ASPECTS OF GALACTOSE AND GLUCOSE TRANSPORT IN ESCHERICHIA COLI.

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PREFACE.

I wish to thank my supervisor, Professor H.L. Kornberg, F.R.S., for his invaluable support, guidance and encouragement throughout the course of this work. My thanks are also due to those members of the Biochemistry and Genetics Departments at Leicester who at various times provided me with advice, organisms, or practical assistance, and in particular to Mr. S.J.Bungard, Dr. P.J.F.Henderson and Dr. M.C.Jones-Mortimer. I also wish to thank Mrs. D.Wagner for typing this thesis, and Miss S.N.Dilks and Miss E.Brown for preparing the Figures.

ABSTRACT.

1. Strains of *E.coli* have been characterised which are galactose-positive despite their failure to express any of the active transport systems for galactose during growth on this sugar. The growth of such strains on galactose occurs at rates that are a function of the galactose concentration of the medium: half-maximal growth rates require more than 2mM galactose to be present. The introduction of a mutation in the glucose phosphotransferase Enzyme II specified by the gene *umg* severely impairs the ability of these strains to grow on galactose; it has been established by a variety of means that this Enzyme II, or a component of it, provides the means of galactose entry into these organisms. However, the uptake of galactose does not require phosphotransferase activity, but occurs by facilitated diffusion on this carrier. The implications of this finding on the current understanding of the mechanism of glucose uptake by the phosphotransferase system are discussed.

2. Although the Umg-system provides the major route of glucose uptake in many strains of *E.coli*, this is not true for all strains. Evidence is presented that suggests the existence of a fourth Enzyme II for glucose, in addition to those specified by the genes *umg*, *ptsX* and *bgl*.

3. The galactose permease specified by the gene *galP* can transport glucose in addition to galactose. A

screening procedure for distinguishing GalP$^+$ from GalP$^-$ strains is described which makes use of this property and which has been used to locate the *galP* lesion close to minute 55 on the *E.coli* chromosome, between the genetic markers *fda* and *lysA*.

ABBREVIATIONS USED IN THIS THESIS.

PT	phosphotransferase
HPr	the small, histidine-containing, protein of the phosphotransferase.
αMG	methyl-α- D-glucopyranoside
DG	2-deoxy-D-glucose
DFG	3-deoxy-3-fluro-D-glucose
TG	5-thio-D-glucose
IPTG	isopropyl-1-thio-β-D-galactopyranoside
ONPG	o-nitrophenyl-β-D-galactopyranoside
TMG	methyl-1-thio-β-D-galactopyranoside
MeGal	methyl-β-D-galactopyranoside
CCCP	m -Cl -carbonylcyanide phenylhydrazone
ATP	adenosine triphosphate
ATPase	Ca^{++} Mg^{++} - adenosine triphosphatase
cyclic AMP	adenosine 3' : 5' - cyclic monophosphate
LDH	lactate dehydrogenase
NAD^+	nicotinamide adenine dinucleotide
NADH	reduced nicotinamide adenine dinucleotide
PEP	phosphoenolpyruvate
UDP	uridine diphosphate

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INTRODUCTION.

General properties of bacterial sugar transport systems.

The first step in the utilisation of any carbohydrate by a bacterium is its passage through the membrane into the cell. In general this process obligatorily requires the participation of one or more specific membrane carrier proteins. This was first realised when "cryptic" bacterial mutants were isolated, which were unable to grow on a particular sugar despite possessing all the intracellular enzymes of its catabolism (Cohen and Monod, 1957). Carrier-mediated transport systems have since been characterised in other microorganisms, as well as in animal cells (for review, see Oxender, 1972). In their natural habitat bacteria are subject to wide variations in environmental conditions to which they must be able to respond rapidly, a fact which is reflected in the observation that most bacterial sugar uptake systems are inducible. This contrasts with the situation in mammalian cells, which are constantly bathed in a medium of relatively unchanging composition, and which synthesise constitutively their transport systems for carbohydrates. Carrier-mediated transport in bacteria can occur by one of three mechanisms: facilitated diffusion, active transport or group translocation.

The characteristics of facilitated diffusion systems have been elaborated by Stein (1967). In brief, facilitated diffusion differs from passive diffusion in that it is stereospecific, is subject to competitive

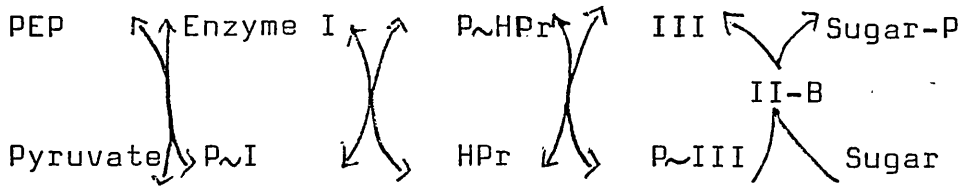
inhibition by structurally similar compounds, and does not occur at a rate directly proportional to the sugar concentration, but reaches a limiting 'saturated' rate. Moreover the process can be 'poisoned' by inhibitors of enzyme activity, and exhibits the high temperature coefficients characteristic of enzyme reactions. Facilitated diffusion does however share with simple diffusion the property of being independent of the input of energy; in this respect it differs from active transport and group translocation, which both require the expenditure of energy by the cell. The uptake of glycerol by E.coli is an example of a bacterial facilitated diffusion system (Sanno et al., 1968).

Sugars taken up by active transport systems appear inside the cell in unchanged form, as do those taken up by facilitated diffusion. However the processes differ in that here, because metabolic energy is expended to bring about the uptake process, cells unable to metabolise the substrate accumulate it to an intracellular concentration greater than that in the surrounding medium. The classical demonstration of this phenomenon in bacteria was that of Rickenberg et al. (1956), who grew a culture of E.coli on lactose and hence induced the β-galactoside transport system. The cells were shaken with [^{35}S]-thiogalactoside and rapidly harvested. Not only was the intracellular concentration of radioactive material 100-fold or more greater than that in the outside medium, but extraction from the cells and analysis of the labelled material showed it to be the unchanged thiogalactoside.

Since then this and other techniques have been used to demonstrate the presence of active transport systems for various sugars in E.coli (Horecker et al., 1960; Pogell et al., 1966; Pouysségur et al., 1974) and other bacteria including A.vinlandii (Barnes, 1972) A.pyridinolis, P.aerugenosa (Kaback, 1974) and a thermophilic bacillus (S.J.Bungard, unpublished results). The mechanism by which energy is used to bring about active transport in bacteria is a subject of considerable debate, and will be discussed later in this thesis. It is apparent though that active transport carriers can act as facilitated diffusion carriers under conditions in which the input of energy is blocked, either by treatment with metabolic inhibitors (Winkler & Wilson, 1966; Wong & Wilson, 1970) or by mutation (Wong et al., 1970; Wilson & Kusch, 1972; Langridge, 1974). Some active transport systems are dependent for their activity on binding proteins which are located in the periplasmic space, and are released if the cells are subjected to osmotic shock (Heppel, 1969). The role of these binding proteins in transport is evident from the observations that mutants lacking a particular binding protein are defective in their ability to take up the sugar which it binds, and that osmotic shock leads to impairment of the transport of sugars for which there are binding proteins. However the mechanism by which binding proteins participate in the uptake process is still not clear (Boos, 1974; Silhavy et al., 1974).

Group translocation is a mode of bacterial sugar

transport which, unlike facilitated diffusion and active transport, brings about covalent modification of the sugar. The enzymes involved were discovered when Kundig et al. (1964) found that extracts of E.coli could catalyse the transfer of the phosphate moiety from phosphoenolpyruvate to certain carbohydrates. These workers purified and characterised the protein components involved (Kundig & Roseman, 1971 a,b) and found that two of them, a small histidine-containing protein termed HPr, and a larger protein designated Enzyme I, both derived from the soluble fraction, were required for the phosphorylation of all substrates of this reaction. Each substrate also required the participation of a second, membrane-bound, enzyme, termed Enzyme II, which was specific for that substrate and in most cases was inducible. Further characterisation of several Enzymes II from E.coli and other bacteria showed that each could be divided into two components. In some cases both were membrane-bound and were termed Enzymes II -A and II - B (Kundig & Roseman, 1971, b), whereas in others, in particular in S.aureus(Simoni et al., 1968) only one component, Enzyme II - B, was present in the membrane fraction, and the other, referred to as Factor III, was soluble. It was shown that the phosphate moiety was sequentially passed from one component to another until finally the sugar was phosphorylated as shown below (from Roseman, 1972).



Sugars which can be phosphorylated by this system have been termed PT-sugars and are thus distinguished from the non-PT sugars which are not substrates for the reaction.

Several pieces of evidence led to the conclusion that this phosphotransferase system (now often referred to as the PT- or Roseman system) was directly involved in the uptake of PT-sugars. Some of these are summarised below.

1. A mutant of S.aureus which was pleiotropically affected in its ability to take up and grow on 11 sugars (Egan & Morse, 1965) was shown to lack Enzyme I activity (Simoni et al., 1968). Mutants with similar properties were isolated from other bacteria including S.typhimurium (Simoni et al., 1967; Saier et al., 1970), A.aerogenes (Tanaka & Lin, 1967) and E.coli (e.g. Tanaka et al., 1967; Wang et al., 1969) and in each case were found to be defective in either HPr or Enzyme I. Studies with these mutants initially implied that the PT-system is also involved in the uptake of certain non-PT sugars, since many of them failed to grow not only on PT-sugars but also on some, but not all, non-PT sugars. More recently though this phenomenon has been ascribed to an extreme susceptibility of Enzyme I mutants to catabolite repression, as will be discussed in Chapter Three.

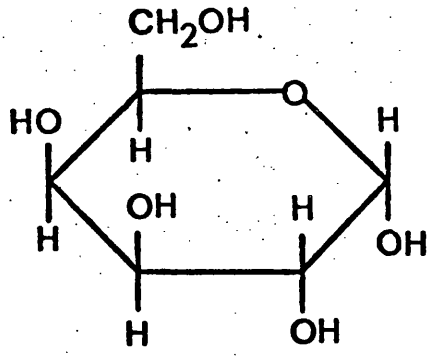
2. Mutants were described (Fox & Wilson, 1968;

Ferenci & Kornberg, 1971; Simoni & Roseman, 1973); which were specifically impaired in their ability to take up and grow on one particular PT-sugar; these were found to lack the relevant Enzyme II activity. Moreover the patterns of inducibilities of the Enzymes II and of the uptake systems for various sugars corresponded both qualitatively and quantitatively (Roseman, 1969).

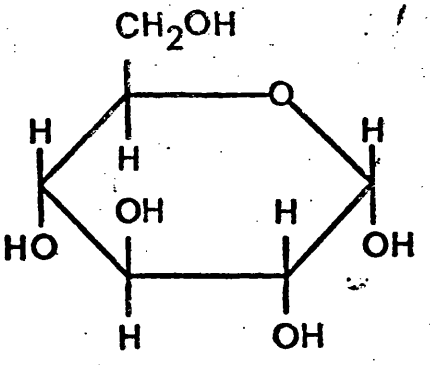
3. Osmotically shocked cells of E.coli were severely impaired in transport activity; this activity was partially restored on addition of purified HPr (Kundig et al., 1966).

4. A role of the PT-system in sugar transport provided a satisfactory explanation for the earlier observation (Rogers & Yu, 1962) that certain sugars were apparently initially accumulated as phosphate esters during transport by whole cells.

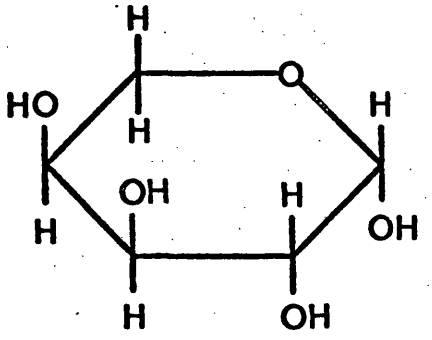
In S.aureus all sugars are apparently taken up by means of the Roseman system (Egan & Morse, 1965; Hengstenberg, Egan & Morse, 1968), but in other bacteria not all sugars are PT-sugars. In E.coli for example, PT-sugars include fructose, mannose, mannitol and sorbitol, but lactose, maltose, melibiose and hexose phosphates are non-PT sugars (Roseman 1969, 1972). The question whether Enzymes II can act as facilitated diffusion carriers if Enzyme I, and hence the phosphorylating capacity of the Roseman system, is absent, is at present a matter of some controversy; it will be considered in detail in Chapter Three.



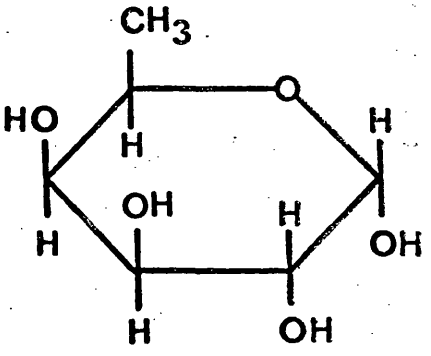
D-galactose



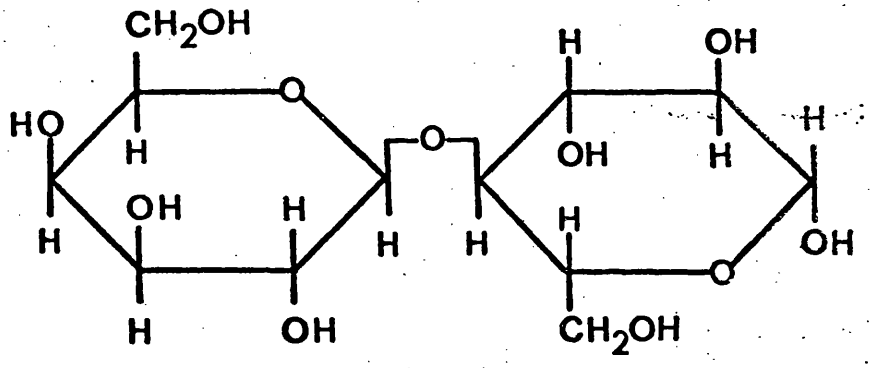
D-glucose



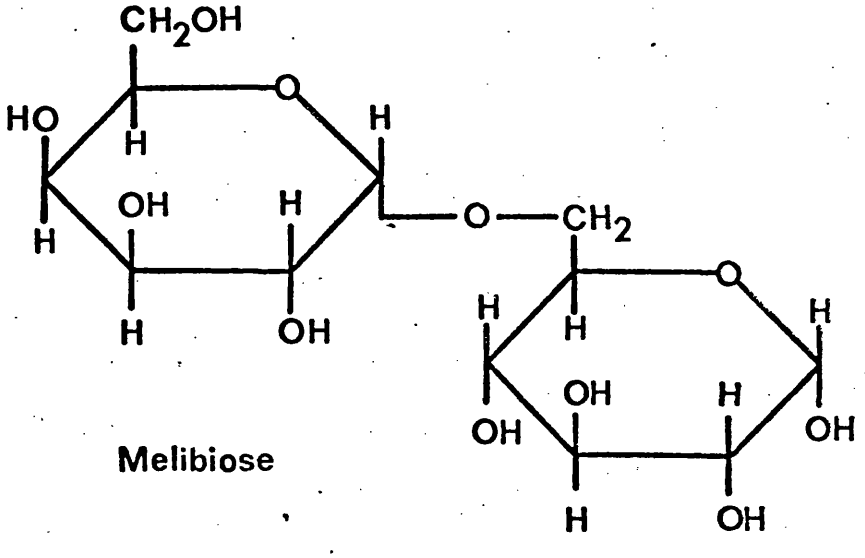
L-arabinose



D-fucose



Lactose



Melibiose

Fig. I.1. The structures of galactose, glucose, and some sugars with which galactose shares active transport carriers.

The uptake of glucose by E.coli

The close similarity between the structures of glucose and galactose (Fig. I.1) might lead one to suppose that membrane-bound transport proteins which have an affinity for one might also transport the other. Indeed this has been shown to be true in mammalian systems, both where sugar uptake is by facilitated diffusion (Lefevre, 1954; Levine et al., 1965) and where the process is one of active transport (Crane 1960). This contrasts with the situation in the bacterium E.coli, in which the well-characterised uptake systems for glucose and galactose not only involve different carrier proteins, but also operate by different mechanisms.

Glucose has been shown to enter wild-type E.coli by the activity of the phosphotransferase system. Purified components of the phosphotransferase system readily phosphorylate glucose (Kundig et al., 1964, 1966; Kundig & Roseman, 1971 a,b) and there is evidence from uptake experiments with whole cells (Rogers & Yu, 1962; Hoffee et al., 1963; Miles & Pirt, 1973) and membrane vesicles (Kaback, 1968) that glucose and its non-metabolisable analogues arrive in the cell in the form of the 6-phosphate ester.

The study of mutants impaired in their ability to take up glucose and its analogues has enabled two Enzymes II for glucose to be identified. The first, specified by the gene umg, located at about minute 23 on the E.coli linkage map and co-transducible with pur B (Kornberg & Smith, 1972),

has a high affinity also for methyl- ∝ - D - glucoside, so that mutants lacking this activity are almost totally unable to take up this compound. Mutants with similar properties to those of umg mutants have been isolated by other workers, and variously designated cat (Tyler et al., 1969), gptA (Epstein & Curtis, 1972) and later by the latter workers gpt (Curtis & Epstein, 1975). Curtis and Epstein (1971) showed that cat and gptA mutants carried defects in the same gene; since this, like umg, was found to be co-transducible with the purB marker (Tyler et al., 1969; Curtis & Epstein, 1975), it seems to be identical with the umg gene. Mutants impaired in the second glucose Enzyme II were first described by Curtis and Epstein (1971), who, by comparing the growth and uptake properties of these organisms with wild-type cells and gptA mutants, demonstrated that this system transports mannose, 2-deoxyglucose, glucosamine and mannosamine in addition to glucose (Epstein & Curtis, 1972; Curtis & Epstein, 1975). The structural gene for this Enzyme II was initially designated gptB, and later mpt, and was located at minute 35.5, contransducible with the marker eda (kga) (Curtis & Epstein, 1975). Kornberg and Jones-Mortimer (1975) found that strains of E.coli lacking the fructose Enzyme II specified by the gene ptsX (Jones-Mortimer & Kornberg, 1974b) were impaired in their ability to utilise glucose, glucosamine and mannose. The similarity in the properties of these mutants with those of the mpt strains described by Curtis and Epstein suggested that their lesions

might be in the same gene; this was supported by the finding (Jones-Mortimer & Kornberg, 1974b) that ptsX is co-transducible with kga. Throughout this thesis the terms umg and ptsX will be used to refer to the different structural genes for the glucose Enzymes II.

The uptake of galactose by E.coli.

There have been reports that galactose is phosphorylated to some extent during its uptake by E.coli (e.g. Rogers & Yu, 1962; Kundig et al., 1965; Kaback,1968; Vorisek & Kepes, 1972), but no galactose-specific Enzyme II has been characterised. Moreover the phosphorylated product, found to be galactose 6-phosphate, appears not to be metabolised by this organism (Vorisek & Kepes, 1972) so the physiological significance of its synthesis is dubious. This contrasts with the situation in S.aureus where galactose is taken up and phosphorylated by the Roseman system, and the resulting galactose 6-phosphate is metabolised by a pathway involving its conversion to tagatose 6-phosphate (Bissett & Anderson, 1974). In addition, it has been found that some, but not all, of the E.coli Enzyme I mutants which have been studied grow on galactose (e.g.Asensio et al.,1963; Kamogawa & Kurahashi, 1967) so phosphotransferase activity cannot be obligatory for galactose uptake. The major route of galactose uptake by wild-type E.coli appears therefore to involve one or more of the active transport systems which have been shown to have an affinity for this sugar. These mediate its entry

Table I.1(a) Summary of the substrate specificities of the galactose transport systems of E.coli K12

Substrate	Permease: Lactose	Melibiose	Arabinose	Methyl-galacto-side	Galactose
Lactose	+	-	+	-	-
TMG	+	+	+	-	-
MeGal	+	+	-	+	-
D-fucose	-	-	+	+	+
D-galactose	+	+	+	+	+

Table I.1(b) Summary of the inducer specificities of the galactose transport systems of E.coli K12.

Inducer	Permease: Lactose	Melibiose	Arabinose	Methyl-galacto-side	Galactose
IPTG	+	-	-	-	-
D-galactose	+†	+†*	-	+	+
D-fucose	-	-	-	+	+
L-arabinose	-	-	+	-	-

† = induction in galK strains only

* = induction only during growth at or below 30°C

The tables summarise data taken primarily from Rotman et al. (1968) but with modifications and additions from Leder and Perry (1967), Brown and Hogg (1972), Messer (1974) and Parsons and Hogg (1974).

into the cell as free galactose; it is then phosphorylated to galactose 1-phosphate by the cytoplasmic galactokinase and further metabolised by the other enzymes of the gal operon, galactose 1-phosphate uridyl transferase and UDP galactose epimerase, to form UDP glucose.

The properties of the various transport systems for galactose have been discussed by several authors (eg. Rotman et al., 1968; Lin, 1970; Kalckar, 1971; Oxender, 1972); some of their substrate and inducer specificities are summarised in Tables I.1a and I.1b. In this Thesis they will be referred to as 'permeases'. It has been suggested (Kepes & Cohen, 1962) that when a transport system has several components, the term 'permease' should refer only to the stereospecific translocating protein component; here, however, the convention of Cohen and Monod (1957) will be adopted, so that the term will encompass the whole transport system, which may consist of more than one gene product. Moreover the expression 'galactose permease' will be used exclusively to refer to the particular active transport system which has been so designated (Rotman et al., 1968) and will not be used in discussing galactose transport systems in general.

Three of the active transport systems which bring about galactose uptake in E.coli are primarily concerned with the transport of other sugars, namely lactose, arabinose and melibiose. The β-galactoside or lactose permease specified by the gene lacY and referred to by some authors as TMG permease I (Prestidge & Pardee, 1965), was one of the first

active transport systems to be described (Rickenberg et al., 1956). Its transport specificity is for α-and β-D-galactopyranosides, β-D-thiogalactopyranosides, and D-galactose (Rickenberg et al., 1956; Pardee, 1957). It is induced by galactose only in strains which do not metabolise this sugar (Kalckar et al., 1959). The melibiose permease, first described by Prestidge and Pardee (1965), and designated by them TMG permease II, is similar to the lactose permease in its substrate specificity except that it has no affinity for lactose. It is not active in cells of E.coli K12 grown at 37°C, but when growth is at 26°C, at which temperature it is active, there is evidence that, like the lactose permease, it may be induced by galactose if the strain lacks galactokinase (Leder & Perry, 1967). These workers found that galactose inhibits the induction of this uptake system in Gal^+ strains.

There are two arabinose permeases in E.coli, specified by the genes araE and araF, and both subject to the positive control mediated by the araC gene product (Brown & Hogg, 1972). The properties of the two systems have been studied mainly in E.coli B/r, but they appear to be essentially the same in K12 strains (Schleif, 1969). The AraF system requires the participation of a periplasmic binding protein (Hogg & Englesberg, 1969; Schleif, 1969; Brown & Hogg, 1972) and has a higher affinity for arabinose than the AraE system. It is this system which, of the two, appears better able to recognise galactose as substrate (Brown & Hogg, 1972).

The arabinose carriers, being under the control of the regulatory gene *araC*, are not induced by growth on, or in the presence of, galactose.

The other two galactose active transport systems seem to be primarily concerned with the uptake of this rather than any other sugar, since they are induced by growth on galactose; however their substrate specificities overlap to some extent with those of the carriers already described (Table 1.1a). Of the two, the methyl-β-D-galactoside permease (MeGal permease) has been subject to the more detailed investigation. The substrate and inducer specificities of this system, whose structural genes are located in the region of the chromosome designated *mgl* (corresponding to the *mglP* region of Taylor and Trotter,1972), have been studied by Rotman *et al.*, (1968). Most of the work on this system has however been aimed at elucidating the nature and the role in galactose uptake of the periplasmic binding protein with which it is associated (reviewed by Silhavy *et al.*,1974). The particular results of these investigations which are important to this study will be discussed in the relevant sections of this Thesis, but it should be made clear here that although both the AraF and Mgl systems have an affinity for galactose, and both require the participation of binding proteins, the two systems are not identical. This was made clear by Parsons and Hogg (1974) who compared the amino acid compositions, ligand binding affinities, and induction characteristics of both binding

proteins, and found significant differences.

The outstanding feature of the MeGal permease is its high affinity for galactose (Km=2 x 10^{-7}M, Vorisek and Kepes, 1972). As a result of this, GalK$^-$ mutants which have MeGal permease activity are constitutive for the expression both of this uptake system and of the other enzymes of the *gal* operon. This is because galactose, derived internally from hydrolysis of UDP galactose, is not allowed to be lost to the medium but is efficiently recaptured such that it reaches a high enough intracellular concentration to maintain the induction (Wu, 1967; Wu *et al.*, 1969). Before this phenomenon was understood, the constitutivity of GalK$^-$ mutants and inducibility of GalK$^+$ transductants of these strains led to the belief that a regulatory gene, controlling the expression of the MeGal permease, was located close to the *galK* gene (Ganesan & Rotman, 1966). More recently though Lengeler *et al.* (1971) have described a different regulatory gene which apparently fulfils this role; it is designated *mglR* and lies between minutes 56 and 74 on the linkage map.

The galactose permease, whose existence as a distinct entity was implied by the ability of the Mgl$^-$ LacY$^-$ GalK$^-$ strain W4345 to accumulate galactose after growth at 37^{o}C in the presence of D-fucose (Ganesan & Rotman, 1966), was studied in detail in this strain by these workers (Rotman *et al.*, 1968). They concluded that D-fucose, galactose, melibiose and TMG induced the system and that its only substrate was galactose, but they did not test melibiose as

a potential substrate. Lengeler (unpublished observations discussed by Kalckar, 1971, and Oxender, 1972) raised some doubts as to the validity of their interpretation of the results they obtained, by pointing out that cells of strain W4345, supposedly induced for galactose permease activity, take up melibiose; thus it might have been that these workers were in fact studying a heat-resistant melibiose permease. Since then, however, galactose accumulation has been demonstrated after growth at 37°C of other Mgl^- $LacY^-$ $GalK^-$ strains which have normal, temperature-sensitive, melibiose permease activity (e.g. the organisms described by Wu *et al.*, 1969); it therefore seems that the galactose permease and melibiose permease are separate systems, each with an affinity for melibiose. Wilson (1974a) provided evidence in support of this view by demonstrating that galactose permease activity is induced by growth on galactose, whereas it had previously been shown that melibiose permease activity is not (Leder & Perry, 1967). Wilson also showed that the structural gene for the galactose permease (*galP*) is controlled by the regulatory gene *galR*, which was first described by Buttin (1963,b) as the gene regulating the expression of the *gal* operon, and was shown by Saedler *et al.* (1968) to be a negative control gene.

Several papers which are concerned with the active transport of galactose, notably those by Horecker *et al.* (1960) and Buttin (1963a,b) were published before the distinction between MeGal permease and galactose permease was

realised. In both these cases galactose uptake was studied in GalK$^-$ cells and was found to be constitutive; MeGal permease activity must therefore have been present in the strains used, but it is probable that galactose permease activity also contributed to the measured uptake. It is clear that to study in isolation any one of the various galactose transport systems, as Rotman et al. (1968) attempted to do, conditions must be chosen such that only that particular system is induced (if possible using a mutant devoid of the other activities), or such that the substrate used is recognised only by that system.

The major portion of the work to be described in this Thesis was carried out in an attempt to understand how galactose enters and supports the growth of strains of E.coli that are effectively devoid of all these galactose active transport systems. It was found that in such strains there is a physiologically important common carrier for glucose and galactose, namely the glucose Enzyme II specified by the gene umg, or a component of it, but that whereas the uptake of glucose by this system is dependent on phosphotransferase activity, the uptake of galactose is not.

MATERIALS AND METHODS.

I MATERIALS.

Commercial materials.

The enzymes, coenzymes, sugar phosphates, and most of the non-metabolisable sugar analogues used in this work were obtained from Sigma Ltd. (London, England) and Boehringer GmbH (Mannheim, Germany); 3-deoxy-3-fluoroglucose was a gift from Professor N.F.Taylor (University of Bath). Sugars, which with the exception of L-arabinose were all of the D-configuration, were in general from British Drug Houses Ltd. (Poole, England) or Fisons Ltd. (Loughborough, England) but glucose-free galactose, which was used to dilute the isotopic galactose and in all experiments with *ptsI crr* strains, was purchased from Sigma Ltd. Growth supplements were obtained from Sigma Ltd. or British Drug Houses Ltd. The uncoupler m-Cl-carbonylcyanide phenylhydrazone was from Calbiochem Ltd. (Hereford, England). Nutrient broth was obtained from Oxoid Ltd. (London, England) and agar either from this source or from Difco Laboratories (Detroit, U.S.A.). Streptomycin sulphate was obtained from Glaxo Ltd. (Greenford, England) and chloramphenicol from Parke-Davies Ltd. (Hounslow, England). Trimethoprim lactate was a gift from Messrs. Burroughs Wellcome & Co. (London, England). [^{14}C]-Methyl-β-D-galactoside was purchased from NEN Chemicals GmbH (Dreieichenhain, West Germany) and all other isotopic material from the Radiochemical Centre (Amersham, England).

TABLE M.1. (Contd)

Strain	[c]Genetic markers	Source or Reference
CLR115	his arg(HBCE) thr leu galK galP mgl pps mal umg	Spontaneously from CLR100
CLR116	arg(HBCE) thr leu umg	[KL16 x CLR113] His^+
CLR121	arg(HBCE) thr leu galK galP pps(?) mal	[KL16 x CLR100] His^+
CLR130	his arg(HBCE) thr leu galP mgl ptsI crrΔ fda^{ts} umg	[JM807 x CLR106T] Thy^+
CLR131	his arg(HBCE) thr leu serA galP mgl ptsI crrΔ umg	CLR130 transduced to Fda^+ with ME100 as donor
CLR132	his arg(HBCE) thr leu mgl ptsI crrΔ fda^{ts} umg	CLR131 transduced to Ser^+ with JM803 as donor

[c] Several of the F^- strains used carry additional carbohydrate markers which have no relevance to this work; to avoid overcrowding of the Table these have been omitted.

TABLE M.I. (Contd).

Strain	°Genetic markers	Source or Reference
CLR101	lacZ galP mgl	20SOK⁻ transduced to Gal⁺ with K2.1t as donor
CLR102	his arg(HBCE) thr leu galP mgl	[K1 x K2.2W] Trp⁺
CLR103	arg(HBCE) thr leu galP mgl ptsI^{ts} pps(?)	[ts19 x K2.1t] His⁺
CLR104	arg(HBCE) thr leu galP mgl ptsI crrΔ pps(?)	[ts19 -1Δ x K2.1t] His⁺
CLR105	his thr leu galK mal(?) galP mgl ptsI crrΔ	[ts19 -1Δ x CLR100] Pps⁺
CLR106	his arg(HBCE) thr leu galP mgl ptsI crrΔ umg	[ts19 - 1Δ x K2.1.22a] Pps⁺
CLR106T	his arg(HBCE) thr leu thyA galP mgl ptsI crrΔ	From CLR106 by trimethoprim resistance
CLR107	arg(HBCE) thr leu thyA galP mgl ptsI crrΔ pps(?)	From CLR104 by trimethoprim resistance
CLR108	arg(HBCE) thr leu mgl ptsI crrΔ pps(?)	CLR107 transduced to Thy⁺ with AT713 as donor
CLR111	his thr leu galP mgl ptsI crrΔ mal(?)	CLR105 transduced to Gal⁺ with K2.1t as donor
CLR112	his arg(HBCE) thr leu mgl ptsI crrΔ umg	[ED1032 x CLR106T] Thy⁺
CLR113	his arg(HBCE) thr leu mgl umg	CLR112 transduced to Pts⁺ with K2.1t as donor
CLR114	lacZ galP mgl umg	Spontaneously from CLR101

TABLE M.I. (Contd).

Strain	°Genetic markers	Source or Reference
CLR12	his arg(HBCE) thr leu galP mgl pps (λ)	K2.1t lysogenised with λc1857
CLR13	his arg(HBCE) thr leu galP mgl pps (λ) mal	From CLR12, λ-resistant spontaneous derivative
CLR14	his arg(HBCE) thr leu bio galP mgl pps mal	λ-educant from CLR13
CLR15	lacZ galK galP mgl umg	Spontaneously from 20SOK$^-$
CLR16	lacZ galP mgl umg	CLR15 transduced to Gal$^+$ with K2.1t as donor
†CLR17	lacΔ galK mgl umg	Spontaneously from W4345
†CLR18	lacΔ mgl	[K1 x W4345] Gal$^+$
†CLR19	lacΔ mgl umg	[K1 x CLR17] Gal$^+$
CLR20	arg(HBCE) thr leu galP pps (?)	[K16 x K2.1.22a] His$^+$
CLR21	leu ptsF ptsX kga kdgR^c umg	Spontaneously from JM479
CLR22	leu ptsF umg	CLR21 transduced to Kga$^+$ with 20SOK$^-$ as donor
CLR23	leu ptsF umg	CLR21 transduced to Kga$^+$ with K2.1t as donor
CLR24	lacZ galK galP mgl thyA	From 20SOK$^-$ by trimethoprim resistance
CLR25	lacZ galK galP(?) mgl ptsI crrΔ	[ts19 - 1Δ x CLR24] Pts$^+$
CLR100	his arg(HBCE) thr leu galK galP mgl pps mal	CLR14 transduced to Bio$^+$ with 20SOK$^-$ as donor

TABLE M.I. (Contd).

Strain	[c]Genetic markers	Source or Reference
0144	purB galK mgl galP	From A.L.Stouthamer
0144.W	purB galK galP mgl umg	Kornberg & Smith (1972)
0144.g$^+$	purB galP mgl	[B11 x 0144] Gal$^+$
0144.Wg	purB galP mgl umg	[B11 x 0144.W] Gal$^+$
AB1325	purB his pro galK galP(?) mgl	From A.L.Taylor
AB1325.αMG^R	purB his pro galK galP(?) mgl umg	From AB1325 by selection for resistance to αMG
2092	purF pheA his arg(HBCE) galP	From M.L.Morse
2092.F4	purF pheA his arg(HBCE) galP umg(?)	From 2092 by selection for resistance to DFG
2092.W4	purF pheA his arg(HBCE) galP umg	"
20S0K$^-$	lacZ galK galP mgl	From W.Boos
†W4345	lacA galK mgl	"
AT713	cysC argA lysA thi(?)	Laboratory stock
JM479	leu ptsX kdgR^c ptsF kga	Jones-Mortimer & Kornberg (1974b)
CLR5	purB his galP(?) kga	[K1 x AB1325] Pro$^+$
CLR6	purB his galP(?) mgl umg	From CLR5 by selection for resistance to αMG
CLR7	purB his galP(?) mgl umg	[K1 x AB1325.αMG^R] Pro$^+$ Gal$^+$
CLR9	arg(HBCE) thr leu galP umg ptsF	[KL16.21 x K2.1.22a] Pps$^+$
CLR11	arg(HBCE) thr leu galP pps	[KL16 x K2.1t] His$^+$

TABLE M.1. (Contd).

Strain	Genetic markers	Source or Reference
CLR133	gnd hisΔ gal bioΔ ptsX mgl fda^{ts}	JM759 transduced to PtsF$^+$ with 20SOK$^-$ as donor.
CLR135	gnd hisΔ gal bioΔ ptsX mgl	CLR133 transduced to Fda$^+$ with 20SOK$^-$ as donor
CLR136	gnd hisΔ gal bioΔ ptsX mgl galP	"

b. F' strain

Strain	Genetic markers	Source or Reference
KLF16/KL110	his argE leu metB thyA lacY strA recA1 F' serA$^+$ lysA$^+$ argA$^+$ thyA$^+$	*CGSC

c. F$^-$ strains (all streptomycin-resistant except those marked +)

Strain	°Genetic markers	Source or Reference
K2	his arg(HBCE) thr leu trp gal(K?) galP mgl	Laboratory stock
K2.1t	his arg(HBCE) thr leu galP mgl pps	Brice & Kornberg 1967
K2.1.22a	his arg(HBCE) thr leu galP mgl umg pps	Kornberg & Jones-Mortimer (1975)
K2.2W	his arg(HBCE) thr leu trp gal(K?) galP mgl umg	Kornberg & Smith (1972)
16/3G	his arg(HBCE) thr leu galP umg pps mgl	From K2.1t by selection for resistance to TG.

*E.coli Genetic Stock Centre.

TABLE M.1. Strains of E.coli used in this work.

a. Hfr strains.

Strain	Genetic markers	Source or Reference
K1.	thy metB	Brice & Kornberg (1967)
K1.1	thy metB pps	" "
B11	metB	Broda (1967)
K10	prototroph	Laboratory stock
KL16	prototroph	*CGSC
KL16.21	pstF ptsX $kdgR^c$	Ferenci & Kornberg (1971)
14/136	umg	Spontaneously from KL16
KL96	prototroph	*CGSC
AB2297	ilv pur	From E.A.Adelberg
AB312	thi thr1 leu6 lacZ4 str8 supE44	*CGSC
ED1032	lac proΔ spc^R	From P.Meacock
ME100	thi serA zwf	From R.A.Cooper
ts19	met $ptsI^{ts}$	Bourd et al (1971)
ts19-1Δ	met ptsI crrΔ	Jones-Mortimer & Kornberg (1974a)
JM759	gnd hisΔ gal bioΔ ptsX pstF fda^{ts}	From M.C.Jones-Mortimer
JM762	gnd hisΔ ptsX fda^{ts} (NAG^R)	" " "
JM803	gnd hisΔ gal bioΔ ptsX ptsF ptsI srl^{rc} fda^{ts}	" " "
JM807	gnd hisΔ ptsX ptsF fda^{ts} cysI	" " "

* E coli Genetic Stock Centre

Organisms used.

The strains of E.coli K12 used in this work are presented in Table M.1, which shows which, if any, auxotrophic markers they carry, and hence their growth requirements. The abbreviations for genetic markers listed by Taylor and Trotter (1972) have been used where possible; markers which have been described since the appearance of this publication are defined in the text. Figure M.1 shows the chromsomal location of the markers which are most frequently referred to in the text.

Maintenance of organisms.

The majority of the organisms used were stored at room temperature on nutrient agar slopes supplemented with thymine and methionine (each at 40μg/ml), but the F' strain KLF16/KL110 was kept on a slope of defined selective medium to ensure that the episome was retained. All strains were maintained by periodic sub-culture, and were routinely checked before use for their possession of the appropriate genetic markers, and for the absence of contaminants, by testing their growth on suitable selective media. Organisms which were in frequent use at any period of time were stored also in the form of single colony isolates on a nutrient agar plate; in such cases inocula could be taken from single colonies rather than directly from the slope.

Media.

The organisms were grown on the basal salts medium

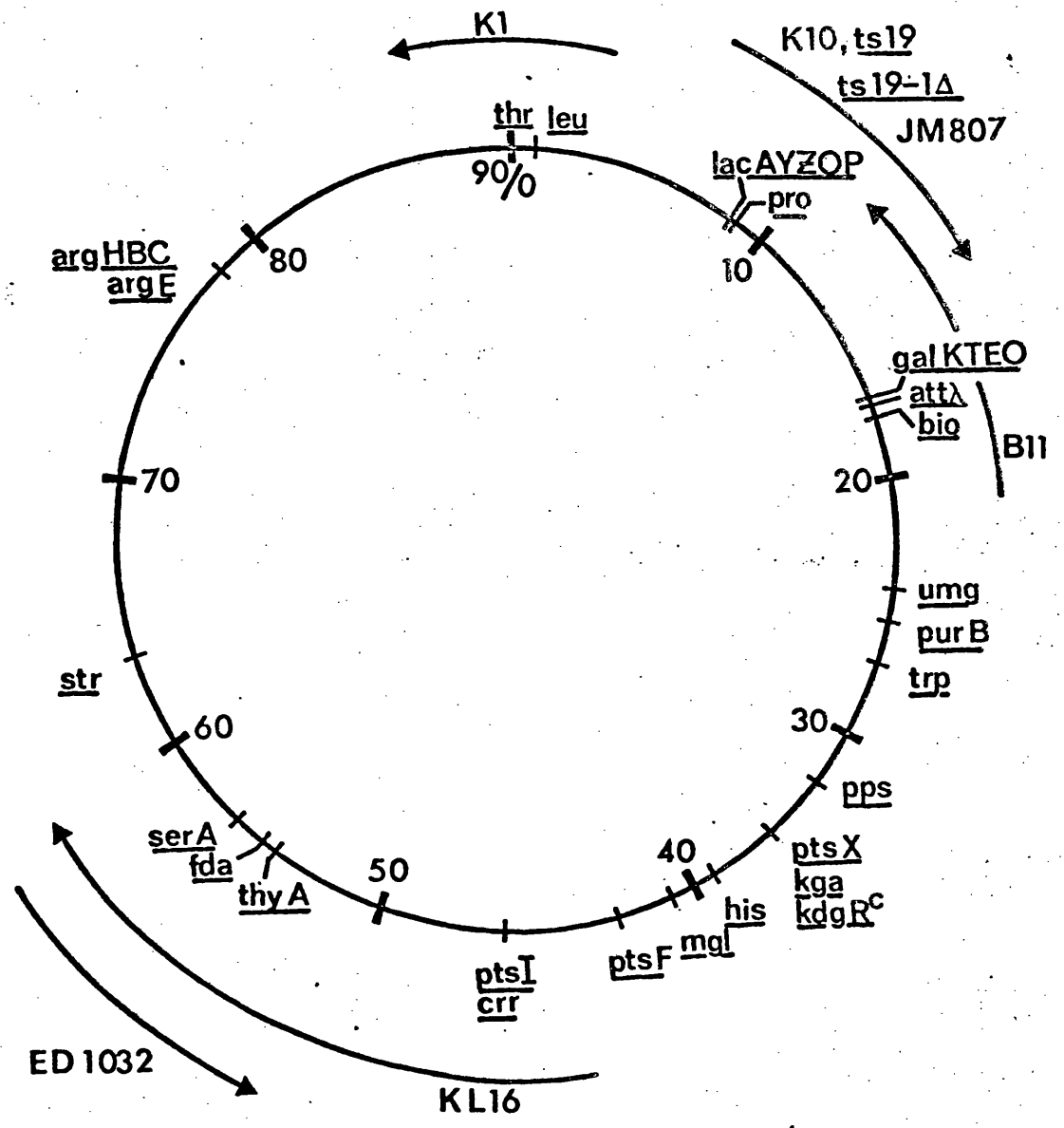


Fig. M.1. Linkage map of E.coli, showing the chromosomal location of markers carried by, and used in the construction of, the F^- strains listed in Table M.1. The origins and directions of genome transfer of the Hfr strains used in the preparation of these F^- strains are also shown.

of Ashworth and Kornberg (1966) supplemented with a carbon source,usually at 0.2% (w/v), and the required growth factors (40 - 100 µg/ml). The basal salts medium was prepared and stored at a concentration five times greater than the required final concentration, and was diluted and sterilised when needed; compounds to be added to the medium were sterilised separately. In cases where a defined medium was not required, the bacteria were grown on double strength nutrient broth.

The media and supplements were in general sterilised by autoclaving at 15 lb./in^2 for 20 minutes; where this would have led to decomposition or alteration of the material (eg sugar phosphates, streptomycin sulphate) the compound was either dissolved in sterile water, or made into solution and subsequently sterilised by membrane filtration. Solid medium was prepared by adding 1.5% (w/v) agar to the medium before autoclaving.

II BIOCHEMICAL AND PHYSIOLOGICAL TECHNIQUES.

Growth of organisms.

Cultures of organisms in liquid defined medium were usually obtained by inoculation from the slope,or from a single colony, directly into the appropriate sterile medium; in some cases though inoculation was first into nutrient broth, and a sample of the resulting stationary-phase culture was used to inoculate the defined medium. Small cultures (1-2ml) were incubated in test tubes held in a revolving holder in a room maintained at the required

temperature, larger cultures in Erlenmeyer flasks in a thermostatically controlled reciprocating or rotary shaker. To ensure that the culture was well aerated, its volume was never in excess of two-fifths of the volume of the vessel containing it.

To measure the rate of growth of an organism on different media, a culture was grown overnight on a carbon source such as fructose or glycerol, utilisation of which does not lead to catabolite repression. The culture was harvested by centrifugation in the bench centrifuge and resuspended in a small volume of salts medium containing no carbon source; samples of this suspension were then used to inoculate the test media, to give an initial cell density of about 0.1mg dry mass. ml^{-1}. The cell density, and hence the rate of doubling of the culture, was measured in terms of the absorbance of the suspension at 680nm in a Unican SP600 spectrophotometer, using 3ml plastic cuvettes of 1cm light path. At this wavelength an absorbance of 1.0 represents 0.68mg drymass.ml^{-1} (Ashworth & Kornberg, 1966). Growth experiments were not performed aseptically because of the large inocula used.

Exponentially-growing cultures which were required for uptake measurements or enzyme assays were routinely obtained in the same way,i.e.by harvesting an overnight culture and resuspending a suitable number of cells in the same, or different medium. In some cases though, the overnight culture was simply diluted into fresh medium. After two or three hours' growth in their fresh media, the

cells were harvested and dealt with as required.

Preparation of membrane vesicles of strain W4345.

A large culture (2.2l) of strain W4345 was grown on glycerol (50mM) in the presence of galactose (2mM) to the mid-logarithmic phase of growth (about 0.4 mg dry mass. ml^{-1}), and was used for the preparation of membrane vesicles by the method of Kaback (1971). In summary this involved harvesting and washing the cells, and then treating them with EDTA and lysozyme to produce osmotically-sensitive cells (spheroplasts). The spheroplasts were diluted into an excess volume of hypotonic medium, a process which Kaback has shown causes them to release their intracellular contents, but the membranes subsequently reanneal to yield closed, empty, membrane vesicles. These were extensively purified from contamination with whole cells and debris by differential centrifugation until no whole cells were visible under microscopic examination; they were then washed several times before finally being resuspended in 0.1M potassium phosphate, pH 6.6. The preparation, containing about 20mg protein. ml^{-1}, was stored in small aliquots in liquid nitrogen. The division of the preparation into several aliquots meant that they could be thawed separately as required, and hence repeated freezing and thawing was avoided.

The amount of protein in the vesicle preparation was assayed by the method of Lowry et al. (1951) using bovine serum albumen as the standard.

A total of 0.9g dry mass of whole cells yielded about 60mg of membrane protein.

Measurements of the uptake of isotopically labelled substrates.

a. Routine measurements of uptake by whole cells.

Cells were harvested in the mid-logarithmic phase of growth (0.2 - 0.6 mg. dry mass. ml^{-1}) by centrifuging them at 20,000g for 5 min. at 15°C. They were washed once with nitrogen-free salts medium (Ashworth & Kornberg, 1966), resuspended in the same medium to the required cell density (usually 1 - 2mg dry mass. ml^{-1}) and stored in ice until required (0-2h). Samples of this suspension were then shaken at the required temperature for 10 min. before the addition of labelled substrate. Unless otherwise stated in the text, this incubation was carried out at 25°C. Where the labelled compound was a non-catabolisable sugar analogue, one-tenth volume of nutrient broth was also included at the start of this incubation period. For the assay of Mgl-activity, [^{14}C]-galactose at 0.5μM(Boos, 1969) or [^{14}C]-methyl-β-D-galactopyranoside at 10μM was used; the concentrations of substrates used for other uptake measurements are given in the text. Samples (0.1ml) were taken rapidly at suitable time intervals, filtered with suction through Millipore filters (0.45μm pore size) and washed twice with 2ml of nitrogen-free salts medium at room temperature (20-22°C). Filters were transferred to vials containing 5ml of Bray's (1960) fluid, and radioactivity was

measured with a Packard model 3385 liquid scintillation spectrometer.

Since in general the rate of uptake of labelled material was of more interest than the absolute amount present in the cells at any time, corrections for non-specific binding of isotope to the cells were not normally made. However it was important to know the contribution made by non-specific binding of [^{14}C]-galactose to the counts obtained with the GalK$^-$ strains with low uptake activity, strains CLR100 and 20S0K$^-$. This information was obtained by keeping the cell suspension on ice, adding ice-cold isotope, sampling as soon as possible after mixing (0-5 sec), and washing the filtered sample with ice-cold salts medium. Since the counts obtained in these samples were not significantly above background, no correction of the test results was necessary.

To enable the counts in each sample to be interpreted in terms of nmoles of substrate taken up, duplicate vials were routinely set up which contained a known volume (5 - 10μl) of isotope solution dissolved in Bray's fluid. A membrane filter, through which 0.1ml of the non-radioactive cell suspension had been filtered, was also included so that any quenching of the counts due to the presence of the filter or cells would be the same here as in the test vials. Background counts were obtained by setting up vials which were the same as these, but omitting the isotope solution.

b. Measurement of the effect of metabolic inhibitors on the uptake process.

To avoid interference with the effect of arsenate on the uptake process by the phosphate present in the salts medium, the effects of arsenate and other metabolic inhibitors were studied in experiments in which the buffer used was phosphate-free. It contained 75mM sodium/potassium chloride, 5mM Hepes and trace elements and calcium chloride at the same concentrations as in the basal medium of Ashworth and Kornberg (1966); it was adjusted to pH 7.1 with sodium hydroxide. The experiments were carried out in the same way as the routine uptake measurements described above, but the Hepes buffer was used throughout, for washing the cell preparation, for finally resuspending the cells, and for washing the filtered samples.

The effects of metabolic inhibitors on uptake were studied also under anaerobic conditions. Here the washed cell suspension was maintained at 25°C and bubbled with argon for 10 min. before the addition of isotopic substrate, and throughout the time over which the uptake process was studied. Argon was passed also into the isotope solution and the washing fluid, and care was taken to see that no air bubbles were trapped in the syringe used to transfer the isotope to the test flask.

c. Measurements of uptake by membrane vesicles.

The vesicle preparation, thawed at room temperature after storage in liquid nitrogen, was stored in ice until required (0 - 4h). It was then diluted (to a final

concentration of about 3mg protein.ml^{-1}) into 50mM potassium phosphate buffer, pH 6.6, containing 20mM sodium D-lactate and 10mM magnesium chloride, and was shaken gently for 10min. at 25°C as a thin film in the bottom of a large flask. In this way maximal aeration of the suspension was achieved. Labelled substrate was then added to the required concentration. Samples (0.1ml) were taken at intervals, diluted into 2.5ml lithium chloride (0.1M), filtered with suction through Millipore filters (0.45µm pore size) and washed with another 2.5ml lithium chloride. The radioactive material retained by the filters was assayed, and the observed counts.min^{-1} were converted into nmoles sugar taken up, in the same ways as described for whole cells.

d. Purification of labelled galactose.

It was found that the [^{3}H]-and [^{14}C]-galactose as purchased were contaminated with traces of radioactive glucose which sufficed to introduce significant errors into measurements of galactose uptake by strains with low transport activity for galactose. The method devised to purify the commercial material involved one such strain, 20SOK^{-}, which has neither galactokinase activity nor an active transport system for galactose. However, the organism possesses constitutive glucose phosphotransferase activity (Kornberg and Reeves, 1972b), and therefore takes up glucose from the medium in preference to galactose and with high efficiency (Kornberg, 1973). A culture of strain

20SOK$^-$, grown on glycerol, was harvested, and the cells were washed and resuspended in nitrogen-free salts medium as for a routine uptake experiment. The impure labelled galactose was added and the suspension incubated until no more radioactive material was taken up (approx. 10 min). The cells were then filtered through a dry Millipore filter; the filtrate was essentially unaltered with respect to galactose concentration, and was substantially glucose-free as judged from the inability of a fresh washed suspension of strain 20SOK$^-$ to incorporate labelled material from this preparation into cellular constituents.

Measurements of the incorporation of isotope into cell material.

To 10ml of medium containing the appropriate carbon source was added sufficient of the [^{14}C] isotopic solution of the same carbon source to give 25,000 - 30,000 cpm. 0.1ml^{-1}. The stock solution of isotope was in every case of a concentration such that this addition did not significantly alter the overall concentration of the sugar in the medium. A sample (0.1ml) of this medium was removed, dissolved in 5ml Bray's fluid, and counted, so that the counts per mole of sugar could be calculated. A large inoculum (about 1 mg) of the required bacteria, derived from an overnight culture, was then added to the medium, and the flask incubated aerobically at 37^{o}C. The growth of the culture was followed, and at each increase in dry mass of 0.1mg. ml^{-1} a sample (0.5ml) was removed.

This was filtered and washed as described for samples from uptake experiments, and the radioactivity it contained was also assessed as previously described.

Assay of PEP-dependent phosphotransferase activity.

Cells were rendered permeable and assayed for phosphoenolpyruvate-dependent phosphotransferase activity according to the method of Kornberg and Reeves (1972b). Phosphotransferase activity was followed at 30°C by measuring the decrease in absorbance at 340nm of NADH, as the pyruvate produced in this reaction was reduced by lactate dehydrogenase to lactate, with concomitant oxidation of NADH.

$$\text{Sugar} + \text{phosphoenolpyruvate} \xrightarrow{\text{PTS}} \text{Sugar phosphate} + \text{pyruvate}$$

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow{\text{LDH}} \text{Lactate} + NAD^+$$

Assay of β-galactosidase.

β-Galactosidase activity was measured as the rate at which toluenised cells hydrolysed o-nitrophenyl-β-D-galactopyranoside (ONPG) at 30°C, as described by Hestrin et al. (1955).

III GENETICAL TECHNIQUES.

Bacterial conjugation.

Conjugation between Hfr and F^- strains was carried out essentially as described by Miller (1972). Where it

was necessary to interrupt the mating process, this was done by diluting a sample (0.5ml) 20-fold in nitrogen-free salts medium and agitating with a vortex mixer for 1 min. Samples of this suspension, as well as suitable dilutions of it, were plated on the selective medium.

Counterselection against the Hfr donor strains was in general achieved by including streptomycin sulphate (100µg/ml) in the selection plates. This was not possible in crosses between the Hfr strain K1 and the Str^s F^- recipients W4345 and its derivatives; here omission from the selection plates of the donor's nutritional requirements, methionine and thymine, provided the necessary counter-selection.

Episome transfers.

Diploid strains were constructed by the transfer of an F' factor from one strain to another as described by Miller (1972).

Phage P1-mediated transduction.

The propagation of transducing phage and subsequent use of the preparation to bring about phage-mediated transduction were as described by Miller (1972). The host range mutant, P1kc (Lennox, 1955) was used, since this plates well on E.coli K12.

Experiments with phage λ.

The λεI857 lysogen of strain K2.1t was prepared

essentially according to Miller (1972), but with all incubations at 30°C. $\underline{\lambda}$ -resistant mutants of this strain were obtained by spreading 0.1ml of an overnight culture onto a nutrient agar plate containing 10mM magnesium sulphate. A drop of a lysate of $\underline{\lambda vir}$ was spotted onto the plate, which was incubated at 30°C until the $\underline{\lambda}$ -resistant clones had grown up. $\underline{\lambda}$ -eductants of the $\underline{\lambda}$ -resistant lysogen were prepared by incubating the organism at 42°C, as described in the test.

The $\underline{\lambda}$ mutants $\underline{\lambda cI857}$ and $\underline{\lambda vir}$ were kindly provided by Dr. B.Wilkins.

Selection for specific spontaneous mutants.

Stacey and Simson's (1965) method of selection for resistance to trimethoprim was employed in the derivation of thyA mutants.

Mutants impaired in glucose transport (some of which were umg mutants) were obtained essentially as described by Kornberg and Smith (1972), by selection for resistance to non-catabolisable glucose analogues during growth on fructose, glycerol or galactose.

Screening of mutants for Umg-activity by autoradiography.

The method of Kornberg and Smith (1972) was used. The organisms to be tested were grown overnight as patches on a nutrient agar plate containing 10μM methyl-α-[^{14}C]-glucoside and transferred to Whatman No. 40 filter paper by pressing this onto the plate. This was left in

contact with Kodak 'Blue Brand' X-ray film overnight. Development of the film revealed black patches which corresponded to the organisms which had taken up the isotopic material (Umg^+); those organisms which did not blacken the film were classed as Umg^-.

Screening of mutants for Mgl-activity.

Strains devoid of galacktokinase were assessed for Mgl-activity by the method of Boos and Sarvas (1960), an autoradiographical method in essence the same as that described above for screening for Umg-activity, but involving instead growth on nutrient agar supplemented with 0.1μM $[^{14}C]$-galactose.

The presence or absence of the Mgl^+ character in $GalK^+$ strains was detected by measuring the uptake of 0.5μM $[^{14}C]$-galactose or 10μM $[^{14}C]$-methyl-β-galactoside by washed cell suspensions.

Screening of mutants for GalP-activity.

The methods used for distinguishing $GalP^+$ from $GalP^-$ strains are described in Chapter 4.

CHAPTER I - A NOVEL TRANSPORT SYSTEM FOR GALACTOSE.

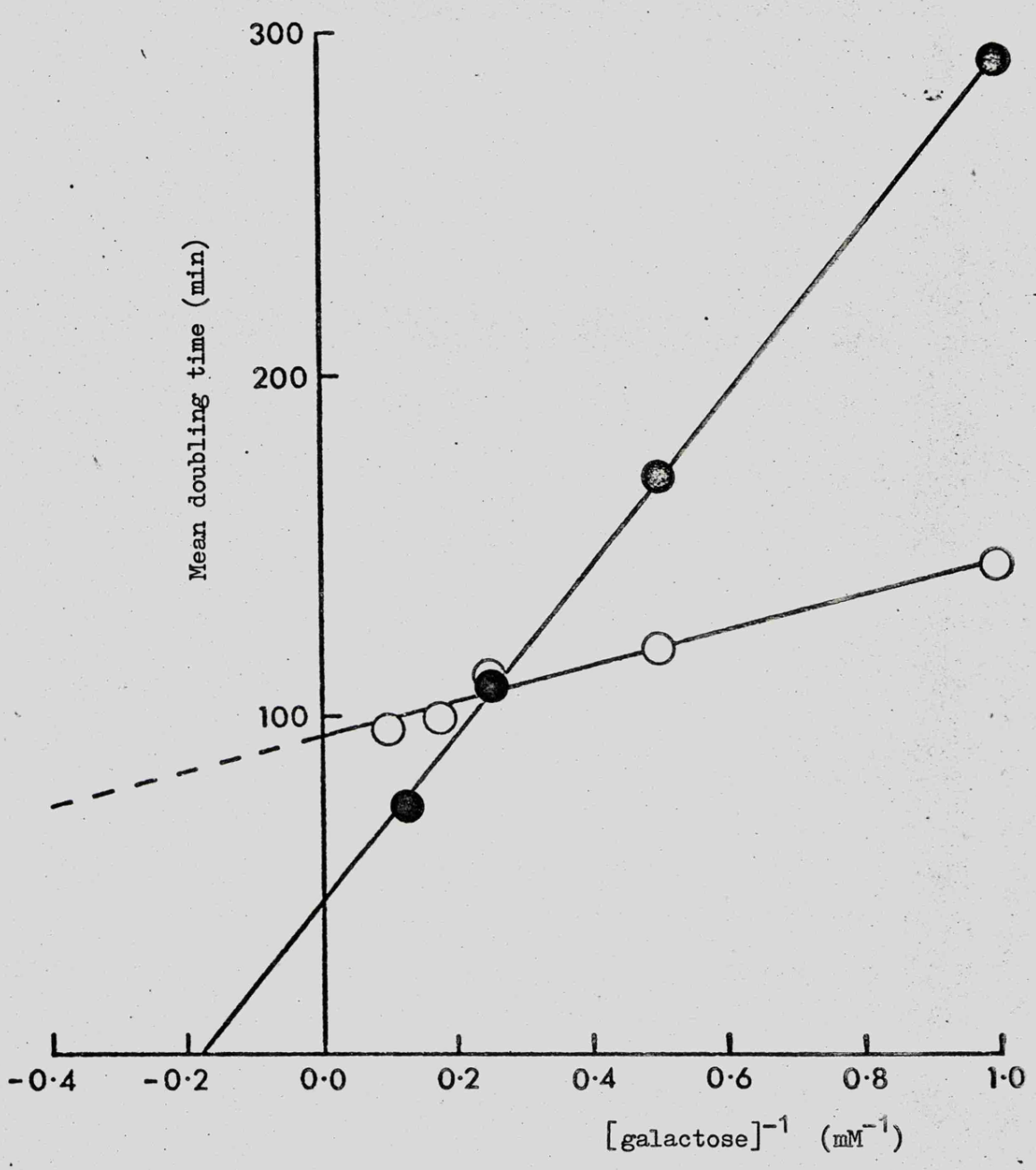


Fig.1.1 The effect of galactose concentration on the growth rate of strains K2.1t (●) and KL16 (o). The mean doubling time is plotted against the reciprocal of the galactose concentration in the medium.

Classification of strains on the basis of their affinity for galactose.

Several of the F^- strains routinely used in the laboratory, e.g. K2, 0144, carry genetic lesions in the gal operon and are therefore unable to metabolise galactose. As might be expected, when these strains are used as recipients in genetic crosses with Gal^+ Hfr donors, such as Kl.1 and Bl1, which transmit gal as an early marker, Gal^+ recombinants may be readily isolated. However it was observed that the growth of these Gal^+ strains on galactose is remarkably concentration-dependent, being very slow when the hexose is present at a concentration of 3mM or less, but fast when it is provided at 10mM or higher. This contrasts with the properties of other strains, typified by the Hfr strains Kl.1, KL16 and KL96, which grow readily on media containing even the lowest concentration of galactose on which measurements of growth rates in batch culture are feasible (1mM); increasing the galactose concentration in the medium to 10mM or more only slightly reduces the doubling times of these strains.

It is possible to express these relationships by means of plots of the mean doubling time against the reciprocal of the initial concentration of galactose in the medium; straight lines are obtained as shown in Fig. 1.1. Such graphs may be viewed as analogous to Lineweaver-Burk plots, in which the reciprocal of the initial rate of an enzyme-catalysed reaction is expressed as a function of the substrate concentration. Thus the extrapolated lines cut the abscissa at points which correspond to the negative

Table 1.1. 'Km' values for growth on galactose of several strains of E.coli

Strain	Gal$^-$ parent	'Km' for growth on galactose (mM)
K2.1t	K2	6
0144g$^+$	0144	10
KL16		0.6
KL96		0.6
K1.1		0.6

The growth rates on galactose as sole carbon source were measured over a range of concentrations. For each strain the data were expressed by means of a double reciprocal plot as in Fig. 1.1, and the Km value was calculated from the point at which the extrapolated line cut the abscissa.

reciprocal of the galactose concentration at which half the maximal growth rate would be achieved; the 'Km' values which these data yield are a measure of the affinity for galactose of the overall growth process. Table 1.1, which presents the 'Km' values for growth on galactose of a variety of strains, shows clearly the distinction between the two classes; those with a high affinity for galactose have a 'Km' of about 0.6mM whereas the 'Km' is at least ten times this value for strains with a low affinity for galactose. It should be pointed out however that at the higher concentrations of galactose used in these experiments the so-called 'low-affinity' strains often grew faster than those classed as having a high affinity for galactose (e.g. Fig.1.1).

Because the limiting step in galactose utilisation is the rate at which the sugar enters the cell (Horecker et al., 1960), these observations suggest that at low concentrations of galactose the two classes of organisms might differ in their ability to take up the hexose. Studies with washed suspensions of galactose-grown cells showed that this is indeed the case. Taking strains K2.1t and KL16 as representative of the two classes, it can be seen from Table 1.2 that the former strain is severely impaired in its ability to take up 0.2mM galactose from the medium relative to the latter. Increasing the concentration of galactose in the medium enabled the 'low-affinity' strain to take it up at a faster rate, but did not alter the rate of sugar uptake by the 'high-affinity' strain.

Table 1.2. The rate of uptake of [^{14}C]-galactose by washed suspensions of galactose-grown cells.

Strain	Rate of uptake of [^{14}C]galactose (nmoles. min. $^{-1}$ mg dry mass $^{-1}$) when present in the medium at:-	
	0.2mM	4mM
K2.1t	1.2	4.8
KL16	8.5	8.0

The absence of β-methylgalactoside permease activity in strain K2.1t

The inability of strain K2.1t to take up galactose rapidly at low concentrations suggests that it is defective in at least one of the active transport systems which have a high affinity for this sugar. The activity of the β-methylgalactoside permease (MeGal permease) could be measured relatively easily because this transport system has features which enable it to be assayed specifically, even under conditions where other galactose transport systems are also active. Because it has such a high affinity for galactose (Km 2 x 10^{-7}M; Vorisek & Kepes, 1972) its activity can be measured by assaying the uptake of labelled galactose at a concentration of 0.5μM (Boos, 1969); no other galactose carrier displays significant transport activity towards such a low concentration of the sugar. The second useful feature of the MeGal permease is its ability to transport methyl-β-D-galactopyranoside (MeGal; Rotman et al., 1968; Boos, 1969; Vorisek and Kepes, 1972); this compound is also a substrate for the lactose and melibiose permeases (Rotman et al., 1968), but it is possible to induce the MeGal permease without inducing either of these other uptake systems. The melibiose permease is not detected in E.coli K12 strains when they are grown at or above 30°C (Buttin, 1968), whereas the strains used in the present study were routinely grown at 37°C. On the other hand the lactose permease, which can be induced at 37°C, is not induced by growth on galactose, or by the addition of D-fucose to growing cultures

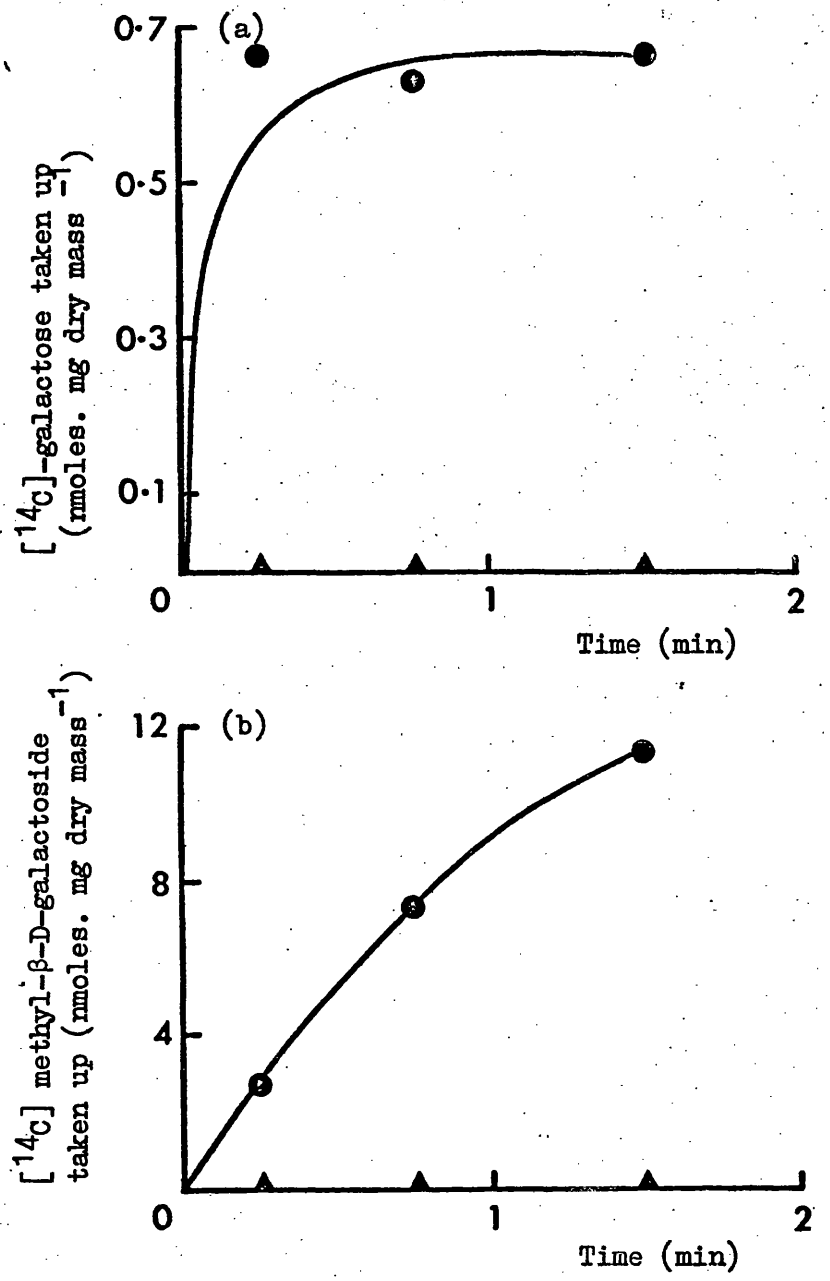


Fig. 1.2. The uptake of (a) 0.5μM [^{14}C] galactose and (b) 10μM [^{14}C] methyl-β-D-galactoside by washed suspensions of galactose-grown cells of strains KL16 (●) and K2.1t (▲).

(Rotman et al., 1968), two conditions which induce MeGal permease activity (Wu et al., 1969; Wilson, 1974a). (There have been reports that cells grown on galactose are only poorly induced for MeGal permease activity (Rotman et al., 1968; Parsons & Hogg, 1974) but no support for this was obtained from control uptake measurements using the Mgl^+ strains KL16, K10 and K1). Routine assays for MeGal permease activity therefore consisted of measurements of the uptake of 0.5 μM galactose or 10 μM MeGal in cells grown on galactose or on glycerol supplemented with D-fucose. Washed suspensions of galactose-grown cells of strain K2.1t failed to take up either 0.5 μM galactose or 10 μM MeGal whereas those of strain KL16 were able to take up both substrates (Fig. 1.2). Clearly, strain K2.1t is Mgl^-.

The question was now asked whether the difference in overall affinity for galactose exhibited by strains KL16 and K2.1t results only from their different Mgl phenotypes. If so, the introduction of the Mgl^+ character into strain K2.1t should enable it to behave like strain KL16 with respect to growth on galactose and the uptake of this sugar. The *mgl* locus, which consists of a cluster of at least three structural genes (Ordal & Adler, 1974a,b; Robbins, 1975), is situated at approximately minute 40 on the *E.coli* linkage map (Taylor & Trotter, 1972) and is cotransducible with the genetic marker *ptsF* (Ordal & Adler, 1974a; Silhavy & Boos, 1974; Robbins, 1975). Strain KL16, which transfers its genome in the order o-*lys*-*mgl*-*his* from an origin at minute 56 (Low, 1972), was

Table 1.3. The contribution of the MeGal permease in increasing the affinity of strain CLR11 for galactose.

Strain	Rate of uptake (nmoles. min.$^{-1}$ mg dry mass^{-1}) by washed galactose-grown cells, of			'Km' for growth on galactose (mM)
	galactose (0.5μM)	galactose (0.2mM)	MeGal (10μM)	
CLR11 (Mgl^+)	0.46	4.0	0.3	4
K2.1t (Mgl^-)	0	1.2	0	6
KL16 (Mgl^+)	0.36	8.5	9	0.6

therefore used as the Hfr donor in a genetic cross to introduce the mgl$^+$ genes into strain K2.1t. His$^+$ Str^R recombinants were selected, and screened for Mgl$^+$ by testing their ability to take up 0.5μM galactose. One such Mgl$^+$ derivative thus isolated, designated CLR11, was chosen for detailed study.

Table 1.3 compares some of the properties of strain CLR11 with those of its F$^-$ and Hfr parents. The introduction of the MeGal permease increased the rate of uptake of 0.5μM and 0.2mM galactose, but raised only slightly the organisms's affinity for galactose for the overall growth process. However neither the rate of uptake of 0.2mM galactose nor the 'Km' for growth on galactose was restored to the high level seen in strain KL16, suggesting that a galactose transport component is still missing from strain CLR11. Table 1.3 also shows that whereas strain CLR11 was able to take up 0.5μM galactose from the medium equally rapidly as strain KL16, the alternative assay for MeGal permease activity, involving measurement of MeGal uptake, did not give such a clear-cut result; although, unlike strain K2.1t, strain CLR11 took up this compound, the rate of uptake was poor compared with the Mgl$^+$ strain KL16. Three possible reasons for this were considered.

1. The [^{14}C]-MeGal used in this work was labelled in the methyl group. Thus it was conceivable that it was hydrolysed by strain CLR11 to produce [^{14}C]-methanol which, being freely diffusible, was lost into the medium. The enzyme β-galactosidase is known to catalyse this hydrolysis,

Table 1.4. The failure of growth on galactose to induce β-galactosidase activity.

Strain	β-galactosidase* activity in cultures grown on -		
	Glycerol (20mM)	Glycerol (20mM)/ IPTG† (2mM)	Galactose (10mM)
CLR11	2.9	>260	1.9
K2.1t	4.6	>260	2.4
KL16	6.5	>260	5.6

* The activity of β-galactosidase is expressed as nmoles o-nitrophenyl-β-D-galactopyranoside (ONPG) hydrolysed. $min.^{-1}$ (mg permeabilised cells)$^{-1}$.

† IPTG = isopropyl-1-thio-β-D-galactopyranoside, a gratuitous inducer of the *lac* operon.

which has been recognised as a possible cause of anomalous results where the conditions used to induce MeGal permease activity also induce the lac operon (Wu, 1967). Measurements of β-galactosidase activity however showed clearly that growth of strain CLR11 on galactose does not induce this enzyme; indeed the basal level present in galactose-grown cells is slightly lower than that in similarly-grown cells of strain KL16 (Table 1.4). Moreover it was found that although the transport activity of strain CLR11 towards MeGal was induced by growth in the presence of D-fucose, which does not induce the lac operon, the rate of uptake of 10 μM MeGal by such suspensions (1.6nmoles.min^{-1}.mg dry $mass^{-1}$) was still significantly below that of similarly-induced suspensions of strain KL16 (7nmoles. min^{-1}.mg dry $mass^{-1}$).

2. The MeGal permease is a complex system, requiring for full activity the products of all three structural genes of the mgl region, mglA, mglB and mglC. (It has been shown by Robbins (1975), that mglD, mutations in which lead to constitutive expression of the MeGal permease, is an operator locus and does not specify a protein.) The precise functions of the various gene products are not yet clear. The gene mglB specifies the galactose binding protein; this has been shown to be essential for complete transport activity (Boos, 1969, 1972; Ordal & Adler, 1974b), although there is some evidence for substrate translocation by the MeGal transport system even in its absence (Robbins & Rotman, 1975). The products of

the genes mglA and mglC have not been identified, but mutants defective in one or both of them show strongly reduced galactose transport activity (Ordal & Adler, 1974b). In the absence of a more complete understanding of the system, it must be considered a possibility that strain CLR11 did not receive the full complement of mgl genes from its Hfr parent and thus has only partial MeGal permease activity. Since strain KL16 has been shown to have all three mgl structural genes unimpaired (Robbins, 1975), this could only have come about if the genetic crossover had by chance occurred in the middle of the mgl region.

3. The third possible explanation for the properties of strain CLR11 is that complete MeGal permease activity requires the participation of another component, specified by a gene located distal to the mgl region, and absent from strain K2.1t and hence also strain CLR11. Wilson's evidence (1974a) that there is an interaction between the galactose permease and the MeGal permease which affects the properties of both gives some support to this idea.

It is difficult to distinguish directly between postulates 2 and 3, i.e. to establish whether the poor uptake activity shown towards MeGal in a strain that does apparently have the high-affinity galactose uptake system is a result of the absence of one of the mgl genes, or of another genetically unlinked component. The observation that other recombinants of strain K2.1t, classed as Mgl^+ on the basis of their ability to transport 0.5μM galactose,

were able to take up MeGal at rates more closely resembling that seen with strain KL16 suggests that the former explanation might be the correct one. However it is evident that the poor affinity towards galactose exhibited by strain CLR11 at the concentrations used for growth was a separate phenomenon, resulting from the absence of some function other than those specified by the mgl genes. This conclusion was supported by a survey of several laboratory strains, which revealed that one, (strain 2092), rapidly takes up MeGal in addition to 0.5μM galactose and must therefore be totally Mgl^+, but takes up galactose at higher concentrations at rates similar to those observed with strain CLR11, and grows on galactose with a similar low affinity for this substrate ('Km' approx. 4mM). In an attempt to determine the nature of the missing function, experiments were designed to test whether or not the galactose permease is present in strain K2.1t..

The absence of galactose permease activity in strain K2.1t.

The galactose permease, specified by the gene galP, was described by Rotman et al. (1968) but has been less well characterised than the MeGal permease; no well-defined criteria are available for establishing its activity in any strain. Rotman et al. found the only compound transported by this carrier to be galactose, but Wilson (1974a) showed that, in some strains of E.coli K12, D-fucose is also a substrate. However no sugar has been found which is a substrate specifically for the galactose permease and which

might therefore be used directly to assay its activity. Thus it could only be ascertained whether strain K2.1t is defective in galactose permease activity by comparing its properties with those of strains of known GalP phenotype. Two strains were available which, like strain K2.1t, are Mgl^-, but which differ in their GalP phenotype; strain W4345 is $GalP^+$ (Rotman et al., 1968) whereas strain 20SOK$^-$ is $GalP^-$ (Buttin, 1963a). Both these strains are devoid of galactokinase (galK), whereas strain K2.1t is $GalK^+$; an unequivocal comparison of their galactose transport properties with those of strain K2.1t could not therefore be made directly. This is because in strains able to metabolise a transported sugar, the uptake of that sugar, measured as the amount of radioactive material retained by the cells, is a function not only of the transport process, but also of the metabolism of the substrate. A $GalK^-$ derivative of strain K2.1t was therefore prepared.

The technique used to obtain the required $GalK^-$ mutant was one of λ-eduction according to the method of Shimada et al. (1972) to make a biotin auxotroph, followed by transduction of this auxotroph to galK. A culture of strain K2.1t was lysogenised with phage λcI857, which makes a temperature-sensitive repressor and therefore can exist as a stable prophage at the permissive temperature (30°C) but enters the lytic cycle at the restrictive temperature (42°C). By screening for resistance to λvir at 30°C, a spontaneous λ-resistant mutant of the lysogen was obtained. The failure of this strain to grow on maltose showed that its

resistance to phage λ was due to a lack of production of the maltose uptake system, a component of which acts as a membrane receptor site for the phage. Samples (0.1ml) of an overnight culture of this organism were spread on nutrient agar plates and incubated at 42°C. For the host to survive under these conditions, the prophage must be unable to enter the lytic cycle successfully, a condition which obtains if phage genes - together, perhaps, with adjacent bacterial genes - have been lost. None of the eductants thus obtained was galactose-negative, but several biotin-requiring (*bio*) mutants were isolated. One of these was infected with phage P1 propagated on strain 20SOK$^-$ (*galK*); selection for Bio$^+$ yielded transductants carrying the defective *galK* allele of strain 20SOK$^-$. One of these, designated CLR100, was purified and used for further experiments. The derivation of strain CLR100 has been described in detail in order to stress the fact that neither mutagenesis nor conjugation was involved; specific genetic changes were introduced only in the region of the *gal* operon, and in either *malB*, which specifies the maltose uptake system, or its positive control gene in the *malA* region. Since none of the genes specifying galactose transport systems is located in any of these areas (Taylor & Trotter, 1972; evidence to be presented in Chapter Four of this Thesis), strains CLR100 and K2.1t may be assumed to be identical in terms of their ability to transport galactose.

The Km for galactose of the galactose permease specified by the gene *galP* is of the order of 1.5×10^{-4}M (Rotman

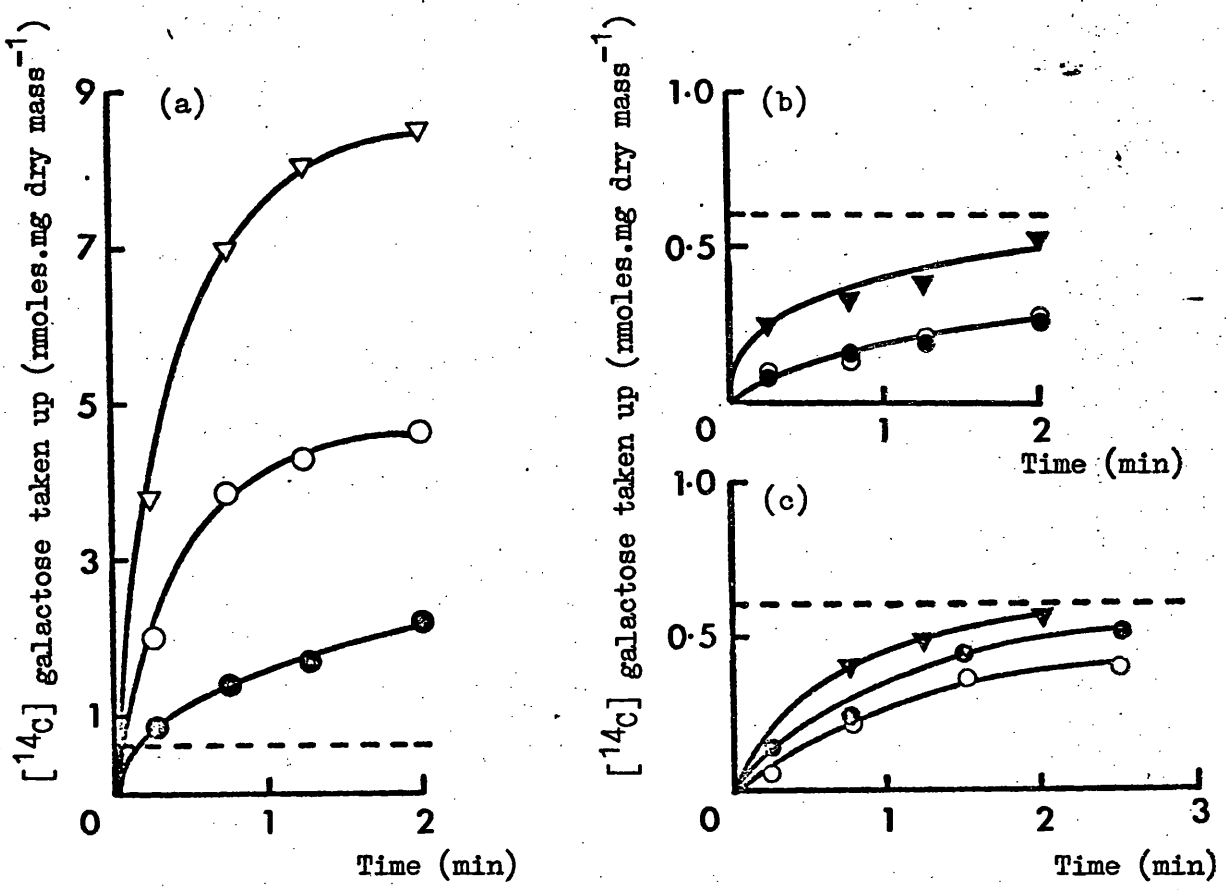


Fig.1.3 The uptake of 0.2mM galactose by washed suspensions of strains (a) W4345, (b) 20SOK⁻ and (c) CLR 100. The cells were grown on 20mM glycerol alone (●) or supplemented with 1mM D-fucose (o), 1mM galactose (▽) and 10mM galactose (▼). The dotted lines show the level of uptake expected if the galactose inside the cells merely equilibrates with that present extracellularly.

et al., 1968; Wilson, 1974a). This means that GalP$^+$ strains induced for this activity should take up galactose from the medium if it is provided at a concentration of 0.2mM, albeit at only just over half the maximal rates. Both galactose and D-fucose are inducers of this transport system (Rotman et al., 1968). Fig. 1.3. shows that cultures of the GalP$^+$ strain W4345 do indeed take up 0.2mM galactose and that this uptake is induced by galactose and D-fucose, the former being the better inducer. The galactose-induced cells of this strain took up the sugar until they contained about 9nmoles/mg dry mass. Horecker et al. (1960) showed that when a galK strain takes up galactose by a combination of Mgl- and GalP-activities, it accumulates inside the cell as the free sugar; the galactose permease must therefore transport galactose without chemically changing it. Taking the intracellular water content of the cells to be 3μl/mg dry mass (Winkler & Wilson, 1966), it is thus apparent that suspensions of strain W4345 actively accumulated galactose to an internal concentration of 3mM, concentrating the sugar 15-fold. The dotted lines in Fig. 1.3 show the level of uptake which would be expected were the galactose not accumulated but merely equilibrated with external sugar.

Clearly, washed suspensions of neither strain 20S0K$^-$ nor strain CLR100 accumulate galactose, but take it up only until the intracellular concentration is equal to that in the medium (Fig. 1.3). Neither D-fucose nor galactose apparently induced an active transport system for galactose in either strain, although growth in the presence of galactose

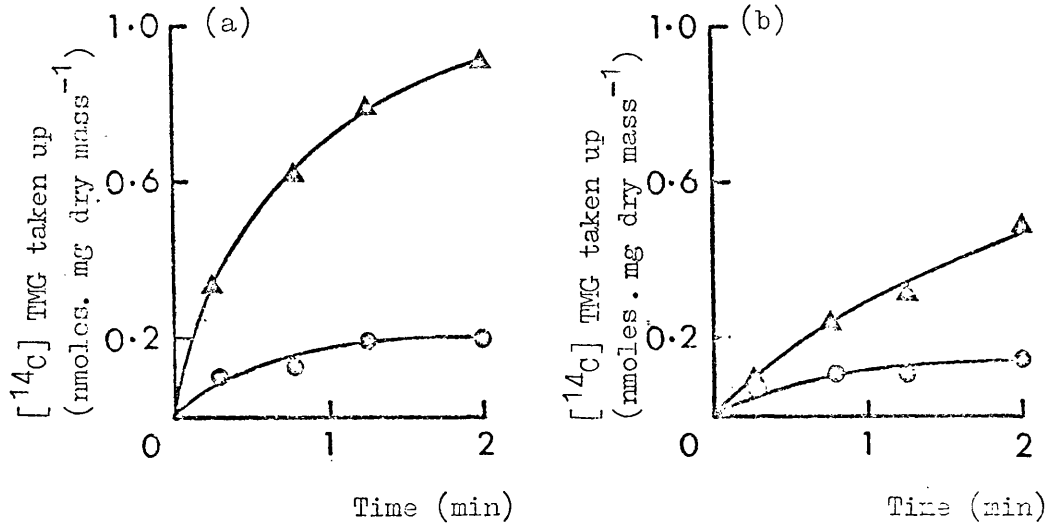


Fig. 1.4 The uptake of 0.1mM TMG by washed suspensions of strains (a) 20SOK⁻ and (b) CLR100. The cells were grown on 20mM glycerol alone (o) or supplemented with 10mM galactose (A).

led to a slight increase in both the rate and extent of galactose uptake, particularly in strain 20SOK$^-$. Kalckar *et al.* (1959) showed that the *lac* operon is induced by galactose in strains of *E.coli* lacking galactokinase, so it was possible that induction of the lactose permease, which itself transports galactose, could account for this increase. Indeed, measurements of the uptake of methyl-1-thio-β-D-galactopyranoside (TMG), an analogue of lactose and a substrate for the lactose permease, in cells of strains 20SOK$^-$ and CLR100 support this hypothesis (Fig. 1.4); galactose induced lactose permease activity, and this effect was more marked in strain 20SOK$^-$. Since strain W4345 carries a deletion of the *lac* operon (Rotman *et al.*, 1968), the lactose carrier cannot have been responsible for any of the galactose transport seen in this organism.

The data illustrated in Fig. 1.3 therefore show that strain CLR100 (and hence also strain K2.1t), like strain 20SOK$^-$, lack galactose permease activity. The particular genetic defect concerned is quite probably a component of the *galb* mutation, a complex mutation whose precise nature is still unclear, but which is known to cover several genes involved in galactose metabolism. The pedigrees of both strains 20SOK$^-$ and K2.1t can be traced back to strain PA678, which carries the *galb* mutation (Bachmann, 1972). Since even 10mM galactose, ten times the amount used to induce the uptake system in strain W4345, failed to induce galactose permease activity in these strains, it is unlikely that they are regulatory mutants, able to express galactose permease activity but requiring a higher inducer concentration than do wild-type strains. The dysfunction appears to be in the permease itself.

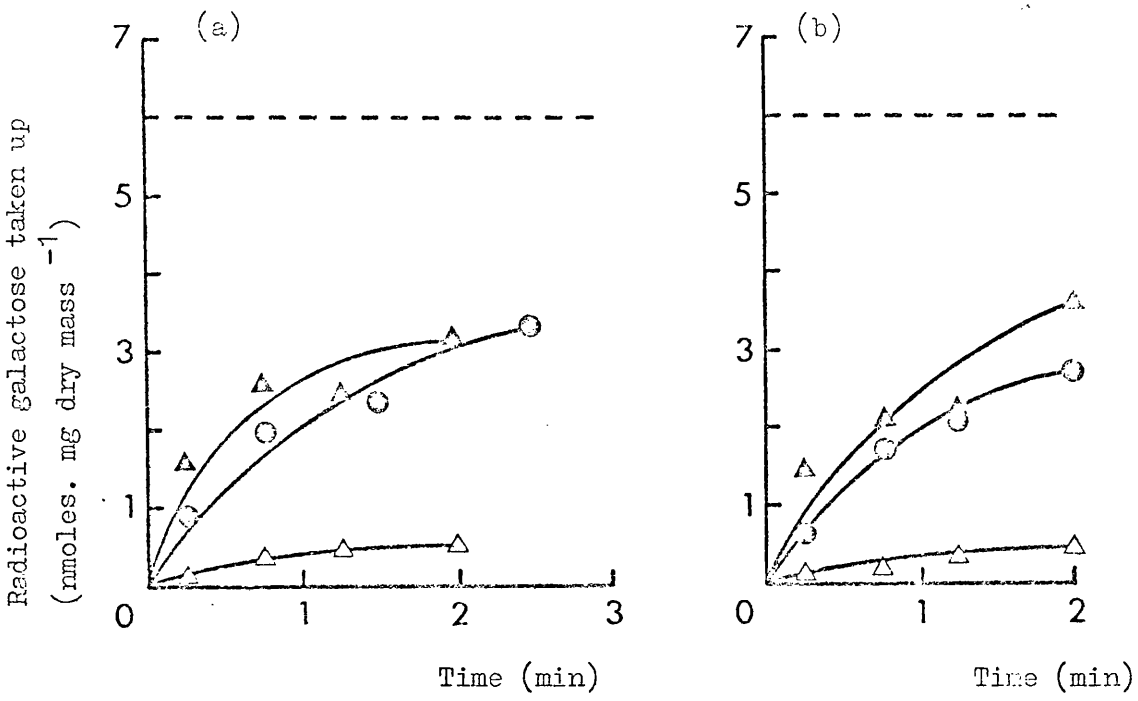


Fig. 1.5. The uptake of 2mM galactose by washed cell suspensions of (a) strain CLR100 and (b) strain 20S0K^{-}. The organisms were grown on 20mM glycerol alone (O) or supplemented with 10mM galactose (▲). The uptakes of 0.2mM galactose by galactose-induced cells (Δ) are reproduced from Fig. 1.3 for comparison. The dotted lines represent the uptake expected if the galactose inside the cells equilibrates with that in the medium, i.e. reaches a concentration of 2mM.

In an attempt to discover whether these strains synthesisea defective galactose permease with an increased Km for galactose, the uptake of 2mM galactose was followed. The presence of such a high-Km uptake system would explain the requirement of strain K2.1t for high concentrations of galactose for fast growth. However, although the initial rate of uptake of 2mM galactose was faster than it had been with 0.2mM substrate, there was still no accumulation above the external concentration, even when the cells were grown in the presence of galactose (Fig. 1.5). It thus appeared that active transport on the galactose permease was totally lacking.

Induction of other galactose transport systems in strain K2.1t.

The failure to detect MeGal and galactose permease activities in strain K2.1t, while highlighting the reason for the organism's low affinity for galactose, at the same time raised another problem; what is the nature of the uptake system which transports galactose during growth on this sugar? It was clearly of interest to know whether the only means of galactose entry into the cell was the one seen in the GalK$^-$ strain CLR100, or whether perhaps a different uptake system operates in Gal$^+$ cells actually growing on galactose medium.

As already stated, there is evidence that the lac operon is not induced by galactose in Gal$^+$ strains, but nevertheless the possible expression of lactose permease in strain K2.1t growing on galactose was tested, together with that of arabinose permease. Measurements of the uptake of TMG and L-arabinose by cells grown under a variety of conditions (Table 1.5) showed that, although uptake

Table 1.5. Induction of the lactose and arabinose permeases in strain K2.1t.

Carbon source for growth	Labelled material taken up (nmoles. mg dry mass^{-1}) after 2 min incubation of washed cell suspensions with	
	[^{14}C]-TMG (0.1mM)	[^{14}C]-arabinose (0.2mM)
Glycerol	0.2	0.6
Galactose	0.2	0.6
Arabinose	nt	3.5
Lactose	2.4	nt

nt = not tested

systems for both sugars may be induced in this strain, they are only present at their basal levels during growth on galactose. Since TMG is also a substrate for the melibiose permease (Prestidge & Pardee, 1965; Buttin, 1968), these observations also show that, as expected, this uptake system is not induced by growth of the organism on galactose at 37°C. Thus strain K2.1t appears not to be able to use for growth any galactose transport system other than that present also in its galK-offspring CLR100.

The lack of active galactose transport in strain CLR100.

The observation that washed cell suspensions of strain CLR100 were unable to accumulate galactose, but took it up only to a concentration equal to that in the surrounding medium, suggested that the uptake process does not involve the input of energy and is therefore not one of active transport. This hypothesis was tested further by looking at the effect of an inhibitor of active transport on galactose uptake by this organism.

The precise mechanism by which energy is coupled to active transport in bacteria remains to be elucidated, but it is generally accepted that energy, derived either directly by electron transfer on the respiratory chain, or from hydrolysis of ATP by the membrane-bound Ca^{++} Mg^{++}-adenosine triphosphatase (ATPase) is conserved by the formation of a 'high-energy intermediate', and withdrawn from here to drive transport as and when required. This intermediate is variously envisaged as an electrochemical gradient of

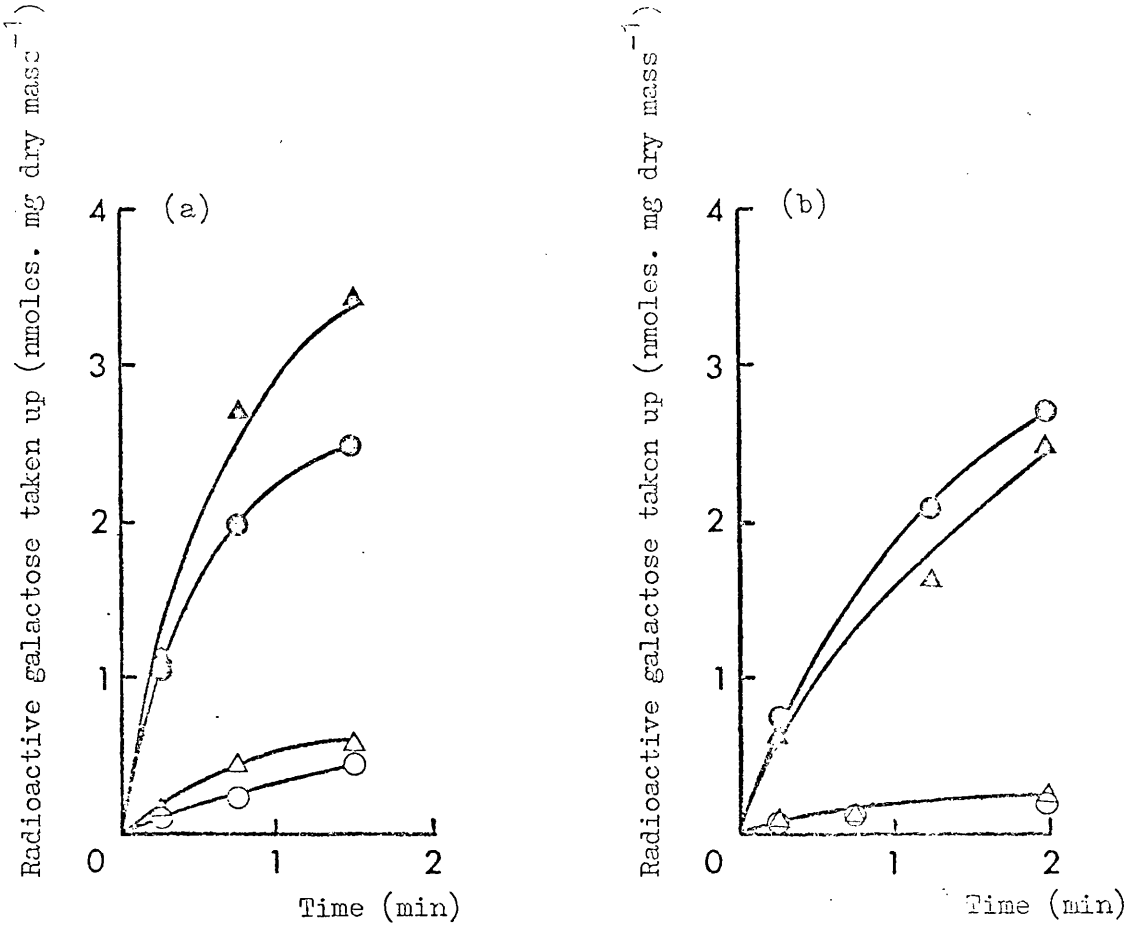


Fig. 1.6. The effect of the uncoupler CCCP on galactose uptake by washed suspensions of glycerol-grown cells of strains (a) CLR100 and (b) 20SOH$^-$. The uptakes of 0.2mM [^{14}C] galactose (open symbols) and 2mM [^{3}H] galactose (closed symbols) were measured after 3 min. incubation with 20μM CCCP (Δ, ▲) and in the absence of uncoupler (o, ●).

ions (Mitchell, 1973), a chemical compound (Slater, 1953) or a conformational state of protein (Kaback & Barnes, 1971; Reeves et al. 1972; Boyer & Klein, 1972); the relative merits of these different viewpoints have been discussed in several reviews (Harold, 1972; Kaback, 1974; Henderson & Kornberg, 1975). The important point as far as the present discussion is concerned is that active transport is characteristically sensitive to uncouplers of oxidative phosphorylation (Harold, 1972), supposedly because they dissipate the energy stored in the high-energy intermediate, although it has been argued that in some cases it is their effect of lowering intracellular ATP levels which brings about the inhibition (Berger & Heppel, 1974).

The uncoupler m-Cl-carbonylcyanide phenylhydrazone (CCCP) when incubated with washed cell suspensions of strain CLR100 at a concentration which severely inhibited galactose accumulation in strain W4345 (cf. Fig. 1.7(a)), did not impair galactose uptake but slightly stimulated it (Fig. 1.6). The uncoupler had no significant effect on galactose uptake in strain 20S0K$^-$. Clearly galactose is not taken up by an active transport process in either strain.

The mechanism of energy coupling to galactose permease activity.

The inhibition by CCCP of galactose uptake in strain W4345 was initially measured as a positive control for comparison with strains CLR100 and 20S0K$^-$ where no inhibition occurred. However, the question of the mechanism of energy coupling to active transport by the

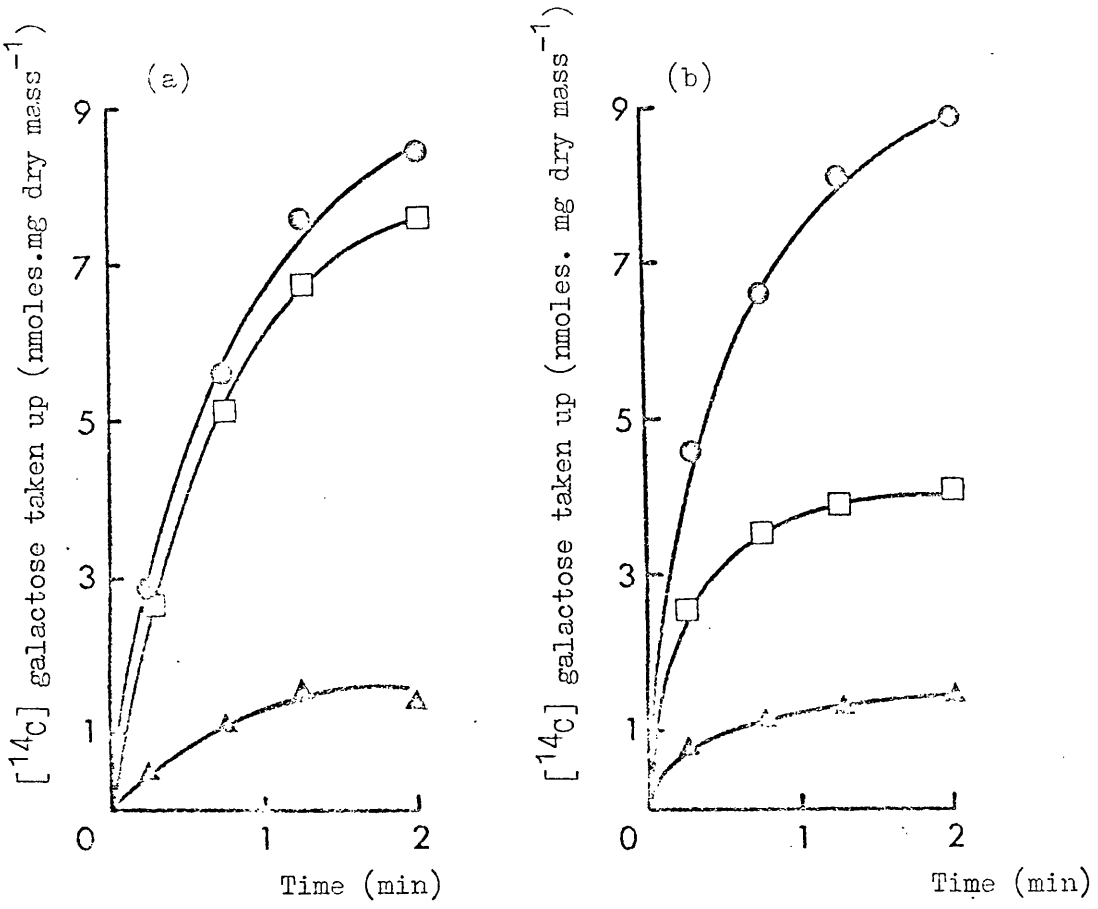


Fig 1.7. The effect of metabolic inhibitors on galactose permease activity. The uptake of 0.2mM [^{14}C] galactose by a washed cell suspension of strain W4345 grown in the presence of 1mM galactose was measured after a 10 minute incubation in the presence of 20μMCCCP (▲), 5mM potassium arsenate (□) or no addition (●). The experiments were carried out in phosphate-free buffer (a) aerobically and (b) under anaerobic conditions.

galactose permease became interesting in itself when it was suggested by Wilson (1974b) that this is not a 'classical' active transport system. It was originally proposed by Berger (1973) and by Berger and Heppel (1974) that those active transport systems which specifically require the participation of periplasmic binding proteins use energy directly from ATP and not via the formation of a high-energy intermediate. Wilson put the galactose permease in this category following experiments in which he measured galactose transport in a wild-type strain and in one devoid of ATPase activity, in the presence of different potential sources of energy. He thus implied that galactose permease activity depends on the presence of a binding protein, although he had previously shown (Wilson, 1974a) that it is independent of the particular galactose binding protein associated with MeGal permease activity. Two pieces of evidence obtained during the present study oppose this view.

1. Incubation of cells of strain W4345 induced for galactose permease activity in medium containing a high arsenate concentration and no phosphate, a treatment which drastically lowers the level of intracellular ATP (Klein & Boyer, 1972), caused significant inhibition of galactose uptake under anaerobic conditions, but had little effect in the presence of oxygen. The uncoupler CCCP was equally inhibitory under both sets of conditions (Fig. 1.7). This is consistent with the obligatory involvement of a high-energy intermediate whose energy is dissipated by the uncoupler. Under aerobic conditions

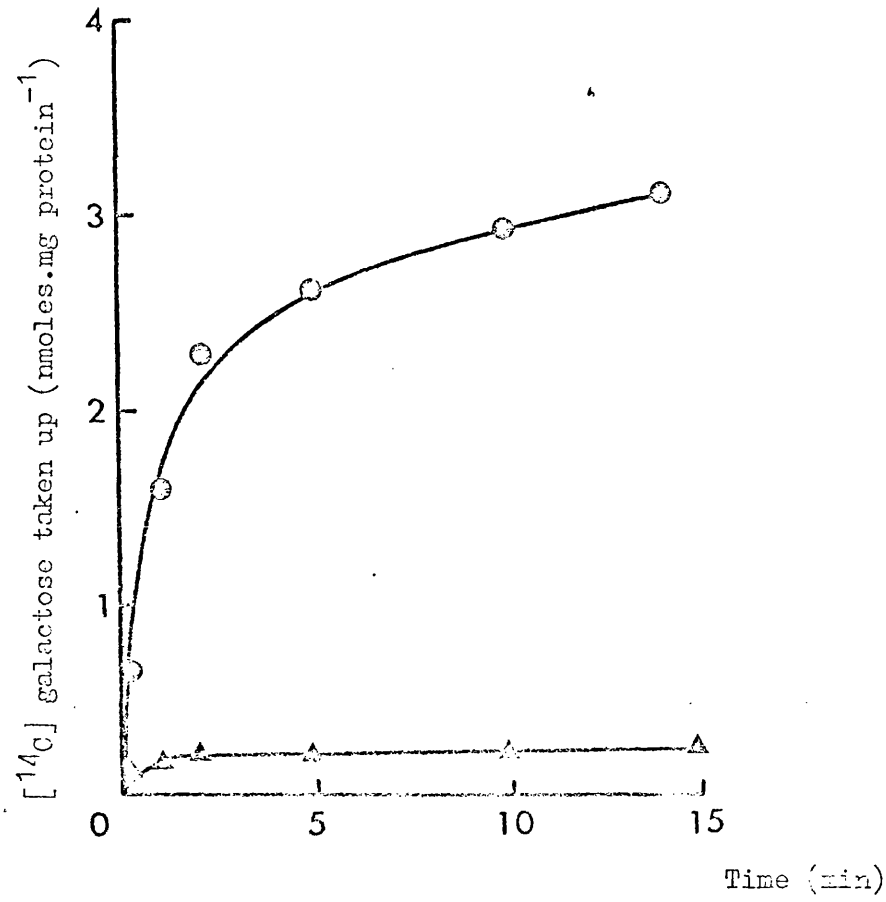


Fig.1.8 The uptake of 0.2mM [^{14}C] galactose by membrane vesicles of strain W4345 in the presence (o) and absence (Δ) of 20mM sodium D-lactate.

respiratory energy can be harnessed to form this intermediate so that ATP hydrolysis is not essential; the deleterious effect of a lowered ATP concentration is only apparent when ATP hydrolysis is the only source of energy. If the energy required to drive uptake had to be derived directly from ATP, arsenate should, like the uncoupler, have been equally effective regardless of whether oxygen was present or not.

2. Kaback (1971) has devised a method of preparing bacterial membrane vesicles which retain their ability to take up and accumulate a variety of substances including amino acids (Kaback & Milner, 1970), sugars (Barnes & Kakack, 1970), and inorganic ions (Lombardi et al., 1973). This technique, which involves an osmotic shock procedure, causes the release of periplasmic binding proteins, so that vesicles are capable of mediating transport only by systems independent of binding proteins. Kerwar et al. (1972) demonstrated the uptake of galactose by E.coli membrane vesicles and attributed this to galactose permease activity, but Wilson (1974b) was unable to reproduce this result using vesicles prepared from cultures of the same strain. When cells of strain W4345, grown in the presence of galactose, provided the starting material for a vesicle preparation, those vesicles were able to take up galactose, and D-lactate, a source of respiratory energy, greatly stimulated this process (Fig. 1.8). It could be argued that the uptake measured here was in fact mediated not by membrane vesicles but by a few whole cells contaminating

the preparation, which would have been able to produce ATP when given D-lactate as an energy source. The poor transport activity in the absence of D-lactate would then be explained by the fact that the vesicle preparation procedure, involving prolonged aerobic incubations in the absence of an energy source, would have effectively depleted these whole cells of their energy reserves (Berger & Heppel, 1974). Assuming that protein constitutes about 60% of the dry weight of E.coli (Roberts et al., 1963), it can be calculated that 15% of the total protein in the preparation would have had to be whole cell protein in order to account for these results on that basis. Because the preparation had been purified until no whole cells could be detected by microscopic examination, this is an unlikely explanation. Moreover, it is improbable that the few whole cells which might have escaped detection would also have survived the osmotic shock procedure and retained the binding protein postulated by Wilson to be required for transport. Thus it seems that the galactose permease is a typical active transport system whose activity is retained in membrane vesicles.

Discussion.

The work described in this chapter has shown that in at least two strains of E.coli that are devoid of both MeGal permease and galactose permease activities, galactose is taken up by a process other than active transport. Since galactose is not accumulated against a concentration

gradient, this process is probably one of diffusion. However, the evidence presented so far does not rule out altogether the participation of a phosphotransferase system. Indeed, the stimulation of galactose uptake by an uncoupler of oxidative phosphorylation seen in strain CLR100 could be taken to support the idea of phosphotransferase involvement. The uptake of the PT-sugar methyl-α-D-glucoside(αMG) has been studied in some detail with respect to its response to metabolic inhibitors, and it has been found that, depending on the experimental conditions, it is either unaffected or stimulated by the addition of uncouplers (Englesberg et al., 1961; Hoffee et al., 1964). The enhancement by uncouplers is partly due to their inhibition of an energy-requiring exit reaction (Hoffee et al., 1964). In addition, it has been shown that the respiration of oxidisable substrates (present endogenously in freshly-harvested cells) suppresses αMG uptake by a mechanism thought to involve the energy-rich intermediate of active transport (Hernández-Asensio et al., 1974; del Campo et al., 1975); uncouplers are believed to overcome this inhibition by dissipating this stored energy. The uptake of galactose could occur by a mechanism similar to that mediating αMG transport; Kaback's observation (1968) that membrane vesicles of strain K2.1t have a low-activity PEP-dependent transport system for galactose lends support to this hypothesis.

Leaving aside for the moment the question whether the galactose uptake process is one of diffusion or group

translocation, if it is assumed that a transport protein is involved (i.e. if the process is not simple diffusion) two of its properties are apparent from the results described in this chapter. Firstly it has a low affinity for galactose, which results in a high 'Km' for growth of these strains, and secondly it is not induced by either galactose or D-fucose, but appears to be present constitutively. Robbins and Rotman (1975) showed that the mglA and mglC gene products, in the absence of the galactose binding protein specified by the gene mglB, form a defective MeGal permease which translocates its substrates by facilitated diffusion at a rate sufficient to allow growth of the organism on these sugars. MeGal permease activity is constitutive in Mgl^+ $GalK^-$ strains because galactose, derived endogenously from uridine diphosphate galactose (UDPGal) is retained by this high affinity uptake system and hence accumulates to an intracellular concentration sufficient to maintain the induction (Wu, 1967; Wu et al., 1969). However in the absence of the galactose binding protein this efficient retention process cannot occur, so the remaining MglA and MglC activities must be inducible. Therefore, since the uptake rate in the galK strain CLR100 was not increased after growth in the presence of inducers of mglA and mglC expression, it is doubtful whether galactose entry in this strain is mediated by this facilitated diffusion system. Moreover the properties of strain CLR11 described in this chapter suggest that strain K2.1t carries a lesion in more than one of the mgl genes; this implies that the organism is unable to

express at least one of the MglA and MglC activities.

The following chapter describes how mutation of a different constitutive transport system was found severely to impair the growth of this strain on galactose.

CHAPTER 2. - STUDIES WITH UMG^{-} MUTANTS.

I The role of Umg-activity in growth on galactose

The preparation of umg mutants and their growth properties

The uptake of a variety of carbohydrates by E.coli is powerfully inhibited by glucose, a phenomenon termed 'catabolite inhibition' by McGinnis & Paigen (1969). Non-catabolisable analogues of glucose, such as methyl-α-D-glucoside (αMG), 2-deoxyglucose (DG), 3-deoxy-3-fluoro-glucose (DFG) and 5-thio-D-glucose (TG) have the same inhibitory effect in many strains, and thus the addition of one of these analogues to a culture growing on a carbohydrate whose uptake is sensitive to catabolite inhibition brings about growth stasis. Mutants resistant to this toxic effect can be readily isolated, and because many of them are found to be impaired in their ability not only to take up the glucose analogues but also glucose itself, this method has been used in the isolation of such transport mutants (Kornberg & Smith, 1972).

This chapter is concerned with some of the properties of organisms defective in the glucose phosphotransferase Enzyme II specified by the gene umg. Since the uptake of αMG specifically requires the activity of this enzyme (Kornberg & Smith, 1972), umg mutants can be recognised by their inability to take up this compound from the medium. Autoradiography of organisms grown on nutrient agar supplemented with 10μM [^{14}C]αMG (see Materials and Methods) provides a convenient means of screening for such mutants. Routine screening of mutants resistant to the various toxic

Table 2.1. (Contd)

Strain	Origin	Umg phenotype	Doubling time (min) on 10mM glucose	Doubling time (min) on 10mM galactose *
AB1325 αMG^R	From AB1325: selected for resistance to αMG during growth on fructose	-	270	-
CLR7	Recombinant from a genetic cross [AB1325. αMG^R x K1] selecting Pro^+ Gal^+ progeny	-	315	340
20SOK$^-$	Laboratory stock	+	62	-
CLR101	From 20SOK$^-$ transduced with phage P1 propagated on K2.1t, and selection for Gal^+ transductants	+	70	90
CLR15	From 20SOK$^-$: selected for resistance to DFG during growth on fructose	-	85	-
CLR16	From CLR15 transduced with phage P1 propagated on K2.1t, and selection for Gal^+ transductants	-	80	350
CLR114	From CLR101: selected for resistance to DFG during growth on fructose	-	80	335

* Strains 0144, AB1325 and 20SOK$^-$ are Gal^- owing to lesions in the *gal* operon; growth rates on galactose could therefore only be measured in their Gal^+ derivatives.

Table 2.1. The growth of umg mutants on glucose and galactose.

Strain	Origin	Umg phenotype	Doubling time (min) on 10mM glucose	Doubling time (min) on 10mM galactose*
K2.1t	Laboratory stock	+	57	69
K2.1.22a	From K2.1t: selected for resistance to DG during growth on fructose	-	183	360
16/3G	From K2.1t: selected for resistance to TG during growth on fructose	-	170	425
0144	Laboratory stock	+	67	-
0144.W	From 0144: selected for resistance to DFG during growth on fructose	-	255	-
0144.g$^+$	Recombinant from a genetic cross, [0144 x B11] selecting Gal$^+$ progeny	+	75	90
0144.Wg	Recombinant from a genetic cross [0144.W x B11] selecting Gal$^+$ progeny	-	255	330
AB1325	Laboratory stock	+	85	-
CLR5	Recombinant from a genetic cross [AB1325. x K1] selecting Pro$^+$ Gal$^+$ progeny	+	70	90
CLR6	From CLR5: selected for resistance to αMG during growth on galactose	-	305	365

Cont.....

glucose analogues commonly revealed that they were of three types: "black" mutants which take up the isotope and consequently blacken X-ray film when, after growth in the presence of [^{14}C]αMG, they are placed in contact with it, "white" mutants which take up no isotope and leave the film unaltered, and "faint" mutants, a class of intermediate activity which cause a slight darkening of the film. Mutants of the first class were presumed to have retained their Umg$^+$ character, whereas those of the second were designated Umg$^-$; the nature of those of the third class will be discussed in a later chapter.

Because the protein specified by the gene umg is a component of the phosphotransferase system that mediates the uptake of glucose, it was to be expected that mutants impaired in Umg-activity would show defective growth on glucose. Studies on a variety of umg mutants indeed bore out this expectation, with only one exception which will be discussed later. In addition to this though, it was found that the umg mutants isolated from strains with a low affinity for galactose were markedly impaired not only in their rates of growth on glucose, but, unexpectedly, also in their rates of growth on galactose. Table 2.1 summarises some of the growth properties of these mutants, and shows that the correlation between the umg mutation and the impairment of growth on galactose was neither limited to one strain nor to one method of isolating the umg mutants.

Bearing in mind the close similarities between the galK strains 20SOK$^-$ and CLR100 reported in the preceding chapter,

it was to be expected that Gal^+ derivatives of strain $20SOK^-$ would behave like strain K2.1t and its derivatives with respect to growth on galactose. It can be seen from Table 2.1 that this is indeed the case, and in addition strain CLR101 ($GalK^+$, Umg^+) was found to exhibit the same high 'Km' for growth on galactose as did strain K2.1t. Strains $20SOK^-$ and K2.1t clearly differ however as far as the uptake of glucose is concerned; the growth of the former strain on this hexose was hardly affected by the introduction of the umg mutation, a phenomenon which had been observed in no other strain. An investigation of the nature of glucose uptake in strain $20SOK^-$ is the subject of the second section of this chapter.

Because the concomitant effects on the utilisation of galactose and the uptake of αMG occurred so frequently, it is doubtful that each results from a separate mutation, particularly as in only one case did the selection procedure involve galactose metabolism. Studies on the properties of revertants of strain 16/3G support this conclusion. Revertants, able to grow rapidly on glucose or on galactose, were isolated by spreading portions (0.1ml) of an overnight culture of this strain on solid medium containing 10mM glucose or 10mM galactose respectively as sole carbon source. All the revertants selected on glucose medium were "black" in the autoradiography test for Umg-activity, and were therefore Umg^+; they had also regained the ability to grow fast on galactose. Similarly, many of the colonies selected for their fast growth on galactose had become

Table 2.2. The effect of the *umg* mutation on growth on different carbon sources.

Growth substrate	Doubling time (min) of strain K2.1t (Umg^+)	K2:1.22a (Umg^-)
10mM glucose	57	183
10mM fructose	72	72
10mM mannose	60	147
10mM galactose	69	360
10mM arabinose	80	95
10mM maltose	105	105
10mM gluconate	63	63
10mM lactose	80	80
10mM glucose 6-phosphate	53	57
10mM fructose 6-phosphate	67	63
20mM succinate	150	105
50mM glycerol	85	85

Umg^+; they were "black" in the autoradiography test and grew rapidly on glucose as well as on galactose. Thus a single mutational event affects both Umg-activity and the utilisation of galactose in this Mgl^- $GalP^-$ strain.

Mutations in the phosphotransferase Enzyme I or HPr have a pleiotropic effect on the growth of E.coli on many non-PT as well as on all PT-sugars (Wang & Morse, 1968; Fox & Wilson, 1968; Bourd et al., 1969; Wang et al., 1969, 1970). In contrast to this, the Enzyme II umg lesion was found to be specific in its effects (Table 2.2). The growth rates of strain K2.1t and its Umg^- derivative, strain K2.1.22a, were measured on a variety of carbon sources, and the umg mutant was found to be significantly impaired in its doubling times only on glucose, mannose and galactose. Mannose uptake in E.coli occurs predominantly by means of the activity of the glucose Enzyme II specified by the gene ptsX; indeed this system has been referred to by Curtis and Epstein (1975) as the mannosephosphotransferase, and the structural gene alternatively designated mpt. The impaired growth of strain K2.1.22a on mannose could therefore be taken to reflect a defect in PtsX- as well as in Umg-activity in this strain; this is feasible since it was derived as a mutant resistant to the toxic effects of DG, which is taken up by the Mpt-(PtsX-)system (Curtis & Epstein, 1975). However Curtis and Epstein also found that mannose utilisation is impaired in umg (or, in their terminology, gpt) mutants; this and other evidence led them to conclude that some mannose uptake is mediated by the Umg-system. Moreover, the

introduction of a well-defined ptsX mutant allele into strain K2.1.22a markedly alters its properties (Kornberg & Jones-Mortimer, 1975), showing that strain K2.1.22a must be PtsX$^+$. It thus appears that in strain K2.1.22a the increased doubling time on mannose, like that on glucose, results from the lack of sugar uptake by the Umg-system; galactose was therefore the only sugar tested whose utilisation was affected by the umg lesion but which was not known to be a substrate for the Umg-system.

Evidence for the involvement of Umg-activity in galactose uptake.

The striking effect of the umg lesion on galactose utilisation suggested that perhaps galactose too is taken up by a process involving Umg-activity. The impaired growth on galactose of umg mutants would then result if this provides a major route of galactose entry into the cell, or if, as proposed by Kaback (1968), the galactose phosphate produced by the phosphotransferase reaction associated with the umg$^+$ gene acts as the true inducer of the gal operon. Several pieces of evidence point to the conclusion that the former explanation is the correct one; the presence of the umg mutation affects the rate of galactose uptake in the strains under consideration but does not affect its subsequent metabolism.

The doubling times quoted in Table 2.2 for strains K2.1t (Umg$^+$) and K2.1.22a (Umg$^-$) were measured in cultures grown overnight on fructose or glycerol. When, however, strain K2.1.22a was grown on lactose, washed, and

resuspended in galactose medium, it initially grew rapidly, slowing down as the enzymes induced by growth on lactose were diluted out in the growing culture. Growth on lactose induces both lactose permease, which can mediate galactose uptake, and the enzymes of the gal operon, but it was evidently the induced galactose uptake system which was the important factor here. This became evident when revertants of strain K2.1.22a, selected for their fast growth on galactose, were examined. It will be recalled that most of the revertants of strain 16/3G (Umg^-) selected in this way were Umg^+; in contrast, of those derived from strain K2.1.22a which were tested (about 20), all were still Umg^-. All but one of these were constitutive for the expression of the lac operon as demonstrated by their ability to grow on phenylgalactoside as sole carbon source; this lactose analogue is a substrate of the lactose permease and of β-galactosidase but does not induce their expression. Since lactose-constitutive mutants are unaltered with respect to the inducibility of the gal operon, the slow growth on galactose of strain K2.1.22a must have been due to poor galactose uptake, which was improved by the provision of the constitutive lactose permease. The other revertant, which had retained both its Umg^- and lactose-inducible properties, was not further characterised; it is conceivable that it had become constitutive for the expression of an arabinose permease, another transport protein which would mediate galactose uptake. The different reversion patterns of strains K2.1.22a and 16/3G, which are both Umg^-

Table 2.3. The rate of galactose uptake by washed cell suspensions of the Umg^+ and Umg^- strains K2.1t and K2.1.22a.

Carbon source for growth of cultures	Rate of galactose uptake (nmoles. min^{-1}. mg dry $mass^{-1}$) at			
	0.2mM		2mM	
	K2.1t	K2.1.22a	K2.1t	K2.1.22a
Galactose (10mM)	1.3	0.25	2.4	0.5
Glycerol (20mM)	0.9	0.15	2.1	0.5
Glycerol (20mM)/ D-fucose (1mM)	1.1	0.15	2.3	0.6

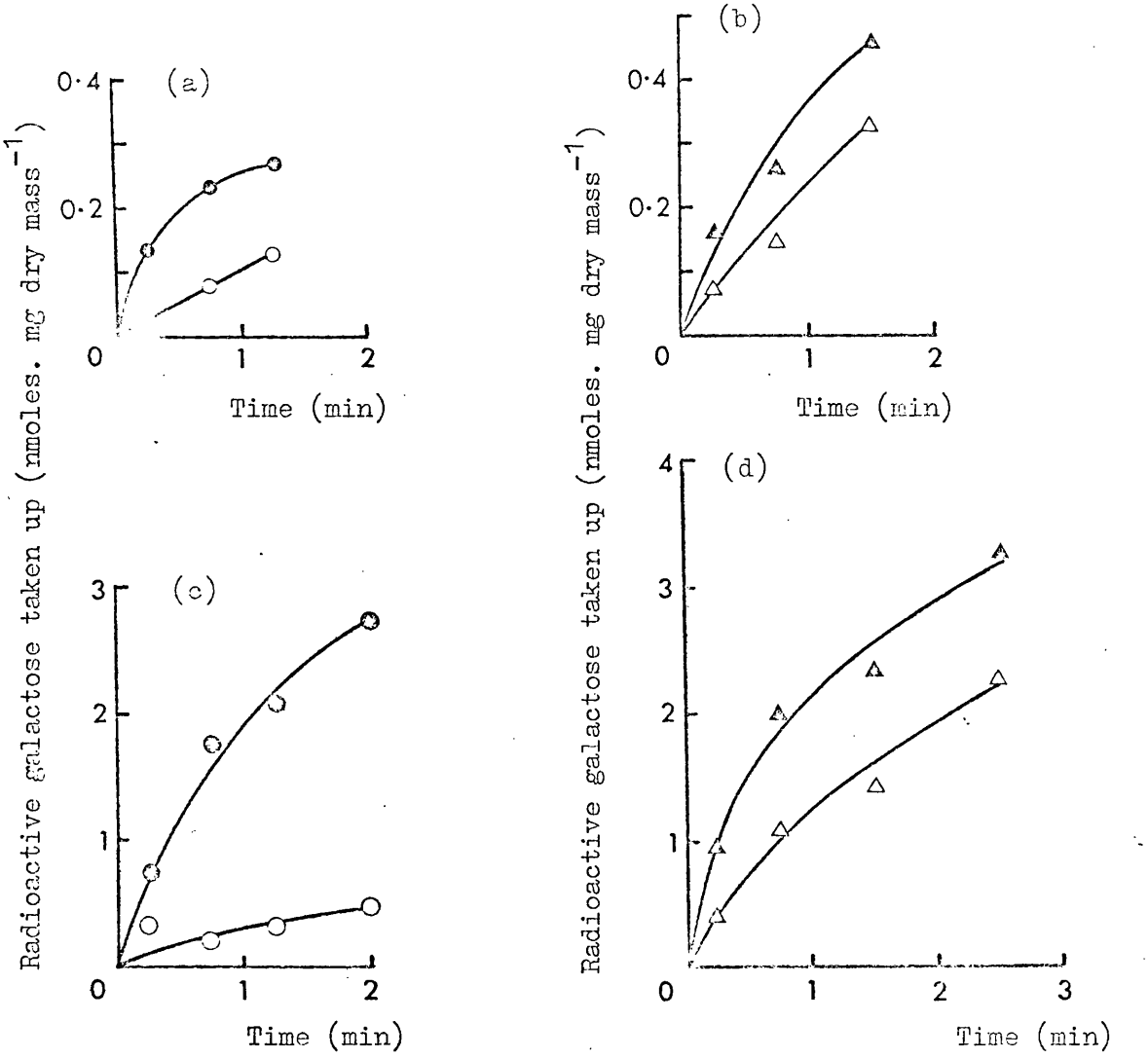


Fig. 2.1. The uptake of galactose by Umg$^+$ and Umg$^-$ strains devoid of galactokinase. The uptake of the sugar was measured at concentrations of 0.2mM (a and b) and 2mM (c and d) in washed suspensions of glycerol-grown cultures of the Umg$^+$ strains 20S0K$^-$ (●) and CLR100 (▲) and their Umg$^-$ derivatives CLR15 (○) and CLR115 (△).

derivatives of strain K2.1t, indicates that the precise mutations present in these strains might be different, or that perhaps strain K2.1.22a carries more than one genetic lesion affecting Umg-expression.

Measurements of galactose uptake by washed cell suspensions showed that, in the absence of MeGal and galactose permease activities, Umg^- strains take up galactose less rapidly than do Umg^+ strains. After growth under a variety of conditions, strain K2.1t (Umg^+) consistently takes up galactose about five times faster than does strain K2.1.22a (Umg^-) (Table 2.3). It could be argued, however, that different levels of activity of the galactose metabolic enzymes affected the measured rates of uptake in these Gal^+ strains, so similar comparisons were made using galK strains in which the uptake process could be looked at in isolation. As shown in Fig. 2.1, the Umg^- strains again took up galactose less readily than their Umg^+ parents,although the difference was more marked between strain 20SOK$^-$ and its Umg^- derivative (graphs a and c) than between the Umg^+ and Umg^- $GalK^-$ derivatives of strain K2.1t (graphs b and d). These experiments contrast with similar ones carried out with the $GalP^+$ strain W4345 and its derivatives. Very little difference in the rate of uptake of galactose was observed either between strain W4345 and its Umg^- derivative CLR17, or between the Gal^+ strains CLR18 (Umg^+) and CLR19 (Umg^-) both of which were derived from strain W4345 (Fig. 2.2).

If Umg^- mutants of $GalP^-$ Mgl^- strains grow poorly on

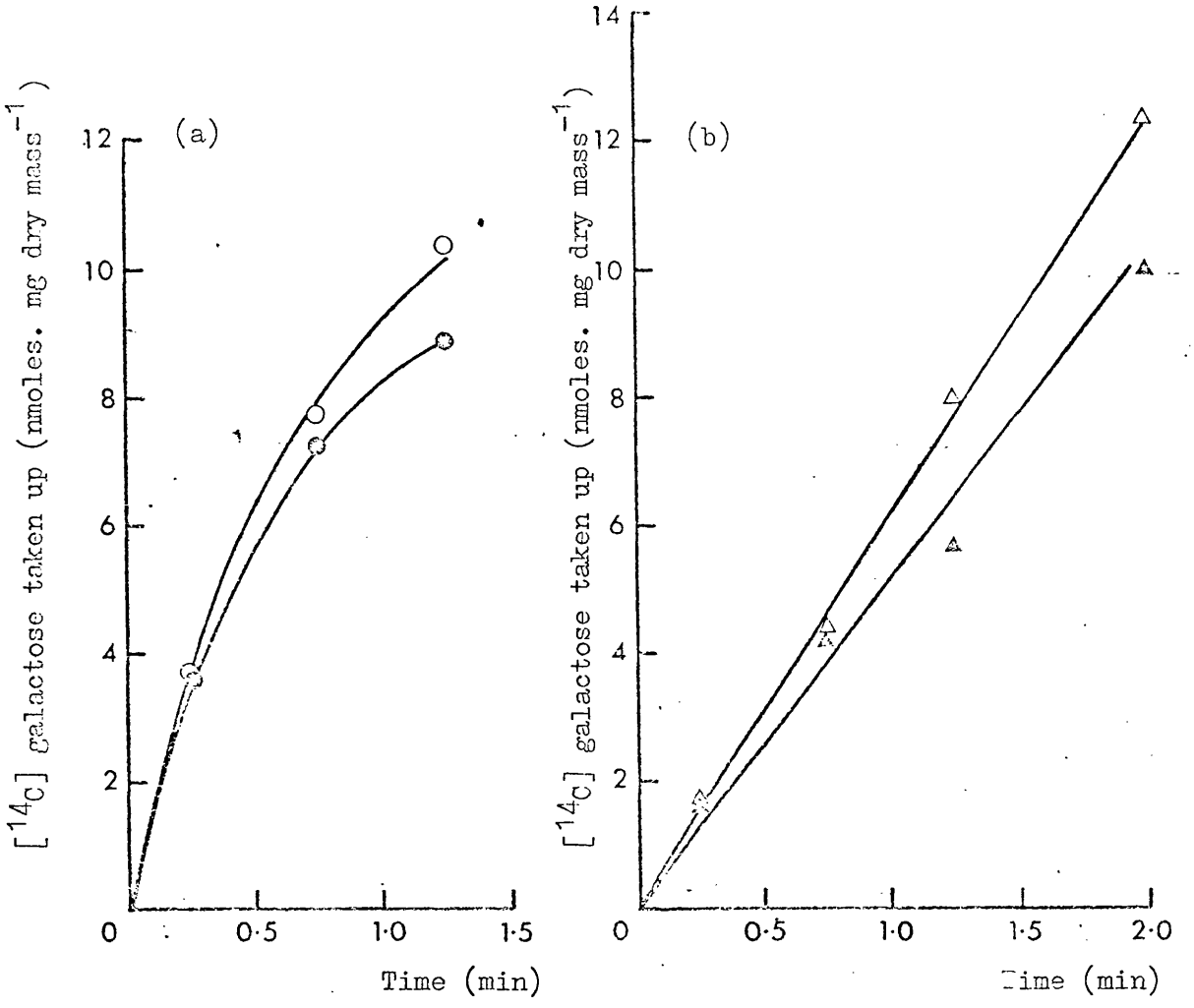


Fig. 2.2 The uptake of 0.2mM galactose by GalP$^+$ strains of Umg$^+$ and Umg$^-$ phenotype. Uptake measurements were carried out in washed suspensions of cells induced for galactose permease activity by growth on, or in the presence of, galactose. The strains used were (a) the GalK$^-$ strains W4345 (o) and its Umg$^-$ derivative CLR 17 (o) and (b) the GalK$^+$ strains CLR 18 (Umg$^+$, Δ) and CLR 19 (Umg$^-$, ▲).

Table 2.4. The effect of MeGal permease activity on the ability of Umg$^-$ cells to take up and grow on galactose.

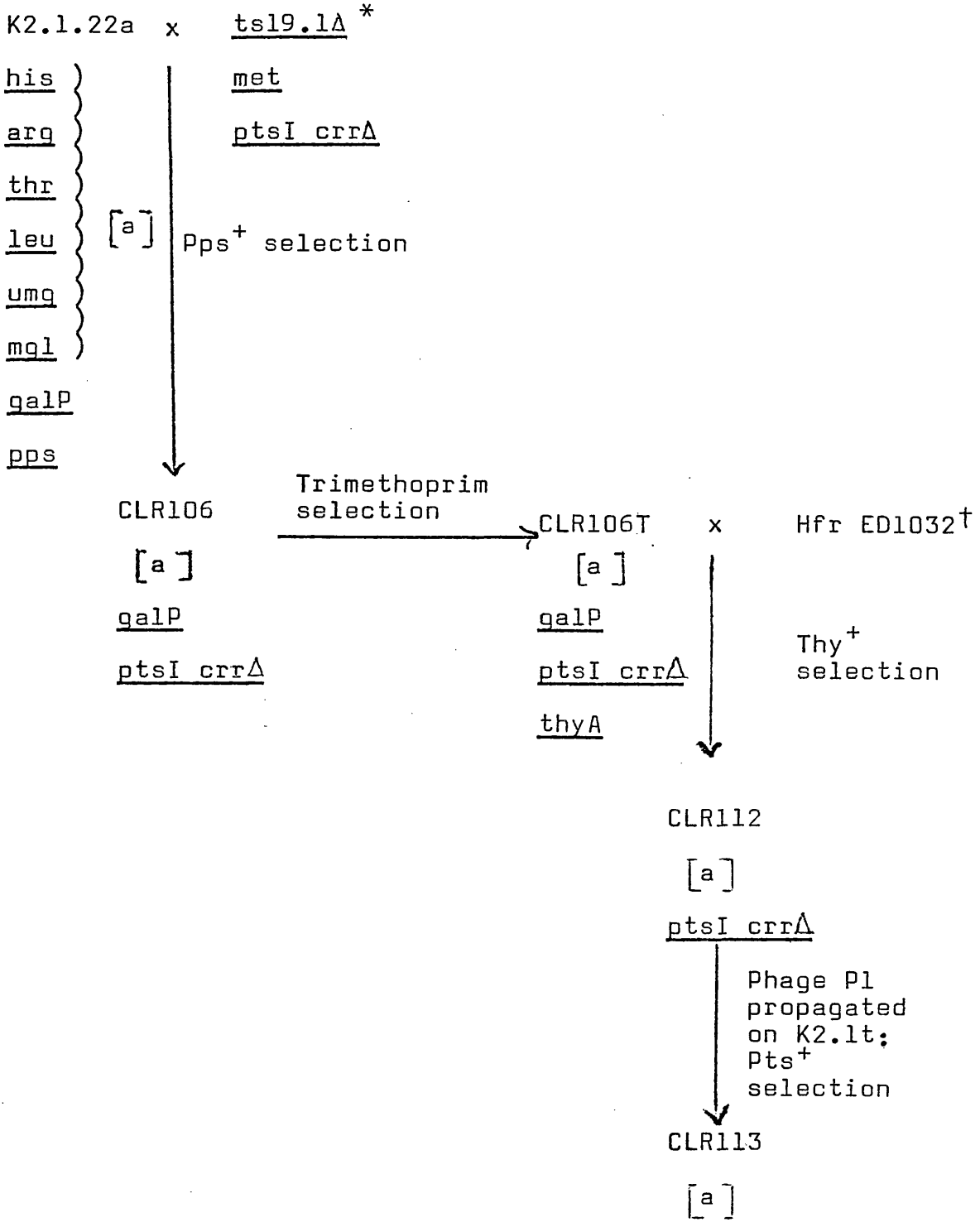
	Strain K2.1.22a (Umg$^-$, GalP$^-$, Mgl$^-$)	Strain CLR9 (Umg$^-$, GalP$^-$, Mgl$^+$)
Doubling time (min) on galactose (10mM)	320	165
Doubling time (min) on glucose (10mM)	183	185
*Uptake of 0.2mM [^{14}C]-galactose by cells grown on:		
glycerol (20mM)	0.15	0.9
glycerol (20mM)/D-fucose (1mM)	0.15	6.0
galactose (10mM)	0.25	6.9
*Uptake of 2mM [^{3}H]-galactose by cells grown on:		
glycerol (20mM)	0.5	1.2
glycerol (20mM)/D-fucose (1mM)	0.6	3.2
galactose (10mM)	0.5	3.3
* Uptake of 0.5μM [^{14}C]-galactose by cells grown on:		
glycerol (20mM)/D-fucose (1mM)	0	0.48
* Uptake of 10μM [^{14}C]-MeGal by cells grown on glycerol (20mM)/D-fucose (1mM)	0	3.2

* The uptakes are expressed as nmoles labelled material taken up. mg dry mass^{-1} min.$^{-1}$

galactose simply because they are unable to take up the hexose as rapidly as their Umg^+ parents, it follows that strains with wild-type activity of the galactose active transport systems should grow well on this sugar irrespective of their Umg character. Experiments with strain KL16 (Mgl^+, $GalP^+$) fulfil this expectation; a Umg^- derivative, designated 14/136, was isolated from this strain, and its doubling time on galactose (85 min) was found not to be significantly different from that of its Umg^+ parent (97 min). By the same argument, the introduction of MeGal permease or galactose permease activity, or both, into strain K2.1.22a should improve its growth on galactose. This too was found to be the case in experiments detailed below.

Strain CLR9, an Mgl^+ derivative of strain K2.1.22a,was obtained from a genetic cross between this F^- strain and the Hfr strain KL16.21. Its growth and uptake properties are summarised and compared with those of its Mgl^- parent in Table 2.4. Although the growth rate of strain CLR9 on galactose is improved relative to strain K2.1.22a, it still does not approach that of the Umg^+ strain, K2.1t (doubling time 69 min) despite its vastly improved ability to take up low concentrations of galactose from the medium. Comparison of the rates of uptake of 0.2mM and 2mM galactose by this strain shows that uptake is slower at the higher concentration (Table 2.4). This suggests that the MeGal permease is substrate-inhibited at the galactose concentrations routinely used in this laboratory for batch culture of bacteria; Wilson (1974a) also observed substrate inhibition

Fig. 2.3. The pedigree of strain CLR113 (Umg$^-$, Mgl$^-$, GalP$^+$)



† Strain ED1032 transfers is chromosome in the order o-thyA -serA from an origin at minute 51.

* Strain tsl9.1Δ is derived from HfrC.

of this transport system at high galactose concentrations. Since the galactose binding protein is required for galactose taxis as well as transport (Hazelbauer & Adler 1971; Ordal & Adler, 1974a, b) the major physiological importance of the proteins specified by the mgl genes seems to lie in the detection and uptake of very small quantities of this sugar.

The task of producing a GalP$^+$ derivative of strain K2.1.22a initially presented a considerable problem, because the chromosomal location of the gene galP was not known, and neither was there available a simple, unambiguous method of distinguishing GalP$^+$ from GalP$^-$ strains. A screening procedure was eventually devised, however, and the galP$^+$ marker located at about minute 55 on the E.coli linkage map. The details of that piece of work will be described in a later chapter, but it should be pointed out at this stage that the nature of the screening process necessitated the introduction of the galP$^+$ allele into a ptsI crr derivative of strain K2.1.22a. (The gene ptsI specifies the phosphotransferase Enzyme I, and the presence of an additional lesion in the gene crr enables ptsI mutants to grow on all non-PT sugars (Jones-Mortimer & Kornberg, 1974a)). The pedigree of the GalP$^+$ Umg$^-$ strain designated CLR113, which was eventually derived, is illustrated in Fig. 2.3. At each stage in its preparation it was tested by measurements of 0.5μM galactose uptake, to ensure that it was still Mgl$^-$; because all ptsI mutants fail to take up αMG its retention of the Umg$^-$ character could only be

Table 2.5. The effect of galactose permease activity on the ability of Umg$^-$ cells to take up and grow on galactose.

	Strain K2.1.22a (Umg$^-$, GalP$^-$ Mgl$^-$)	Strain CLR113 (Umg$^-$, GalP$^+$ Mgl$^-$)
Doubling time (min) on galactose (10mM)	320	170
Doubling time (min) on glucose (10mM)	183	180
*Uptake of 0.2mM [^{14}C]-galactose by cells grown on: glycerol (20mM)	0.15	0.3
glycerol (20mM)/ D-fucose (1mM)	0.15	1.3
galactose (10mM)	0.25	1.6
*Uptake of 2mM [^{3}H]-galactose by cells grown on: glycerol (20mM)	0.5	1.0
glycerol (20mM)/ D-fucose (1mM)	0.6	2.9
galactose (10mM)	0.5	2.9
*Uptake of 0.5μM [^{14}C]-galactose by cells grown on glycerol (20mM)/D-fucose (1mM)	0	0

*The uptakes are expressed as nmoles labelled material taken up.mg dry mass^{-1}. min^{-1}.

Table 2.6. The growth on and uptake of galactose by strains of different Umg, GalP and Mgl phenotypes.

Strain	Doubling time (min) on galactose (10mM)	Rate of uptake of galactose (nmoles. min^{-1}. mg dry $mass^{-1}$) at 0.2mM	2mM
KL16 (Umg^+, Mgl^+, $GalP^+$)	97	8.5	n.t.
K2.1t (Umg^+, Mgl^-, $GalP^-$)	69	1.3	2.4
K2.1.22a (Umg^-, Mgl^-, $GalP^-$)	320	0.25	0.5
CLR113 (Umg^-, Mgl^-, $GalP^+$)	170	1.6	2.9
CLR9 (Umg^-, Mgl^+, $GalP^-$)	165	6.9	3.3
CLR116 (Umg^-, Mgl^+, $GalP^+$)	120	6.6	5.8

n.t. = not tested

ascertained when the ptsI lesion was finally removed.

Like strain CLR9, strain CLR113 took up galactose faster than did strain K2.1.22a, and as a result of this was able to grow more quickly on this sugar. Again though (Table 2.5) its doubling time on galactose of 180 min. was still significantly higher than the 69 min. measured in the Umg^+ strain K2.1t.

Finally, a Umg^-, Mgl^+, $GalP^+$ strain, designated CLR116, was obtained by subjecting strain CLR113 to conjugation with the Hfr strain KL16, and screening the His^+ recombinants for their ability to take up 0.5μM galactose. Some of the properties of this strain are given in Table 2.6, together with data already presented for a variety of other strains. It is quite clear that the combined activities of the MeGal and galactose permeases mediate faster uptake of and growth on galactose by Umg^- strains, at high substrate concentrations, than can either alone; they do not however exhibit quite such a high level of activity in the K2 background as they do in strain KL16. Neither of the Mgl^+ $GalP^+$ strains studied grows as fast on 10mM galactose as strain K2.1t, suggesting that the uptake dependent on the umg^+ allele present in strain K2.1t is faster at this concentration than that mediated by the galactose active transport systems.

The Umg-system has both regulatory and structural components (Kornberg & Reeves, 1972a, b) so that different strains of E.coli may be either inducible by glucose or constitutive for the expression of Umg-activity. Strain

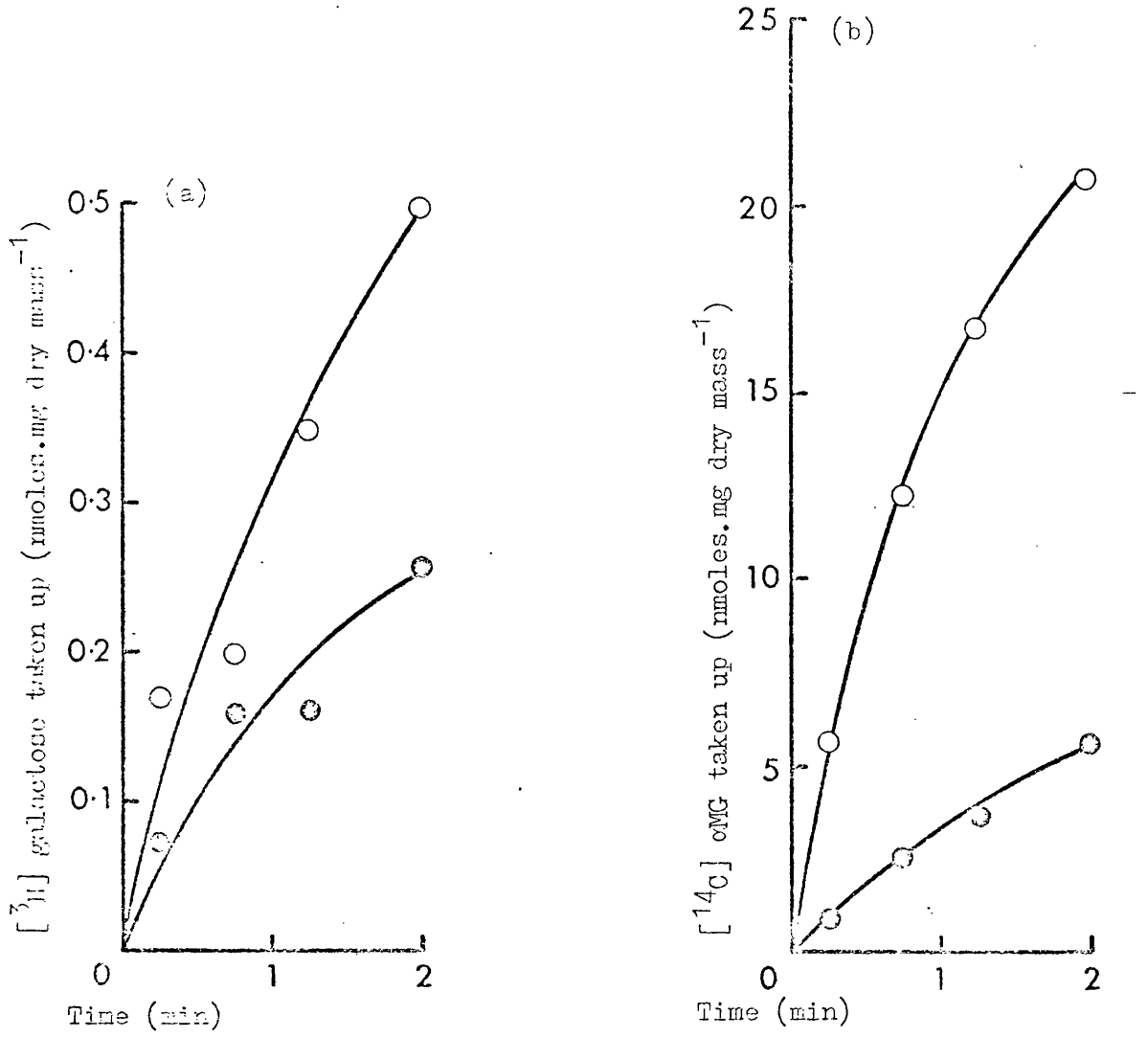


Fig.2.1 The induction of galactose and αMG uptake activities in strain CLR 102 ($GalK^+$ Mgl^- $GalP^-$ Umg^+-inducible). The uptakes of (a) 2mM galactose and (b) 0.1mM αMG were measured using washed suspensions of cells grown on 10mM glucose (o) and 10mM sodium gluconate (●).

KL16 is in the former category (Kornberg & Jones-Mortimer, 1975) whereas strain K2.1t is constitutive in this respect (Kornberg & Reeves, 1972b). Thus the slower growth of strain KL16 on 10mM galactose probably reflects the fact that in cultures of this strain growing on galactose, the Umg-system is not expressed at high enough levels to contribute significantly to galactose transport. This was illustrated when the Umg^+ character was transferred from strain KL16 to strain K2.1.22a by conjugation. The recombinant studied, designated CLR20, had also received the $\underline{mgl}^+$ genes from its Hfr parent; its doubling time on galactose of 195 min. showed no improvement over that of the Mgl^+ Umg^- strain CLR109 (doubling time 165 min.). But the Umg-system present in inducible strains does support galactose transport when it is induced. Strain CLR102 (Umg^+, $GalK^+$, Mgl^-, $GalP^-$), a derivative of strain K2 which had received its Umg^+ character from the Umg-inducible Hfr strain K1, took up galactose only poorly; the rate of uptake was, however, increased under conditions which led to the induction of Umg-activity as measured by αMG uptake (Fig. 2.4).

The nature of the role of Umg-activity in galactose uptake.

Phosphotransferase Enzymes II are membrane-bound proteins (Kundig & Roseman, 1971b; Roseman, 1972). It is therefore conceivable that the protein specified by the umg gene supports galactose transport either by itself acting as a galactose carrier, or by stabilising a different membrane component which effects galactose

Table 2.7. The inhibition of galactose uptake by substrates of the Umg-system in strain K2.1t.

Inhibitor	Rate of uptake of 0.2mM galactose (nmoles. mg dry mass^{-1} min^{-1})
0	1.7
0.2mM glucose	0.05
0.2mM αMG	0.2

The uptakes were measured in washed suspensions of cells of strain K2.1t grown on 10mM galactose. Inhibitors were added 10 sec before the addition of [^{14}C]-galactose.

transport. Since the Umg-substrates glucose and αMG strongly inhibit the uptake of galactose by strain K2.1t (Table 2.7),it appears that the sugar must actually be transported by this system. This is further supported by the observation that when a culture of this strain was allowed to grow on 10mM [^{14}C]-galactose and the incorporation of isotope into cell material followed, the addition of only 2mM glucose immediately and totally abolished galactose utilisation. It is clear that the affinity of galactose for the Umg-system must be significantly lower than that of glucose. It could be argued that these results could equally be interpreted in terms of the second postulate proposed above, that galactose is taken up by a system genetically distinct from, but stabilised by, the Umg-system, and that this system is subject to catabolite inhibition and hence inactivated by the addition of glucose and αMG. Catabolite inhibition has been shown to depend on the uptake and phosphorylation of glucose and its analogues (Winkler & Wilson, 1967; Miles & Pirt, 1973; McGinnis & Paigen, 1973; Kornberg, 1973); it therefore cannot explain the observed inhibition by glucose and αMG of galactose uptake and utilisation in strain K2.1t because, as will be shown in the following chapter, it still occurs in a derivative lacking this phosphotransferase activity. Moreover a direct competition between galactose and the substrates of the Umg-system is supported by reports that galactose inhibits, albeit weakly, glucose and αMG transport and phosphorylation by this system (Hoffee et al., 1964; Gachelin, 1970; Epstein & Curtis, 1972).

Discussion.

It was shown in Chapter One that galactose transport in strain K2.1t is mediated by a constitutive uptake system with a high Km for the sugar, by a mechanism involving either phosphotransferase activity or facilitated diffusion. The evidence presented in this section shows that the Umg-system has all the properties required of this transport system; it is constitutive in strain K2.1t, transports galactose at a rate fast enough to allow rapid growth at high sugar concentrations, and has only a low affinity for galactose.

Since the protein specified by the gene umg is a component of the glucose phosphotransferase, it might be expected that galactose too is phosphorylated during Umg-mediated uptake. Kaback's (1968) evidence for galactose phosphorylation by membrane vesicles of strain K2.1t has already been cited. It has been reported that in other strains too, galactose is phosphorylated during the uptake process both by whole cells (Rogers & Yu, 1962) and by membrane preparations (Kundig et al., 1965; Roseman, 1969); in these cases the product has been identified as galactose 6-phosphate. This presents a paradox because there appears to be no metabolic role in E.coli for galactose 6-phosphate (Vorisek & Kepes, 1972), so it is difficult to see how the organism can grow on galactose if all the sugar enters the cell in the form of this phosphate ester. On the other hand it cannot be that galactose 1-phosphate, which can be metabolised, is produced by the phosphotransferase reaction, because if it were, such a reaction would bypass the formation

of this product from galactose and ATP. This would mean that mutants devoid of galactokinase activity, but possessing the other enzymes of the gal operon intact, would grow on galactose, whereas it is quite clear from the properties of the galK strains used in this study, and those described by other workers (e.g. Horecker et al., 1960; Wu, 1967) that they do not. The paradox was resolved by a series of experiments which will be described in Chapter Three.

II The roles of Umg- and PtsX-activities in growth on glucose.

General properties of the glucose transport systems in strains 20SOK⁻ and CLR15

Epstein and Curtis (1972) and Curtis and Epstein (1975) have described in some detail the properties of the phosphotransferase Enzymes II specified by the genes they termed *gptA* (otherwise described as *umg*, *gpt*, or *cat*) and *gptB* (now called *mpt* by them, and *ptsX* by Jones-Mortimer and Kornberg (1974b)). They showed the former, which they call glucosephosphotransferase, to act predominantly on glucose and αMG, and the latter, mannosephosphotransferase, on glucose, mannose, DG, glucosamine and mannosamine, although the specificities of the two enzymes overlap to some extent. The PtsX-system, as evident from the way in which its activity was first discovered by Ferenci and Kornberg (1974) also mediates fructose transport. It has been established that these two Enzymes II mediate almost all the uptake of glucose by several strains of *E.coli* growing on this sugar. Thus *ptsX umg* double mutants either fail to grow on glucose at all, or grow very slowly in a process relying on limited glucose uptake by active transport systems, and its subsequent phosphorylation by the intracellular ATP-dependent glucokinase (Epstein & Curtis, 1972; Kornberg & Jones-Mortimer, 1975; Curtis & Epstein, 1975). These authors also provided good evidence that the Umg-system is of primary importance for the growth on glucose of these strains; Umg^- $PtsX^+$ mutants are impaired in their ability to grow on glucose whereas Umg^+ $PtsX^-$ mutants are not.

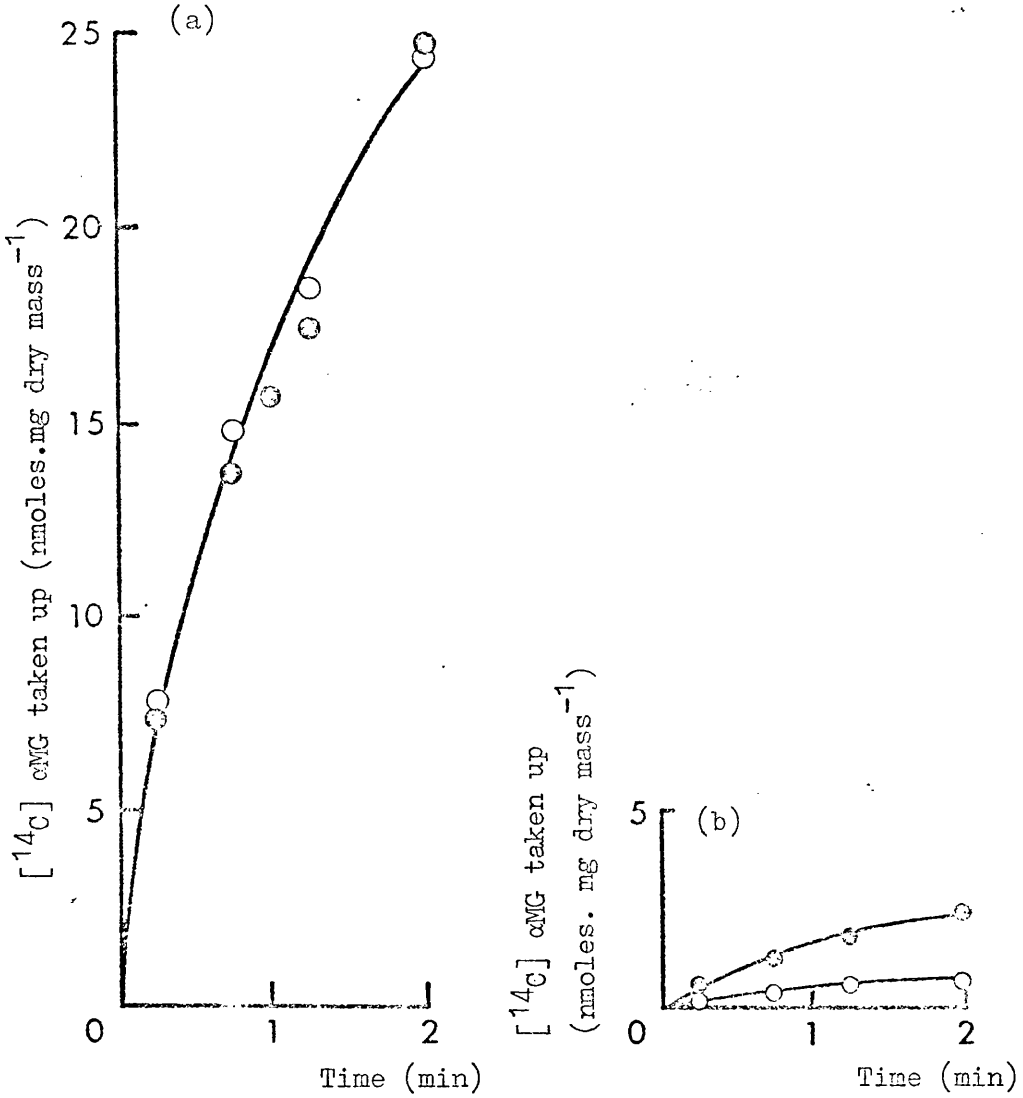


Fig. 2.5 The uptake of 0.1mM αMG by washed cell suspensions of strains (a) 20SOK$^-$ (Umg$^+$) and (b) CLR15 (Umg$^-$) grown on 10mM glucose (o) and 20mM glycerol (o).

This implies that the regulation of Umg and PtsX activities is complex; either PtsX-activity is expressed during growth on glucose only in Umg^- strains, or both systems are so regulated that compensatory changes can be made in one when the other is lost through mutation. As shown earlier, Umg^- derivatives of strain $20SOK^-$ were found to be unusual in that they are only very slightly impaired in their ability to grow on glucose (Table 2.1). This suggests that the strain is altered in this regulatory function, or has another means of transporting glucose. Tyler *et al*. (1969) described certain strains of *E.coli* which were phenotypically similar to strain $20SOK^-$ in that they grew well on glucose even if they carried the *cat* (*umg*) mutation. These workers showed that the gene responsible for this good growth on glucose was not located close to the *cat* gene, but they did not characterise it further. In an attempt to investigate the cause of this difference in phenotype from that of other Umg^- strains, the nature of glucose uptake in strain $20SOK^-$ and its Umg^- derivative, strain CLR15,was examined.

Kornberg and Reeves (1972a) made use of the fact that the Umg-system can be assayed specifically by measurements of αMG uptake, to show that glucose induces this activity in some strains whereas others are constitutive. Using this method it was found that Umg-activity is constitutive in strain $20SOK^-$, and is quite clearly impaired in strain CLR15 (Fig.2.5). However glucose induces a low level of αMG uptake activity in the Umg^- strain. Measurements of the

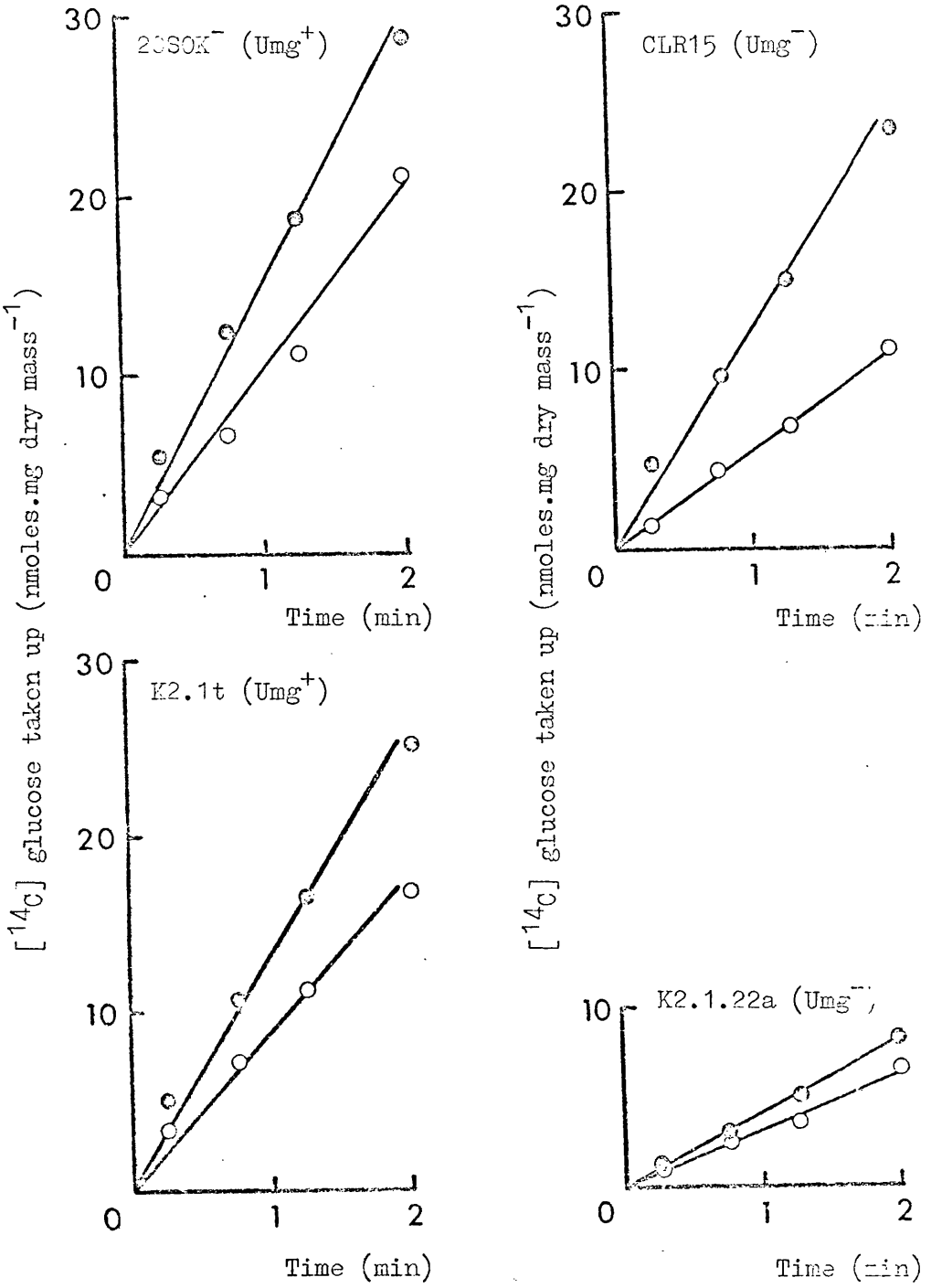


Fig.2.6 The uptake of 0.1mM glucose by washed cell suspensions of strains 20SOK⁻ and K2.1t and their Umg⁻ derivatives CLR 15 and K2.1.22a. The organisms were grown on 10 mM glucose (●) and on 20 mM glycerol (o).

ability of washed cell suspensions to take up glucose (Fig. 2.6) showed that growth on glucose induces the activity of a glucose transport system in the Umg^- strain; presumably this system is also responsible for the glucose-induced αMG transport. The system which is induced by glucose appears also to be expressed in the Umg^+ strain, $20SOK^-$, but because of the intact Umg-system, the basal level of glucose uptake is higher here. Comparison with strains K2.1t and K2.1.22a shows that these strains too have a glucose-inducible glucose transport system, but its activity is much lower in the Umg^- strain K2.1.22a than in strain CLR15.

Epstein and Curtis (1972) reported that PtsX-activity is not induced by glucose. Conflicting evidence comes however from the observation of Kornberg and Reeves (1972b) that both glucose and fructose induce PEP-dependent mannose-phosphotransferase activity in E.coli. Since the PtsX-system transports mannose, and has also been shown to be induced by fructose (Ferenci & Kornberg, 1974), it is quite likely that Kornberg and Reeves were measuring PtsX-activity. A possible explanation for the observed properties of strain CLR15 is therefore that the PtsX-system is so regulated in this strain as to be expressed at higher levels than in the other Umg^- strains so far examined. However although it was clearly demonstrated that strain CLR15 takes up glucose by phosphotransferase activity, there was no direct evidence that the Enzyme II responsible for this uptake was that specified by the gene ptsX. Indeed some observations

Table 2.8. Phosphotransferase activities in strains 20SOK$^-$ and CLR15.

Strain	Carbon source for growth	Assayed phosphotransferase activity (nmoles pyruvate formed.min^{-1} mg dry mass toluenised cells^{-1}) Glucose	∝MG	Fructose
20SOK$^-$ (Umg$^+$)	Glucose	68	61	22
	Fructose	93	94	17
CLR15 (Umg$^-$)	Glucose	56	25	28
	Fructose	23	15	14

Cells were grown on 10mM carbon source, harvested and toluenised as described in 'Materials and Methods'. PEP-dependent phosphotransferase activities were measured at a substrate concentration of 5mM.

placed serious doubt on such a hypothesis.

The dependence of glucose transport in strain CLR15 on phosphotransferase activity.

When ptsI crr mutants of strain 20SOK$^-$ were isolated from among the Thy$^+$ Str^R progeny of a genetic cross between a thyA derivative of strain 20SOK$^-$ and the Hfr strain ts19.1Δ (ptsI crrΔ), they were found to be severely impaired in their growth on glucose (doubling times $7\frac{1}{2}$ - 14h). Thus the major routes of glucose uptake in strain 20SOK$^-$ must involve phosphotransferase activity. This was confirmed by experiments in which the phosphotransferase reaction was measured by following the sugar-dependent conversion of phosphoenolpyruvate to pyruvate in the reaction

phosphoenolpyruvate + hexose ⟶ hexose phosphate + pyruvate

in cells made permeable to the assay compounds. This technique, devised by Kornberg and Reeves (1972a), enables phosphotransferase uptake systems to be distinguished from those of active transport and facilitated diffusion in the activity of which phosphoenolpyruvate is not directly involved. Table 2.8 shows that although the Umg$^-$ strain CLR15 is clearly impaired in its ability to phosphorylate glucose and αMG compared with the Umg$^+$ strain 20SOK$^-$, its phosphorylating activity is still significant, and indeed cells of strain CLR15 grown on glucose phosphorylate this hexose at nearly the same rate as those of strain 20SOK$^-$. It is clear though that the particular Enzymes II mediating the phosphorylation are different in the two strains, since not only do they display different relative affinities

towards glucose and αMG, but they are also differently regulated. Thus, just as the experiments involving the uptake of radioactive sugars implied (Figs. 2.5 and 2.6), the phosphotransferase activity towards glucose and αMG is highest in the Umg^- strain in glucose-grown cells. This contrasts with the Umg^+ organism in which fructose stimulated both glucose and αMG phosphotransferase activities to levels above those in glucose-grown cells. This phenomenon, observed also by Kornberg and Reeves (1972a, b) and termed by them the 'fructose kick', might be thought to be due to the induction by fructose of PtsX-activity; however, it seems doubtful whether this can be the true explanation, because the PtsX-system displays little activity towards αMG, whereas the 'fructose kick' stimulates αMG phosphorylation as much as that of glucose. Moreover, an increased level of expression of PtsX-activity in fructose-grown cells of strain 20SOK$^-$ is not evident from the measurements of phosphorylation of fructose, another substrate of the PtsX-system. The results displayed in Table 2.8 suggest rather that growth on fructose somehow stimulates Umg-expression, and thus the 'fructose kick' is lost in the Umg^- strain.

Glucose phosphorylation by a phosphotransferase system other than that depending on Umg-activity therefore appears to occur at a high enough rate in strain CLR15 to account for its fast growth on glucose. It was stated earlier that *ptsX* and *umg* are the only genes known to specify glucose Enzymes II in wild-type *E.coli*. The

organism can however mutate to express an aryl-β-D-glucoside uptake activity (Schaefler, 1967a, b; Buttin, 1968), which is a phosphotransferase system with an affinity for the substrates of the other glucose Enzymes II as well as for aryl-β-D-glucosides. These mutants, designated Bgl^+, can be distinguished from wild-type (Bgl^-) strains by their ability to grow on salicin (o-hydroxymethylphenyl-β-D-glucoside). Strains 20SOK$^-$ and CLR15 do not grow on this compound, so must be Bgl^- and unable to use the Bgl-system for glucose uptake. Thus, in the Umg^- strain, CLR15, the Enzyme II mediating glucose uptake must be either that specified by the gene ptsX, or another, as yet unrecognised, glucose Enzyme II.

Characterisation of the ptsX allele present in strain 20SOK$^-$

The hypothesis that the PtsX-system plays the major role in mediating glucose uptake in the Umg^- strain CLR15 was not supported by transduction experiments, which showed that the phenotype of rapid growth on glucose displayed by this strain was not highly cotransducible with $PtsX^+$. The gene ptsX is 53% cotransducible with kga, which specifies phospho-2-keto-3-deoxygluconate aldolase (Jones-Mortimer & Kornberg, 1974b). A kga ptsF ptsX umg derivative of strain K2 (designated CLR21) was constructed, and transduced to kga^+ in separate experiments using phage P1 propagated on strains 20SOK$^-$ and K2.1t respectively. The gene ptsF specifies the high affinity fructose phosphotransferase Enzyme II (Ferenci & Kornberg, 1974); because strain CLR21 lacks this activity as well as that of PtsX, it cannot grow on fructose,

Table 2.9. The growth on glucose of some strains of different Umg and PtsX phenotype.

Strain	Phenotype	Doubling time (min) on 10mM glucose
*JM479	Kga^- Umg^+ $PtsX^-$	75
CLR21	Kga^- Umg^- $PtsX^-$	500
CLR22	Kga^+ Umg^- $PtsX^+$ (from 20SOK$^-$)	200
CLR23	Kga^+ Umg^- $PtsX^+$ (from K2.1t)	210

* Strain JM479 is the strain from which strains CLR21, 22 and 23 were derived.

and PtsX$^+$ transductants could be recognised by their ability to grow on this sugar. Both phage preparations yielded the same types of glucuronate-positive (*kga*$^+$) transductants; those which were fructose-positive (*ptsX*$^+$) grew fairly well on glucose, whereas those which were fructose-negative (*ptsX*$^-$) were also essentially glucose-negative. The *ptsX*$^+$ transductants all appeared to grow at the same rate on solid medium containing glucose as sole carbon source; none of the 35 organisms which had received the *ptsX*$^+$ allele from strain 20SOK$^-$ grew significantly faster than those in which the *ptsX*$^+$ allele was derived from strain K2.1t. When the growth rates on glucose were measured in liquid medium (Table 2.9), it was clear that the PtsX$^+$ transductants derived from both phage preparations grew at the slow rate typical of PtsX$^+$ Umg$^-$ derivatives of strain K2, rather than at the rapid rate exhibited by the Umg$^-$ derivative of strain 20SOK$^-$ designated CLR15. The differences in growth rate on glucose between strain CLR15 and other Umg$^-$ strains do not therefore reflect differences in the *ptsX* structural gene. If the strains differ in the regulation of PtsX-activity, then the regulatory gene involved must be located on the chromsome at a site distant from *ptsX*. This is feasible, since there are other transport systems, e.g. those for galactose (Buttin, 1963b; Lengeler *et al.*, 1971) and arabinose (Schleif, 1969), the levels of expression of which are controlled by genes located quite separately from the structural ones; if true, however, the regulation would be different from that of the Umg-system, where the

gene which defines the inducibility or constitutivity of enzyme expression is highly cotransducible with the structural gene (Kornberg & Smith, 1972).

Discussion

Bourd *et al.* (1974a; 1975) have recently described mutants which are similar to strain CLR15 in some respects; they are defective in their ability to take up radioactive αMG from the medium, but take up and grow on glucose at approximately wild-type rates. However the ability of extracts of these mutant cells to phosphorylate αMG is not impaired, but in fact is slightly increased relative to that of extracts of the parent strain. Bourd *et al.* have suggested that these mutants, which they designate Tgl^-, lack a component whose activity is required in conjunction with phosphorylation by the Umg Enzyme II for full glucose and αMG transport on the Umg-system. Strain CLR15 is defective in phosphoenolpyruvate-dependent αMG phosphorylation as well as in overall αMG transport activity, so is unlikely to be a mutant of this type. If though, as these workers propose, the Tgl^+ function is essential for complete Umg-mediated transport activity, the fast uptake of glucose and growth on this sugar of their Tgl^- strains still remains to be explained. The work described in this section has shown that there seems to be a glucose Enzyme II, different from that specified by the gene *umg*, which is capable of mediating rapid glucose uptake in the Umg^- mutant CLR15; perhaps it is also present in these Tgl^- strains. The

nature of this Enzyme II remains to be elucidated; there is no convinving evidence that it is that specified by the gene *ptsX*. An investigation of the rate of growth on glucose of *ptsX umg* double mutants of strain 20SOK$^-$ would show clearly how important the PtsX-system is in mediating the growth on glucose of its Umg$^-$-derivatives. However, the construction of such double mutants is not easy since strain 20SOK$^-$ lacks genetic markers in the *ptsX* region of the chromsome.

CHAPTER 3. THE MECHANISM OF GALACTOSE UPTAKE BY THE UMG-SYSTEM.

The independence of galactose uptake in Mgl⁻ GalP⁻ strains from phosphotransferase activity

The Enzyme II specified by the umg gene is the final component of the phosphotransferase system that brings about the translocation and phosphorylation of glucose and αMG. Thus, when its activity was shown to be directly involved in the uptake of galactose by GalP⁻Mgl⁻ strains of E.coli, the question arose whether galactose uptake in these strains also depends on phosphotransferase activity. The problems concerning the nature and utilisation of the galactose phosphate such an uptake system would produce have been discussed; however, several experimental approaches yielded the evidence, summarised below, that galactose transport on the Umg-system is not obligatorily coupled to its phosphorylation.

1. Morgan and Kornberg (1967) showed that the addition of pyruvate to cultures of E.coli strains devoid of phosphoenolpyruvate synthetase (pps; Cooper & Kornberg, 1967; Brice & Kornberg, 1967) causes a significant decrease in their rate of growth if the growth substrate is a sugar (such as glucose, fructose or mannitol) whose uptake necessarily involves the activity of the phosphotransferase system. If however the culture is growing on a sugar which is taken up by some other means (such as gluconate, glucose 6-phosphate or lactose) the growth rate is unaffected by the addition of pyruvate. It was found that in strain K2.1t, which is Pps⁻, galactose was in the latter category; neither the growth of the organism on galactose, nor the rate of uptake of this sugar was impaired by the presence

Table 3.1. The effect of pyruvate on the uptake of and growth on different sugars.

Substrate	Addition	* Rate of sugar uptake(nmoles. min^{-1} mg dry $mass^{-1}$)	† Doubling time (min)on 10mM sugar
Galactose	None	1.1	67
	+Pyruvate	1.1	70
Glucose	None	12	60
	+Pyruvate	3	180
Fructose	None	19	80
	+Pyruvate	4.2	210

* The rates of uptake of 0.2mM $[^{14}C]$- galactose and of 0.1mM $[^{14}C]$ - glucose and fructose were measured, in washed suspensions of galactose-grown cells, alone, and after 3 minutes incubation in the presence of 2mM pyruvate.

† Growth rates were measured of cells growing on 10mM sugar either as sole carbon source or in the presence of 5mM pyruvate. In the latter case, the recorded doubling times are those which had been established 90 min after the addition of pyruvate.

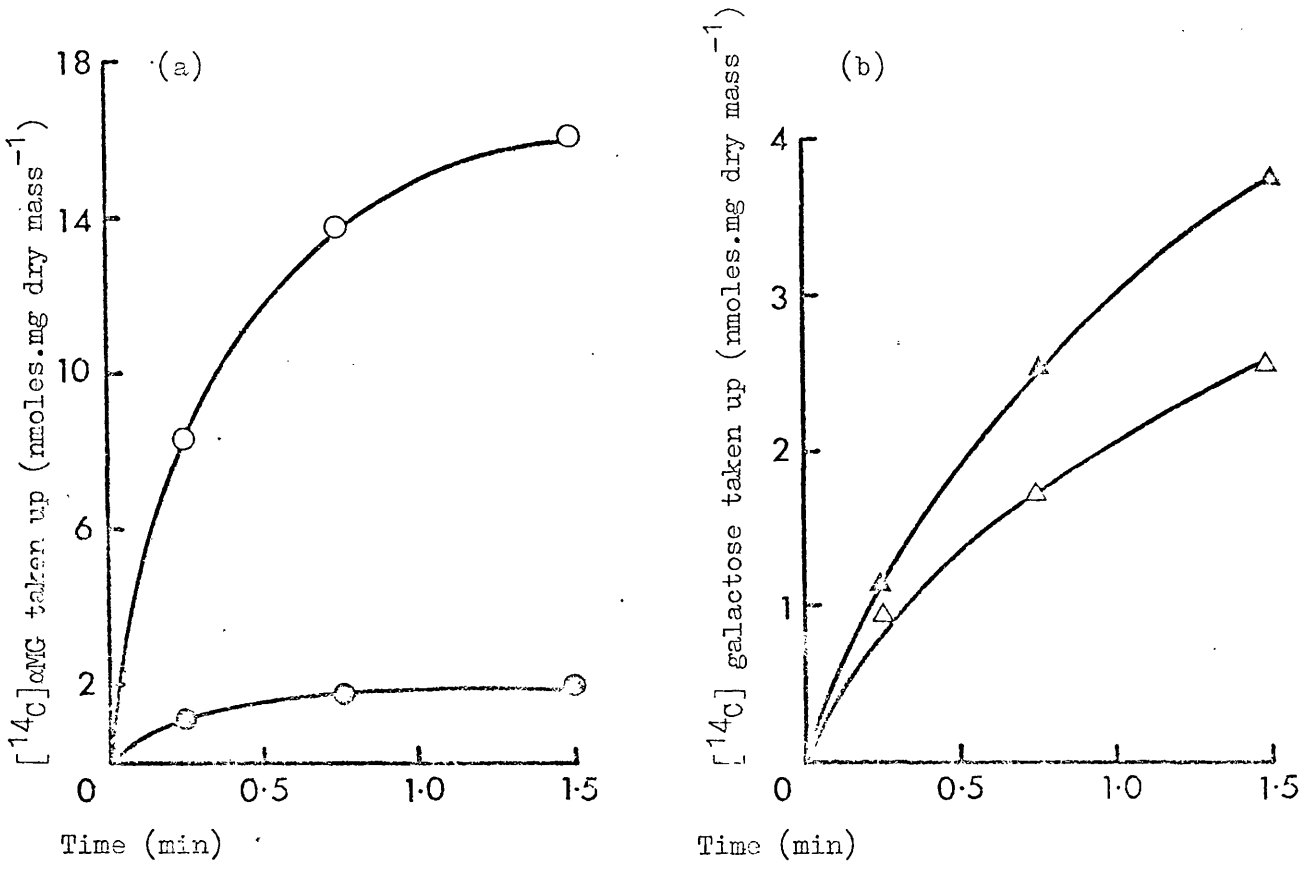


Fig.3.1 The uptake of (a) 0.1mM αMG and of (b) 0.2mM galactose by suspensions of strain CLR 103, grown on galactose at 30°C. The suspensions were incubated with the labelled substrates at 30°C (open symbols) and at 42°C (closed symbols).

of pyruvate (Table 3.1), although both these parameters were adversely affected when the substrate was a PT-sugar.

2. Enzyme II-mediated phosphorylation cannot occur in the absence of Enzyme I, which maintains the supply of phosphate donor, phospho-HPr, so it was of interest to know whether galactose transport on the Umg-system is impaired in mutants devoid of Enzyme I activity. Strain CLR103 is a His^+ recombinant from a genetic cross of the F^- strain K2.1t (Mgl^- $GalP^-$) with the HfrC strain ts19, which is a mutant with a temperature-sensitive phosphotransferase Enzyme I (*ptsI*^{ts}; Bourd *et al.*, 1971). Because this strain, like its Hfr parent, grew on PT-sugars at 30°C but not at 42°C, it must have received the *ptsI*^{ts} lesion from the donor strain; the inability of cell suspensions of the recombinant to take up either 0.5μM galactose or 10μM MeGal showed however that it had retained its Mgl^- character. It was believed also to have remained $GalP^-$, because the rate of uptake at 25°C of 0.2mM galactose by suspensions of this strain, which had been grown on galactose at 30°C, closely resembled that exhibited by galactose-grown cells of its $GalP^-Mgl^-$ parent, strain K2.1t.

Suspensions of strain CLR103 readily take up 0.1mM [^{14}C]-αMG if the cells are grown on galactose at 30°C and subsequently exposed to the labelled substrate at this temperature; they do not, however, take up αMG to a significant extent at 42°C. In contrast, the same cell suspensions take up galactose somewhat more rapidly at 42°C than they do at 30°C (Fig. 3.1). Moreover if cells

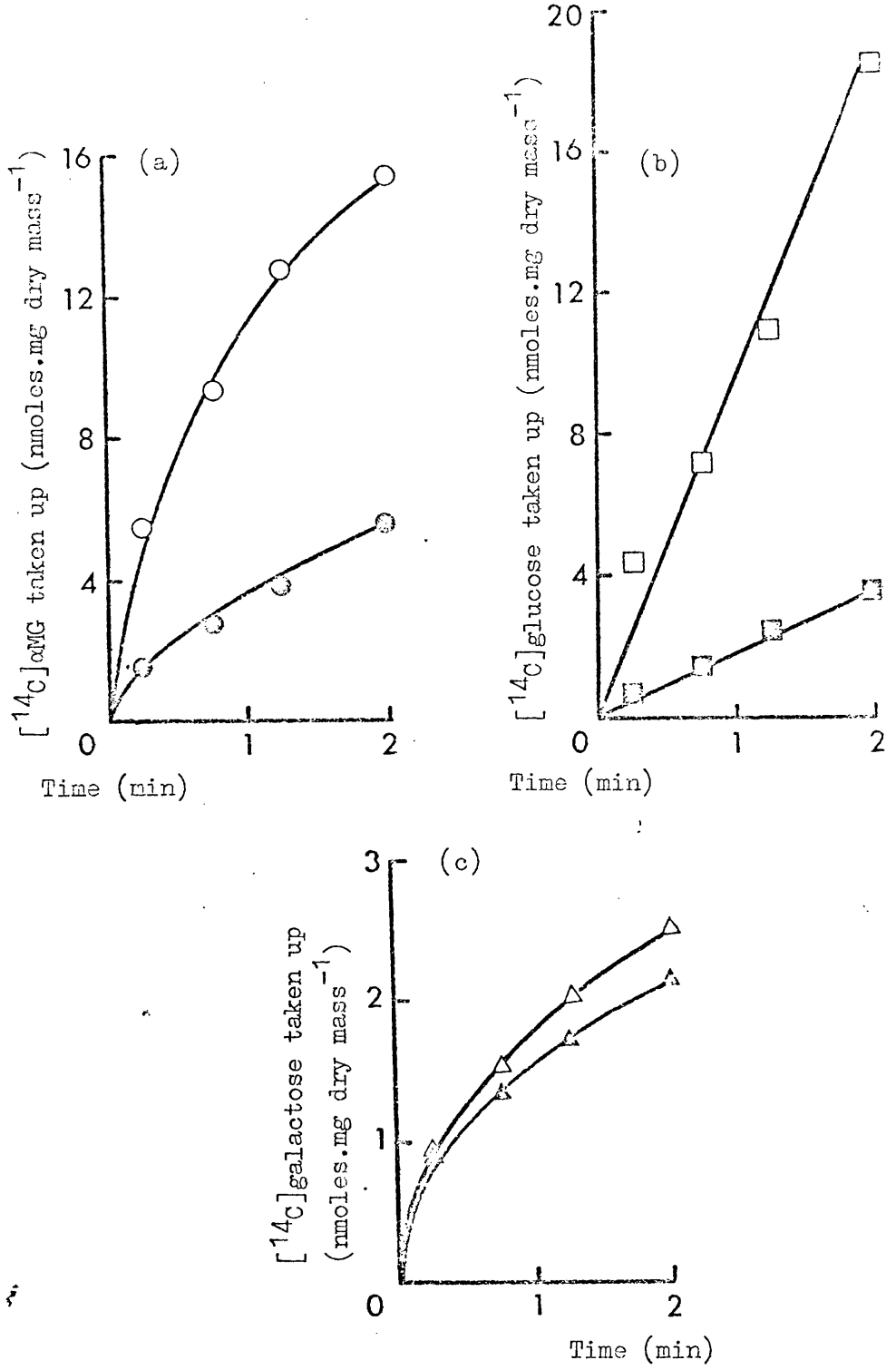


Fig.3.2 The uptake of (a) 0.1mM αMG, (b) 0.1mM glucose, and (c) 0.2mM galactose by washed cell suspensions of strain CLR 103 grown on galactose at 30°C. The measurements were made at 25°C using suspensions which had been incubated at 42°C for 30 min (closed symbols) and control cells which were stored in ice for this length of time (open symbols).

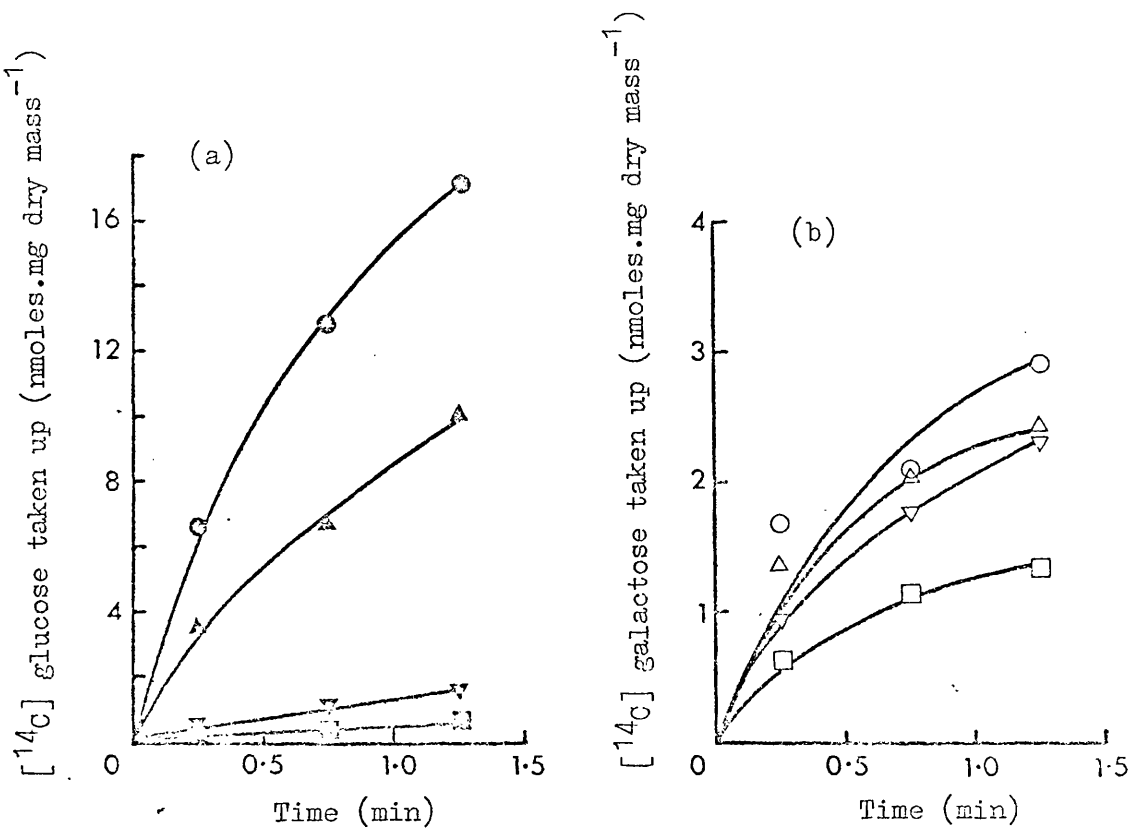


Fig 3.3. The uptake of (a) 0.1mM glucose (closed symbols) and of (b) 0.2mM galactose (open symbols) by suspensions of strain CLR103 grown on galactose initially at 30°C and then transferred to 42°C. Samples were taken (●, ○) immediately, (▲, △) 30 min, (▼,▽) 90 min and (■,□) 120 min after transfer to the higher temperature. The medium with which the cells were washed and in which they were resuspended contained chloramphenicol, 40μg/ml.

of this strain are grown on galactose at 30°C, washed, incubated at 42°C for 30 minutes, and then tested for their ability to take up radioactive substrates at 25°C, galactose again manifests itself as a non-PT sugar. The uptake of the PT-sugars αMG and glucose is severely impaired in consequence of the heat-denaturation of Enzyme I activity by this treatment, whereas that of galactose is little affected (Fig. 3.2). Fig. 3.3 shows that the same phenomenon applies also to cells growing on galactose; a culture of strain CLR103, growing on galactose at 30°C, was transferred to 42°C, and cells, sampled at different times after this transfer, were assayed at 25°C for their ability to take up various sugars. The switch to the restrictive temperature resulted in a rapid loss of uptake activity towards αMG (not shown) and glucose, whereas the galactose uptake system showed a much slower and less dramatic heat denaturation.

3. The experiments with strain CLR103 outlined above show that galactose uptake in this Mgl^- $GalP^-$ strain is apparently independent of the activity of Enzyme I of the phospho-transferase; it might be expected therefore that the organism should grow on this sugar even when Enzyme I is inactivated. However, ambiguous results might have been obtained if this prediction had been tested using the *ptsI*^{ts} strain CLR103, because as has already been mentioned, mutants lacking Enzyme I activity are known to be unable to grow on several sugars, including galactose, whose uptake does not normally involve phosphotransferase activity (Wang & Morse, 1968; Wang *et al.*, 1969; Bourd *et al.*, 1969;

Wang et al., 1970). Since the inability of such mutants to grow on any one particular non-PT sugar can be overcome by mutation to constitutivity for its uptake (Wang et al., 1970) or by the addition to the culture of cyclic AMP or a good inducer of the sugar's uptake system (Pastan & Perlman, 1969; Berman et al., 1970; Kornberg, 1970) it has been suggested that ptsI mutants are extemely susceptible to catabolite inhibition, with the result that the growth substrate fails to induce the enzymes required for its uptake and metabolism (Saier & Roseman, 1972;Roseman, 1975). Saier and Roseman (1971, 1972) described a mutation in S.typhimurium at a locus designated crr which renders ptsI mutants able to grow on non-PT sugars while still failing to grow on PT-sugars. The crr gene was located on the Salmonella genome contiguous to the genes ptsI and ptsH specifying the Enzyme I and HPr components respectively of the phosphotranferase system. Similarly a mutant of E.coli designated ts19-1Δ was isolated from strain ts19 (Jones-Mortimer & Kornberg, 1974a); this mutant,whose properties are similar to those of the S.typhimurium crr mutants, was shown to carry a deletion covering the genes ptsI and crr.

A cross of strain ts19-1Δ with the F^- strain K2.1t (Mgl^- $GalP^-$) yielded recombinants which carried the ptsI crr deletion of the Hfr donor. One of these, designated CLR104, was chosen for detailed study. It was assessed as Mgl^-on account of its failure to take up either 0.5μM galactose or 10 μM MeGal, and was assumed also to be $GalP^-$ because galactose

Table 3.2. Some properties of strain CLR104 (ptsI crrΔ, mgl, galP) and of its parent, strain K2.1t (ptsI$^+$ crr$^+$, mgl, galP

a.

Carbon source for growth	Rate of uptake of 0.2mM, [^{14}C]-galactose (nmoles.min.$^{-1}$ mg dry mass^{-1}) by washed suspensions of	
	CLR104	K2.1t
Galactose (10mM)	0.95	1.05
Glycerol (10mM)/ D-fucose (1mM)	0.5	0.8
Glycerol (20mM)	0.5	0.8

b.

Carbon source for growth	Doubling time (min) of strain	
	CLR104	K2.1t
Fructose (10mM)	ng	72
Glucose (10mM)	ng	57
Galactose (10mM)	120	69
Glycerol (20mM)	90	85
Lactose (5mM)	120	80
Gluconate (10mM)	85	65

ng = no growth

uptake at a concentration of 0.2mM was neither induced by D-fucose, an inducer of galactose permease activity, nor improved relative to that of the GalP$^-$ parent,strain K2.1t (Table 3.2a). The GalP$^-$ phenotype of strain CLR104 was later confirmed when the location of the galP marker was established, and it proved possible to convert this strain to GalP$^+$ by conjugation or transduction with suitable GalP$^+$ donor strains (Chapter 4). Some of the properties of these GalP$^+$ derivatives of strain CLR104 will be discussed later in this Chapter.

Strain CLR104 grows well on galactose despite its inability to grow on PT-sugars such as glucose and fructose. Its growth rate on galactose is somewhat slower than that of its PtsI$^+$ Crr$^+$ parent, strain K2.1t (Table 3.2b), but since the doubling times on other non-PT sugars are also increased to various extents in this mutant, it is unlikely that this reflects a specific effect on galactose utilisation.

4. The ability of Mgl$^-$ GalP$^-$ strains to grow on galactose despite the presence of the ptsI crr deletion and consequent absence of Enzyme I activity was further demonstrated by experiments with strain CLR105. This is a recombinant, derived from a genetic cross between strain ts19-1Δ (ptsI crrΔ) and the GalK$^-$ derivative of strain K2.1t, strain CLR100; its inability to grow on glucose or fructose showed that it had inherited the ptsI crr deletion. The organism was classed as Mgl$^-$ GalP$^-$ when it was shown (Fig. 3.4) that cell suspensions were unable to accumulate galactose to a level above the extracellular concentration, even when the culture

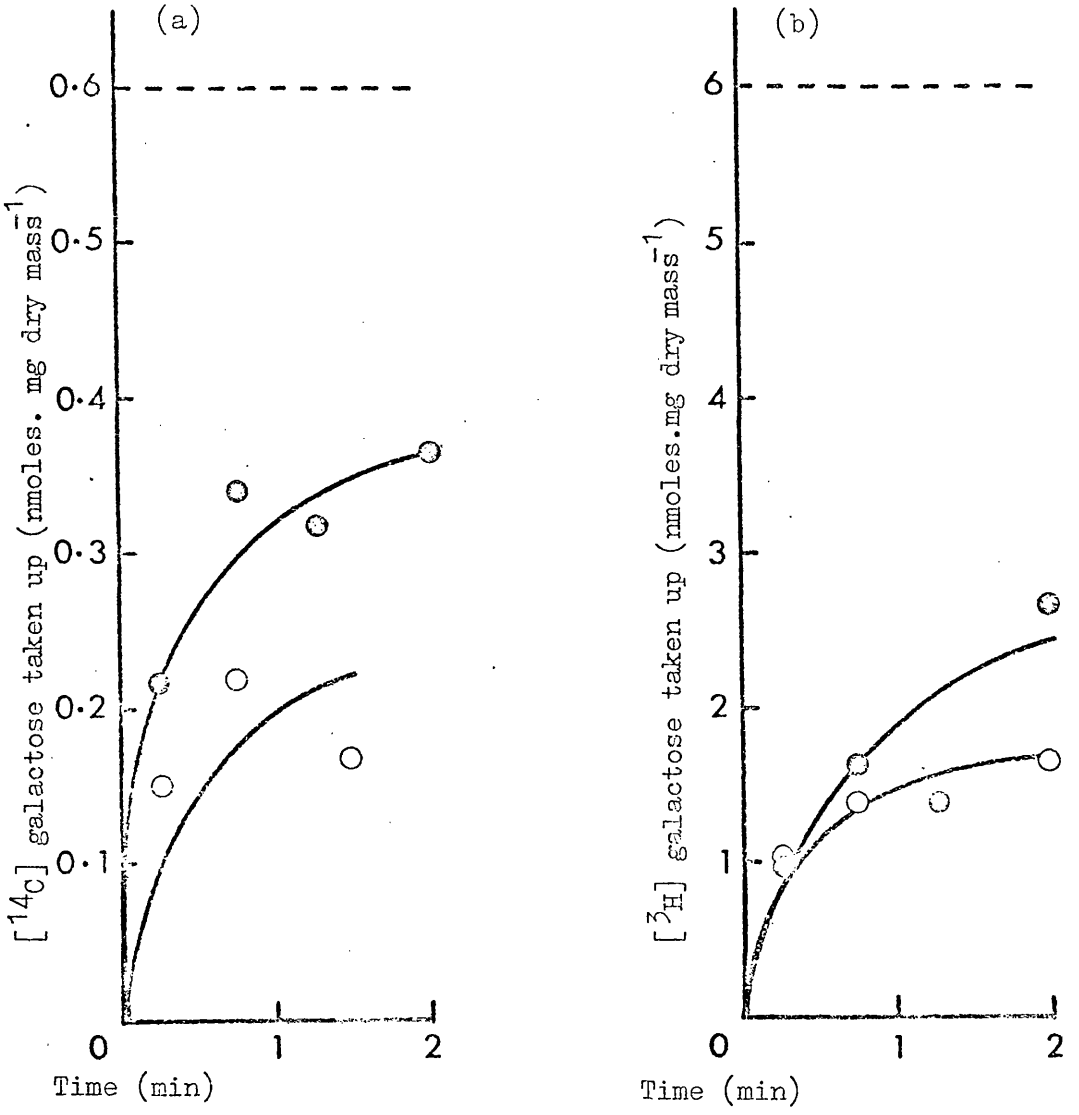


Fig.3.4 The uptake of (a) 0.2mM galactose and (b) 2mM galactose by suspensions of strain CLR 105 (galK, ptsI crr Δ) grown on glycerol (20mM) (o) or glycerol (20mM)/galactose (10mM)(o). The dotted lines represent the level of uptake to be expected if the cells took up the sugar to equal the concentration present in the medium.

had been grown in the presence of 10mM galactose. As was shown in Chapter One, this behaviour is characteristic of mutants which lack, in addition to galactokinase, both MeGal permease and galactose permease activities.

A culture of strain CLR105 was infected with phage P1 which had been propagated on strain K2.1t ($GalK^+$, Mgl^-, $GalP^-$) and samples were plated out on both galactose- and fructose-agar plates. There were equally as many galactose-positive (*galK*$^+$) transductants as there were fructose-positive (*ptsI*$^+$ *crr*$^+$) ones. Since the galactose-positive clones had remained fructose-negative, and those selected as being fructose-positive had retained their galactose-negative phenotype, this provides good evidence that the only lesion preventing strain CLR105 from growing on galactose was the absence of galactokinase activity. When one of the *galK*$^+$ *ptsI crr*Δ transductants, designated CLR111, was grown on 10mM galactose in liquid medium, its doubling time (135 min) was found to be similar to that of strain CLR104 (120 min). This confirms that strains which are Mgl^- $GalP^-$ $PtsI^-$ Crr^- can take up galactose at high concentrations at a rate fast enough to support growth.

The dependence of galactose uptake in Mgl^-GalP^- $PtsI^-$ strains on the Umg-system.

The evidence that $PtsI^-Crr^-$ Mgl^-GalP^- derivatives of strain K2.1t take up galactose by a process involving Umg-activity can be summarised as follows:-

1. Recombinants derived from a cross of strain *ts19-1*Δ with the Umg^- recipient strain K2.1.22a, which carried the

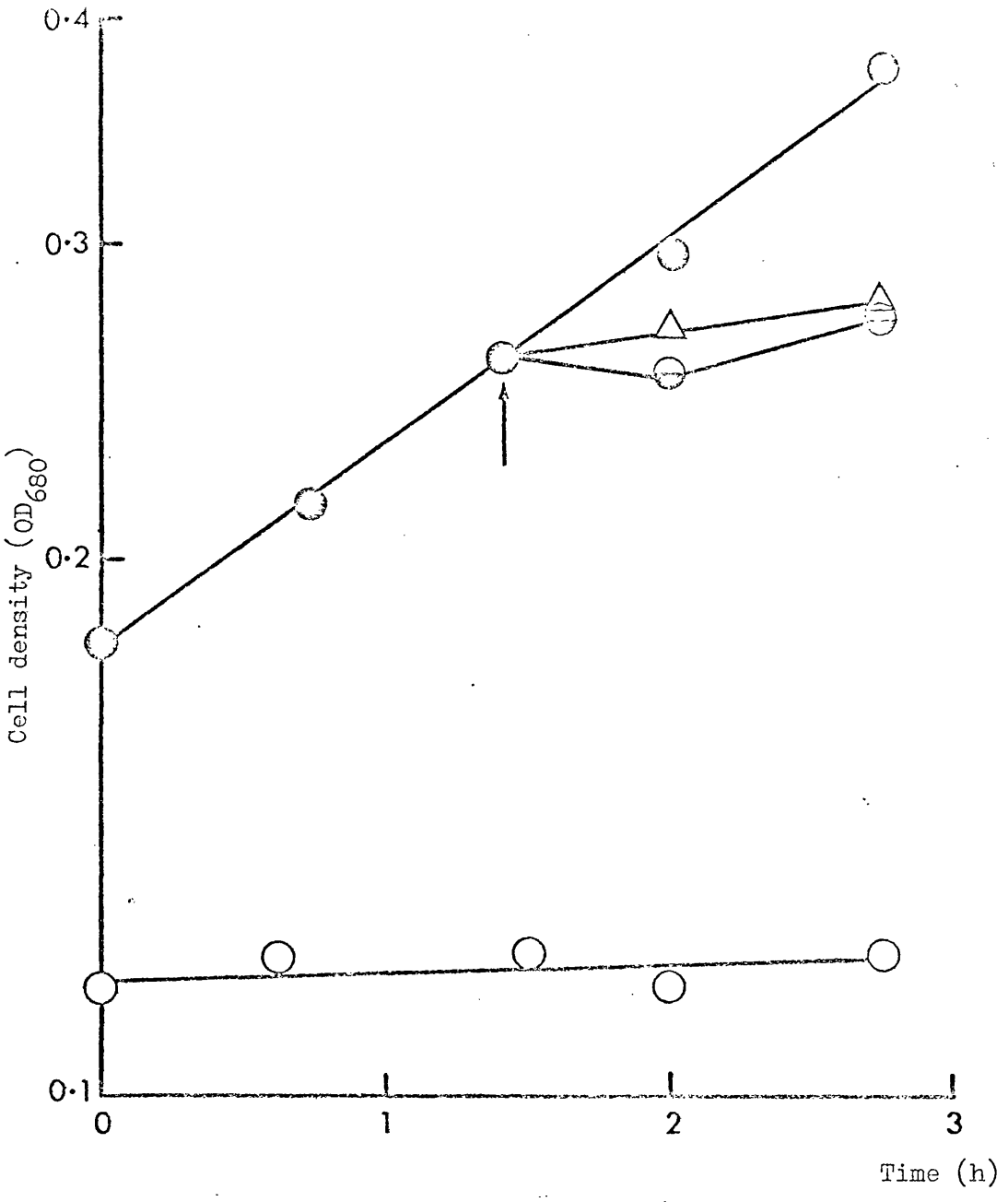


Fig.3.5 The growth of strain CLR104 (galP, mgl, ptsI crr Δ) on 10mM galactose (○), 10mM galactose + 0.5mM glucose (●), 10mM galactose + 0.5mM αMG (△), and 10mM glucose (○). The inhibitors were added to the cultures growing on galactose at the time indicated by the arrow.

ptsI crr deletion and were still Mgl^- $GalP^-$, were galactose-negative, showing no growth on galactose-agar plates after 2 days' incubation at 37°C. The only recombinants derived from this cross which grew on galactose were able to take up 0.5 μM galactose and must therefore have become Mgl^+.

2. The addition of glucose or αMG to cultures of strain CLR104 growing on galactose severely inhibited their growth (Fig. 3.5). Only small quantities of inhibitor were required to mediate this effect; indeed strain CLR104 would not grow on the commercial galactose routinely used in the laboratory, but only on Sigma 'glucose-free' galactose. Since the organism is severely impaired in its ability to take up glucose and αMG, to the extent that it fails to grow on the former compound, this must be a competitive effect taking place on the outside of the cell membrane, and cannot be attributed to catabolite inhibition or repression. The nature of the inhibitory sugars implies that they are competing for a component of the Umg-system. That this was a specific rather than a general phenomenon was shown by the observation that cultures growing on other non-PT sugars such as glycerol, glucose 6-phosphate, ribose, gluconate and lactose were all unaffected by the addition of these compounds. There was one exception, however, in that the doubling time of strain CLR104 on arabinose was, like that on galactose, increased when glucose was added to the growing culture; the possible significance of this will be discussed later. The growth properties of

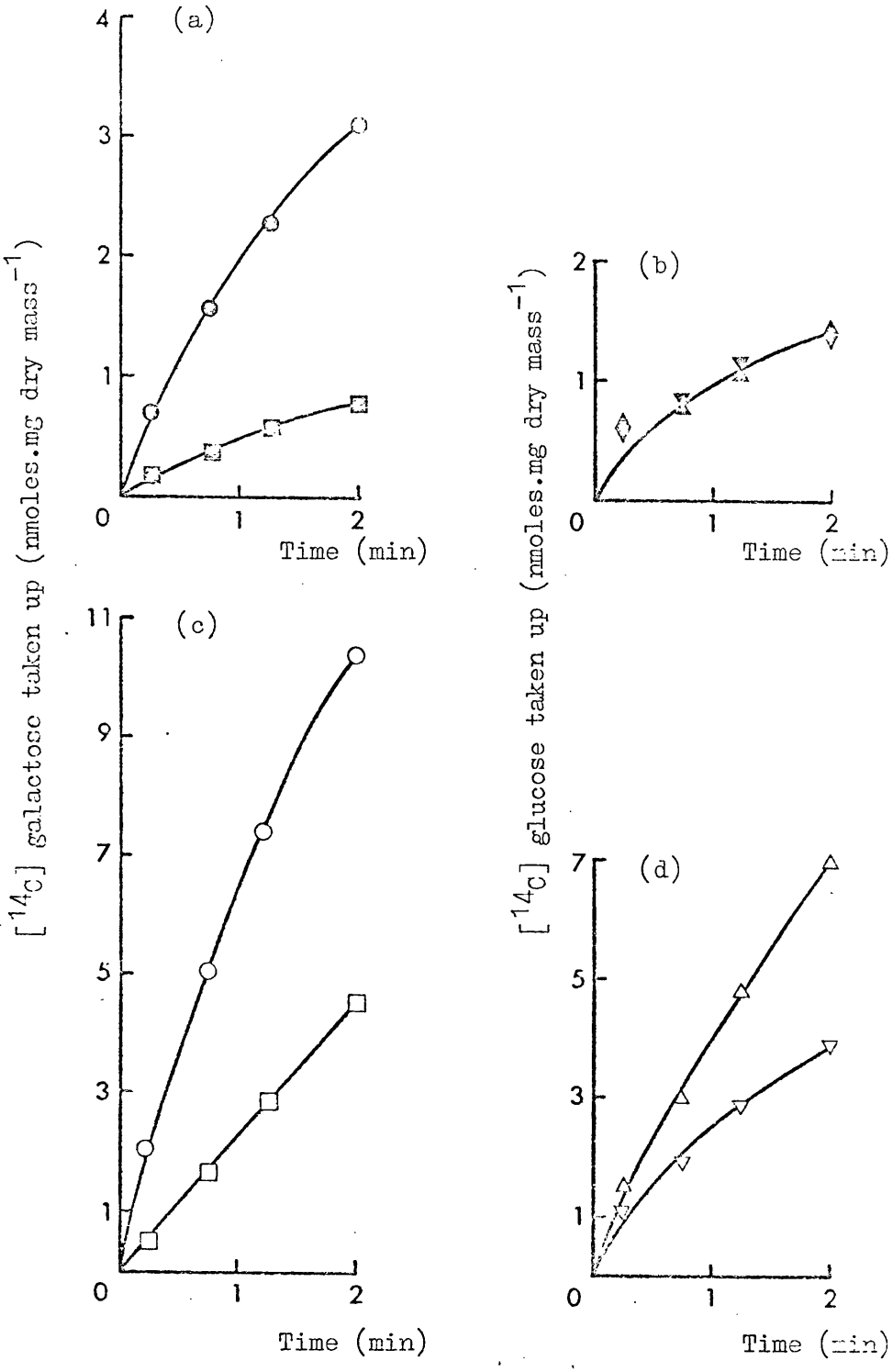


Fig 3.6. Competition for uptake between glucose and galactose in suspensions of strains (a, b) CLR104 (ptsI crr$^{\Delta}$, mgl, galP; closed symbols) and (c, d) CLR108 (ptsI crr$^{\Delta}$, mgl, galP^{+}; open symbols) grown on galactose. The uptake of 0.2mM [^{14}C] galactose was measured alone (●, ○) and in the presence of 0.1mM glucose (■,□), and that of 0.1mM [^{14}C] glucose alone (▲, △) and in the presence of 0.2mM galactose (▼,▽). Inhibitors were added 15 sec. before the isotope.

the GalK$^+$ derivatives of strain CLR105 (GalK$^-$, Mgl$^-$, GalP$^-$, PtsI$^-$, Crr$^-$) were not investigated in such detail, but it was observed that none of these organisms, selected as being galactose-positive on Sigma 'glucose-free' galactose, grew on agar plates containing as carbon source Fison's (glucose-contaminated) galactose.

Competition between glucose and galactose for a common transport protein was also manifested in measurements of the uptake of these sugars by cells of strain CLR104. As shown in Fig. 3.6 (a and b), glucose severely impairs galactose uptake by suspensions of this organism, although its own rate of uptake at the concentration used for inhibition is minimal, and is unaffected by the presence of galactose. This is consistent with competition between the sugars for a carrier to which glucose binds with a higher affinity than galactose, but which, in the absence of Enzyme I activity, cannot effectively mediate the transport of this high-affinity substrate. It contrasts with the behaviour, under the same conditions, of suspensions of strain CLR108, a GalP$^+$ derivative of strain CLR104 (GalP$^-$) which received the *galP*$^+$ allele from strain AT713 by phage P1-mediated transduction. (The procedures used in this transduction and selection for GalP$^+$ are described in Chapter Four). Since glucose can be transported by the galactose permease (see Chapter Four), the two sugars were competing in this GalP$^+$ strain for a system which has a high affinity for galactose as well as for glucose, and the competition could be seen to be reciprocal (Fig. 3.6,c and d). As might be predicted from their

possession of an active transport system for galactose, GalP$^+$ derivatives of strain CLR104 were found to be able to grow on a mixture of glucose and galactose, albeit somewhat less rapidly than on galactose alone.

These competition experiments are particularly important because they argue against an alternative hypothesis which could be put forward to account for the growth on galactose of the ptsI crrΔ mutant CLR104. This strain was shown to lack complete MeGal permease activity, but it could conceivably have received the mglA$^+$ and mglC$^+$ but not mglB$^+$ alleles from its Hfr parent, and hence be able to take up galactose by facilitated diffusion on the resulting defective MeGal permease (Robbins & Rotman, 1975). Kamogawa and Kurahashi(1967) described a PtsI$^-$ mutant, strain W2243A, which appears from its pedigree (Bachmann, 1972), to be Mgl$^+$ GalP$^+$, and whose growth on galactose was inhibited by the addition of glucose; they showed that glucose was taken up by a galactose transport system and subsequently brought about the repression of the synthesis of both the permease itself and the enzymes of galactose metabolism. It could be envisaged that the inhibition by glucose of the growth of strain CLR104 on galactose might be brought about by a mechanism of this sort, if galactose uptake is mediated by the postulated defective MeGal permease. Three of the above results provide particularly strong evidence against this view.

a. Kamogawa and Kurahashi observed only a reduction in growth rate, not a total abolition of growth, even when glucose was added at a concentration of 20mM to a culture

growing on 10mM galactose. In contrast only 0.5mM glucose was sufficient virtually to abolish the growth of strain CLR104 on 10mM galactose.

b. The glucose analogue, αMG, which cannot be taken up by PtsI$^-$ strains, also inhibited the growth on galactose of strain CLR104. This effect was not observed in the PtsI$^-$ strain studied by Kamogawa and Kurahashi, and therefore provides strong evidence for the involvement of the Umg-system in galactose uptake in the K2.1t derivative.

c. The proposed mechanism requires that both galactose and glucose are transported into the cell by the facilitated diffusion system formed by the mglA and mglC gene products. But galactose did not inhibit the limited glucose uptake in strain CLR104, so such a system cannot have been responsible for this uptake.

Galactose transport by facilitated diffusion.

The experiments described above show that strains devoid of MeGal permease and galactose permease activities grow on galactose even in the absence of PEP-dependent phosphotransferase activity, but that this growth appears nevertheless to depend on galactose uptake via the Umg-system and does not occur in Umg$^-$ strains. This implies that the Umg-system (or a component of it) can facilitate the diffusion of galactose into the cells without demanding the input of energy, either for accumulating the sugar against a concentration gradient, or for converting it to a phosphorylated product. Further evidence in support of this view was provided by experiments in which Mgl$^-$ GalP$^-$ GalK$^-$

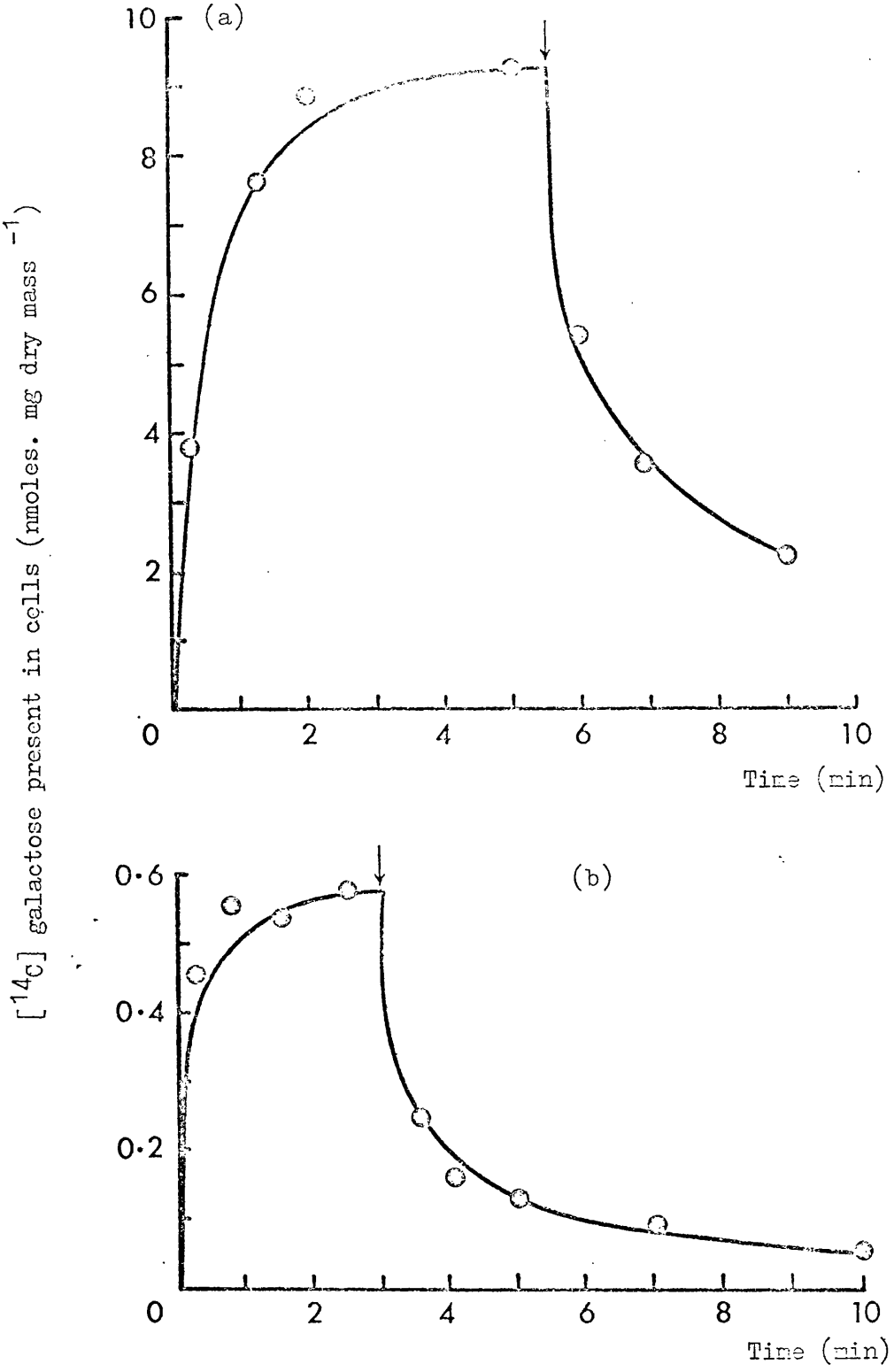


Fig. 3.7. The uptake of [^{14}C] galactose by active transport systems and its displacement from the cells. The uptakes were measured of (a) 0.2mM galactose by a suspension of strain W4345 (GalP$^+$, Mgl$^-$,GalK$^-$) induced with galactose, and (b) 0.5μM galactose by a suspension of strain CLR121 (GalP$^-$, Mgl$^+$, GalK$^-$) induced with D-fucose. At the points indicated by the arrows unlabelled galactose was added to give extracellular concentrations of (a) 20mM and (b) 0.2mM.

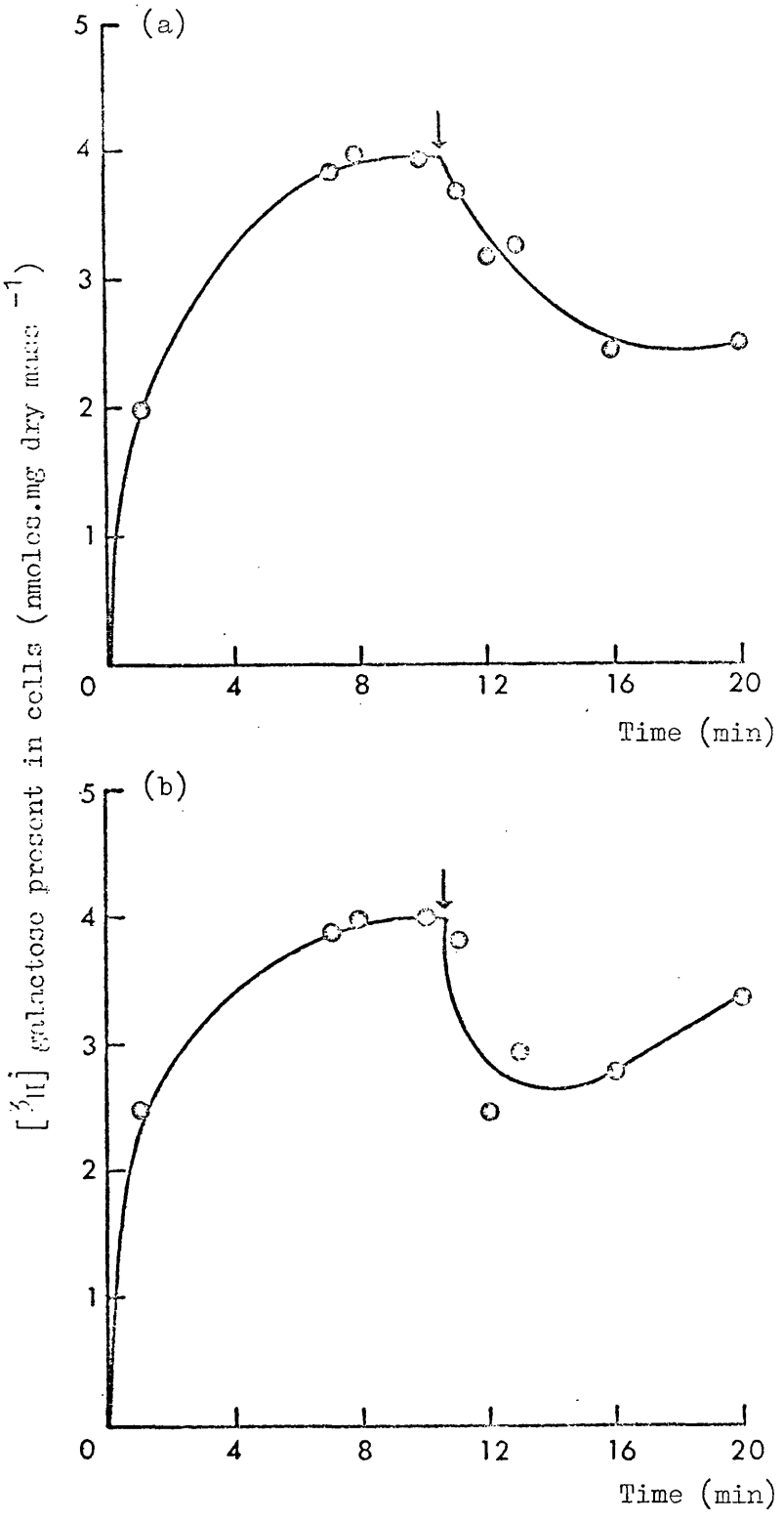


Fig. 3.8. The uptake of 2mM [^{3}H] galactose by suspensions of (a) strain CLR100 (GalP$^-$, Mgl$^-$, GalK$^-$) and (b) strain 20S0K$^-$ (GalP$^-$, Mgl$^-$, GalK$^-$) grown on glycerol. At the points indicated by the arrows unlabelled galactose was added to an extracellular concentration of 50mM.

cells were loaded with radioactive galactose and the kinetics of the loss of labelled material under different conditions was studied.

Horecker et al. (1960) showed that the addition of an excess of unlabelled galactose to a suspension of cells which had actively accumulated radioactive galactose resulted in a rapid and essentially complete displacement of isotopic material from the cells; he attributed this to the fact that the steady state level of galactose accumulated is the result of a balance between uptake from the medium and return of internal galactose to the medium. Other actively-transported sugars such as L-arabinose (Henderson & Kornberg, 1975) and gluconate (Pouysségur et al.,1974) have since been shown to be 'washed out' of cells in a similar way. [^{14}C]-galactose, accumulated by means of galactose permease activity in strain W4345, or by MeGal permease activity in strain CLR121 (a Mgl^+ $GalP^-$ $GalK^-$ recombinant from a cross between strain CLR100 ($GalK^-$ $GalP^-$ Mgl^-) and the Mgl^+ Hfr strain KL16) showed similar patterns of rapid displacement (Fig. 3.7). In contrast, the labelled galactose taken up by strains CLR100 and 20SOK$^-$ ($GalP^-$ Mgl^- $GalK^-$) was lost only slowly from the cells on addition of unlabelled material and did not fall below 60% of its original level (Fig. 3.8). This is to be expected if the uptake mechanism involved is one of facilitated diffusion, because after the initial upset of the equilibrium caused by the addition of excess substrate, the system must tend to return to a state such that both labelled and unlabelled galactose molecules are evenly

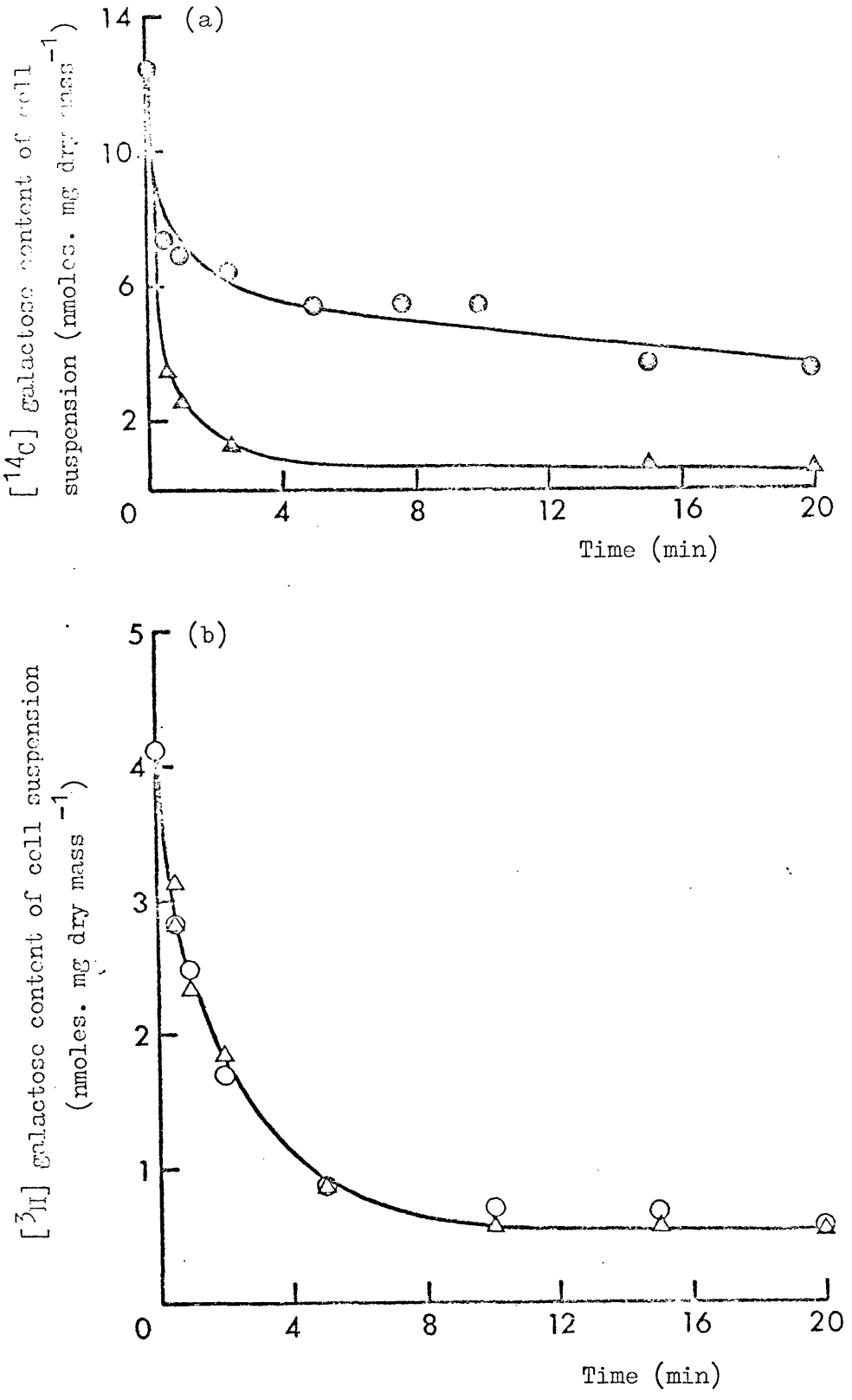


Fig. 3.9. The efflux of labelled galactose from suspensions of strains (a) W2345 ($GalP^+$, $GalK^-$) and (b) CLR100 ($GalP^-$, $GalK^-$). For (a), galactose-induced cells were loaded by incubation with 0.4mM [^{14}C] galactose, and then diluted at 30°C into 10 vol of salts solution (o) or 4mM unlabelled galactose (Δ). For (b), glycerol-grown cells were loaded by incubation with 2mM [^{3}H] galactose and then diluted at 30°C into 100 vol of salts solution (o) or 2mM unlabelled galactose (Δ).

distributed on both sides of the cell membrane. The situation is analogous to the phenomenon of 'exit counterflow' described by Wong and Wilson (1970). Here metabolically poisoned cells are allowed to take up the radioactive substrate of an active transport system, which, under these conditions, can act only as a facilitated diffusion carrier; the addition of excess unlabelled substrate brings about an initial loss of label from the cells followed by a return to approach its original level.

If this is the correct view of the galactose transport mechanism operating in strains CLR100 and 20S0K$^-$, it follows that if cells of these strains are loaded with labelled sugar and then diluted into an excess volume of medium, they will rapidly lose their labelled material until again its concentration inside the cells is roughly equal to that in the medium. Fig. 3.9. shows that this is indeed the case. Cell suspensions of the GalP$^+$ Mgl$^-$strain W4345 and of the GalP$^-$ Mgl$^-$strain CLR100 were allowed to take up labelled galactose to a plateau level; they were then diluted into salts medium alone, and into salts medium containing unlabelled galactose at approximately the concentration present inside the cells at the plateau. The efflux of labelled galactose from the GalP$^+$ strain occurred much more rapidly when the cells were diluted into galactose medium than into medium containing salts only; in contrast the GalP$^-$ cells lost their labelled galactose with equal rapidity whether diluted into medium containing, or not containing, galactose. The GalP$^-$ strain is thus unable to use energy to maintain a galactose concentration gradient;

moreover the virtually total efflux of internal galactose from these cells shows that the labelled sugar had not been phosphorylated during or after its uptake.

It is thus evident that galactose enters E.coli strains that are devoid of MeGal and galactose permease activities on a carrier that is also involved in the uptake of glucose and of its analogue, αMG, but which, contrary to the generally accepted view of sugar uptake by group translocation (Roseman, 1975) does not effect its simultaneous phosphorylation. Rather its mode of action supports the prediction (Tanaka & Lin, 1967) made when the phosphotransferase system had only recently been discovered, that an Enzyme II complex may catalyse the facilitated diffusion of its substrate across the membrane. This implies that two distinct steps are involved in the overall reaction catalysed by the Umg-system; the facilitated passage of the substrate across the membrane, followed by its phosphorylation. After the diffusion step, the high affinity substrates, glucose and αMG, are phosphorylated by this Enzyme II complex; however, although some galactose must be phosphorylated in this way to account for the galactose 6-phosphate isolated by several workers as described in Chapter Two, the major portion of it is acted upon by the enzyme galactokinase.

The properties of mutants with partially defective Umg-activity.

Clearly if the above description represents the true mechanism of action of the Umg-system, then it should be

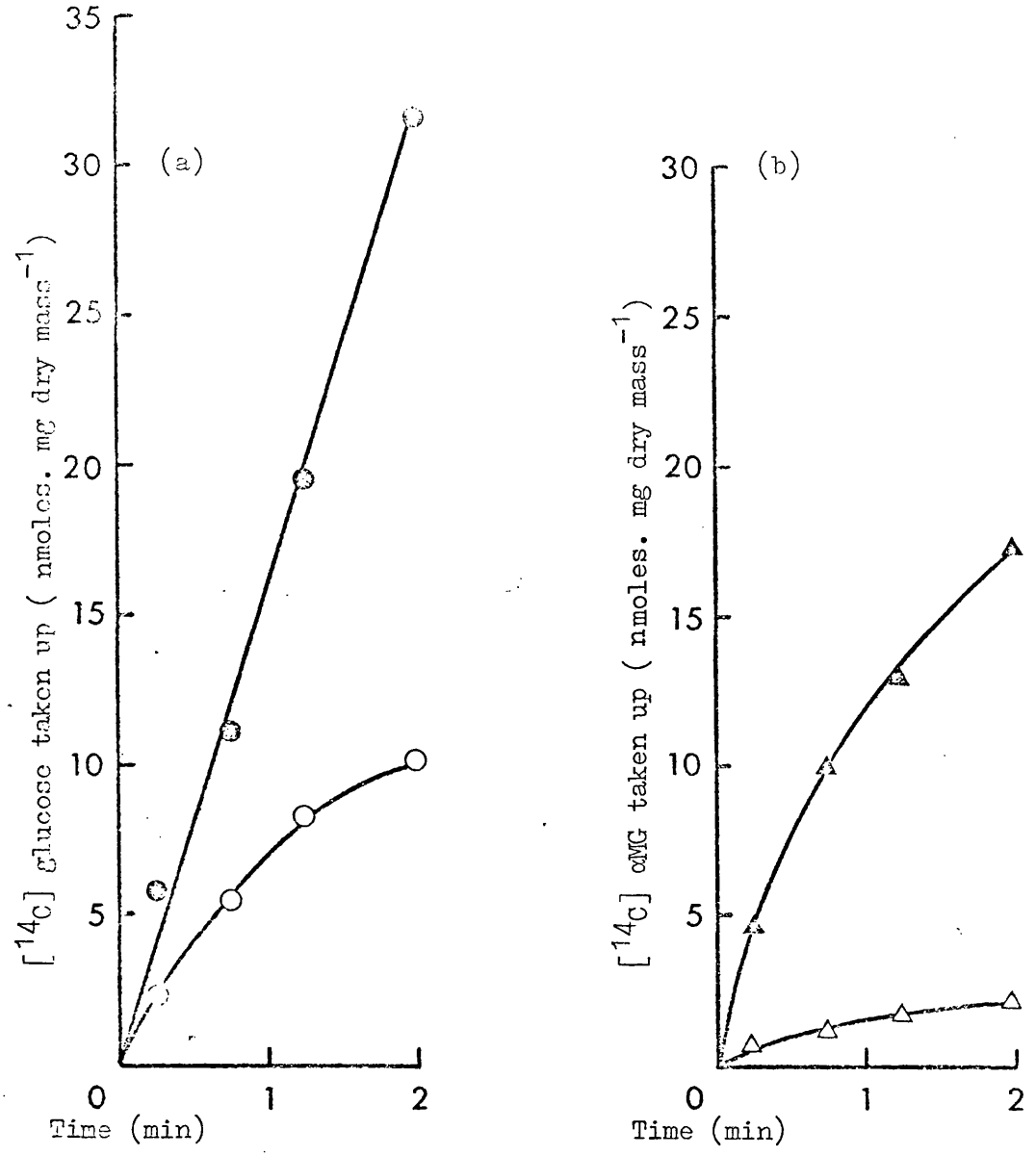


Fig.3.10. The uptake of (a) 0.1mM glucose and (b) 0.1mM αMG by suspensions of strains 2092 (●, ▲) and 2092.F4 (○, △) grown on galactose.

possible to isolate mutants which are defective in only one or other of its component steps, translocation or phosphorylation. The former would be expected to grow poorly on all catabolisable Umg-substrates including (if the strain is Mgl^- $GalP^-$), galactose, whereas the latter would grow well on galactose but not on glucose. The Umg^- mutants so far described are clearly defective in both respects; the transport of the "diffusion" substrate, galactose, is impaired, and in addition phosphotransferase assays in permeabilised cells showed that PEP-dependent phosphorylation of αMG is also severely reduced. However, examination of several mutants which were resistant to DFG during growth on fructose but which were only partially impaired in their ability to take up αMG,(classed as "faint" colonies on the basis of their effect on X-ray film after growth in the presence of [^{14}C]αMG),led to the isolation of one, strain 2092.F4, which appeared to lack only the phosphorylating activity.

Strain 2092, the parent of strain 2092.F4, lacks galactose permease activity but is Mgl^+. It has been shown (Chapter Two) that Umg^- derivatives of strains of this phenotype are commonly impaired in their growth on galactose, and this was no exception; several Umg^- mutants were obtained which grew poorly not only on glucose but also on galactose. Strain 2092.F4, however, which arose from the same starting culture as these Umg^- mutants, differed from them in that despite its impairment in glucose and αMG uptake (Fig.3.10) and consequent poor growth on glucose, it grew well on galactose.

Table 3.3. Growth properties of strain 2092.F4.

Carbon source for growth	*Added glucose analogue	Doubling time (min)
Galactose (10mM)	None	65
"	αMG (2mM)	525
"	DFG (0.2mM)	125
Galactose (5mM)	None	75
"	αMG (2mM)	ng
"	DFG (0.2mM)	180
Fructose (5mM)	None	75
"	αMG (2mM)	75
"	DFG (0.2mM)	75
Glucose (10mM)	None	330

* Glucose analogues were added to the cultures when they had reached the exponential phase of growth on the relevant carbon source.

ng - no detectable growth

Table 3.4. The effect of glucose on the incorporation of fructose and galactose by strains 2092 and 2092.F4.

Carbon Source(s)	Incorporation of labelled material (μmoles.mg increase in cell dry mass^{-1}) by growing cultures of strains	
	2092	2092.F4
10mM [^{14}C]-fructose	4.0	4.0
*10mM [^{14}C]-fructose + 2mM [^{12}C]-glucose	1.2	3.7
10mM [^{14}C]-galactose	4.0	4.0
*10mM [^{14}C]-galactose + 2mM [^{12}C]-glucose	0	0

* The unlabelled glucose was added to the culture when it had already reached the exponential phase of growth on the radioactive carbon source.

This growth on galactose was extremely sensitive to inhibition by glucose analogues which are transported by the Umg-system (Table 3.3). Since these compounds were themselves taken up only slowly by this strain, and indeed failed to bring about the catabolite inhibition of growth on fructose (Table 3.3), this, like the similar inhibitory effect already described for strain CLR104 (MgI^-, $GalP^-$, $PtsI^-$, Crr^-), was assumed to be due to a competition phenomenon taking place on the outside of the cell membrane. This conclusion was supported by the fact that when the galactose concentration in the growth medium was lowered, the inhibition by analogues of glucose became more severe. Experiments in which the incorporation of labelled sugars was followed in the presence and absence of glucose provided more evidence in favour of this hypothesis (Table 3.4). Strain 2092 uses glucose as carbon source in preference to fructose, so that the addition of a small amount of glucose to cells growing on fructose inhibits the incorporation of this sugar into cell material. This effect is not seen in the mutant with impaired glucose uptake, strain 2092.F4; here glucose has little effect on fructose utilisation. In contrast the same concentration of glucose totally abolishes galactose utilisation in both strains, despite the fact that in strain 2092.F4 it is a much poorer growth substrate than galactose (Table 3.3) and consequently brings about a significant reduction in growth rate.

Phosphotransferase assays in permeabilised cells showed (Table 3.5) that strain 2092.F4 was unable to mediate the PEP-dependent phosphorylation of αMG or glucose; in

Table 3.5. Phosphotransferase assays in strain 2092 and its derivatives.

Strain	PEP-dependent phosphorylation (nmoles. min^{-1}.mg dry_1 mass of permeabilised $cells^{-1}$) of	
	Glucose (5mM)	∝MG (5mM)
2092 (Umg^+)	8.1	5.9
2092. W4 (Umg^-)	0	0
2092.F4	0	0

The assays were carried out at 30°C on cells grown on glycerol.

this respect its behaviour was identical to that of the Umg$^-$ strain 2092.W4. It was concluded therefore that strain 2092.F4 is defective in Umg-mediated phosphorylation activity, but has retained the substrate translocation activity of this system. As was the case with strain CLR104, it is possible alternatively to explain some of the observed properties of strain 2092.F4 by postulating a scheme in which galactose uptake is assumed to be mediated solely by the MeGal permease, which takes up and is then repressed by glucose. Again though, a mechanism of this sort cannot account for the inhibition of growth on galactose by glucose analogues such as αMG and DFG which are not taken up by the MeGal permease. Moreover it implies that all mutants derived from strain 2092 which are defective in Umg-activity should behave the same way, since all must be Mgl$^+$; in fact most of them, like strain 2092.W4, grow poorly on galactose and are thus quite different from strain 2092.F4.

The proposed nature of the mutation in strain 2092.F4 is supported also by the recent isolation of a mutant derivative of strain K2.1t, designated 16/3-X1, which has markedly similar properties (H.L.Kornberg, personal communication). This organism was isolated as a mutant resistant to TG during growth on fructose, and it was shown to be defective in Umg-activity measured both as the uptake of αMG or glucose by whole cells, and as the PEP-dependent phosphorylation of these substrates in cells rendered permeable with toluene. The mutant grows poorly on glucose but grows well on galactose, and this growth on galactose

is inhibited by glucose and ∝MG, which are substrates of the Umg-system. Since this strain was derived directly from strain K2.1t, which is known to be Mgl^- $GalP^-$, its properties cannot be due to the activity of the MeGal permease. It appears therefore that mutants of the predicted type, i.e. with defective Umg-dependent phosphorylation but impaired Umg-dependent substrate translocation, do exist, and that strains 2092.F4 and 16/3-X1 are in this category. As far as their growth on galactose is concerned, they are thus phenotypically similar to strain CLR104, in which the Umg-system is intact but unable to catalyse substrate phosphorylation because of the lack of Enzyme I activity.

Discussion.

Roseman (1969) proposed that phosphotransferase Enzymes II might act by bringing about the passage of their substrates across the bacterial membrane, and then phosphorylating them in a subsequent step. However he and his co-authors later abandoned this theory, largely as a result of evidence obtained with S.aureus: mutants devoid of phosphotransferase Enzyme I do not appear to be able to equilibrate sugars in the medium with those in the cells (Simoni & Roseman, 1973). The evidence presented in this chapter supports Roseman's earlier hypothesis by demonstrating that the Umg-system mediates the facilitated diffusion of galactose into the cell; this must carry the implication that other substrates of the Umg-system are dealt with in the same way. It is thus in agreement with the work

of Gachelin (1970), who studied the uptake of glucose and its analogues by a variety of E.coli strains, and concluded that the phosphorylation of αMG is an event subsequent to the passage of the glucose analogue across the membrane by facilitated diffusion. It is interesting to note that Gachelin's results showed galactose to be a competitive inhibitor of the diffusion step, despite its having little affinity for the phosphorylating enzyme.

Mutants devoid of Enzyme I activity, or of Umg-dependent sugar phosphorylation, have been described and shown to have retained their ability to translocate and grow on galactose; mutants that lack entirely the translocating activity of the Umg-system, but retain the ability to mediate PEP-dependent substrate phosphorylation, have not yet been isolated and characterised. Bourd et al. (1974a; 1975) described mutants, designated Tgl⁻, which in some respects appear to be of this type. They are resistant to catabolite repression by glucose, and take up αMG only poorly; extracts of these organisms however show a level of PEP-dependent αMG-phosphorylating activity which is somewhat greater than wild-type. On the basis of this evidence these authors too have proposed that the mechanism of Umg-activity cannot be one of simultaneous uptake and phosphorylation catalysed by a single enzyme, but must be divisible into two discrete steps. It was predicted earlier that mutants devoid of Umg-dependent substrate translocation but active in Umg-dependent phosphorylation should be defective in their ability to grow on glucose.

Contrary to this expectation, the Tgl$^-$ mutants isolated by Bourd *et al.* grow well on glucose, but, as discussed in Chapter Two, this could be due to the presence of another high-affinity glucose Enzyme II in this strain. Since these mutants were derived from an HfrC strain, they are GalP$^+$ Mgl$^+$, so the dysfunction in Umg-activity caused by the *tgl* mutation would not be expected to affect galactose utilisation.

There is evidence however that *tgl* mutants are not devoid of the diffusion carrier of the proposed Umg-transport scheme. A mutant which is resistant to the toxic effect of TG during growth on fructose, and which appears to be Tgl$^-$, has been isolated from strain K2.1t (H.L.Kornberg, personal communication). Like the organisms isolated by Bourd *et al.*, this strain, designated 16/3-X2, exhibits impaired uptake of [^{14}C]-αMG despite the fact that toluene-treated cells, rendered permeable to PEP, phosphorylate both αMG and glucose at wild-type rates. It was shown earlier (Chapter Two) that strain K2.1t possesses only the glucose Enzymes II specified by the genes *umg* and *ptsX*, and that the total abolition of Umg-activity severely impairs this organism's ability to grow on glucose. Therefore if strain 16/3-X2 lacks a component which is essential for Umg-activity towards glucose, its uptake of and growth on glucose should be impaired. But that is not the case; both the uptake of [^{14}C]glucose by this strain and its growth on this hexose are rapid. Similarly the organism grows well on galactose, a property which would not be expected of the postulated 'diffusion carrier' mutant. The precise

nature of the Tgl$^-$ mutants remains to be established, but it appears that the translocation step of Umg-mediated transport is not absent, but might be altered in its specificity towards different substrates.

Since diffusion, facilitated by the carrier component of the Umg-system, appears to be sufficient to support the growth on galactose of Mgl$^-$GalP$^-$ strains that are unable to phosphorylate Umg-substrates, it remains to be explained why such a process cannot support rapid growth on glucose. Glucose uptake by the complete Umg or PtsX system leads to its entry into the cell in the form of glucose 6-phosphate, whereas clearly facilitated diffusion would introduce it as free glucose which must then be phosphorylated by the soluble glucokinase. The poor growth on glucose of strains lacking phosphotransferase activity cannot however be attributed to a low level of glucokinase activity, but is related to the sugar's rate of uptake; as Chapter Four will show, the introduction of galactose permease activity into an Mgl$^-$GalP$^-$ PtsI$^-$ Crr$^-$ strain provides an alternative means of glucose uptake and leads to much improved growth on this hexose. The problem could be simply one of kinetics. Thus glucose, having a high affinity for the Umg carrier, would be rapidly transported not only into the cell, but also out again, whereas the lower affinity substrate, galactose, would not reach a high enough intracellular concentration to cause rapid efflux, and the net inward flow of this substrate would be greater. This apparently paradoxical

situation, in which a substrate saturating at high concentration has a higher overall rate of transport into the cell than one for which the Km is small, has been discussed by Stein (1967). An explanation of this sort is supported by the observation (Asensio et al., 1963) that when a lactose-constitutive Enzyme I mutant is exposed to lactose, the glucose resulting from lactose hydrolysis rapidly appears in the medium, but is utilised very slowly even when all the galactose has been used. This suggests that there is a carrier which can transport glucose out of the cell, but cannot mediate its net transport back in at a rate fast enough to support rapid growth.

The proposed mechanism of action of the Umg-system might also apply to other PT-systems. No attempt has been made to investigate this, but the finding (Solomon et al., 1973) that the rapid efflux of mannitol from ptsI mutants of E.coli occurs only if the Enzyme II for that sugar is present, implies that at least one other Enzyme II complex can catalyse the facilitated diffusion of its substrate across the membrane.

Having established that Umg^+ Mgl^- $GalP^-$ strains take up galactose by means of facilitated diffusion on the Umg-system, the question remains unanswered how the sugar is transported into Umg^- strains, which still grow, albeit slowly, on galactose. Since, in general, glucose uptake in Umg^- strains is mediated by the PtsX-system, it was tempting to suppose that the same might apply to galactose. However, it was shown that Umg^- $PtsX^+$

strains which carry the ptsI crr deletion fail totally to grow on galactose, implying that if the PtsX-system does mediate galactose transport, the mechanism is not one of diffusion, but depends on phosphotransferase activity. This would raise again the problems concerning the nature and metabolism of the galactose phosphate product. An alternative hypothesis, for which there is some supporting evidence, is that Umg$^-$ Mgl$^-$ GalP$^-$ strains make use of the basal level of an arabinose uptake system to bring about galactose transport. Arabinose uptake in strain K2.1t does not depend on Umg-activity; strains K2.1t (Umg$^+$) and K2.1.22a (Umg$^-$) grow at similar rates on this sugar (Table 2.2). However, when the ptsI crr deletion is introduced, it appears that this is no longer the case; not only is the growth on arabinose of the Umg$^+$ strain CLR104 (ptsI crrΔ) inhibited by substrates of the Umg-system, but it was also observed that the growth rate of this strain on arabinose in the absence of inhibitor was significantly faster (doubling time 80 min) than that of the Umg$^-$ PtsI$^-$ Crr$^-$ strain CLR106 (doubling time 210 min). This implies that one or both of the arabinose uptake systems is impaired by the introduction of the ptsI crr deletion so that this sugar, a structural analogue of galactose, now also depends to some extent on the Umg-system for uptake. No attempt has been made to identify the arabinose carrier(s) involved or to explain why it should be so affected; however if, in the Umg$^-$ PtsI$^+$Crr$^+$ strain K2.1.22a, the basal level of this transport system

mediates galactose uptake, then the failure to grow on galactose of strain CLR106, in which this system is impaired, can readily be understood.

CHAPTER 4. - THE CHROMOSOMAL LOCATION OF THE GENE *galP*.

The transport of glucose by the galactose permease.

Various workers have described ptsI mutants of E.coli and S.typhimurium which can grow on galactose (Asensio et al., 1963; Kamogawa & Kurahashi,1967; Simoni et al., 1967). They found that under conditions in which the enzymes for galactose uptake and metabolism were induced, for example if the cells had been grown on galactose or if D-fucose was included in the growth medium, these organisms could grow on glucose. This demonstrated that at least one of the galactose transport systems could take up glucose at a rate fast enough to support growth, but it was not established precisely which galactose transport system was responsible for this effect. The independent observations of Boos (1969) and Vorisek and Kepes (1972), who found that both the galactose binding protein and the uptake system with which it is associated have a high affinity for glucose, suggested that it was likely to be the MeGal permease. But the properties, described in Chapters Two and Three, of mutants in which the MeGal permease provides the only means of galactose uptake showed that substrate inhibition of this system, at the sugar concentrations normally used for growing cultures, prevents its allowing more than a slow rate of growth, even on its natural substrate, galactose. Experiments, detailed below, using the $GalP^+$ Mgl^- strains W4345 and its derivatives showed that the galactose permease also supports glucose uptake, and hence could be responsible, at least in part, for the growth on glucose of the above-mentioned ptsI strains.

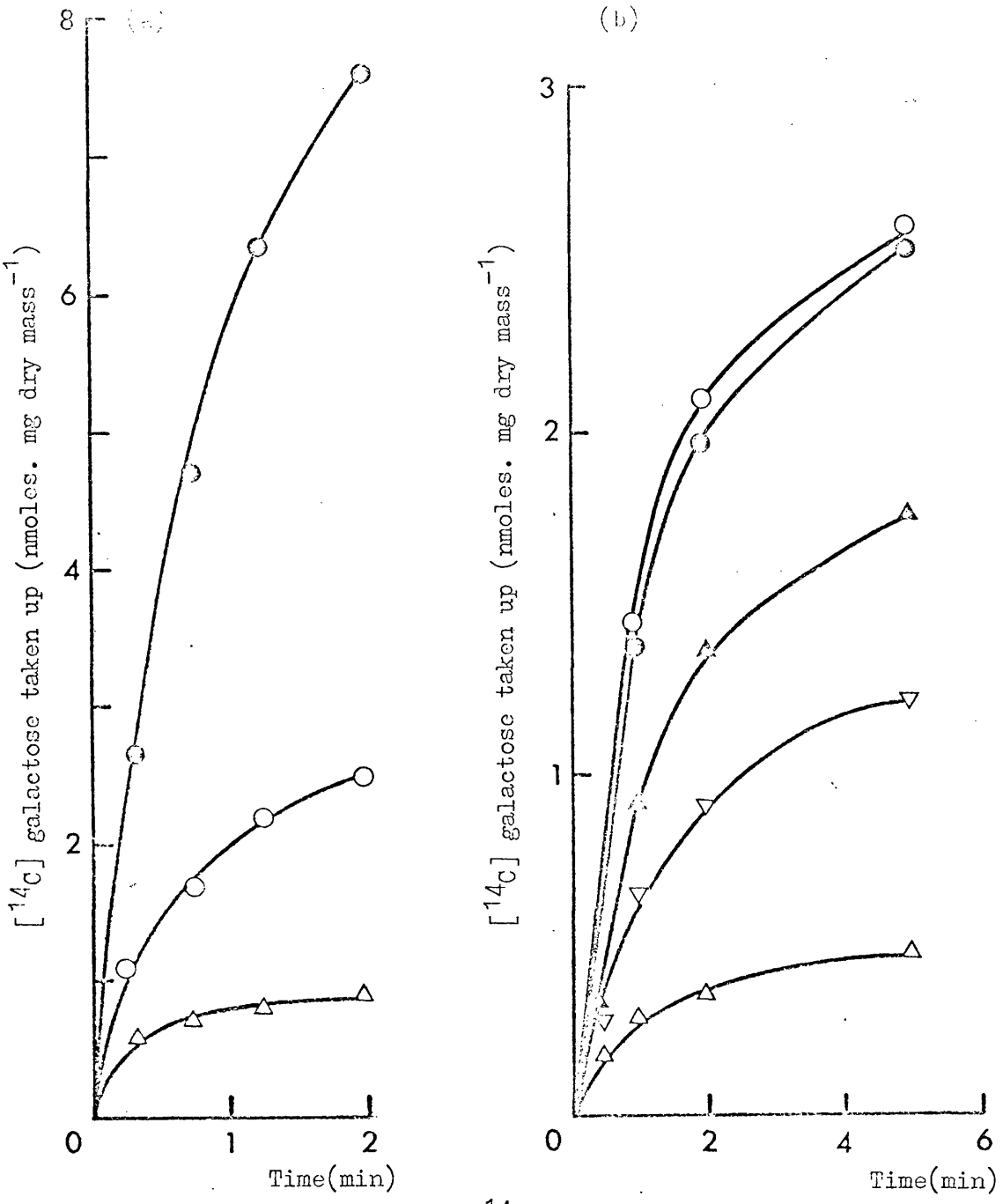


Fig. 4.1. The uptake of 0.2mM [^{14}C] galactose by (a) washed cell suspensions and (b) membrane vesicles prepared from strain W4345 grown on glycerol (50mM)/galactose (2mM). Galactose uptake was measured alone (o) and in the presence of 1mM αMG (o), 0.05mM glucose (Δ), 0.2mM glucose (▽) and 1mM glucose (△). Where membrane vesicles were used,(b), 20mM D-lactate was provided as an energy source.

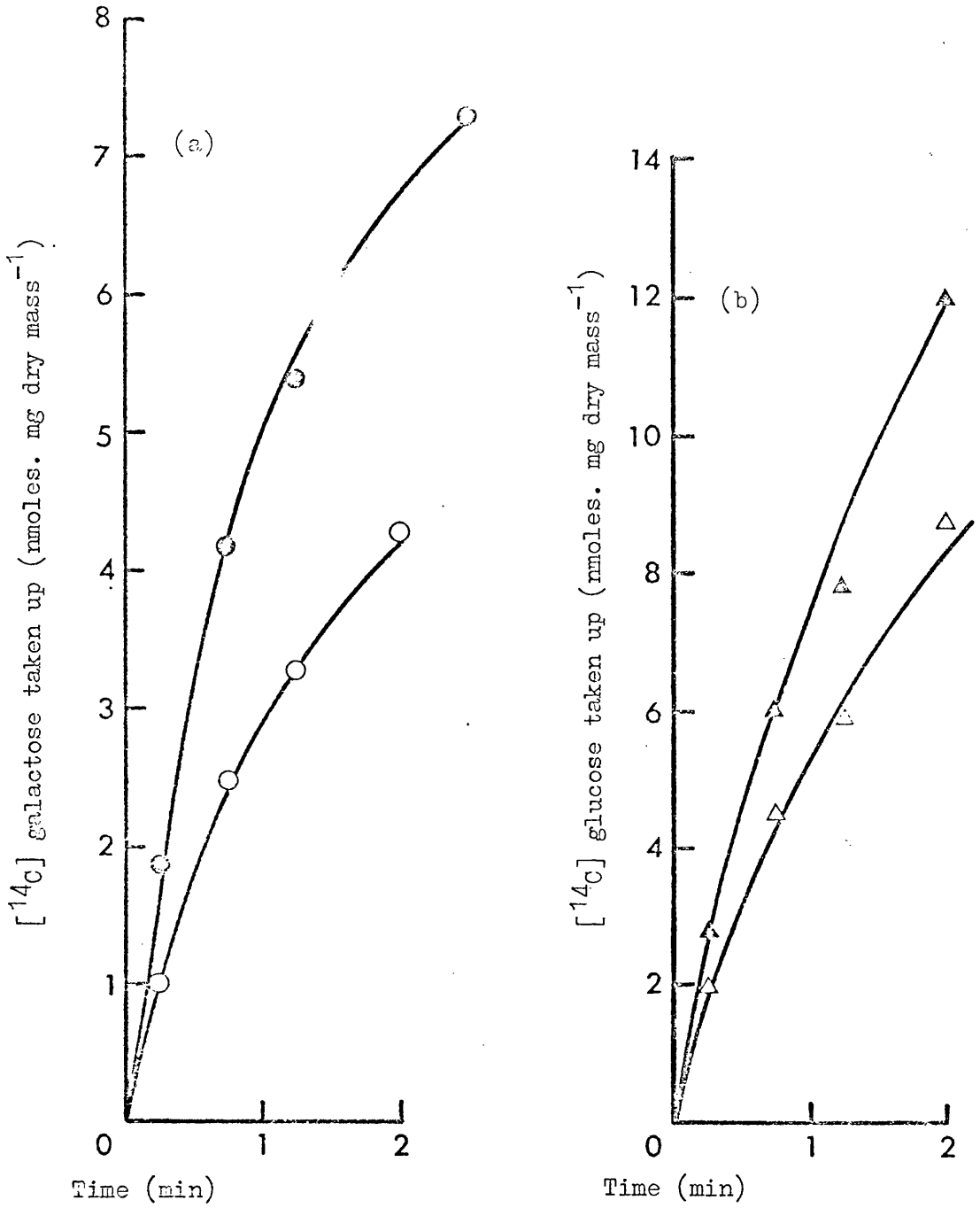


Fig.4.2 The uptake of (a) 0.2mM galactose and (b) 0.1mM glucose by suspensions of strain CLR 17 (Umg^-, $GalK^-$, Mgl^-) grown on 25mM glycerol alone (open symbols) and supplemented with 5mM galactose (solid symbols).

The active uptake of galactose by strain W4345 occurs exclusively by galactose permease activity (Chapter One). Galactose transport in this strain was found to be inhibited by glucose (Fig. 4.1). This was true even when the uptake was measured in membrane vesicles, in which the intracellular enzymes of glucose metabolism are absent, and glucose is not taken up without the addition of PEP (Kaback, 1968); under these conditions catabolite inhibition of galactose permease activity by a product of glucose metabolism was impossible. Moreover the Umg-substrate, ∝MG, which can mediate catabolite inhibition equally as well as glucose in whole cells, did not inhibit the uptake of galactose by these membrane vesicles (Fig. 4.1). These observations suggested that glucose was bringing about inhibition of galactose uptake by competing for a site on the galactose permease. Studies with the Umg^- derivative of strain W4345, strain CLR17, in which the major phosphotransferase-mediated route of glucose uptake has been abolished, supported this conclusion. Glucose uptake in this strain was induced under conditions which led to galactose permease induction (Fig. 4.2), although the uptake of 0.1mM ∝MG was minimal (less than 0.1 nmole. min^{-1}. mg dry $mass^{-1}$), even after growth on galactose. Galactose uptake was still sensitive to inhibition by glucose despite the failure of ∝MG to display any inhibitory effect. Finally it was observed that the uptake of glucose, in cells

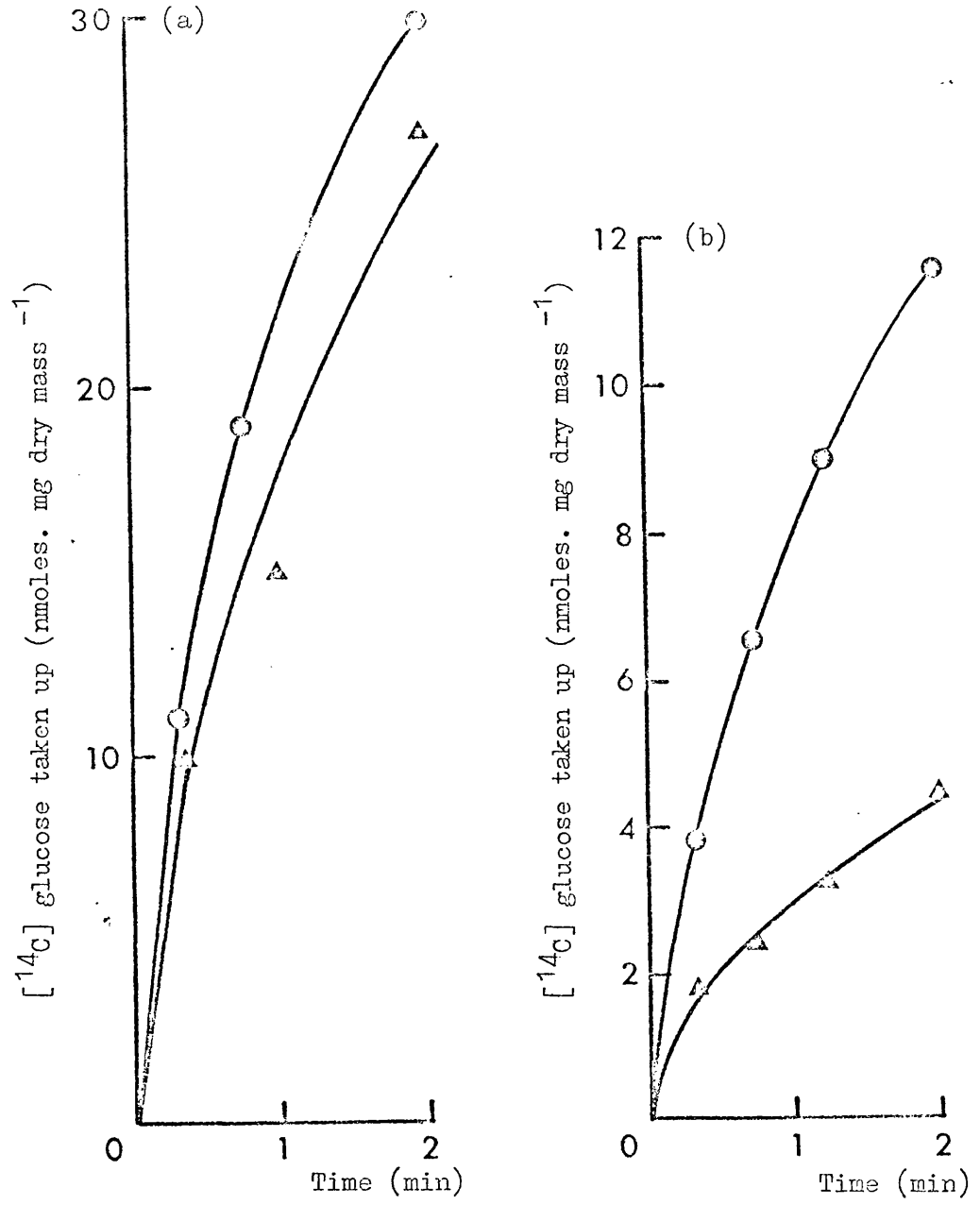


Fig. 4.3. The inhibition of glucose uptake by galactose in suspensions of (a) strain W4345 (Umg^+) and (b) strain CLR17 (Umg^-), grown on glycerol (50mM)/galactose (2mM). The uptake of 0.1mM [^{14}C] glucose was measured alone (○) and in the presence of 1mM galactose (△).

induced for galactose permease activity, was now inhibited by galactose at a concentration which had no significant effect on uptake in the Umg$^+$ parent strain (Fig. 4.3). This finding that the E.coli galactose permease recognises and transports glucose supports the similar conclusion which was reached for the S.typhimurium galactose permease by Saier et al., (1973). These workers isolated ptsI mutants of this organism which had regained the ability to grow on glucose, and found that they exhibited constitutive galactose uptake by virtue of a mutation in the regulatory gene, galR, that controls galactose permease, but not MeGal permease, activity. The galactose permease was therefore held to be responsible for the glucose uptake in these mutants.

A screening procedure for GalP$^+$ strains.

A method was devised in which the ability of the galactose permease to mediate glucose uptake was used in screening for GalP$^+$ strains. Strain CLR104 (GalP$^-$ Mgl$^-$) fails to grow on glucose because it carries the ptsI crr deletion and cannot therefore take up glucose by phospho-transferase activity. D-fucose has been shown to induce galactose permease activity in GalP$^+$ strains (Chapter One) and yet cannot be metabolised by E.coli. Accordingly, strain CLR104 was crossed with a GalP$^+$ Hfr donor strain, and the question asked whether galP$^+$, ptsI crrΔ recombinants could be distinguished from those which had retained the galP allele of their F$^-$ parent, by their ability to grow on a mixture of glucose and D-fucose. Clearly PtsI$^+$ recombinants

Table 4.1. Galactose uptake properties of strain CLR104 and some GalP$^+$ recombinants.

Strain	Rate of uptake of 0.2mM [^{14}C]-galactose (nmoles. min.$^{-1}$mg dry mass^{-1}) by cells grown on			Rate of uptake of 0.5μM [^{14}C]-galactose (nmoles. min^{-1}.mg dry mass^{-1}) by cells grown on Glycerol/Fucose
	Glycerol	Glycerol/Fucose	Galactose	
CLR104	0.35	0.4	0.75	0
Recombinant 1	0.8	1.8	2.25	0
Recombinant 2	0.75	2.4	2.6	0
Recombinant 3	0.75	1.9	2.25	0
Recombinant 4	0.7	1.45	1.7	0

Recombinents 1-4 were derived from a genetic cross of strain CLR104 (*galP mgl ptsI crr*Δ) with the Hfr strain ED1032; unlike the parent strain CLR104 they were able to grow on 10mM glucose supplemented with 1mM D-fucose although, like CLR104, they did not grow on glucose in the absence of D-fucose.

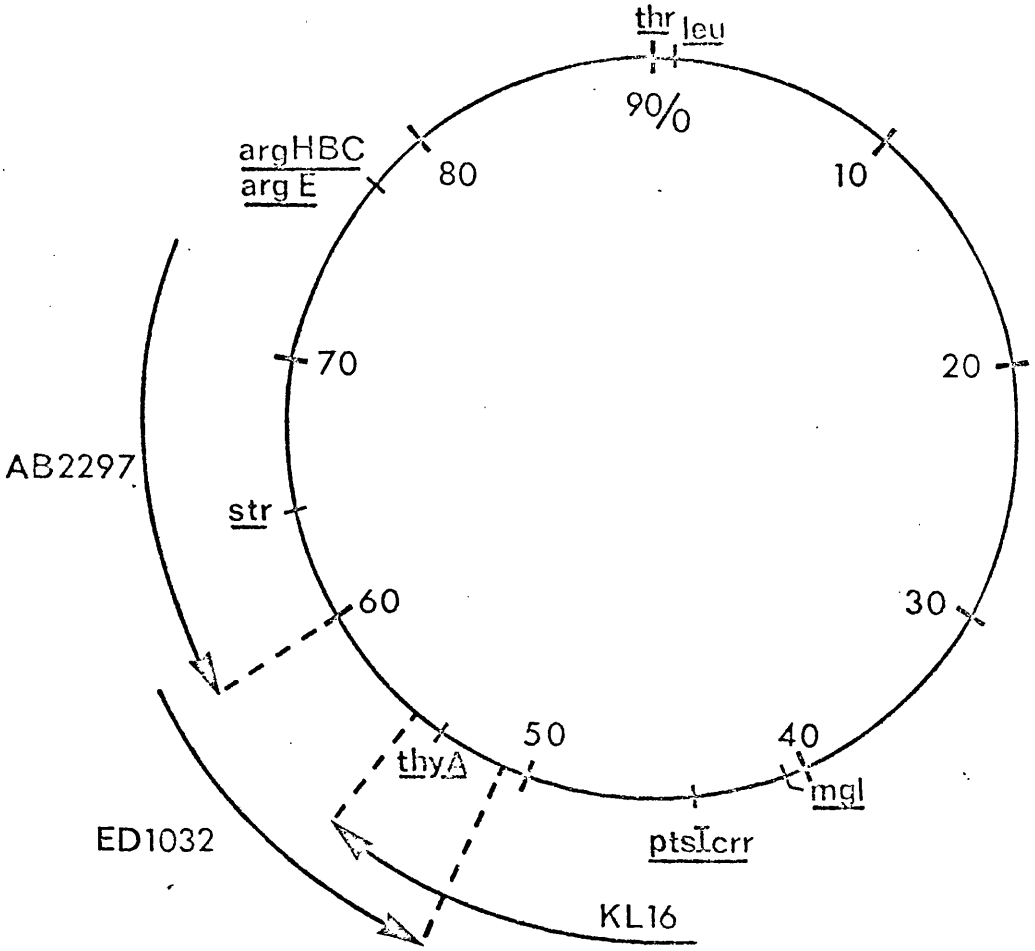


Fig. 4.4. Linkage map of E.coli, showing the location of some of the markers mentioned in this Chapter, and the origins and directions of genome transfer of the Hfr strains used.

would grow on glucose whether they were GalP$^+$ or GalP$^-$, and in addition it was expected that Mgl$^+$ recombinants would show the same phenotype as GalP$^+$ clones in their response to the glucose/fucose mixture. Accordingly, strain ED1032, which transfers mgl and ptsI as late markers (Fig. 4.4) was chosen as the Hfr donor in this test. Arg$^+$ and Thr$^+$ Leu$^+$ recombinants respectively were selected on gluconate medium (growth on which does not involve the phosphotransferase system) supplemented with streptomycin to select against the Hfr strain. The recombinants from each selection plate were then screened for their ability to grow on solid medium containing either 10mM glucose alone, or 10mM glucose supplemented with 1mM D-fucose. Strain CLR104 grew on neither glucose medium, but both selections yielded two types of recombinants: parental colonies which grew on neither test medium, and putative GalP$^+$ colonies which, after 3 days' incubation at 37^{o}C, showed no growth on the simple glucose medium and were therefore still PtsI$^-$, but showed good growth on the D-fucose-supplemented medium. Several of the latter type were purified and compared with strain CLR104 for their ability to take up galactose. They all expressed greater galactose uptake activity than their F$^-$ parent, the transport system responsible being induced by both galactose and D-fucose (Table 4.1). Since none of them was able to take up 0.5μM [^{14}C]-galactose, they could not be Mgl$^+$ and consequently were judged to be GalP$^+$; the validity of the screening procedure was thus confirmed.

The success of the experiment outlined above depended

Table 4.2. The effect of phosphotransferase activity on the expression of the galactose permease.

(a) Growth characteristics of strains of different ptsI and galP genotype*

Carbon source for growth	Doubling time(min) of CLR106 (galP,ptsI)	CLR112 ($galP^+$,ptsI)	CLR113 ($galP^+$ $ptsI^+$)
Galactose(10mM)	ng	180	170
Glycerol(20mM)	95	90	100
Fructose(10mM)	ng	ng	100

ng = no growth

(b) Galactose uptake characteristics of strains of different ptsI and galP genotype*

Carbon source for growth	Uptake of 0.2mM $[^{14}C]$-galactose (nmoles. mg dry $mass^{-1}$. min^{-1}) by CLR106 (galP, ptsI)	CLR112 ($galP^+$,ptsI)	CLR113 ($galP^+$,$ptsI^+$)
Galactose(10mM)	nt	2.0	1.8
Glycerol(20mM)	0	1.0	1.0
Glycerol(20mM)/ D-fucose(1mM)	0	nt	nt

nt = not tested

*Strains CLR106, CLR112 and CLR113 all carry mgl and umg mutations.

on the fact that galactose permease activity could be expressed in the PtsI$^-$Crr$^-$ background of the F$^-$ strain used as recipient. Since the galactose permease is an active transport system rather than a phosphotransferase, and the catabolite repression phenomena associated with *ptsI* mutants are absent in this *crr* strain, there was initially no reason to doubt this. However Bourd *et al.* (1974b), comparing galactose uptake by a *galK*, *mgl*, *ptsI*^{ts} strain under conditions where Enzyme I was active or inactivated, provided evidence that the absence of the phosphotransferase Enzyme I severely reduces galactose permease activity. During the course of the present investigation, strains of *E.coli* were constructed which enabled this to be tested in a slightly different way. Strain CLR112 is a recombinant derived from a genetic cross between the Hfr strain ED1032 and the F$^-$ strain CLR106T (*galP*, *mgl*, *umg*, *ptsI crr*Δ, *thyA*). It, like the recombinants described above, was classed as GalP$^+$ on the basis of its ability to grow on glucose supplemented with 1mM D-fucose whilst being unable to grow on glucose alone. This mutant was transduced to PtsI$^+$Crr$^+$ with phage P1 propagated on strain K2.1t, fructose-positive transductants being selected. One of these, designated strain CLR113, was purified and compared with its PtsI$^-$ parent, strain CLR112. These two strains, which are isogenic apart from the region of the chromosome close to and including the *ptsI crr* gene cluster, were found to be virtually identical in terms of their ability to take up and to utilise galactose (Table 4.2), a fact which did not support the observations of

Bourd *et al*. It is conceivable that these workers, who purchased their [^{3}H]-galactose from Amersham, had not removed the traces of contaminating [^{3}H]-glucose before using the isotope; since glucose is a PT-sugar this would account for the dependence on Enzyme I activity which they observed.

The results of the above experiment also showed that strain CLR106T was a suitable *galP* strain for use in mapping experiments. It had an advantage over strain CLR104 in that, being Umg$^-$ as well as PtsI$^-$,Crr$^-$,Mgl$^-$, GalP$^-$, it was, like its Thy$^+$ parent, strain CLR106 (Table 4.2),totally galactose-negative, and early experiments showed that GalP$^+$ derivatives of this strain were positive not only on glucose/fucose medium but also on galactose medium. Since the growth on galactose of such strains was faster than that on glucose plus fucose (colonies easily visible on plates after 48 hours at 37^{o}C compared with 72 hours) the use of this organism and the galactose screening procedure enabled results to be obtained more rapidly.

Location of the *galP* marker between minutes 54.3 and 60 the *E.coli* chromosome.

The experiments already described with strain CLR104 showed that the Hfr strain ED1032, with an origin of genetic transfer at minute 51 (M.C.Jones-Mortimer, unpublished observation), transfers the *galP*$^+$ allele such that it is present in F$^-$ recombinants selected either for their Arg$^+$ or Thr$^+$ Leu$^+$ phenotype. This was a preliminary indication that the *galP* marker is situated between minute 51 and minute 78.5 (the *arg* locus which specifies the functions absent in

Table 4.3. Comparison of the ability of the Hfr strains ED1032 and AB2297 to transfer the galP$^+$ gene in crosses with strain CLR107.

Hfr parent	Selected marker	Total tested	GalP$^+$ Recombinants
AB2297	*arg*$^+$	207	0
AB2297	*thr*$^+$*leu*$^+$	322	0
ED1032	*thy*$^+$	500(approx.)	350(approx.)
ED1032	*arg*$^+$	161	12

Duplicate cultures of strain CLR107 (*galP*,*mgl*,*ptsI crr*Δ, *arg*(HBCE), *thr*, *leu*, *thyA*) were incubated for two hours with cultures of either the Hfr strain AB2297 or the Hfr strain ED1032. Samples were plated out on gluconate medium containing streptomycin and lacking the relevant amino acid or purine supplements. Strain AB2297 carries a lesion in the *ilv* region (min. 75); so that there should be no selection against Ilv$^-$ recombinants, the selection medium for recombinants derived from this strain was supplemented with isoleucine and valine. Recombinants classed as GalP$^+$ were those which failed to grow on glucose alone, but did grow on glucose medium supplemented with 1mM D-fucose.

strain CLR104). In order to obtain more precise information, a *thyA* derivative of strain CLR104, designated CLR107, was prepared by selection for resistance to trimethoprim (see Materials and Methods). This was used as the F^- recipient in crosses with the Hfr donor strains ED1032 and also AB2297, whose origin of transfer is at minute 60 (Fig 4.4). Unlike strain ED1032, strain AB2297 did not confer the Thy^+ or the $GalP^+$ phenotype onto its Arg^+ or Thr^+ Leu^+ progeny; however a large number of the Thy^+ recombinants and several of the Arg^+ recombinants whose Hfr parent was strain ED1032 were $GalP^+$ (Table 4.3). To check that strain AB2297 is itself $GalP^+$, and had not failed to give rise to $GalP^+$ progeny merely because it too carries the *galP* marker, galactose uptake was measured in this strain. Its ability to take up 0.5μM galactose showed it to be Mgl^+, and its rate of uptake of 0.2mM galactose was similar to that of known $GalP^+$ Mgl^+ strains. It was therefore confirmed as being $GalP^+$. The results presented in Table 4.3 are thus consistent with the location of the *galP* gene between minutes 51 and 60, the origins of transfer of the two Hfr strains; in addition they indicate that it might be linked to the *thyA* marker.

The Hfr strain KL16 transfers *thyA*$^+$ as an early marker (Fig.4.4). If then the gene *galP* is situated close to *thyA* on the *E.coli* chromosome, as the previous experiment suggested, it was to be expected that many of the Thy^+ recombinants derived from a cross of strain KL16 with a $GalP^-$ recipient might also be $GalP^+$. The testing of this prediction was complicated by the fact that the *ptsI*$^+$ *crr*$^+$ and *mgl*$^+$ alleles

Table 4.4 The inability of strain KL16 to transfer the *galP*$^+$ gene to its Thy$^+$ progeny

Time of interruption of conjugation	Total Thy$^+$ recombinants tested	*PtsI$^+$ recombinants	†PtsI$^-$ GalP$^+$ recombinants
20 min.	169	1	0
30 min.	293	4	0

Cultures of strains KL16 and CLR106T (*thyA*, *thr*, *leu*, *arg*, *his*, *umg*, *mgl*, *galP*, *ptsI crr*Δ) were mixed, and incubated at 37^{o}C until, at the times indicated, samples were withdrawn and the conjugation interrupted by dilution and vigorous agitation. Thy$^+$ recombinants were selected on gluconate medium containing streptomycin, arginine, threonine, leucine and histidine.

*PtsI$^+$ recombinants were those which grew on galactose, fructose, glucose and glucose/fucose media. It was impossible to tell from these growth characteristics whether they were *galP*$^+$ or *galP*.

†PtsI$^-$ GalP$^+$ recombinants were expected to grow on galactose or glucose/fucose media, but not on fructose or glucose.

which, if present in the recombinants, would interfere with the GalP screening procedure, are also transferred quite early by this Hfr strain (Fig. 4.4). For this reason the incubation mixture was sampled only a short time after mixing the F^- and Hfr strains, and the conjugation was interrupted by dilution and vigorous agitation. It was hoped that in this way the entry of the *ptsI*$^+$ *crr*$^+$ and *mgl*$^+$ genes would be prevented. Strain CLR106T, a *thyA* derivative of the Umg$^-$ GalP$^-$ Mgl$^-$ PtsI$^-$Crr$^-$ strain CLR106, was used as the F^- recipient, so that data from screening the recombinants for growth on galactose medium could be used to supplement the results of the glucose/fucose growth test. As Table 4.4 shows, the only Thy$^+$ recombinants which were able to grow on galactose or on glucose supplemented with fucose were those which had become PtsI$^+$, and were able to grow also on fructose and on glucose alone. This might be taken to indicate that strain KL16 does not transfer *galP*$^+$ as an early marker and that this gene must therefore lie on the far side of the origin of this Hfr strain, i.e. between minutes 56 and 60. But Low (1965) has shown that genes which lie close to the origin of transfer of an Hfr strain are only infrequently expressed in its progeny. This has been attributed to the fact that the recombination event requires a genetic crossover on both sides of the relevant gene; clearly the nearer the gene lies to the end of the inserted segment, the shorter is the length of chromsome available for one of these events to occur. It is conceivable therefore that the *galP*$^+$ gene could be transferred

Table 4.5 The low cotransduction frequency of galP with thyA

Strain on which phage P1 was propagated	Number of Thy^+ transductants obtained and tested	Number of $GalP^+$ transductants
W4345	531	0
"	196	0
AT713	85	1
"	1600(approx.)	4
ED1032	88	0

Strain CLR107 (ptsI crrΔ, galP, mgl, thyA) was infected with phage P1 propagated on different $GalP^+$ strains as indicated, and Thy^+ transductants selected. Those transductants which were classed a $GalP^+$ were able to grow on glucose medium supplemented with 1mM D-fucose, but not on glucose alone or on fructose.

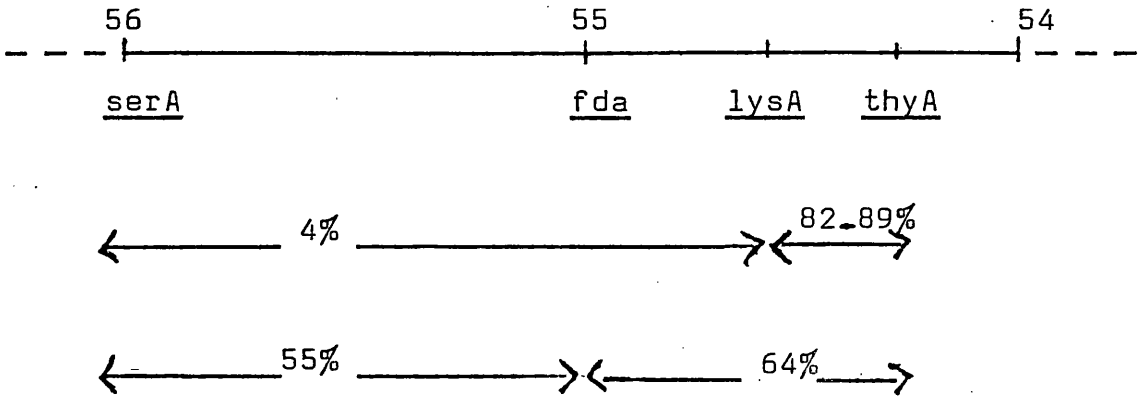


Fig. 4.5. Partial linkage map of E.coli, showing some of the genetic markers which lie between thyA and minute 60, and their cotransduction frequencies as reported by Taylor and Trotter (1967) and Ruffler and Böck (1973).

by strain KL16 before *thyA*$^+$ (min. 54.3) but does not appear in the recombinants for this reason. If it were close to *thyA*$^+$ but transferred by strain KL16 *after* this marker, the above argument would not apply and many of the Thy$^+$ recombinants should have been GalP$^+$. It was therefore tentatively located between minute 54.3 (the *thyA* locus) and minute 60.

Location of the *galP* marker between *fda* and *lysA* on the *E.coli* chromosome.

The linkage between *thyA* and *galP*, which had been suggested by the data of Table 4.3, was investigated further by transduction experiments. Phage P1 was propagated on a variety of GalP$^+$ strains, and used to infect strain CLR107 (*thyA*, *galP*, *mgl*, *ptsI crrA*). Thy$^+$ transductants were selected and screened for their expression of the GalP$^+$ phenotype. As shown in Table 4.5, the markers *thyA* and *galP* were cotransduced with a frequency of less than 1%, an observation which suggested that they are separated by a map distance of 1.5 - 1.8 minutes (Taylor & Trotter, 1967). Fig. 4.5 shows some of the genetic markers which lie between *thyA* and minute 60. Since the gene *galP* was believed to lie in this region, it was planned to introduce one or more of these markers into the *galP mgl ptsI crrA* background of strain CLR107 (Umg$^+$) or strain CLR106T (Umg$^-$) by making use of their cotransduction with *thyA*$^+$. The cotransduction frequencies of *galP*$^+$ with the positive alleles of these markers, some of which must be higher than that with *thyA*$^+$, could then be ascertained. Unfortunately though the transduction experiments aimed at introducing these other markers were largely unsuccessful.

When phage P1 was propagated on strain AT713 (*lysA*) and used to infect strain CLR107 (*thyA*), only 28 of the 85 Thy^+ transductants selected were Lys^-; this cotransduction frequency of 33% compares poorly with the reported cotransduction of these markers of 82 - 89% (Taylor & Trotter, 1967). Moreover it proved impossible to make use of these Lys^- derivatives of strain CLR107 in further investigating the chromsomal location of the gene *galP*; the mutation was unstable so that the organisms reverted readily to the Lys^+ phenotype and quantitative examination of Lys^+ transductants was therefore ruled out.

Strains JM762 and JM803 are both temperature-sensitive for the expression of fructose 1,6-diphosphate aldolase (*fda*^{ts}). Thus at the restrictive temperature of 42°C, the growth of these strains on, or in the presence of, compounds such as glucose, fructose, galactose or glucose 6-phosphate, whose metabolism gives rise to fructose 1,6-diphosphate, is prevented by the accumulation of this now toxic product. Growth on these sugars occurs normally at 30°C, the permissive temperature. The *fda* marker has been reported as being 64% cotransducible with *thyA* (Ruffler & Böck, 1973); however of numerous derivatives of strain CLR106T (*thyA*) which had been transduced to Thy^+ by phage P1 propagated on strains JM762 or JM803 respectively, none carried the *fda*^{ts} lesion. (Strain CLR106T cannot grow on PT-sugars because of its *ptsI crr* deletion; for this reason Thy^+ derivatives of this strain were tested for temperature-sensitivity of the aldolase by

comparing their growth at 30°C and 42°C on the non-PT sugars glucose 6-phosphate or gluconate). The failure to reproduce the published cotransduction frequencies of *lysA* and *fda* with *thyA* suggested that there was severe non-homology in this region of the chromosome between the derivatives of strain K2.1t used as transduction recipients and the strains on which the phage preparations were propagated. It is interesting to note that Josephson and Fraenkel (1969) also encountered difficulties in transduction experiments in this region of the chromosome; they were unable to demonstrate cotransduction of the gene *tkt* with *serA*, *lysA*, *argA* or *cysC*, despite conjugation experiments which clearly pointed to its mapping between minutes 53 and 56.5.

The problems arising from these transduction experiments were overcome by introducing the *fda*^{ts} marker into strain CLR106T not by transduction, but by conjugation. The *fda*^{ts} Hfr donor strain was JM807, a derivative of HfrC, which also carries the *cysI* marker (52.5 min). Thy^{+} recombinants were selected on glycerol medium containing streptomycin, the required amino acid supplements of the recipient, and thiosulphate, which mediates growth of *cysI* mutants. They were screened for growth on glucose and on fructose at 30°C to test for the presence of the *ptsI crr* deletion, and those which had retained it (89%) were further screened for dependence on the presence of thiosulphate (*cysI*), growth on glucose 6-phosphate at 30°C but not at 42°C (*fda*^{ts}), and failure to grow on

galactose at 30°C (galP). Because some of the galactose-positive recombinants were likely to be Mgl$^+$ rather than GalP$^+$, it was not possible to carry out an unambiguous quantitative analysis of the frequency of coinheritance of galP$^+$ with fda^{ts} or cysI. It was observed however that 37% of the Thy$^+$ recombinants had become Cys$^-$, whereas only 14% had received the fda^{ts} lesion, despite the fact that cysI is located further from thyA than is fda. This supported the earlier conclusion that genetic recombination events in the thyA - lysA - fda region of the chromosome are not favoured in strain CLR106T. The required galP, mgl, ptsI crrΔ, fda^{ts}, umg strain, designated CLR130, was isolated and purified.

Strain CLR130 was now infected with phage P1 propagated on the serA strain ME100. Selection at 42°C for fda$^+$ transductants was carried out on glucose 6-phosphate medium because gluconate, the other non-PT sugar which might have been used, was likely to have given ambiguous results. This is because the metabolism of gluconate gives rise to fructose 1,6-diphosphate only when it occurs via the pentose phosphate pathway; mutants which lack 6-phospho-gluconate dehydrogenase (gnd), and hence metabolise gluconate only via the Entner-Doudoroff pathway, are thus insensitive to the fda lesion during growth on gluconate. Selection for growth on gluconate at 42°C would therefore have yielded not only fda$^+$ transductants, but also fda^{ts} gnd secondary mutants. The fda$^+$ transductants were screened for the presence of the galP and serA alleles. The results

Table 4.6 Analysis of the *fda*$^+$ transductants obtained by infecting strain CLR130 (*fda*^{ts} *mgl umg galP ptsI crr*Δ) with phage P1 propagated on strain ME100 (*fda*$^+$ *galP*$^+$ *serA*)

Genotype	Number of transductants
serA galP	10
serA galP$^+$ (donor type)	0
serA$^+$ *galP* (recipient type)	237
serA$^+$ *galP*$^+$	147

Fda$^+$ transductants were selected at 42^{o}C on glucose 6-phosphate medium supplemented with histidine, arginine, threonine, leucine and serine. All were fructose negative (*ptsI crr*Δ). Galactose positive and negative transductants were classed as *galP*$^+$ and *galP* respectively, and failure to grow on gluconate in the absence of serine was taken to indicate the presence in the strain of the *serA* lesion.

Table 4.7 Analysis of the *serA*$^+$ transductants obtained by infecting strain CLR131 (*serA galP umg mgl ptsI crr*Δ) with phage P1 propagated on strain JM803 (*fda*^{ts})

Genotype	Number of transductants
fda^{ts} *galP*	171
fda^{ts} *galP*$^+$ (donor type)	193
fda$^+$ *galP* (recipient type)	503
fda$^+$ *galP*$^+$	25

SerA$^+$ transductants were selected at 37°C on glycerol medium supplemented with histidine, arginine, threonine and leucine. All were fructose-negative (*ptsI crr*Δ). Organisms classed as *galP*$^+$ were galactose-positive at 30°C; *fda*^{ts} organisms were those which grew on gluconate at 30°C but not at 42°C.

(Table 4.6) indicate that the galP marker is about 38% cotransducible with fda, but since the coinheritance of serA with fda$^+$ was only 2.5% whereas Ruffler and Böck (1973) reported a cotransduction frequency of 55% for these two markers, there was clearly still considerable non-homology between the donor and recipient strains. It was therefore not feasible to attempt to calculate the distance in time units between fda and galP from the observed frequency of their joint transduction. The fact that no transductants of the donor type (fda$^+$, serA, galP$^+$) were obtained suggested that perhaps the selected marker, fda$^+$, is located between the other two, so that cotransduction of all three donor alleles would require the insertion of a large chromosome segment.

To test this hypothesis, a similar experiment was carried out, but this time selection was made for the presence of a different marker. The recipient was strain CLR131 (serA, galP, mgl, umg, ptsI crrΔ), one of the serA transductants derived from strain CLR130 in the previous experiment. It was infected with phage P1 propagated on strain JM803 (fda^{ts}) and serA$^+$progeny were selected on glycerol medium lacking serine. The transductants were screened for growth on gluconate and galactose respectively at 30^{o}C and 42^{o}C. Table 4.7, which details the frequencies of the different classes of recombinants, shows clearly that one class (galP$^+$ fda$^+$)was significantly smaller than the other three.Transductants of this class, as discussed by Wu (1966) must therefore be assumed to have resulted from

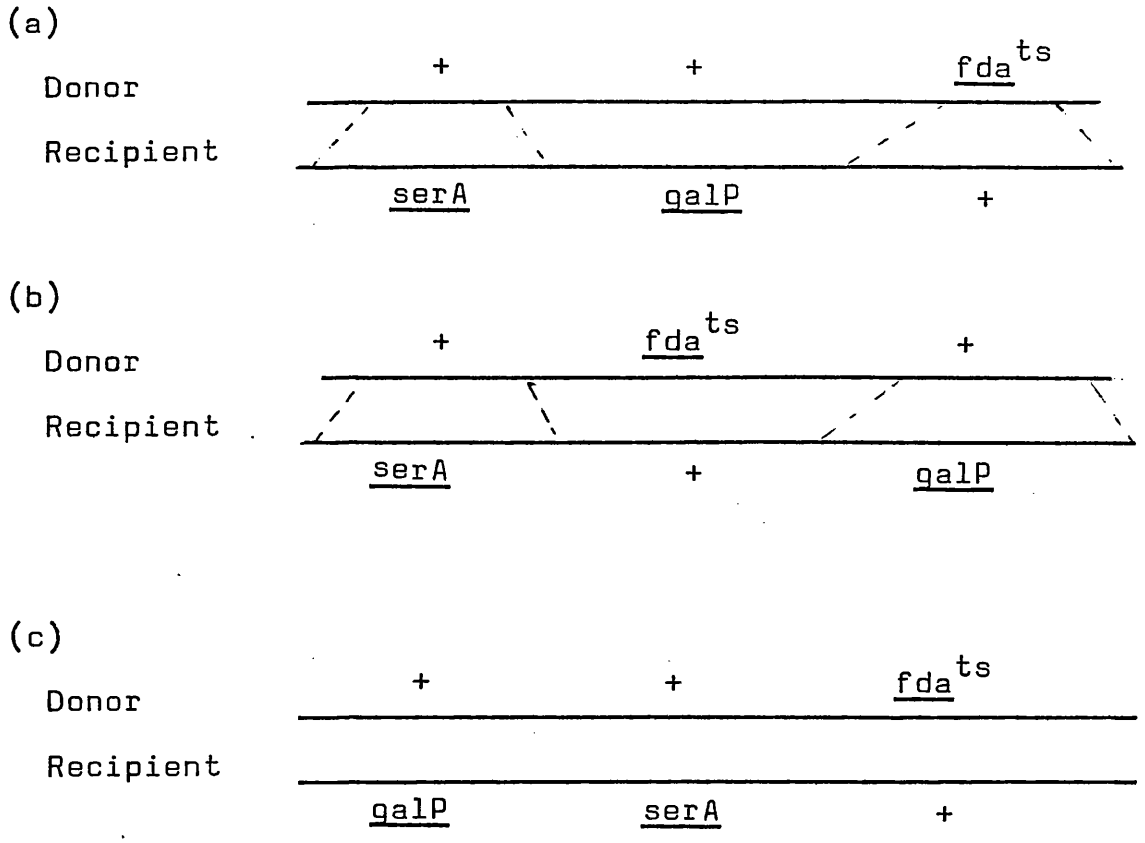


Fig. 4.6. The three possible orders for the genes serA, galP and fda. Strain CLR131 (serA, galP, fda^{+}) was infected with phage P1 grown on strain JM803 (serA^{+}, galP^{+} fda^{ts}) and serA^{+} transductants selected. The diagrams show the alignment of the relevant sections of the donor and recipient genomes, considering each of the three possibilities. Order (a) would require a quadruple crossover to give rise to serA^{+} galP fda^{ts} transductants. Order (b) would require a quadruple crossover to give rise to serA^{+} galP^{+} fda^{+} transductants. Order (c) would not require a quadruple crossover to give rise to any class of serA^{+} transductants.

a quadruple crossover event. As illustrated in Fig. 4.6, the gene order which would require a quadruple crossover to give *serA*$^+$ *galP*$^+$ *fda*$^+$ transductants in this experiment is *serA* - *fda* - *galP*. Experiments have been described which showed that with strain CLR107 as the *galP thyA* recipient and strain AT713 as the *galP*$^+$ *lysA thyA*$^+$ transduction donor, cotransduction of *lysA* with *thyA*$^+$ occurred much more frequently than that of *galP*$^+$ with *thyA*$^+$; the *galP* locus must therefore be further from *thyA* than is *lysA*. This sets the order of the genes on the chromosome as *serA* - *fda* - *galP* - *lysA* - *thyA*.

Transduction of the *galP* allele into a *galP*$^+$ Hfr strain.

The experiments described so far have all been concerned with the introduction of the GalP$^+$ phenotype into derivatives of strain K2.1t. If the conclusions drawn from them are correct, it should be possible to transfer the GalP$^-$ character into a strain that was formerly GalP$^+$, by making use of the cotransduction of the *galP* marker with *fda*$^+$. This was indeed found to be the case. Strain JM759, a derivative of HfrC, has the genotype *fda*^{ts} *gal bio*Δ *ptsF ptsX*. It actively takes up galactose by a combination of galactose permease and MeGal permease activities, and because galactokinase activity is absent it accumulates the sugar in unchanged form. The organism is fructose-negative because it lacks both Enzymes II of the phospho-transferase that effect the uptake of fructose, i.e. those specified by the genes *ptsF* and *ptsX*. Strain JM759 was

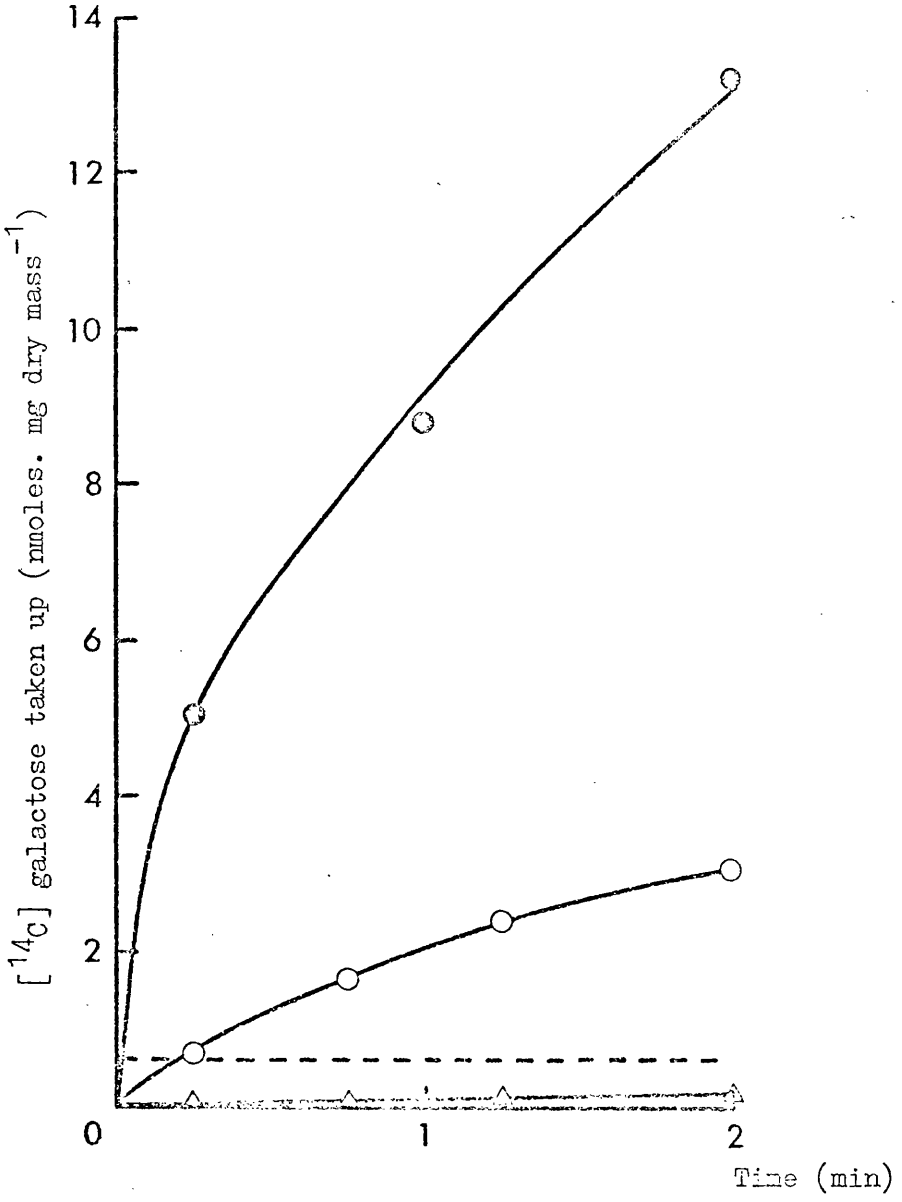


Fig. 4.7 The uptake of 0.2mM galactose by washed cell suspensions of JM 759 ($GalP^+$, Mgl^+, ●) and its derivatives CLR 135 ($GalP^+$, Mgl^-, o) and CLR 136 ($GalP^-$, Mgl^-, Δ). The organisms were grown on glycerol supplemented with 1mM galactose (JM 759) or with 1mM D-fucose (CLR 135 and CLR 136). The dotted line shows the level of uptake which would be attained if the galactose was taken up to an intracellular concentration equal to that in the medium.

infected with phage P1 propagated on strain 20SOK$^-$ (galP, mgl) and fructose-positive transductants selected at 30^{o}C . It was argued that many of these would be ptsF$^+$, and, because mgl is cotransducible with ptsF, there would be some which had also received the Mgl$^-$ character. The transductants were screened for their ability to take up 0.5μM galactose; one which was unable to mediate this transport and was therefore classed as Mgl$^-$, strain CLR133, was isolated and purified. This strain, which could now actively transport galactose only by galactose permease activity, was reinfected with the phage P1 prepared from strain 20SOK$^-$, and fda$^+$ transductants were selected on fructose medium at 42^{o}C. Several of these were tested for their ability to take up 0.2mM galactose, and it transpired that they could be divided into two classes on the basis of their activity in this test. The first class, typified by strain CLR135, took up and accumulated the sugar, but both the rate and extent of uptake were lower than with strain JM759 (Fig. 4.7); they were taken to be GalP$^+$ Mgl$^-$. The second type of transductant, of which strain CLR136 is an example, failed to accumulate galactose at all (Fig. 4.7) and must therefore have become GalP$^-$.

Apparent dominance of the GalP$^-$phenotype.

An F' strain, KLF16/KL110, was available in which the episome covers the area between minutes 53 and 60 (Low, 1972), and hence includes the region containing the galP gene. The complete bacterial chromosome in this

Table 4.8. Comparison of the properties of transductants whose transduction donor was strain AB312 with those for which it was strain 20SOK$^-$ (*galP*) or W4345 (*galP*$^+$)

Donor		Recipient					
Strain	Phenotype	Strain	Phenotype	Selected marker	Number analysed	Number GalP$^+$	Frequency GalP$^+$
AB312	Fda$^+$SerA$^+$	CLR131	SerA$^-$GalP$^-$	*serA*$^+$	290	70	24%
W4345	Fda$^+$SerA$^+$GalP$^+$	"	"	"	439	94	21%
20SOK$^-$	Fda$^+$SerA$^+$GalP$^-$	"	"	"	>1000	0	0%
AB312	Fda$^+$SerA$^+$	CLR132	Fda^{ts}GalP$^+$	*fda*$^+$	284	284	100%
20SOK$^-$	Fda$^+$SerA$^+$GalP$^-$	"	"	"	1216	994	82%

diploid strain is that of the F^- strain KL110 (*thyA*, *leu*, *met*, *argA*, *his*) whereas the episome carries the $thyA^+$ allele. Taking strain CLR106T (*thyA galP mgl umg ptsI crrΔ*) as recipient, and strain KLF16/KL110 as F' donor, diploids, containing the whole chromosome of the former strain and the episome from the latter, were selected as Thy^+ clones on glycerol medium supplemented with threonine, leucine, histidine and arginine; the absence of the methionine required by the donor strain provided an effective selection against it. All the resulting diploids were phenotypically $GalP^-$.

The episome present in strain KLF16/KL110 was derived from the Hfr strain AB312 (Low, 1972). Since phage P1 propagated on strain AB312 could convert strain CLR131 ($SerA^-$, $GalP^-$) to $GalP^+$ by transduction, but could not convert strain CLR132 (Fda^{ts}, $GalP^+$) to $GalP^-$ (Table 4.8), it was assumed that this strain, and hence the episome derived from it, carries the positive allele of the mutant gene. If this is correct it implies that the $GalP^-$ phenotype is dominant over $GalP^+$, since the diploids derived from strain CLR106T must have contained both $galP^+$ and *galP* alleles and yet were phenotypically $GalP^-$.

Discussion.

Saedler *et al.* (1968) showed quite unequivocally that the gene *galR* specifies a repressor protein that mediates the negative control of the expression of the *gal* operon, by showing that deletions of *galR* lead to constitutive

production of the enzymes specified by this operon. The gene is located on the E.coli chromosome at a site close to lysA (min. 54.7; Buttin, 1963b; Saedler et al., 1968). Buttin (1963b) proposed, on the basis of indirect evidence, that the galR product also controls the synthesis of the galactose permease; this was later supported by evidence provided by Saier et al. (1973) and was confirmed by Wilson (1974a). The evidence presented in this chapter has shown that the genetic lesion which prevents the expression of galactose permease activity in strains K2.1t and and 20SOK$^-$, and which has been referred to throughout as galP, is also located near lysA. This could be taken as support for Buttin's (1968) prediction that the regulatory gene,galR,might be closely linked to the structural gene for the galactose permease, galP. Alternatively it could be argued that the lesion in strains K2.1t and 20SOK$^-$ is actually in galR,and that the true structural gene for the galactose permease might be located elsewhere on the chromosome. Clearly a regulatory mutation of the 'super-repressor' (galR^s) type could result in a lack of functional galactose permease activity equally as well as could a structural gene mutation. According to this hypothesis, the mutant regulatory gene would specify a repressor protein with little or no affinity for the inducer, so that the structural gene for the permease would be permanently repressed. The introduction of the wild-type galR$^+$ gene on an episome would do nothing to overcome the repression, and only when the galR^s gene was replaced

on the chromosome by the *galR*$^+$ allele, as in transduction experiments, would the structural gene be transcribed. This was precisely what was observed; GalP$^+$ organisms were obtained by transduction, but not by the introduction of an episome believed to carry the wild-type allele of the mutant gene.

The hypothesis that the lesion which confers the GalP$^-$ phenotype onto strains K2.1t and 20SOK$^-$ is a *galR*^{s} mutation cannot however be readily accepted as correct when other consequences of the presence of such a mutation are considered. As stated earlier, the *galR* regulatory gene was originally discovered and described on the basis of its control of the expression of the *gal* operon, which specifies the intracellular enzymes of galactose metabolism (Buttin, 1963b). Thus *galR*^{s} mutants would be expected not only to be unable to express galactose permease activity, but also to fail to metabolise galactose which has entered the cell by means of a different uptake system; Saedler *et al*. (1968) showed that this is indeed the case. Therefore if strains K2.1t and 20SO (the *galK*$^+$ parent of strain 20SOK$^-$) are *galR*^{s} mutants, their ability to grow on galactose, taken up by facilitated diffusion on the Umg-system, can only be explained if they carry, in addition to this lesion, a mutation at the *gal* operator locus, conferring constitutive expression onto the *gal* operon, and rendering it insensitive to the *gal* repressor. But strain 20SO has been shown quite clearly to be inducible, not constitutive, for *gal* operon expression and yet to be phenotypically GalP$^-$

(Buttin, 1963a). Thus, if the above argument is correct, the lesion which prevents GalP expression in this strain, and hence also in its derivative strain 20SOK$^-$, cannot be a *galR*^{s} mutation; if the strain were *galR*^{s} *galO*$^+$ it would be galactose-negative, and if it were *galR*^{s} *galO*^{c} it would express the *gal* operon constitutively. The lesion seems therefore to be in the structural gene for the permease. Since this *galP* lesion in strain 20SOK$^-$ was shown to be cotransducible with *fda*, the location of the structural gene, originally proposed on the basis of experiments with derivatives of strain K2.1t, is apparently confirmed. Moreover since, as pointed out in Chapter One, strains 20SOK$^-$ and K2.1t have a common ancestry and are likely to carry the same lesions in those genes concerned with galactose uptake, the properties of strain 20SO also imply that strain K2.1t is defective in the structural gene *galP* rather than the regulatory gene *galR*. If this is indeed the case, the failure of *galP*/*galP*$^+$ diploids to express galactose permease activity could perhaps be attributed to their having two copies of the regulatory gene *galR* but only one of the structural gene *galP*; thus there are potentially twice as many repressor molecules controlling transcription from the *galP* gene as in the wild-type strains. Alternatively it is possible that the *galP*$^+$ gene might have been lost from the episome KLF16 on sub-culture so that the diploids studied did not in fact carry this positive allele.

In conclusion it can be stated that the mapping

experiments described in this chapter were successful in so far as they enabled the GalP$^+$ strains, required for other work of relevance to this Thesis, to be constructed. They also provided strong evidence that the structural gene for the galactose permease is located near minute 55 on the chromosome, close to its regulatory gene galR, but direct confirmation that the gene which was mapped was in fact the structural gene and not galR itself was not obtained.

REFERENCES

ASENSIO, C., AVIGAD, G. & HORECKER, B.L. (1963) Archs Biochem. Biophys. 103, 299-309

ASHWORTH, J.M. & KORNBERG, H.L. (1966) Proc. Roy. Soc. Ser.B 165, 179 - 188

BACHMANN, B.J. (1972) Bacteriol. Rev. 36, 525-557

BARNES, E.M. Jr. (1972) Archs Biochem. Biophys. 152, 795-799

BARNES, E.M. Jr. & KABACK, H.R. (1970) Proc. Nat. Acad. Sci. U.S. 66, 1190-1198

BERGER, E.A. (1973) Proc. Nat. Acad. Sci. U.S. 70, 1514-1518

BERGER, E.A. & HEPPEL, L.A. (1974) J.Biol. Chem. 249, 7747-7755

BERMAN, M., ZWAIG, N. & LIN, E.C.C. (1970) Biochem. Biophys. Res. Commun. 38, 272-278

BISSETT, D.L. & ANDERSON, R.L. (1974) J. Bacteriol. 119, 698-704

BOOS, W. (1969) Eur. J.Biochem. 10, 66-73

BOOS, W. (1972) J. Biol. Chem. 247, 5414-5424

BOOS, W. (1974) Annu. Rev. Biochem. 43, 123-146

BOOS, W. & SARVAS, M.O. (1970) Eur. J. Biochem. 13, 526-533

BOURD, G.I., BOLSHAKOVA, T.N., SAPRYKINA, T.P., KLYUTCHOVA, V.V. & GERSHANOVITCH, V.N. (1971) Molek. Biol. 5, 384-389

BOURD, G.I., BOLSHAKOVA, T.N. & GERSHANOVITCH, V.N. (1974b) Biokhimiya 39, 608-613

BOURD, G.I., ERLAGAEVA, R.S., BOLSHAKOVA, T.N. & GERSHANOVITCH, V.N. (1974a) Dokl. Akad. Nauk. USSR 215, 1243-1246

BOURD, G.I., ERLAGAEVA, R.S., BOLSHAKOVA, T.N. & GERSHANOVITCH, V.N. (1975) Eur. J. Biochem. 53 419-427

BOURD, G.I., SHABOLENKO, V.P., ANDREEVA, I.V., KLYUTCHOVA, V.V. & GERSHANOVITCH, V.N. (1969) Molek. Biol. 3, 256-266

BOYER, P.D., & KLEIN, W.L. (1972) in Energy Coupling Mechanisms in Transport (Fox, C.F. & Keith, A.D., eds.), pp. 323-344, Sinauer Associates, Stamford, Connecticut.

BRAY, G.A. (1960) Anal. Biochem. 1, 279-285

BRICE, C.B. & KORNBERG, H.L. (1967) Proc. Roy. Soc. Ser.B 168, 281-292

BRODA, P. (1967) Genet. Res. 9, 35-47

BROWN, C.E. & Hogg, R.W. (1972) J.Bacteriol. 111, 606-613

BUTTIN, G. (1963a) J.Mol. Biol. 7, 164-182

BUTTIN, G. (1963b) J.Mol. Biol. 7, 183-205

BUTTIN, G. (1968) Adv. Enzymol. 30, 81-137

DEL CAMPO, F.F., HERNÁNDEZ-ASENSIO, M., & RAMIREZ, J.M. (1975) Biochem. Biophys. Res. Commun. 63, 1099-1105

COHEN, G.N. & MONOD, J. (1957) Bacteriol. Rev. 21, 169-194

COOPER, R.A. & KORNBERG, H.L. (1967) Proc. Roy. Soc. Ser.B 168, 263-280

CRANE, R.K. (1960) Physiol. Rev. 40, 789-825

CURTIS, S.J. & EPSTEIN, W. (1971) Fed. Proc. 30, 1123

CURTIS, S.J. & EPSTEIN, W. (1975) J. Bacteriol. 122, 1189-1199

EGAN, J.B. & MORSE, M.L. (1965) Biochim. Biophys. Acta, 97, 310-319

ENGLESBERG, E., WATSON, J.A. & HOFFEE, P.A. (1961) Cold Spring Harbor Symp. Quant. Biol. 26, 261-274

EPSTEIN, W. & CURTIS, S.J. (1972) in Role of Membranes in Secretory Processes (Bolis, L., Keynes, R.D. & Wilbrandt, W., eds) pp.98-112, North-Holland Publishing Co., Amsterdam.

FERENCI, T. & KORNBERG, H.L. (1971) FEBS Lett. 13, 127-130

FERENCI, T. & KORNBERG, H.L. (1974) Proc. Roy. Soc. Ser.B 187, 105-119

FOX, C.F. & WILSON, G. (1968) Proc. Nat. Acad. Sci. U.S. 59, 988-995

GACHELIN, G. (1970) Eur. J. Biochem. 16, 342-357

GANESAN, A.K. & ROTMAN, B. (1966) J. Mol. Biol. 16, 42-50

HAROLD, F.M. (1972) Bacteriol. Rev. 2, 172-230

HAZELBAUER, G.L. & ADLER, J. (1971) Nature N.Biol. 230, 101-104

HENDERSON, P.J.F. & KORNBERG, H.L. (1975) in Energy Transformation in Biological systems, Ciba Foundation Symposium 31, pp.243-269, ASP, Amsterdam.

HENGSTENBERG, W., EGAN, J.B. & MORSE, M.L. (1968) J. Biol. Chem. 243, 1881-1885

HEPPEL, L.A. (1969) J. Gen. Physiol. 54, 95s-113s

HERNÁNDEZ-ASENSIO, M., RAMIREZ, J.M. & DEL CAMPO, F.F. (1975) Archs Microbiol. 103, 155-162

HESTRIN, S., FEINGOLD, D.S. & SCHRAMM, M. (1955) Methods Enzymol. 1, 231-257

HOFFEE, P., ENGLESBERG, E. & LAMY, F. (1964) Biochim. Biophys. Acta 79, 337-350

HOGG, R.W. & ENGLESBERG, E. (1969) J. Bacteriol. 100, 423-432

HORECKER, B.L., THOMAS, J. & MONOD, J. (1960) J. Biol. Chem. 235, 1580-1585

JONES-MORTIMER, M.C. & KORNBERG, H.L. (1974a) FEBS. Lett. 48, 93-95

JONES-MORTIMER, M.C. & KORNBERG, H.L. (1974b) Proc. Roy. Soc. Ser.B 187, 121-131

JOSEPHSON, B.L. & FRAENKEL, D.G. (1969) J. Bacteriol. 100, 1289-1295

KABACK, H.R. (1968) J. Biol. Chem. 243, 3711-3724

KABACK, H.R. (1971) Methods Enzymol. 22, 99-120

KABACK, H.R. (1974) Science (Wash. D.C.) 186, 882-892

KABACK, H.R. & BARNES, E.M. Jr. (1971) J. Biol. Chem. 246, 5523-5531

KABACK, H.R., & MILNER, L.S. (1970) Proc. Nat. Acad. Sci. U.S. 66, 1008-1015

KALCKAR, H.M. (1971) Science (Wash. D.C.) 174, 557-565

KALCKAR, H.M., KURAHASHI, K. & JORDAN, E. (1959) Proc. Nat. Acad. Sci. U.S. 45, 1776-1786

KAMOGAWA, A. & KURAHASHI, K. (1967) J. Biochem. 61, 220-230

KERWAR, G.K., GORDON, A.S. & KABACK, H.R. (1972) J. Biol. Chem. 247, 291-297

KEPES, A. & COHEN, G.N. (1962) in The Bacteria vol. 5 (Gunsalus, I.C. & Stanier, R.Y., eds.), pp.179-221, Academic Press, New York

KLEIN, W.L. & BOYER, P.D. (1972) J. Biol. Chem. 247, 7257-7265

KORNBERG, H.L. (1970) in Metabolic Regulation and Enzyme Action, FEBS Symp. vol. 19 (Sols, A. & Grisolia, S., eds.) pp.5-18, Academic Press, New York and London.

KORNBERG, H.L. (1973) Symp. Soc. Exp. Biol. 27, 175-193

KORNBERG, H.L. & JONES-MORTIMER, M.C. (1975) FEBS Lett. 51, 1-4

KORNBERG, H.L. & REEVES, R.E. (1972a) Biochem. J. 126, 1241-1243

KORNBERG, H.L. & REEVES, R.E. (1972b) Biochem. J. 128, 1339-1344

KORNBERG, H.L. & SMITH, J. (1972) FEBS Lett. 20, 270-272

KUNDIG, W., GHOSH, S. & ROSEMAN, S. (1964) Proc. Nat. Acad. Sci. U.S. 52, 1067-1074

KUNDIG, W., KUNDIG, F.D., ANDERSON, B.E. & ROSEMAN, S. (1965) Fed. Proc. 24, 658

KUNDIG, W., KUNDIG, F.D., ANDERSON, B.E. & ROSEMAN, S. (1966) J. Biol. Chem. 241, 3243-3246

KUNDIG, W. & ROSEMAN, S. (1971a) J. Biol. Chem. 246, 1393-1406

KUNDIG, W. & ROSEMAN, S. (1971b) J. Biol. Chem. 246, 1407-1418

LANGRIDGE, J. (1974) Aust. J. Biol. Sci. 27, 331-340

LEDER, I.G. & PERRY, J.W. (1967) J. Biol. Chem, 242, 457-462

LEFEVRE, P.G. (1954) Symp. Soc. Exp. Biol. 8, 118-135

LENGELER, J., HERMANN, K.O., UNSÖLD, H.J. & BOOS, W. (1971) Eur. J. Biochem. 19, 457-470

LENNOX, E.S. (1955) Virology 1, 190-206

LEVINE, M., OXENDER, D.L. & STEIN, W.D. (1965) Biochim. Biophys. Acta 109, 151-163

LIN, E.C.C. (1970) Annu. Rev. Genet. 4, 225-262

LOMBARDI, F.J., REEVES., J.P. & KABACK, H.R. (1973) J. Biol. Chem. 248, 3551-3565

LOW, B. (1965) Genet. Res. 6, 469-473

LOW, K.B. (1972) Bacteriol. Rev. 36, 587-607

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951) J. Biol. Chem. 193, 265-275

McGINNIS, J.F. & PAIGEN, K. (1969) J. Bacteriol. 100, 902-913

McGINNIS, J.F., & PAIGEN, K. (1973) J.Bacteriol. 114, 885-887

MESSER, A. (1974) J. Bacteriol. 120, 266-272

MILES, R.J. & PIRT, S.J. (1973) J. Gen. Microbiol. 76, 305-318

MILLER, J.H. (1972) Experiments in Molecular Genetics, Cold Spring Harbor Laboratory

MITCHELL, P. (1973) J. Bioenerg. 4, 63-91

MORGAN, M.J. & KORNBERG, H.L. (1967) Biochem. J. 103, 57p

ORDAL, G.W. & ADLER, J. (1974a) J.Bacteriol.117,509-516

ORDAL, G.W. & ADLER, J. (1974b) J. Bacteriol. 117, 517-526

OXENDER, D.L. (1972) Annu. Rev. Biochem. 41, 777-814

PARDEE, A.B. (1957) J. Bacteriol. 73, 376-385

PARSONS, R.G. & HOGG, R.W. (1974) J. Biol. Chem. 249, 3608-3614

PASTAN, I. & PERLMAN, R.L. (1969) J. Biol. Chem. 244, 5836-5842

POGELL, B.M., MAITY, B.R., FRUMKIN, S., & SHAPIRO, S. (1966) Archs Biochem. Biophys. 116, 406-415

POUYSSÉGUR, J.M., FAIK, P. & KORNBERG, H.L. (1974) Biochem. J. 140, 193-203

PRESTIDGE, L.S. & PARDEE, A.B. (1965) Biochim. Biophys. Acta 100, 591-593

REEVES, J.P.,LOMBARDI, F.J. & KABACK, H.R. (1972) J. Biol. Chem. 247, 6204-6211

RICKENBERG, H.V., COHEN, G.N., BUTTIN, G. & MONOD, J. (1956) Ann. Inst. Pasteur 91, 829-857

ROBBINS, A.R. (1975) J. Bacteriol. 123, 69-74

ROBBINS, A.R. & ROTMAN, B. (1975) Proc. Nat. Acad. Sci. U.S. 72, 423-427

ROBERTS, R.B., ABELSON, P.H., COWIE, D.B., BOLTON, E.T. & BRITTEN, R.J. (1963) Studies of Biosynthesis in Escherichia coli, Carnegie Inst.Wash. Publ., Washington, D.C.

ROGERS, D. & Yu, S-H (1962) J. Bacteriol. 84, 877-881

ROSEMAN, S. (1969) J. Gen. Physiol. 54, 138s - 180s

ROSEMAN, S. (1972) in The Molecular Basis of Biological Transport (Woessner, J.F., Jr. & Huijing, F., eds.), pp. 181-245, Academic Press, New York and London.

ROSEMAN, S. (1975) in Energy Transformation in Biological Systems, Ciba Foundation Symposium 31, pp.225-241, ASP, Amsterdam.

ROTMAN, B., GANESAN, A.K. & GUZMAN, R. (1968) J. Mol. Biol. 36, 247-260

RUFFLER, D. & BÖCK, A. (1973) J. Bacteriol. 116, 1054-1055

SAEDLER, H., GULLON, A., FIETHEN, L. & STARLINGER, P. (1968) Mol. Gen. Genet. 102, 79-88

SAIER, M.H., BROMBERG, F.G. & ROSEMAN, S. (1973) J. Bacteriol. 113, 512-514

SAIER, M.H. & ROSEMAN, S. (1971) Fed. Proc. 30, 1097

SAIER, M.H. & ROSEMAN, S. (1972) J. Biol. Chem. 247, 972-975

SAIER, M.H., SIMONI, R.D. & ROSEMAN, S. (1970) J. Biol. Chem. 245, 5870-5873

SANNO, Y., WILSON, T.H. & LIN, E.C.C. (1968) Biochem. Biophys. Res. Commun. 32, 344-349

SCHAEFLER, S. (1967a) J. Bacteriol. 93, 254-263

SCHAEFLER, S. (1967b) J. Bacteriol. 93, 264-272

SCHLEIF, R. (1969) J. Mol. Biol. 46, 185-196

SHIMADA, K., WEISBERG, R.A. & GOTTESMAN, M.E. (1972) J. Mol. Biol. 63, 483-503

SILHAVY, T.J. & BOOS. W. (1974) J. Bacteriol. 120, 424-432

SILHAVY, T.J., BOOS, W. & KALCKAR, H.M. (1974) in The Biochemistry of Sensory Functions, 25. Mosbacher Colloquium der Gesellschaft für Biologische Chemie, (Jaenicke, L. ed.), pp.165-205, Springer-Verlag, Berlin, Heidelberg, New York

SIMONI, R.D., LEVINTHAL, M., KUNDIG, F.D., KUNDIG, W., ANDERSON, B., HARTMAN, P.E. & ROSEMAN, S. (1967) Proc. Nat. Acad. Sci. U.S. 58, 1963-1970

SIMONI, R.D. & ROSEMAN, S. (1973) J. Biol. Chem. 248, 966-976

SIMONI, R.D., SMITH, M.F. & ROSEMAN, S. (1968) Biochem. Biophys. Res. Commun. 31, 804-810

SLATER, E.C. (1953) Nature (London) 172, 975-978

SOLOMON, E., MIYAI, K. & LIN, E.C.C. (1973) J. Bacteriol. 114, 723-728

STACEY, K.A. & SIMSON, E. (1965) J. Bacteriol. 90, 554-555

STEIN, W.D. (1967) The Movement of Molecules Across Cell Membranes, Academic Press, New York and London.

TANAKA, S., FRAENKEL. D.G. & LIN, E.C.C. (1967) Biochem. Biophys. Res. Commun. 27, 63-67

TANAKA, S. & LIN, E.C.C. (1967) Proc. Nat. Acad. Sci. U.S. 57, 913-919

TAYLOR, A.L. & TROTTER, C.D. (1967) Bacteriol. Rev. 31, 332-353

TAYLOR, A.L. & TROTTER, C.D. (1972) Bacteriol. Rev. 36, 504-524

TYLER, B., WISHNOW, R., LOOMIS, W.F. Jr. & MAGASANIK, B. (1969) J. Bacteriol. 100, 809-816

VORISEK, J. & KEPES, A. (1972) Eur. J. Biochem. 28, 364-372

WANG, R.J. & MORSE, M.L. (1968) J. Mol. Biol. 32, 59-66

WANG, R.J., MORSE, H.G., & MORSE, M.L. (1969) J. Bacteriol. 98, 605-610

WANG, R.J., MORSE, H.G., & MORSE, M.L. (1970) J. Bacteriol. 104, 1318-1324

WILSON, D.B., (1974a) J. Biol. Chem. 249, 553-558

WILSON, D.B., (1974b) J. Bacteriol. 120, 866-871

WILSON, T.H. & KUSCH, M. (1972) Biochim. Biophys. Acta 255, 786-797

WINKLER, H.H. & WILSON, T.H. (1966) J. Biol. Chem. 241, 2200-2211

WINKLER, H.H. & WILSON, T.H. (1967) Biochim. Biophys. Acta 135, 1030-1051

WONG, P.T.S., KASHKET, E.R. & WILSON, T.H. (1970) Proc. Nat. Acad. Sci. U.S. 65, 63-69

WONG, P.T.S. & WILSON, T.H. (1970) Biochim. Biophys. Acta 196, 336-350

WU, H.C.P. (1967) J. Mol. Biol. 24, 213-223

WU, H.C.P., BOOS, W. & KALCKAR, H.M. (1969) J. Mol. Biol. 41, 109-120

WU, T.T. (1966) Genetics 54, 405-410

ASPECTS OF GALACTOSE AND GLUCOSE TRANSPORT IN ESCHERICHIA COLI.

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ABSTRACT.

1. Strains of E.coli have been characterised which are galactose-positive despite their failure to express any of the active transport systems for galactose during growth on this sugar. The growth of such strains on galactose occurs at rates that are a function of the galactose concentration of the medium: half-maximal growth rates require more than 2mM galactose to be present. The introduction of a mutation in the glucose phosphotransferase Enzyme II specified by the gene umg severely impairs the ability of these strains to grow on galactose; it has been established by a variety of means that this Enzyme II, or a component of it, provides the means of galactose entry into these organisms. However, the uptake of galactose does not require phosphotransferase activity, but occurs by facilitated diffusion on this carrier. The implications of this finding on the current understanding of the mechanism of glucose uptake by the phosphotransferase system are discussed.

2. Although the Umg-system provides the major route of glucose uptake in many strains of E.coli, this is not true for all strains. Evidence is presented that suggests the existence of a fourth Enzyme II for glucose, in addition to those specified by the genes umg, ptsX and bgl.

3. The galactose permease specified by the gene galP can transport glucose in addition to galactose. A

screening procedure for distinguishing GalP$^+$ from GalP$^-$ strains is described which makes use of this property and which has been used to locate the *galP* lesion close to minute 55 on the *E.coli* chromosome, between the genetic markers *fda* and *lysA*.