Genetics in the Study of Carbohydrate Transport by Bacteria

Sixth Griffith Memorial Lecture

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On 26 August 1927, a weighty parcel was delivered to the desk of the Editor of the *Journal of Hygiene*. When opened, this parcel was found to contain a manuscript which was remarkably lengthy even by the standards of those more literate days of scientific publication, for it was to occupy 46 pages of the *Journal* when it was duly published. This paper was by Dr Frederick Griffith, who, in the address, described himself simply as 'A Medical Officer in the Ministry of Health'. Neither the title of the paper – 'The significance of pneumococcal types' – nor its opening paragraph:

'Since communicating my report on the distribution of pneumococcal types in a series of 150 cases of lobar pneumonia occurring in the period from April, 1920 to January, 1922, I have not made any special investigation of this subject. In the course, however, of other inquiries and of the routine examination of sputum during the period from the end of January, 1922, to March, 1927, some further data have been accumulated'

gave any indication that the results reported therein, and the interpretation placed on these results, were in the nature of a time-bomb. As Hayes (1966) remarked in the first Griffith Memorial Lecture, Griffith's paper 'proved, had he but known it, to be a delayed-action fuse which, 25 years after its publication, triggered off an explosion of biological knowledge, comparable only to that ignited a century ago by the work of Mendel'. The nucleus of this explosive device lay in the observation that suspensions of virulent (capsulated) pneumococci, that had been killed by heat, contained some substance that could not only cause live avirulent (non-capsulated) pneumococci of the same strain to give rise to pathogenic (capsulated) progeny, but could also cause non-capsulated organisms of a different strain to yield capsulated progeny that exhibited the serological characteristics of the heat-killed donor organisms. This phenomenon, to which Griffith gave the name 'transformation', was ultimately shown to be due to the DNA present in the cell-free extract made from the heat-killed bacteria (Avery, MacLeod & McCarty, 1944); later work, on highly purified extracts, particularly by Hotchkiss (1952), made it virtually certain that the ability to change the genotype of one strain to that of another by transformation resided wholly within this substance. This work, and Hershey & Chase's (1952) clear demonstration that DNA was the infectious molecule transmitted by bacteriophages to *E. coli*, established that DNA was indeed the genetic material.

The Society is well aware of the significance of these studies, for both Professor MacLeod and Professor Hotchkiss, who played such prominent roles in the discoveries, were invited to give (respectively) the fourth and the fifth Griffith Memorial Lectures: it was sad indeed that Professor MacLeod's untimely death in February 1972 robbed us of the opportunity of hearing his account of the events spanning this important episode in the history of our discipline. The researches that led from Griffith's original publication in 1928 to the estab-

lishment of the identity of the transforming principle as DNA, and (in 1953) to the postulate of the double-helical structure of this material (Watson & Crick, 1953*a*) which explained at one dazzlingly brilliant stroke three of its properties – the ability of DNA to carry genetic information, to replicate itself accurately, and to undergo inheritable mutations (Watson & Crick, 1953*b*) – have been described fully and well by previous Griffith Memorial Lecturers (Hayes, 1966; Pollock, 1970; Downie, 1972; Hotchkiss, 1974). Any attempt by me now to rehearse these events could only be regarded as a work of supererogation: I would, in any case, be incompetent to do so. Indeed, the only justification that I can find for the Society's choice of me as the sixth Griffith Memorial Lecturer is that Griffith's paper was apparently published on the day on which I was born...

Recombination by transformation

Although I do not propose to discuss the relationship of Griffith's work to the establishment of the identity of the genetic material, I must refer to his original paper again as it bears on the phenomenon of recombination in bacteria, that is, the exchange of at least portions of genomes between partners in a cross. Perhaps I might be allowed to let both my biochemical prejudices and the clear light of hindsight play upon Griffith's observations in order to interpret the results he described.

Pneumococci usually form smooth, glistening colonies when growing on solid media. This appearance, which often also indicates the ability of the organisms to withstand bodily defences and hence to cause disease, is due to the presence of complex heteropolysaccharides in the capsule that forms the outer layer of the organism. These polysaccharides elicit the synthesis of specific antibodies when the bacteria are inoculated into animals and are of a great chemical diversity: over 80 serological types have been described. This diversity is, of course, the consequence of the specific arrangement of the various sugar and sugar-acid moieties in the polymer. These components are synthesized from the UDP derivatives of central metabolites by enzymic steps that are either found in all pneumococci ('common') or found to occur only in those strains that synthesize a particular capsular polysaccharide ('type specific'). For example, UDPglucose, UDPgalactose and UDP-*N*-acetylglucosamine are involved in a variety of reactions other than capsule formation: the enzymes for their synthesis are therefore 'common'. In contrast, the NAD-linked UDPglucose dehydrogenase that catalyses the formation of UDPglucuronic acid (reaction 1)

and the enzyme that epimerizes UDPglucuronic acid to UDPgalacturonic acid (reaction 2)

are concerned in the synthesis of, for example, type I polysaccharide and are found only in pneumococci which elaborate capsules containing glucuronic and galacturonic acid moieties.

Fig. 1. An interpretation of some of Griffith's transformations of pneumococci in terms of recombinational events. In the upper part of the diagram, the function of a defective gene *A* is restored by replacement of *A1*⁻ with its allele *A2*⁺; adjacent genes *B* and *C* are unaffected and the pneumococcal type is thus unchanged. In the lower part of the diagram, the cross-over results not only in the restoration of function of gene *A* but also in the replacement of genes *B* and *C* by *X*, *Y* and *Z*: the capsulated recombinant is thus of a type different from that of the uncapsulated recipient.

These enzymes are therefore 'type specific'; their absence causes the normal capsulated and hence smooth strain to appear as uncapsulated rough mutants (Mills & Smith, 1965).

If such mutants are now transformed with DNA derived from another strain and the transformed organisms are found to have acquired the serological characteristics of that other strain, not only must the previously defective gene have been replaced, but others, specific for a different type of polysaccharide, must have been simultaneously introduced. The implication, that there is close physical contiguity (linkage) between at least some of the genes involved in polysaccharide assembly, has received experimental support (Bernheimer, Wermundsen & Austrian, 1968; Austrian *et al.*, 1959). This phenomenon is illustrated in Fig. 1.

As Hayes (1966) perceptively pointed out, the transformations of pneumococcal types recorded by Griffith (1928) are striking illustrations of the ability of these bacteria to undergo exchanges of genetic material by recombination. Unfortunately, pneumococci appear to be limited to this type of exchange: no sexual transfer of DNA has yet been demonstrated in them. Since the amount of DNA involved in transformation is only 0.1 to 1 % of the whole genome (Mäkelä & Stocker, 1969), only closely linked markers can be thus transferred. Even if many different mutants were available, the construction of a genetic map by these means would be laborious and the map would probably remain incomplete. It was this lack

of a convenient method for genetical analysis that caused the late Harriett Ephrussi-Taylor (who first demonstrated the recombinational events underlying Griffith's observations) to write, in 1951, '...Griffith's discovery exerted virtually no influence in biological thought until nearly twenty years later, for the absence of sexual reproduction in bacteria was sufficient to discourage any geneticist from studying these induced transformations' (Ephrussi-Taylor, 1951). To understand what happened nearly 20 years after Griffith's original paper to change this situation, we shall have to leave the pneumococci and turn to another family of bacteria, the Enterobacteriaceae.

Recombination by conjugation and transduction

The relationship between genetic constitution and biochemical function had emerged from the combination of biochemical and genetical analysis that Beadle & Tatum (1941) had so successfully employed with *Neurospora crassa*. In order to apply similar techniques to bacteria, Lederberg & Tatum (1946*a, b*) used mutants derived from cultures of the K12 strain of *E. coli* that had been treated with X-rays, to show that such mutants could restore each other's function and yield a small but reproducible proportion of prototrophic progeny. Thus, for example, when a mutant that carried the wild-type alleles *A, B, C* but the mutated forms of three others, *x, y* and *z*, was mixed with another mutant affected in the reverse sense (*abcXYZ*), about one cell arose from about ten million in the mixture that was stably *ABCXYZ* and had thus lost all nutritional requirements. This phenomenon required the participation of intact live cells (Tatum & Lederberg, 1947): neither heat treatment nor filtration gave active extracts, which therefore immediately distinguished this type of genetic exchange from both transformation and phage-mediated transduction. Indeed, in a series of brilliant studies that followed up these observations, Lederberg was able amply to establish the sexual character, though not the mechanism, of this phenomenon.

The analogy between this bacterial process and the conjugal events that occurred in higher organisms became clear through the important work of our first Griffith Memorial Lecturer, W. Hayes, and, as one of his former students, I am particularly glad to give a brief account of this. In 1952, Hayes noticed that the feasibility of transfer of genetic material depended on the ability of one, but not the other, of the partners in a cross to survive on selective media (Hayes, 1952). Thus, when streptomycin-sensitive cells of one type (A) and streptomycin-resistant cells of another (B) were mixed, and streptomycin was used as an agent for the selection of recombinants on minimal media, it was found that recombinants were obtained only from this distribution of the streptomycin marker: none was obtained from a cross of streptomycin-resistant A with streptomycin-sensitive B. Hayes interpreted this observation as indicating that the A type of cell acted as donor and the B type as recipient. The donor (A) was presumably no longer needed after it had transferred some genetic material to the recipient (B), but no progeny could arise if that recipient was prevented from multiplying. Hayes (1953*a*) further showed that the transfer of genetic material from A to B was mediated by a sex factor, F, which was absent from the recipient: again, this transfer from F⁺ to F⁻ cells requires physical contact and results in the conversion of the recipient (F⁻) type to the donor (F⁺) state (Cavalli-Sforza, Lederberg & Lederberg, 1953; Hayes, 1953*b*). However, Hayes (1953*b*) also discovered that it was possible to isolate cells from F⁺ cultures that not only acted as genetic donors to produce recombinants with a much higher frequency than did the parent organism – and such extraordinarily fertile strains were therefore designated Hfr – but also did not convert F⁻ recipients to the F⁺ state. Moreover, Hfr strains always transferred chromosomal markers to recipient F⁻ cells from the same point [designated O for *origine* by Wollman & Jacob (1955, 1958)] and in the same direction.

Thus, for example, the original Hfr strain isolated by Hayes (1953*b*), called HfrH after him, always transfers the markers for threonine and leucine biosynthesis very early and before those specifying the enzymes of lactose uptake and cleavage, whereas the HfrC strain described by Cavalli-Sforza (1950) always transfers these markers in the reverse order. This reproducibility of chromosome transfer made it possible to time the entry of successive markers into recipient cells. The isolation of many more Hfr strains with different *origines* led rapidly to the construction of increasingly complete linkage maps, such as that compiled by Taylor & Trotter (1972), for markers located on the circular chromosome of *E. coli*. Since, under favourable conditions, the chromosome of the Hfr-donor can be completely transmitted to the F⁻ recipient in about 90 min, it became convenient to express genetic loci in terms of minutes; with the *thr* marker arbitrarily chosen to be at 0/90 min, the *leu* marker lies at min 1, *lac* at min 10, etc.

The accuracy with which genetic loci can be established, of course, depends on the sensitivity with which one genetic marker can be distinguished from another close to it. Genetical analysis by periodic interruption of conjugal chromosome transfer, from Hfr to F⁻ strains, does not have the discrimination required for fine structure analysis: a scalpel, rather than a broadsword, is needed here. The existence of a scalpel convenient for this purpose was first recognized through studies with another enteric bacterium, *Salmonella typhimurium*, by Lederberg *et al.* (1951). They showed that prototrophic recombinants could arise also when a culture of an auxotrophic mutant was mixed with a *filtrate* derived from a suspension of another mutant. The vector of genetic transfer present in such a filtrate was not naked DNA, but a discrete, particulate bacteriophage (PLT-22) that could be propagated on one strain and was able to infect the other (Zinder & Lederberg, 1952). This process of phage-mediated genetic exchange, involving an amount of DNA of the same order of magnitude as that involved in the transformation of *Pneumococcus*, was termed transduction. Transducing phages for *E. coli* were isolated soon after they had been discovered for *Salmonella*. In consequence of the combination of these techniques of Hfr-mediated conjugation and phage-mediated transduction, over 800 markers have now been located on the genome of *E. coli*. These include genes that specify proteins for the uptake of carbohydrates. It is the essential contribution that genetical analysis is making to our knowledge of the identity, function and control of these proteins that provides the reason for discussing carbohydrate transport in the context of this lecture.

Recognition of systems effecting the uptake of carbohydrates

Although I hope shortly to justify my use of the word 'essential' in describing the application of genetical analysis to the study of carbohydrate transport by micro-organisms, it would be foolish to deny that the existence of such systems, and the kinetic parameters that characterize their specific functions, were established without such detailed genetical studies.

Two main lines of evidence led, in the 1950s, to the realization that micro-organisms possess the ability to select which substances to admit from their environment, and when to admit them. The pioneer work of Gale (1947) had established that *Streptococcus faecalis* could accumulate large amounts of different amino acids, which could be readily recovered if the cells were crushed, but which could not be washed out of the intact organisms. With some amino acids this accumulation occurred only if the bacteria were supplied with a utilizable energy source, such as glucose. It was thus probable that the cells were relatively impermeable to these amino acids but that they could be taken up by a unidirectional energy-requiring process.

A second line of evidence arose from the recognition that, although micro-organisms may not utilize a variety of substances when those substances are added to suspensions of whole cells, extracts of these cells contain the enzymes necessary for their metabolism. This might have merely indicated that the microbial envelope is impermeable to certain classes of compounds. While generally true, this relatively trivial explanation could not account for the stereospecificity of the exclusion of many metabolites, nor for the observation that a substance excluded by bacteria under some circumstances was admitted under others. For example, bakers' yeast readily ferments the disaccharide sucrose but does not attack the disaccharide maltose, even though extracts of that yeast are rich in maltase. A mutant of *E. coli* was described over 30 years ago that did not ferment lactose although β -galactosidase was present in extracts of dried cells (Deere, Dulaney & Michelson, 1939). Doudoroff and his colleagues in 1949 described an even more remarkable mutant that would metabolize glucose if that sugar was generated internally through the breakdown of maltose, but could not use glucose supplied externally (Doudoroff *et al.*, 1949). Similarly, work on a variety of bacteria showed that whole cells oxidized some added intermediates of the tricarboxylic acid cycle only after prolonged lag periods, yet extracts of these cells contained the enzyme required for the oxidation of these intermediates at all times (Campbell & Stokes, 1951; Stone & Wilson, 1952*a, b*; Repaske & Wilson, 1953). The puzzling nature of these latter phenomena was resolved by the studies of Kogut & Podoski (1953) and the confirmatory work of Barrett, Larson & Kallio (1955), who showed that the observed lags in oxidation were associated with the elaboration of systems that catalysed the transport of substrates from the medium into the cells. These transport systems had the characteristics of inducible enzymes: they were elicited in response to specific substrates, and their formation was arrested or abolished by ultraviolet irradiation, and by agents such as amino-acid analogues, that were known to inhibit protein biosynthesis.

But it was the realization that *E. coli*, grown upon lactose as sole carbon source, could take up not only lactose but also a number of non-catabolizable analogues containing the β -galactoside linkage, that provided the impetus for the definition of the specificity of an uptake system and for study of the kinetics of this uptake process; it also set the stage for the analysis of its genetic control. In their now-classic early work, Rickenberg *et al.* (1956) found that washed suspensions of lactose-grown cells would accumulate methyl [^{35}S]-thiogalactoside (TMG) to such an extent that it formed nearly 4 % of the total dry mass of the organism. Wild-type cells catalysed this uptake only if they had been previously exposed to lactose or to a number of analogues that induced the formation of this uptake system. The affinity of the cells for the labelled TMG was found to be strikingly similar to that of an enzyme for its substrate: a double reciprocal plot of the levels of TMG accumulated versus the external TMG concentration showed a linear relation, as in the familiar Lineweaver-Burk treatment of enzyme saturation. Furthermore, a number of unlabelled thiogalactosides competitively inhibited the uptake of labelled TMG. These observations suggested that the binding of a galactoside to a limited number of specific sites was an essential step in the uptake and accumulation of these materials.

It was also realized that two types of mutant could be obtained that were impaired in their ability to grow upon lactose: type Lac^- took up only traces of labelled TMG but could be induced to form β -galactosidase whereas type Lac^+ took up labelled TMG normally but formed no β -galactosidase (Monod, 1956). Clearly, the gene (*y*) that specifies the uptake system, and that is altered in the 'cryptic' Lac^- mutant, is different from the gene (*z*) that is non-functional in the mutant Lac^+ , and that specifies the structure of β -galactosidase.

This pioneer work was quickly followed by the discovery of other 'active transport'

systems. Mutants devoid of the enzymes that normally catalyse the entry of galactose and of maltose into the central metabolic routes were used by Horecker, Thomas & Monod (1960) to show that galactose accumulated as such against a concentration gradient, and by Wiesmeyer & Cohn (1960) to demonstrate that maltose behaved similarly. Similar studies have also been made with mutants that are impaired in the catabolism of charged substances like glucose 6-phosphate (Dietz & Heppel, 1971; Essenberg & Kornberg, 1975) and 6-phosphogluconate (Pouysségur, Faik & Kornberg, 1975; Robin & Kepes, 1975) but that can still take up glucose 6-phosphate or gluconate. In each case, the accumulated isotopically-labelled material exchanged rapidly with the same, but unlabelled, substrate when that was supplied externally. Such studies have been most useful in defining the kinetics and stereo-specificity of active transport, and have also revealed one way in which metabolic energy may power this process. For example, West (1970) showed that, in the absence of an energy source, the uptake of lactose by a mutant devoid of β -galactosidase was accompanied by the uptake of H^+ ions; my colleague Peter Henderson has found a similar proton uptake concomitant with the active transport of D-arabinose by suspensions of a mutant unable, through lack of arabinose epimerase, to catabolize this sugar (Henderson, 1974). In both cases, the accumulation of the sugars and the uptake of protons were arrested by agents that uncouple oxidative phosphorylation. Findings such as these support strongly the view, first formulated by Peter Mitchell (1963), and elaborated 10 years later (Mitchell, 1973), that the inward movement of the sugar is energized by a trans-membrane gradient of protons and/or of electrical charge.

Illuminating though such experiments have been and are continuing to be, they must be complemented by genetical analysis in order to answer questions that hinge on the *identity* of the proteins involved in uptake processes. Kinetic analysis, by revealing possible discontinuities in the Lineweaver-Burk plots relating (rates of uptake) $^{-1}$ to the (concentrations of substrate) $^{-1}$, may suggest that more than one protein participates in the uptake process; this suggestion may be strengthened by observations of different affinities for the uptake of various substrate analogues if such analogues are available. But only the demonstration that these different proteins are specified by genes located at different loci can remove lingering doubts, and only in the possession of such information can one be confident about the minimum number of protein components of any one uptake system, about the number of doors through which a given substrate can enter the cell, about the variety of substrates that can enter through any one door, and about the nature of the changes that can restore a phenotype without repairing the original dysfunction: this latter information is of possible evolutionary significance as well as of immediate physiological interest. I shall take advantage of my privileged position at this lectern to illustrate these points with some examples drawn largely from the work of our laboratory.

The uptake of galactose: seven types of ambiguity

Nowhere is the need for some independent means of disentangling the complexities of the active transport of a carbohydrate as apparent as in the multiplicity of ways in which D-galactose can enter *E. coli* (Rotman, Ganesan & Guzman, 1968). The lactose uptake system will effect the entry of a wide variety of β -galactosides including lactose and free galactose (Rickenberg *et al.*, 1956; Pardee, 1957). The uptake system for melibiose, an α -galactoside of glucose, does not transport the β -galactoside lactose but does effect the uptake of the non-catabolizable lactose analogues transported by the lactose system, and also of galactose. To make matters worse, both the lactose and melibiose uptake systems are induced at 26 °C by galactose in strains devoid of galactokinase activity (GalK $^-$),

Fig. 2. Linkage map of *E. coli* (after Taylor & Trotter, 1972). The location of genes concerned in galactose utilization is indicated by arrows; that of genes involved in the uptake of glucose and of fructose (see later) by asterisks.

which accumulate galactose (Leder & Perry, 1967). Such GalK⁻ strains may also form constitutively a high-affinity uptake system for galactose that also transports methyl β -galactoside but not lactose or other β -galactosides: this system is usually described as the methyl- β -galactoside permease. Fourth and fifth in this assemblage are two permeases for arabinose. One of these, AraF, requires the participation of a periplasmic binding protein and appears to have a higher affinity both for arabinose and for galactose than has the other, AraE, system, (Brown & Hogg, 1972). (Fortunately, these systems are under the positive control of a regulatory gene activated by arabinose, and are not induced by growth in the presence of galactose.) Sixth in this series (and physiologically one of the two most important) is an uptake system, the existence of which was recognized by Ganesan & Rotman (1965) in a strain of *E. coli* which was devoid of galactokinase activity, of the lactose permease, and of the methyl- β -galactoside permease. This strain had been grown at 37 °C, at which temperature the melibiose permease is not formed by the K12 strain of *E. coli*. It readily took up galactose (but not β -galactosides) after induction by galactose or by its 6-deoxy derivative, D-fucose; this must therefore have been due to the presence of a distinct galactose permease. My colleague Claudia Riordan and I have now added to this multiplicity by showing that strains of *E. coli* devoid of all these six means of taking up galactose actively can still grow on galactose, but do so by permitting that sugar to enter by facilitated diffusion on a carrier normally involved in the uptake of glucose which also

specifically mediates the uptake of its analogue, methyl α -D-glucoside (Kornberg & Riordan, 1976).

These various galactose transport systems have overlapping specificities for the uptake of galactose and analogues such as fucose, methyl β -galactoside, other β -galactosides and lactose, and cannot be readily distinguished by this means. Only the methyl-galactoside permease can be assayed uniquely, by measurements of the uptake of galactose supplied at less than $1\text{ }\mu\text{M}$, or of methyl galactoside at $10\text{ }\mu\text{M}$ (Boos, 1969), at which concentrations other systems for galactose transport are barely active. Furthermore, and as already mentioned, the inducing agents for these various transport systems are not sufficiently specific to avoid overlap. Clearly, studies of galactose transport are unlikely to yield unambiguous, or even interpretable, results in the absence of genetical analysis. However, knowledge of the location on the linkage map (Fig. 2) of the genes specifying these various uptake systems, and especially the methyl-galactoside permease (*mgl*) at min 41 (Boos, 1969) and the galactose permease (*galP*) at min 54 (C. Riordan & H. L. Kornberg, unpublished results), enables mutants in these systems to be characterized. Such knowledge is now bringing to light other proteins specified by components of the Mgl system. For example, a galactose-binding protein (Boos, 1969) and other proteins involved in galactose chemotaxis (Ordal & Adler, 1974*a, b*), as well as a regulatory component (Robbins, 1975), have been found to map in the *mgl* gene. The study of GalP in the absence of Mgl, and vice versa, is also providing information on the immediate sources of energy used for the uptake of galactose and of its analogues: for example, uptake of such materials via the Mgl system is not accompanied by a simultaneous uptake of protons (Henderson, Dilks & Giddens, 1975) and there is evidence that the energy for this active transport is supplied by direct cleavage of ATP (Wilson, 1974).

The uptake of glucose and fructose: un embarras des richesses

In contrast to the manner in which galactose and lactose are actively transported by *E. coli*, to appear inside the cells chemically unchanged, glucose and fructose are usually converted to phosphate esters in the course of their uptake (Rogers & Yu, 1962). This is due to the operation of the phosphotransferase (PT) system, in which metabolic energy in the form of phosphoenolpyruvate (PEP) is utilized to effect the uptake of the sugars and their retention in the cell in phosphorylated forms.

The PT system, discovered by Dr Saul Roseman and his colleagues (Kundig, Ghosh & Roseman, 1964), is a multicomponent one closely associated with the membrane(s) of Gram-negative organisms that dissimilate carbohydrates predominantly via the Embden-Meyerhof pathway (Romano *et al.*, 1970). It comprises (at least) three components that play a role in the uptake of many sugars (reactions 3 and 4), and other components that exhibit some specificity for a relatively restricted range of sugars (reactions 5 and 6).

The main pleiotropic components are (reaction 3) an Enzyme I that is phosphorylated by PEP at a nitrogen atom of a histidine residue (Stein *et al.*, 1974), and (reaction 4) a small protein, also containing histidine and capable of being phosphorylated at that residue, and designated HPr (histidine-containing protein) for that reason (Kundig *et al.*, 1964).

The sugar-specific components that participate in reactions 5 and 6 are (reaction 5) the

appropriate Enzyme(s) II that catalyse the transfer of phosphate from the phosphorylated HPr to a terminal hydroxyl group of the appropriate sugar (reaction 6) and, of course, the sugars themselves. Although much valuable information has been obtained from biochemical analysis of purified components of this system, and the (at least partial) reconstitution of its function from such components (Roseman, 1969, 1975), it will be evident that the precise role that these components (and doubtless others, yet to be discovered) play in the uptake of individual sugars can be revealed only by the study of organisms with unambiguously identifiable lesions.

This can best be illustrated by considering the manner in which glucose and fructose enter *E. coli* via the PT system. Akin to the ability of lactose-grown cultures to take up non-catabolizable β -galactoside analogues of lactose, suspensions of glucose-grown *E. coli* readily take up, and retain as 6-phosphate esters, analogues of this sugar such as methyl α -D-glucoside, 2-deoxyglucose and 3-deoxy-3-fluoroglucose (Miles & Pirt, 1973). They also take up mannose and glucosamine, and the uptake of all these materials is inhibited to various degrees by the simultaneous provision of glucose. Interpretation of such competition experiments in terms of one or several glucose carriers is very difficult, but becomes remarkably simple if genetical analysis is also brought to bear. Mutants of *E. coli* that no longer take up methyl α -[14 C]glucoside are easily isolated and recognized; the gene specifying this defective Enzyme II of the PT system (designated *umg* = uptake system for methyl glucoside on purely operational grounds) is located at about min 24 on the linkage map and is cotransducible with *purB*. Although *Umg*⁻ mutants fail totally to take up methyl α -glucoside, they are not totally unable either to grow on glucose (Kornberg, 1976) or to incorporate [14 C]glucose when growing on a neutral carbon source like glycerol. Clearly, some other uptake system, usable by glucose but not usable by methyl α -glucoside, must still be functional. This second Enzyme II (for, again, the PT system is obligatorily involved) is specified by a gene located at about min 36 on the linkage map, cotransducible with the *kdgR* and *kga* markers (Jones-Mortimer & Kornberg, 1974). This gene had been recognized originally as specifying an Enzyme II that could participate in fructose utilization (Ferenci & Kornberg, 1974) but the Enzyme II had such poor affinity for this sugar that the gene was designated *ptsX*, to indicate uncertainty about its physiological role. Later work (Kornberg & Jones-Mortimer, 1975; Curtis & Epstein, 1975) revealed that *ptsX* specified the second glucose uptake system, and effected also the uptake and phosphorylation of glucosamine and mannose. Removal of *PtsX* activity from cells that are already *Umg*⁻ virtually abolishes both growth on glucose and the ability to take up [14 C]glucose (Kornberg & Jones-Mortimer, 1975). It must be emphasized, however, that this clear-cut result was obtained with strains of *E. coli* that lacked both *GalP* and *Mgl* activities: when either of these active transport systems for galatose is induced, glucose can also be taken up, by-passing the PT system by being phosphorylated by hexokinase and ATP (Curtis & Epstein, 1975). Again the interrelationship of these various transport proteins with each other and with the other components of the PT system cannot be studied unambiguously unless the presence or absence of individual gene products can be deduced from recombination analysis.

In describing the experiments that led to the view that glucose is normally taken up by two Enzymes of the PT system, it was mentioned that the *PtsX* system has been originally identified as playing a part – albeit a rather inefficient part – in the uptake of fructose. This had emerged from study of the properties of two types of mutant impaired in a high-affinity Enzyme II for fructose (Kornberg, 1972; Ferenci & Kornberg, 1974). Neither mutant took up [14 C]fructose when this was supplied at the usual concentration used for assays of carbohydrate uptake (0.1 mM). However, one mutant took up [14 C]fructose when it was present

Fig. 3. Alternative routes for fructose utilization in *Escherichia coli*. The abbreviations designate: Enz I, Enzyme I of the PEP-phosphotransferase system; HPr, histidine-containing protein of that system; PtsF and PtsX, fructose-specific Enzymes II of that system; Fdp, fructose 1,6-bisphosphatase; Pfk, fructose-6-phosphate kinase; Fpk, fructose-1-phosphate kinase. [Reproduced from Ferenci & Kornberg (1974) with permission of The Royal Society.]

at > 2 mM, whereas the other did not. Genetical analysis (Jones-Mortimer & Kornberg, 1974) showed that both mutants lacked a functional gene located at about min 41, designated *ptsF* to indicate its role in the PT system-mediated uptake of fructose, but the mutant impervious to even high concentrations of fructose was also impaired in *ptsX*. With the aid of mutants also affected in subsequent steps of fructose metabolism, a 'fail-safe' mechanism of fructose utilization (Fig. 3) became apparent (Ferenci & Kornberg, 1974). Normally, fructose is phosphorylated to fructose 1-phosphate by PEP and the PtsF component of the PT system; this product is then phosphorylated to fructose 1,6-bisphosphate by ATP and a specific fructose-1-phosphate kinase. In the absence of either of these enzymes, *E. coli* can still grow on high concentrations of fructose by using the PtsX system to phosphorylate fructose to its 6-phosphate, and converting this also to fructose 1,6-bisphosphate but by ATP and fructose-6-phosphate kinase. It is difficult to see how this ingenious mode of fructose utilization could have been brought to light in the absence of appropriate mutants, the lesions in which could both be precisely ascertained and be suitably combined by genetical procedures.

This point is perhaps brought out most strongly by considering the properties of further mutants, derived from parents that lack both PtsF and PtsX activities. When such organisms are plated on media containing high concentrations of fructose as sole carbon source, occasional cells grow up that do not grow on, or take up, fructose supplied in low concentrations (and are therefore still PtsF⁻) but are phenotypically indistinguishable from revertants to PtsX⁺. However, by chance the *ptsF ptsX* mutant that had been used happened to be one that carried a deletion spanning both this gene and the neighbouring *kdgR* (Jones-Mortimer & Kornberg, 1976): reversion of this *ptsX* allele to *ptsX*⁺ was thus highly unlikely. This paradox was resolved by recombination analysis: introduction of a wild-type

umg-mutants no longer exhibit this preference for glucose, which suggests that either the process of phosphorylation of glucose, or the level of glucose 6-phosphate formed, are important to this 'fine' control (Kornberg, 1972).

Abolition of the ability of glucose to inhibit the uptake of fructose, by removal of *Umg* function, is not fructose-specific: in *umg* mutants, glucose is no longer preferred to any other carbon source (Kornberg, 1973). A more specific 'fine' control was revealed through study of *E. coli* mutants that were resistant to hitherto toxic analogues of glucose (such as 2-deoxyglucose and 3-deoxy-3-fluoroglucose) when growing on fructose, but were still sensitive to these compounds when growing on mannitol, glycerol or lactose (Amaral & Kornberg, 1975). The mutation that gave rise to this phenotype, designated *cif* (catabolite inhibition of fructose uptake), might be envisaged as the consequence of an alteration in either the Enzyme II for glucose (*umg* or *ptsX*) or in a pleiotropic component of the PT system, since *cif* mutants grow normally on glucose and appear to be unaltered in their ability to take up [¹⁴C]glucose or methyl α -[¹⁴C]glucoside in the absence of fructose. But this is not so: the *cif* marker is highly cotransducible with the *ptsF* marker that specifies the preponderant Enzyme II for fructose. Since *cif* mutants, unlike their parent organisms, can be induced by fructose to synthesize this Enzyme II even in the presence of glucose, it is further apparent that the utilization of glucose in preference to fructose when both sugars are present is at least in part due to the exclusion of fructose from the cell, by interaction of glucose (or glucose 6-phosphate) with a site on the PtsF system that is not directly involved in fructose phosphorylation. This view is, of course, not novel but confirms for the PT system the conclusions reached for active transport by Lengeler (1966).

Concluding remarks

In this lecture I have attempted to trace the development of methods of recombination analysis in micro-organisms, and have exemplified their peculiar utility in studies of carbohydrate transport. In doing so, I have used as my starting point Griffith's demonstration of recombination between the genome of *Pneumococcus* and the transforming DNA. It will no doubt be asked – as has been asked of Griffith's contribution to the elucidation of the nature of the hereditary material (Hayes, 1966) – whether Griffith realized the significance of this particular aspect of his work. I did not have the privilege of knowing Dr Griffith (he was killed in an air raid on London 35 years ago this month) and thus have no means of knowing the answer to that question. Indeed, except in so far as it allows men wiser than I am to speculate on the factors that often prevent a breakthrough being recognized as such at the time (Stent, 1972; Wyatt, 1975), I do not think that the question is relevant to the importance of his contribution. After reading his paper, I am profoundly aware of the great debt that we owe to Frederick Griffith, who in 1928 so modestly and honestly recorded and discussed his observations on the significance of pneumococcal types and who thus opened a new chapter in microbiology. The Society does well to commemorate him.

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