the conclusion that there was no significant relationship between growth inhibition and inhibition of sugar transport. The major activities of all three analogues appear to be exerted intracellularly.

This report presents the results of an examination of the physiological, biochemical and genetical characteristics of mutants selected specifically for resistance to growth inhibition by deGlc.

2. MATERIALS AND METHODS

(i) *Organism.* The strain used for the induction of mutants was the haploid wild-type number BC9/6,6 (mating type A_6B_6). Mating type recombinants of resistant mutants were prepared by crossing them with the haploid wild-type number ZBw601/40,40 (mating type $A_{40}B_{40}$) and isolating resistant progeny with the $A_{40}B_{40}$ mating-type specificities. For linkage tests a number of strains carrying known auxotrophic markers of the established linkage groups (Moore, 1967) were also used.

(ii) *Media.* Three media were used: the Coprinus minimal (MM) and complete (CM) media (Moore, 1968) and the NCM medium (Moore, 1969*a*) with the addition of 0·5 mM-$MgSO_4$. The latter is a medium which lacks all potential sources of carbon (the usual Coprinus media contain organic nitrogen sources). Where necessary, media were solidified with 1·5% (w/v) Difco Bacto-Agar. As a general rule carbohydrates were sterilized independently of other medium constituents.

(iii) *Isolation and characterization of mutants.* Mutants were induced by treating oidia (haploid, asexually produced spores) with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) using the treatment conditions and techniques described by Moore (1969*b*). Resistant mutants were selectively isolated by plating the treated suspen-

sion onto medium containing deGlc. Linear growth rates of the mutants were determined using the methods detailed previously (Moore & Stewart, 1971). For dry-weight determinations oidia of the strain under test were inoculated to liquid medium contained in medical flats (either 20 ml per 6 oz bottle or 50 ml per 16 oz) which were then laid flat and incubated for 6 days at 37 °C. Mycelium was harvested by filtration through Whatman GF/A glass fibre filter disks, washed and dried to constant weight at 40 °C. Genetic analyses were carried out using standard techniques of tetrad and random spore isolation and progeny testing (Moore, 1966, 1967).

(iv) *Preparation of enzyme extracts.* Mycelium was grown in liquid NCM medium containing either 50 or 75 mM maltose. Extracts were prepared by grinding frozen mycelial pads with acid-washed sand. The extraction buffer consisted of 100 mM Tris-chloride (pH 7·5) containing 0·5 mM EDTA. Crude extract was filtered through glass-fibre paper (Whatman GF/C) and stored in an ice-bucket until required. Enzyme activity was determined in a temperature-controlled (30 °C) recording spectrophotometer by measuring the change in E_{340} due to the reduction of $NADP^+$. Preliminary experiments indicated that reaction velocities were proportional to the amount of extract used. Blanks were carried out by adding extract to assay media which lacked substrates. Enzyme assay media were as follows:

(A) For hexokinase (EC 2.7.1.1), 100 μmoles Tris-chloride buffer, 10 μmoles $MgCl_2$, 5 μmoles ATP, 10 μmoles glucose or fructose, 2 μmoles $NADP^+$, 50 μg glucose-6-phosphate dehydrogenase, 50 μg glucose phosphate isomerase; pH was 7·5.

(B) For glucose phosphate isomerase (EC 5.3.1.9), 100 μmoles Tris-chloride buffer, 10 μmoles $MgCl_2$, 2 μmoles $NADP^+$, 5 μmoles fructose-6-phosphate, 50 μg glucose-6-phosphate dehydrogenase; pH was 7·8.

(C) For glucose-6-phosphate dehydrogenase (EC 1.1.1.49), 100 μmoles Tris-chloride buffer, 15 μmoles $MgCl_2$, 20 μmoles glucose-6-phosphate, 2 μmoles $NADP^+$; pH was 8·0.

(D) For 6-phosphogluconate dehydrogenase (EC 1.1.1.44), 100 μmoles Tris-chloride buffer, 15 μmoles $MgCl_2$, 15 μmoles 6-phosphogluconate, 2 μmoles $NADP^+$; pH was 8·0.

(v) *Experiments with isotopically labelled sugars.* The accumulation of glucose and fructose, uniformly labelled with ^{14}C, by oidia and mature mycelium was examined by suspending the washed cells in liquid NCM at 37 °C. The initial total sugar concentration was 3·6 mM and the suspension initially contained ^{14}C at 0·14 μCi/ml. At the conclusion of the experiment the cells were filtered off on to Millipore filter disks, rapidly washed with isotopically normal medium and then dried at 90 °C. The harvesting procedures typically occupied less than 20 sec. In some experiments a stream of air was passed through the suspension and the evolved CO_2 was precipitated in barium hydroxide. The barium carbonate was filtered off, washed and dried. The absolute radioactivities of the dry filters were measured with a Packard Model 3380 Tri-Carb spectrometer.

3. RESULTS

(i) *Mutant induction and selection.* Mutants were isolated from three different experiments. In each a suspension of BC9/6, 6 oidia was exposed to NTG at a final concentration of 15 μg/ml for 70 min at 37 °C, a treatment which typically leads to the death of at least 98 % of the cells. After being filtered and washed free of NTG the treated oidia were resuspended in buffer for plating on to selection medium. The only important difference between the three experiments was the composition of this selection medium. In the first experiment (NTG 1) this was NCM + 5 mM fructose + 5 mM deGlc (NCM + 5 mM fructose as control); in the second (NTG 2) it was NCM + 5 mM fructose + 0·5 mM deGlc (NCM + 5 mM fructose as control); and in the third (NTG 3) NCM + 5 mM Acetate + 1 mM deGlc (NCM + 5 mM Acetate as control). Subsequent tests have not revealed any consistent differences between mutants isolated from these media so for the purposes of this description no further distinction will be made between the experiments. Following the mutagen treatment oidia were plated on to the selection medium at an average density of $8{\cdot}5 \times 10^6$ treated oidia/plate; parallel platings were made on control medium. Completed plates were incubated for 7 days at 37 °C. At the end of the incubation period all control plates were uniformly covered with dense mycelial growth. Some selection plates were completely free of growth but many had small, thin and ragged colonies. Microscopical examination of such plates showed a heavy background of ungerminated oidia against which the growing colonies of the presumed resistants stood out clearly. The presence of ungerminated oidia obviously presented a danger of contamination when transfers of the presumed resistants were made. A number of plates were given an overlay of CM medium, re-incubated and microscopically examined to test the viability of these oidia. None were observed to invade the overlay.

A total of $1{\cdot}9 \times 10^9$ oidia were subjected to mutagenesis and selection, and 505 presumed resistant mutants were initially isolated. Subsequent tests showed that some of these were not truly resistant with the result that only 388 (77 % of selectants) were accepted for further study. The mutants were given the stock numbers Z1/6, 6 to Z59/6, 6 (selected from NTG 1), Z60/6, 6 to Z353/6, 6 (from NTG 2), and Z 354/6, 6 to Z505/6, 6 (from NTG 3). Since all of the isolated mutants could not be tested in detail, a sample of 35 original mutants was chosen at random and $A_{40}B_{40}$ mating-type recombinants prepared. Unless otherwise stated, all of these strains were subjected to the tests detailed below. This test sample consisted of mutants having the stock numbers 15, 32, 33, 60, 65, 70, 77, 79, 83, 88, 98, 154, 163, 167, 168, 169, 182, 183, 197, 205, 206, 211, 215, 222, 336, 361, 398, 412, 421, 435, 463, 492, 496, 500 and 505.

(ii) *Physiological characteristics of the mutants.* There was no appreciable difference, judging by eye, between the growth of the mutants and that of their parent wild-type strain on media which contained either 5 mM glucose or 5 mM acetate. There was a striking difference, however, in growth on 5 mM fructose. Although, on the latter medium, the linear growth rates were more or less normal,

in all cases the quality of the growth made differed greatly from the quality of wild-type growth in being extremely thin and sparse. Thus it was quite obvious even from tests on solid media that the mutants produced far less mycelium in the presence of fructose than did the wild-type and far less than they themselves produced on media containing 5 mM glucose.

Mutants were selected on medium containing deGlc. Not surprisingly therefore they were all able to grow on NCM + 5 mM fructose + 0·5 mM deGlc, NCM + 5 mM acetate + 1 mM deGlc, and, slowly but definitely, on NCM + 5 mM deGlc. All of these media completely inhibited growth of the wild-type. An unexpected discovery, however, was that the deGlc-resistant mutants were cross-resistant to both sorbose and glucosamine. Growth of the wild-type was completely inhibited on NCM + 5 mM sorbose whereas all of the deGlc-resistants appeared to be free of this inhibition. When grown on NCM + 5 mM sorbose the mutant mycelium was thin and scanty but the rate of extension growth was about normal and the hyphae had an entirely normal morphology. Similarly, although the extension growth rate of the wild-type was 80·7 % inhibited on media containing 5 mM fructose + 0·25 mM glucosamine, the growth rates of the mutants were inhibited by only 0·0 to 3·5 %.

No differences were evident between mutant and wild-type responses to mannose, pyruvate, malate, sucrose, lactose, or galactose. Also, the mutants were still as sensitive as the wild-type to growth inhibition by such agents as dehydroacetate, citrate and glyceraldehyde.

Table 1 summarizes results of experiments in which the rates of linear growth of three resistant mutants were compared with wild-type and with mating-type recombinants of the mutants. Two points are immediately obvious from these data. First, the mutants were definitely much more resistant to inhibition by sorbose than they were to inhibition by deGlc. Furthermore, resistance to all three agents was expressed to a much lesser extent on glucose-based media than on fructose- or acetate-based media. Indeed some of the mutants showed no resistance to deGlc on glucose media although resistance to sorbose was still significant, and this, together with the absence of morphological abnormalities on sorbose, demonstrates that resistance was not entirely limited to fructose and acetate media.

There were clear variations in extent of resistance between the mutants, as would be expected if they all resulted from independent mutational events. Tests have shown, for example, that among the 35 randomly chosen mutants the linear growth rate was inhibited from 0 to 90·8 % (mean = 40·4 %) on NCM + 5 mM acetate + 1 mM deGlc. There was also some evidence that the background genome had some effect for, as Table 1 shows, significant changes occurred in the detailed pattern of resistance (particularly in resistance to deGlc) when the resistance character was transferred to the different genetic environments of the mating type recombinants. These $A_{40}B_{40}$ recombinants were selected for the high linear growth-rate characteristic of the ZBw601/40,40 wild-type in addition to its mating-type specificities. In consequence it may reasonably be assumed that the genetic background into which the resistance character was introduced differed fundamentally

from that in which it was induced. Thus it would seem that while the general features of resistance may be expressible in any genome, the detailed expression of that resistance is nevertheless subject to modification by background genetic effects.

Table 1. *Effect of hexose analogues on the linear growth rate of representative mutants, their mating type recombinants and parental wild types*

(Percentage inhibition of the growth rate of strains with the indicated isolation numbers.)

	A_6B_6 mating types				$A_{40}B_{40}$ mating types			
Medium*	Wild†	15	215	505	Wild†	15	215	505
5 mM glucose	0	0	0	0	0	0	0	0
+ 10 mM deGlc	82·9	76·4	32·4	52·3	77·6	64·1	29·1	77·8
+ 20 mM deGlc	81·5	84·2	43·5	86·8	87·2	78·6	58·6	83·2
+ 20 mM GA	87·7	89·7	90·3	93·3	73·8	42·9	30·8	72·5
+ 75 mM Sor	69·1	13·7	0	15·5	83·6	14·5	15·5	14·0
+ 100 mM Sor	94·5	18·4	0	0·7	88·9	26·7	22·3	16·9
5 mM fructose	0	0	0	0	0	0	0	0
+ 0·1 mM deGlc	52·5	68·1	1·0	41·9	89·0	62·0	0	62·8
+ 0·25 mM deGlc	100·0	73·3	1·4	35·2	100·0	63·7	12·8	64·4
+ 0·25 mM GA	80·7	3·5	2·1	0	—	0	1·9	0
+ 2·0 mM Sor	91·9	0	0	0	94·2	2·5	0	0
+ 5·0 mM Sor	98·4	0·7	0	0	100·0	5·7	0	0
5 mM acetate	0	0	0	0	0	0	0	0
+ 0·5 mM deGlc	100·0	68·3	11·7	45·4	100·0	61·5	26·5	68·9
+ 1·0 mM deGlc	100·0	71·7	8·4	57·3	100·0	59·7	60·3	68·7
+ 0·4 mM GA	84·9	0	1·2	1·9	56·6	7·7	0	0
+ 2·0 mM Sor	60·8	2·5	0	0	95·9	0	0	0
+ 5·0 mM Sor	100·0	0	0	0	100·0	1·8	0	1·9

* Abbreviations used: deGlc, 2-deoxy-D-glucose; GA, D-glucosamine; Sor, L-sorbose.
† The wild-types used were BC9/6,6 and ZBw601/40,40.

One last observation which was made during experiments with solid inhibitor media was that although the mutants were free of sorbose-induced morphological aberrations they still appeared to be subject, at least to some extent, to deGlc induced abnormalities. This feature has yet to be analysed in detail.

(iii) *The response to fructose.* As stated above, when grown on solid media containing fructose as sole carbon source the mutants appeared to produce a very sparse mycelial growth. Tests in which measurements were made of dry weights of mycelium produced in liquid medium confirmed that the mass of growth made by mutants was usually very considerably less on fructose than on glucose media. The 35 strains of the test sample were screened for this characteristic by inoculating oidia to 20 ml quantities of NCM containing either 5 mM fructose or 5 mM glucose. The dry weights of mycelium formed on fructose varied among this set of mutants from 3 to 45% (mean = 19·9%) of the dry weight formed on glucose. It seems likely therefore that a general character of these mutants was that they made no use or only sparing use of fructose. Growth yields of some selected mutants growing on various concentrations of glucose, fructose and other carbon sources are com-

pared in Tables 2 and 3. Data in these Tables illustrate the paucity of growth on fructose. They also show that this character was to some extent concentration-dependent in that growth yield increased as the fructose concentration was increased, although the growth made by the mutants was still poor in comparison with that made by the wild-type.

Table 2. *Growth yields of some representative mutants grown on medium containing fructose or glucose*

	Mycelial dry weight (mg)			
Strain no.	150 mM glucose	150 mM fructose	5 mM glucose	5 mM fructose
Wild (BC9/6, 6)	75·7	104·5	19·5	20·7
Z15/6, 6	79·2	5·5	27·8	4·3
Z33/6, 6	90·8	26·8	18·8	3·2
Z77/6, 6	70·8	30·0	20·3	3·3
Z78/6, 6	62·2	15·2	24·5	26·0
Z183/6, 6	81·8	10·8	13·8	2·3
Z205/6, 6	76·5	9·5	12·7	1·8
Z215/6, 6	89·8	7·5	16·8	1·8
Z361/6, 6	80·3	9·3	14·8	1·0
Z492/6, 6	81·8	10·2	15·7	2·3
Z505/6, 6	67·2	12·2	15·5	2·2

Each strain was tested by inoculating identical quantities of the same oidial suspension to 50 ml portions of NCM medium + hexose contained in 16 oz medical flats, which were then incubated at 37 °C for 6 days. Mycelium was harvested by filtration through glass-fibre filters and dried to constant weight. Three replicates were made of each test.

Table 3. *Growth yields of wild-type and mutants from media containing different carbon sources*

	Dry weight of mycelium (mg)		
Medium	BC9/6, 6	Z215/6, 6	Z505/6, 6
No carbon source	4·2	2·5	2·5
50 mM glucose	51·2	44·2	73·5
50 mM xylose	21·7	0·8	19·0
50 mM acetate	34·2	27·7	25·5
50 mM maltose	116·3	62·8	127·5
15 g/l inulin	20·0	5·0	5·5
50 mM fructose	30·8	2·5	10·0
150 mM fructose	110·5	7·3	17·8
300 mM fructose	156·5	20·7	48·3
500 mM fructose	155·5	36·3	43·3

Methods as detailed in footnote to Table 2.

(iv) *Correlation between the physiological characters.* The relationships between resistance to deGlc, glucosamine and sorbose have already been discussed and illustrated. Analysis of many thousands of progeny from numerous crosses (during the isolation of mating-type recombinants) has not revealed a single instance of

segregation of the three resistance characters; segregation of the fructose non-utilizer characteristic from the resistance feature has never been observed either. It must be concluded therefore that these are phenotypic characteristics of a single mutational event. In addition, although only 35 mutants have been tested in sufficient detail, it does appear that resistance and the ability to utilize fructose are negatively correlated; the most resistant strains forming the least amount of mycelium on fructose medium.

(v) *Complementation tests.* The fact that so many randomly chosen and independently induced mutants shared the physiological characteristics outlined above suggested that the mutants might be alleles. An extensive series of complementation tests confirmed this. These tests were performed in the established manner, dikaryons being synthesized from appropirate resistant monokaryons and then tested for resistance to growth inhibition by deGlc and sorbose in both fructose and acetate media. In such tests dikaryons would be expected to be resistant if the mutations were allelic, not resistant (and therefore not able to grow) if they were not alleles (i.e. resistance follows from the lack of complementation). All the mutants proved to be recessive to wild-type. All possible dikaryon combinations of the sample set of mutants were successfully tested and all the dikaryons were resistant. More limited testing of a further 16 mutants indicated that they too were members of the same allelic series.

The tests were not entirely straightforward, however, for the mutant + mutant dikaryons showed an unusual readiness to resolve into their monokaryotic components with the result that non-complementation could not simply be scored by the presence of a colony. Microscopical examination of all colonies was necessary, a test being scored only when very extensive areas of dikaryotic growth were observed. Many of the results obtained with solid media have been confirmed using an entirely different testing regime which consisted of determining the growth yield of dikaryons inoculated (as mycelial plugs) to liquid medium containing 5 mM fructose. However, even these tests were subject to error because of the selection of wild-type revertants. While we are satisfied that the complementation data as it now stands reliably indicates that the mutants are allelic, it is clear that the more detailed analysis of the complementation pattern among the alleles will depend on either the identification of an improved testing regime or the use of auxotrophic forcing of dikaryosis. The conclusion that these mutants are allelic is supported by the results of crosses between the mutants. Out of $1{\cdot}67 \times 10^8$ viable progeny analysed from 122 crosses (involving 19 different mutants) only 33889 proved to be wild-type recombinants, giving a mean recombination value of 0·04%. A fine structure map of this cistron will be presented at a later date.

The resolution of these complementation dikaryons is of interest in itself, though, as it may shed some light on the nature of resistance. As already stated, resistant monokaryons were not entirely free of deGlc-induced morphological aberrations, though they were not subject to sorbose-induced abnormalities. Homozygous dikaryons (i.e. mutant + mutant) growing on deGlc media were very aberrant morphologically, much more so than their component monokaryons.

Such dikaryons frequently resolved; but strangely enough heterozygous (mutant + wild-type) dikaryons, though completely unable to grow on inhibitor media, resolved much less frequently. On sorbose, heterozygous dikaryons never resolved, homozygous ones did but only rarely. It would seem unlikely therefore that the dikaryons resolved simply through outgrowth of monokaryon on to medium the dikaryon could not invade. Rather, the observations suggest that resolution was related to, and dependent upon, both active growth of the dikaryon and the aberrance of that growth. Thus it can be argued that despite their resistance the mutants still fall victim to deGlc-mediated alterations in cell-wall structure. The mutation which confers resistance must therefore be at an early stage in carbohydrate metabolism. It must be a stage which is obligatory for the utilization of fructose and important (rather than obligatory) for the introduction of the analogues into the metabolic sequence.

Table 4. *Hexose uptake by mutant and wild-type oidia and mycelium*

(*a*) Oidia, exposed to labelled sugar for 10 min

	Hexose uptake (d.p.m./10^6 oidia)	
Strain	Glucose	Fructose
BC9/6,6	2794·7	1103·0
Z215/6,6	1785·4	137·4
Z505/6,6	3456·3	0·0

(*b*) Mycelium, exposed to labelled sugar for 30 min

	Glucose medium		Fructose medium	
Strain	Mycelium (d.p.m./mg dry weight)	CO_2 (d.p.m./mg dry weight of mycelium)	Mycelium (d.p.m./mg dry weight)	CO_2 (d.p.m./mg dry weight of mycelium)
BC9/6,6	1468·8	300·4	592·1	36·5
Z215/6,6	707·9	100·2	196·8	13·1
Z505/6,6	911·1	394·6	108·5	21·6

(vi) *The biochemical basis of resistance.* Despite this suggestion that the mutation must have occurred in a cistron controlling an early step of carbohydrate metabolism it was found that extracts of the mutants, analysed as detailed in Methods, contained the enzymes necessary for the utilization of fructose as a carbon source. However, by the use of sugars labelled with ^{14}C it was demonstrated that the mutants were virtually unable to accumulate fructose either within the oidium or the mature mycelium. The amount of labelled CO_2 produced was correspondingly reduced (Table 4). It thus appears that the most probable explanation is that these mutants are defective in some component of the fructose transport system. The gene symbol *ftr* is therefore proposed for this cistron.

(vii) *Chromosomal localization.* One mutant was chosen and tested for linkage to genes which mark the centromeres of each of the known linkage groups. This strain, number Z336/6,6 was chosen because it also carries a useful morphological

mutation which arose concurrently with the resistance mutation and which confers a feathery morphology to the colony margin; it has been termed 'ostrich' (gene symbol *ost*). Linkage studies with random spores have shown that *ost* is 15·5 units from *paba*-2 in linkage group VI. However, *ftr* showed independent segregation with each of the centromere markers (Table 5). Only six of the twelve chromosomes are marked with reliable centromere markers (the group G marker is very distant from its centromere so that the independent status of this group is in doubt) (Moore, 1967). It is quite likely therefore that *ftr* belongs to an entirely new linkage group. A small number of tetrads were analysed from a cross between

Table 5. *Summarized results of crosses between* ftr *and auxotrophic mutant markers of known centromeres*

Marker allele	Linkage group	Total progeny analysed	Total recombinants	*P* for independent segregation
ad-8	I	122	60	0·8
chol-2	II	276	145	0·4
pdx	III	276	141	0·7
nic-4	IV	405	222	0·05
me-8	V	405	220	0·05
paba-2	VI	271	146	0·2
ad-3	G	95	50	0·5

the wild type and a strain which carried the genes *paba*-2, *ost* and *ftr*. The tetrad distributions obtained were: for the *paba*/*ost* pair, 8 parental ditypes, 0 non-parental ditypes and 3 tetratypes; for the *ost*/*ftr* pair, 6 parental ditypes, 3 non-parental ditypes and 2 tetratypes; and for the *ftr*/*paba* pair, 5 parental ditypes, 1 non-parental ditype and 5 tetratypes. Second-division segregation frequencies and standard sampling errors calculated from these data were: *ost*, $7{\cdot}1 \pm 25{\cdot}0\,\%$; *paba*-2, $34{\cdot}4 \pm 13{\cdot}5\,\%$; *ftr*, $22{\cdot}9 \pm 10{\cdot}4\,\%$. Linkage between *ost* and *paba*-2 is thus confirmed and there are indications that both genes are on the same chromosome arm, *ost* being nearest to the centromere. However, in view of the indicated close centromere linkage and the large sampling error a much larger number of tetrads would be required to position *ost* with accuracy. It is also clear that *ftr* is quite closely linked to its centromere (between about 2 and 20 units), and this fact, taken together with the random spore data showing free recombination between *ftr* and reliable markers of each of the known linkage groups, qualifies the gene *ftr* for rank as a marker of the centromere of an entirely new linkage group – linkage group VII.

4. DISCUSSION

The experiments reported above clearly show that when slection is made for growth on media containing either fructose or acetate together with inhibitory concentrations of 2-deoxy-D-glucose the mutants which arise are defective in some function associated with fructose transport and appear to be alleles of a single cistron. It must therefore be that the resistance phenotype results from a corres-

ponding block to the transport of the inhibitory analogues. The transport system involved is evidently of wide specificity, since in the normal state it must be involved in the transport of at least four quite different hexoses. Even wider specificity may be deduced from the poor growth made by *ftr* mutants on xylose, but this has yet to be rigorously examined. Lack of resistance to deGlc on glucose media coupled with the apparently unaffected accumulation of ^{14}C-labelled glucose by the mutants (the differences which do exist may simply be due to background genetic effects) suggests the occurrence of a completely independent glucose transport system. The kinetics of sugar transport by both mutant and wild-type obviously warrants further attention.

The deGlc-resistant mutants discussed here differ quite markedly from those similar mutants obtained in yeasts and HeLa cells, for in no other case was sugar transport specifically involved. In the case of *Saccharomyces* (Heredia & Sols, 1964) and of HeLa cells (Barban, 1962) almost tenfold increases in the activities of phosphatases with fairly high specificity against deGlc-6-phosphate can adequately account for the resistance observed. Such a possibility is ruled out in *Coprinus* because the mutants are cross-resistant to sorbose and this sugar is not phosphorylated by extracts of the *Coprinus* wild-type (Stewart, unpublished observations). In *Schizosaccharomyces* (Megnet, 1965) a fivefold decrease in the specific activity of hexokinase was shown to be characteristic of deGlc-resistant cells. No such alteration occurred in the *Coprinus* mutants.

The differences between the mutants in the various organisms may represent basic differences in metabolism, primarily, perhaps, those stages directly affected by deGlc. Alternatively they may reflect the different techniques of mutant isolation. Although Heredia & Sols (1964) selected mutants in medium containing fructose and deGlc, they used a three-step clonal isolation technique which involved three successive increases in deGlc concentration. Barban (1962) used a similar technique but with a glucose + deGlc medium, while Megnet (1965) used a one-step initial challenge with gluconate and deGlc, making a subsequent selection for resistant colonies which could not utilize glucose. It may be, therefore, that the type of resistance character obtained can be controlled by altering the selection conditions. With respect to *Coprinus* it will be particularly interesting to investigate the resistance mechanism revealed by selection on medium containing deGlc and glucose. Limited experiments have been performed with mutants selected for resistance to either sorbose or glucosamine in the presence of either fructose or acetate. So far all such mutants have proved to possess the characters of established *ftr* strains.

Intriguing contrasts between this and previous work are also evident in relation to sorbose resistance. Sorbose-resistant mutants obtained in *Neurospora crassa* were shown to be defective in sorbose uptake. However, even here differences are apparent, for although only 19 mutants were isolated they represented mutations in at least six different genes, four of which were involved in transport. Furthermore, although sorbose was not transported, the uptake of normal hexose was unaffected (Klingmüller, 1967*a*, *b*). In *Aspergillus nidulans* sorbose resistance was

shown to result from mutation in two distinct loci (Elorza & Arst, 1971). Mutation in one of these loci conferred cross-resistance to deGlc and resulted in a defect in sugar uptake. Like the *Neurospora* case, however, while sorbose uptake was prevented the utilization of normal sugars was not affected. Mutation in the second locus caused the loss of phosphoglucomutase and was not followed by cross resistance to deGlc.

While the *ftr* mutants are of considerable interest in themselves, particularly with regard to the mechanism of sugar transport, they also have potential as experimental material for other studies. For example, on the basis of present knowledge it appears that selection for mutation in a single cistron can be made in two directions (forwards towards resistance, and in the reverse direction towards fructose utilization), so that detailed studies of mutation kinetics should be possible. Similarly there seems to be no impediment to fine-structure analysis of the cistron since allelic crosses are adequately fertile and selection can readily be made for the wild type recombinant class.

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