to the opposite polar regions of the two mantles, thus causing the distance between the two centers of charge to be somewhat larger than 13 f.

There is no structure for an elongated core intermediate between that shown in Fig. 11, with three inner-core spherons, and that shown in Fig. 12, with four. The transition between these structures is calculated by use of Eq. 1, with $n_i = 22$, to occur at $n_t = 69$, that is, at $N = 138$. It is accordingly an expectation from the close-packed-spheron theory that, as observed, ${}_{90}Ac_{138}{}^{227}$ (formed by bombardment of Re^{226} with 11-Mev protons) gives a three-humped fission product distribution curve (*23*), which has been interpreted (*24*) as showing that both symmetric fission and asymmetric fission occur.

Asymmetric fission is observed in the spontaneous decomposition of ${}_{98}Cf_{156}{}^{254}$ and other very heavy nuclei. We may ask when the transition to symmetric fission would begin. The next elongated core, in the series represented in Figs. 11 and 12, would contain 31 spherons, and the transition to it should occur for 28 spherons in the core of the undistorted nucleus, that is, at $N = 163$ (calculated with use of Eq. 1). We conclude that ${}_{103}Lw_{163}{}^{266}$ and adjacent nuclei should show both asymmetric and symmetric fission.

Conclusion and Summary

The close-packed-spheron theory of nuclear structure may be described as a refinement of the shell model and the liquid-drop model in which the geometric consequences of the effectively constant volumes of nucleons (aggregated into spherons) are taken into consideration. The spherons are assigned to concentric layers (mantle, outer core, inner core, innermost core) with use of a packing equation (Eq. 1), and the assignment is related to the principal quantum number of the shell model. The theory has been applied in the discussion of the sequence of subsubshells, magic numbers, the proton-neutron ratio, prolate deformation of nuclei, and symmetric and asymmetric fission.

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Galactose Metabolism and Cell "Sociology"

Galactose, one of the freaks of evolution, furnishes a simple illustration of the extravagances of nature.

Herman M. Kalckar

"Lettre de M. Pasteur à M. Biot [Lille, 11 février 1856]:

". . . Mais, en realité, le sucre de lait modifié par les acides est tout autre que le glucose. Je propose de le nommer *lactose*. On reserverait le nom du sucre de lait ou de lactine pour le sucre cristallisable du lait. . ."

"Le lactose cristallise beaucoup plus facilement que le glucose. . ." [From a communication appearing in the 11 February 1856 issue of *Comptes Rendus*]

The molecular basis for cell-surface patterns governing the "social characteristics" of a cell has become a great chapter in general biology. It began, as did so many other adventures in biology, with Louis Pasteur's discovery of asymmetric molecules and their relevance to the function of the living cell. However, Pasteur's work is involved in a more direct way with the topic discussed here. A concrete example can best illustrate this. Studies on the molecular basis of human blood-group specificity (*1*) have taught us that a number of peculiar cell-surface sugars (like amino sugars, L-fucose, sialic acid) determine this specificity. For instance, the difference between blood groups A and B resides solely in the terminal sugar, *N*-acetylgalactosamine in A and D-galactose in B; otherwise, the chains are identical.

It was Pasteur who early in 1856 first pronounced D-galactose, this peculiar enanthiomorph of glucose, something "*tout autre que le glucose*," at a time when he began to focus his interest on the study of sugar fermentation. This period of Pasteur's life coincided with circumstances which, according to Dubos (*2*), contrib-

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uted to Pasteur's turning from his grandiose dreams of inducing asymmetric forces to create life anew and directing his interest in the chemistry of life toward more attainable goals. In 1856 Pasteur was professor of chemistry and Dean of Sciences of the newly created Faculty of Lille in northern France (*2*). Although he was directing his major efforts toward the study of alcohol fermentation of beet sugar, one of the most important industries of Lille, he apparently also found time to study milk sugar, as is shown by the little excerpt from his communication to *Comptes Rendus* (*3*) for 11 February 1856.

His speed of communicating his finding of a new sugar from milk surpasses present-day instant communications in *Comptes Rendus* from Institut Pasteur, or in the "Bioquickies," whether from Boston or Buenos Aires.

In his communication directed to his old mentor, academician Biot, Pasteur mentions a communication on milk sugar by Dubrunfaut which appeared in the 4 February issue of *Comptes Rendus* but first reached Lille "aujourd'hui"—that is, 11 February, one week later, on which day Pasteur submitted an account of his own studies on milk sugar.

Pasteur's observations do not vary so much from those of Dubrunfaut, whose studies Pasteur mentions with great respect. However, his formulation is very different from Dubrunfaut's. Dubrunfaut wonders about the fact that a readily crystallizable sugar from acid hydrolyzates of milk sugar is fermentable but has stronger dextrorotatory power than does the usual form of glucose. Pasteur wonders likewise about his findings of the high dextrorotation of the readily crystallizable sugar, but his intuition tells him immediately that this is not another form of glucose. This is a new sugar, and he introduces a new name for this hydrolysis product, "lactose."

Pasteur's term "lactose" applies to our present-day "galactose." It was Tanret who in an article in the *Bulletin de la Societé Chimique* in 1902 (*4*) correctly described the structure of lactose, which contains glucose, as well as the peculiar new hexose which he called galactose. Pasteur, however, fully realized the novelties of his "lactose" with respect to cell physiology. With his characteristic fervor he posed a number of questions about physiological research in connection with the new sugar.

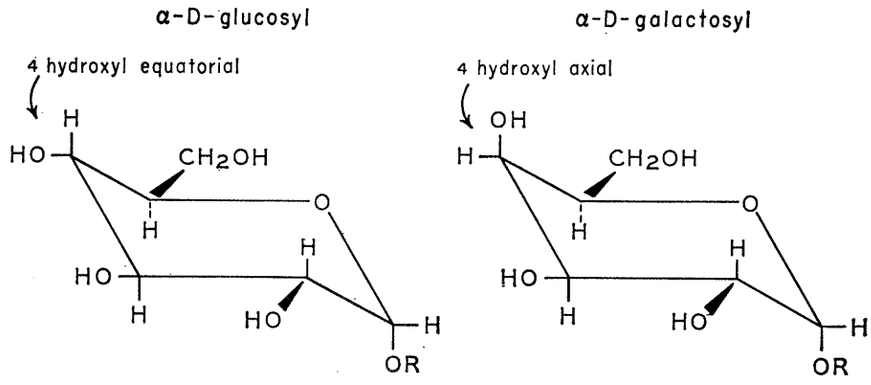

Fig. 1. Chair models of glucose and galactose.

As mentioned, the biological importance of galactose went beyond its importance as a nutrient and a metabolite. The pioneer work by Avery, Heidelberger, and Goebel about 40 years ago (*5*) first demonstrated that galactose is a component of polysaccharides, and that it can act as a specific determinant in the pneumococcal antigens.

Biosynthesis of "Active" Galactose

The difference between glucose and galactose is best illustrated by Fig. 1, which shows chair molecular models of the pyranose hexoses. No chemist has managed to achieve a conversion of glucose to galactose. Moreover, how the living cell manages this conversion is still not known. Kosterlitz (*6*) isolated galactose-1-phosphate (Gal-1-P) from liver-assimilating galactose. The conversion of this ester to α-glucose-1-phosphate (Cori ester) remained a puzzle until Leloir and his co-workers (*7*) discovered a new type of nucleotide, uridine diphosphoglucose (UDP-glucose), in which the phosphate of glucose-1-phosphate (G-1-P) is esterified to the phosphate of 5′-uridylic acid. Leloir then showed that the inversion of the 4-hydroxyl group, which he called the galacto-Walden inversion, is catalyzed by an enzyme, specific for the nucleotide as well as for the hexose. The reactions UDP-glucose ⇌ UDP-galactose are catalyzed by the specific enzyme "galactowaldenase." Since the mechanism of this inversion is not known as yet, the more neutral name "UDP-galactose 4-epimerase," or simply "4-epimerase," is being used. Moreover, the word *galactowaldenase* has been used to describe an enzymatic reaction which catalyzes the overall conversion of glucose-1-phosphate to galactose-1-phosphate. For this formulation, UDP-glucose, in the presence of galactowaldenase, is described as acting somewhat like a cofactor. This formulation, as we shall see later, is ambiguous and misleading.

It has been established that UDP-glucose is one of the crucial glycosyl donors in polysaccharide biosynthesis (*8*). UDP-galactose is active as galactosyl donor for many galactosyl compounds (*9*). UDP-glucose as well as UDP-galactose can be synthesized from glucose-1-phosphate. UDP-galactose can also be synthesized from galactose-1-phosphate. The various biosynthetic pathways can be expressed by the reactions (*10*) illustrated in Table 1, in which (Gal*) signifies 1-^{14}C-labeled galactose. It may be seen from Table 1 how the formation of labeled glucose-1-phosphate or (G*-1-P)—these are abbreviations for 1-^{14}C-labeled α-glucose-1-phosphate—depends on a number of enzymes.

In this article I refer mainly to the enzymes catalyzing steps 1, 2, 3, and 4, using the abbreviations *kinase, transferase, epimerase*, and *UDPG synthetase*, respectively. It is evident from the nature of the pathway that labeled galactose will not appear as labeled glucose-1-phosphate as early as step 2. The 4-epimerase reaction (step 3) and the release of the glucose ester from the nucleotide by a pyrophosphorolytic fission (*10*) are steps required in order to label the carbon of the glucose metabolic pool.

In yeast the biosynthesis of the enzymes catalyzing steps 1, 2, and 3 requires prolonged induction by galactose, whereas the UDP-glucose synthetase is constitutive (*11, 12*). The induction of steps 2 and 3 takes place as well in mutants of yeast or *Escherichia coli* with defective galactokinase (*11–13*). It is therefore unlikely that the induction is of the sequential type.

As may be seen from the reactions of Table 1, UDP-galactose can be synthesized either from galactose-1-phosphate (with UDP-glucose or UTP as uridyl partner) or from glucose-1-phosphate (with UTP as uridyl partner), provided there is an epimerization at the carbon-4 position (see *10*). It is important to realize here that, in order to synthesize UDP-galactose from exogenous galactose, organisms need both kinase and transferase, but not epimerase. In contrast, *only* epimerase and UDP-glucose synthetase are needed in order to make UDP-galactose from endogenous carbohydrates, since enzymes that convert reserve polysaccharides (glycogen, starch) into glucose-1-phosphate are ubiquitously present.

Furthermore, the hexose bound to such a nucleotide can undergo inversion (UDP-glucose ⇌ UDP-galactose), dehydrogenation (UDP-glucose → UDP-glucuronic acid) (*14*), or another type of reaction sequence first recognized by Ginsburg (*15*), that of reduction and rearrangement; the pathway from guanosine diphosphate mannose leading to guanosine diphosphate fucose, or related pathways (see *15, 16*), illustrates this. Mutations leading to defects in the synthesis of some of these sugars do not jeopardize cell viability and growth. This is in contrast to the phenotypic expression of mutants such as diaminopimelic acid (*17*), in which the biosynthesis of basal-layer components of the cell wall is affected.

Transferase and Epimerase in Yeast and Escherichia coli

The highest concentrations of galactose-1-phosphate uridyl transferases or UDP-galactose epimerase are obtained by growing the yeast *Saccharomyces fragilis* on nutrient medium with galactose as the only sugar. My co-workers and I have found the purified *S. fragilis* epimerase to be brilliantly fluorescent (*18*). It is possible to release DPN as well as 1.4 DPNH from the purified fluorescent enzyme (*18*). In the native *S. fragilis* epimerase, reduced DPN is incorporated in a form which brings about a greatly enhanced fluorescence and makes the enzyme operate independently of the addition of DPN (*18*). The mammalian epimerase, in contrast, must have additional DPN in order to function (*19*). The mechanism of reaction has so far remained obscure. An oxidation-reduction involving DPN and giving rise to a 4-keto sugar seems a reasonable theory, but it is as yet unproved.

Table 1. The main pathway of galactose metabolism.

Conversion	Group transfer	Specific enzyme	Step No.
Gal* + ATP → Gal*-1-P + ADP	Phosphoryl	Galactokinase	1
Gal*-1-P + UDPG ⇌ G-1-P + UDPGal*	Uridyl	Gal-1-P, G-1-P, uridyl transferase	2†
UDPGal* ⇌ UDPG*	Intramolecular transfer of H?	UDPGal 4-epimerase (galactowaldenase)	3
UDPG* + PP ⇌ G*-1-P + UTP	Uridyl	UDPG pyrophosphorylase (UDPG synthetase)	4

† An alternative pathway of minor capacity which we might call 2*a* has been described (*10*). The type of conversion is as follows: Gal*-1-P + UTP ⇌ UDPGal* + PP. The group transferred is a uridyl group. The enzyme catalyzing the conversion is Gal-1-P,PP, uridyl transferase (or UDPGal pyrophosphorylase).

The idea that an electrophilic catalysis is brought about by the uracil ring has been discussed (*10*). In such a reaction mechanism the hydrogen ion would go to the uracil ring and the hydrogen (or hydride) to the DPN. Robichon-Szulmajster has elaborated on this theory and has shown that molecular models compatible with such a reaction mechanism can be built. Figure 2 is one of her ingenious sketches. It is possible that hydrogen (or hydride) likewise goes to the uracil ring, giving a transitory dihydrouridine diphospho-4-ketohexose. This suggestion deserves more attention in view of recent experiments with UDP-glucose and UDP-galactose tritiated specifically in the hydrogen at the carbon-4 position (*18*). In these experiments it was shown that the tritiated galactose compound is converted about three times as fast to the glucose compounds as are the ordinary hexose compounds (*20*). Such a reverse isotope effect has been observed only in connection with transfer of hydrogen to nitrogen (*20*). Knowledge of the three-dimensional structure of UDP-glucose and UDP-galactose would be of great interest.

The relation of epimerase function to fluorescence is puzzling. It is possible to "make" artificially a nonfluorescent (dark) catalytically active epimerase from a fluorescent form (*18*). The dark epimerase requires DPN for catalytical activity and does not permit enhanced fluorescence although it still retains its DPNH. The epimerase from *E. coli* strain K-12 is not fluorescent, yet it contains bound DPN and DPNH (*21*). In *E. coli* strain K-12, the genes for galactose metabolism were found, by Lederberg and his coworkers, to be coupled together (*22*). The three "galactose genes" program the enzymes catalyzing steps 1, 2, and 3 in the UDP-galactose pathway (*11, 12*).

The importance of the UDP-galactose pathway in cell biology and the complex problems encountered may be particularly well illustrated by a discussion of galactose-sensitive mutants in the human species.

"Galactose-Sensitive" Human Mutants

Work initiated by Holzel and Komrower in Britain (*23*) has made it clear that congenital galactosemia is a hereditary disorder. Disease ensues if the individual ingests galactose or lactose (or other galactose-containing compounds). Tissue damage is seen most dramatically in the lens of the eye, in the form of a pure white cataract. The liver is also affected, and fatty degeneration appears often after a few months' ingestion of milk. In several instances mental retardation is known to have developed, in many cases as a result of continued ingestion of galactose. Hence, early diagnosis of a case of galactosemia is imperative. If a strict galactose-free diet is instituted early, further tissue damage is prevented and mental retardation can usually be completely prevented (see *23*).

The galactosemic organism not only spills galactose in the urine and has elevated concentrations of galactose in blood (hence the name "galactosemia") but has an accumulation of galactose-1-phosphate in the red blood cells (*24*). A lack of galactokinase can therefore be eliminated as a cause of galactosemia. It has been proposed that the disease may be due to a lack of galactowaldenase (*24*). This suggestion is justified in the sense that a block in step 3 would give rise to an accumulation of galactose-1-phosphate and would likewise block any conversion of galactose to glucose or its derivatives (glycogen, glucuronic acid, lactic acid) or block any oxidation to CO_2.

Demonstration of galactose-1-phosphate accumulation in erythrocytes in

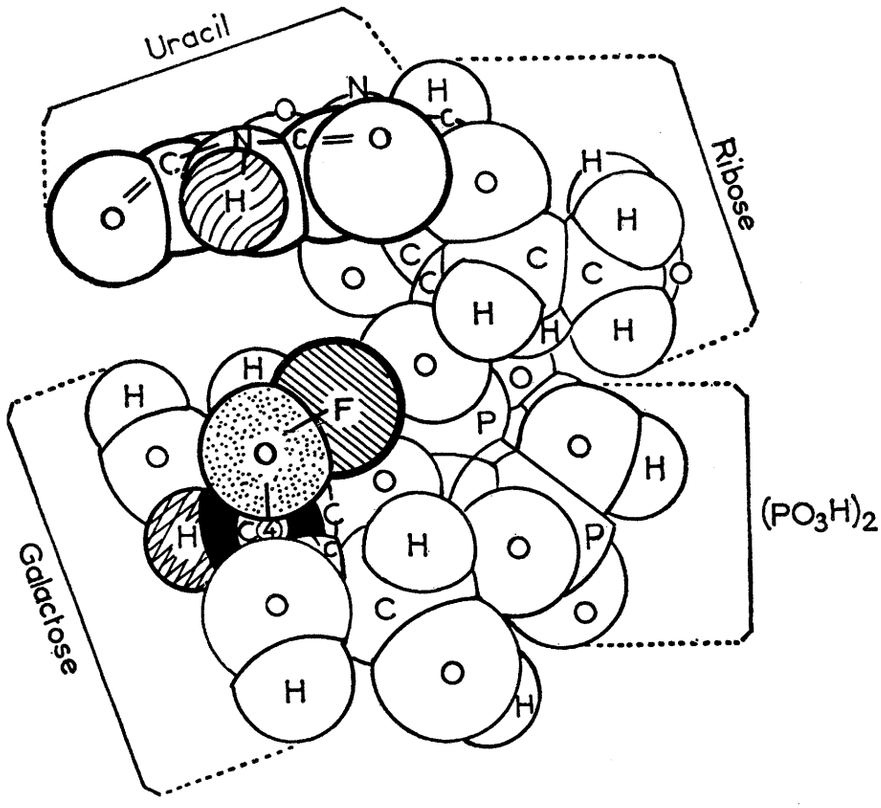

Fig. 2. Molecular model of UDP-galactose. In order to discriminate between the hydrogens on the carbon-4 of the hexose, fluorine instead of hydrogen has been placed on the 4 oxygen of the hexose. [From H. de Robichon-Szulmajster, "Contribution a l'Etude Génétique et Physiologique du Métabolisme du Galactose Chez la Levure" (Institute National de la Recherche Agronomique, Paris, 1960)]

vitro is frequently used as a basis for a diagnosis of galactosemia (*24*). It has also become customary to incubate red blood cells with ^{14}C-labeled galactose-1-phosphate and to determine the generation of ^{14}C-labeled CO_2 (*25*) as a means of diagnosis. Finally, quantitative or semiquantitative field methods of measuring respiration have been used in order to detect heterozygotes (*26*). In some of these field methods specific tests are employed (*26*); in others only the rate of generation of ^{14}C-labeled carbon dioxide from 1-^{14}C-labeled galactose is measured.

The value of the quick tests cannot be disputed. A screening of a population or a routine checking of patients would scarcely be feasible without simple methods. It is obvious from the scheme of Table 1, however, that many of the findings would be the same regardless of whether the block occurred in step 2, step 3, or step 4. I want to stress why a block in step 2 (that is, in the transferase) would be particularly relevant to an explanation of the pathogenesis of galactosemia. This may seem more obvious if I summarize some earlier observations.

Galactosemic children maintained on a strict galactose-free diet are found to have a much better chance of developing normally than those not on such a diet, although their galactose metabolism remains abnormal. On such a diet they do not develop cataracts, and their intelligence quotients are normal or close to normal (see *23*). In any case, the I.Q.'s are much higher than those of galactosemic children on an ordinary milk diet (see *27*).

In this connection, one should bear in mind the fact that the central nervous system, especially the brain, contains large amounts of galactolipids, most of which are deposited during the first months after birth (*28*).

Several authors have formulated the galactowaldenase reaction in a manner which is most confusing, or at least not very discriminating. I refer to formulations such as the following, mentioned earlier:

$$\text{Gal-1-P} \xrightarrow[\text{galactowaldenase}]{\text{UDPG}} \text{G-1-P} \quad (5)$$

Reaction 5 indicates that the conversion of galactose-1-phosphate to glucose-1-phosphate is catalyzed by one enzyme, galactowaldenase, with UDP-glucose serving as a "coenzyme." It appears that methods of diagnosing galactosemia are frequently based on reaction 5. A block in reaction 5 would in some way not only jeopardize the catabolism of galactose and cause accumulation of galactose-1-phosphate but would also interfere with the deposition of galactose in oligosaccharides and in galacotolipids. A galactosemic infant on a galactose-free diet who had a block in reaction 5 might have difficulty in depositing the proper amount of brain galactolipids. Glucolipid synthesis might compensate, albeit with possible disastrous results in terms of mental development.

The fatal drawback in the summarized formulation of the galactowaldenase reaction (reaction 5) is its inability to discriminate deficits in uridyl transferases from deficits in epimerase, which would produce entirely different types of alternations as regards polysaccharides.

In 1955 my associates and I introduced a number of specific enzyme assays (*28*), for galactose-1-phosphate, UDP-glucose uridyl transferase, 4-epimerase, and UDP-pyrophosphorylase, respectively, to be used on human blood cells.

By these methods the various blocks in the galactose pathway may be discerned. In all the individuals with congenital galactosemia tested so far by these specific methods we have found a deficit only in Gal-1-P,G-1-P, uridyl transferase—that is, a blockage in step 2, (*28, 29*) and not in step 3 (involving 4-epimerase). We also investigated the concentration of UDP-glucose pyrophosphorylase and found no deficit (*29, 30*). The blockage in step 2 accounts much better for the fact that a galactose-free diet has a favorable effect on these human mutants than a blockage in steps 3 or 4 would. This favorable effect is due to the fact that these individuals have not lost the capacity to synthesize "active" galactose from glucose by way of epimerase, thus circumventing an accumulation of galactose-1-phosphate.

If the specific enzyme assays were to be entirely abandoned in favor of quicker but less specific methods, a possible occurrence of human hereditary deficits of epimerase or of UDPG-pyrophosphorylase would very likely be overlooked. A mixing up of galactose-metabolism defects would be so much more likely, because many of the symptoms due to galactose-1-phosphate accumulations are strikingly similar,

whether we are dealing with deficits in transferase, epimerase, or UDPG-pyrophosphorylase (*31, 32*). It is also desirable to use specific enzyme tests in tissue-culture studies (*33*).

Existence of Galactose-Defective Mammals?

Let me sketch a little science fiction to illustrate the various fundamental aberrations which might occur in an epimeraseless organism. I will choose as an example a lactating human female with an epimerase deficit, in order to ponder the possible consequences and manifestations of such an enzyme deficit. This human mutant would obviously be unable to metabolize galactose, and this inability would be apt to cause cell damage because of the ensuing accumulations of precursors (galactose-1-phosphate, UDP-galactose). Hence, if the female mutant had been recognized early in life as a sibling from a "galactose-sensitive" family she would have been on a strictly galactose-free diet right from birth.

This might, however, have produced a new and different type of affliction. Owing to the lack of epimerase, she would be unable to synthesize "active" galactose from endogenous carbohydrates. For this reason her brain would presumably not have been able to form galactolipids, or it would have formed an insufficient amount, with perhaps partial replacement of galactolipids by glucolipids. Her brain might in time contain mainly glucolipids, with no galactolipids, or only trace amounts (*34*). Such a state might bring about a cessation of normal brain development, hence an extreme case of mental retardation. The effect on the brain is obviously a most unpredictable feature. The intelligence of the unhappy (or merry?) mutant might be lower than a mermaid's or as high as one of Szilard's dolphins. Her "glucolipid brain" might even reach heights of development undreamt-of. As an adult, she might be able to give birth to children (normal or abnormal), but her milk would probably be grossly lacking in lactose. The milk sugar might be completely or partly composed of glucose, or of cellobiose, the glucose analog of lactose. Her blood group picture might be grossly defective, with complete or partial loss of blood groups A, B, and so on. It is of course also difficult to make extrapolations concerning her transplantation pattern, since no one knows as yet whether sugars play any role in the specificity of antigens of the type involved. But if they do, spleen transplants of such an epimeraseless individual might be hazardous for any normal siblings she might have, since the recipients might not be able to discard the epimeraseless transplant. Such a transplant might, rather, react against the normal host in a graft-versus-host reaction [as in runt disease (see *35*)]. In any case, if the cell sociology and cell-immunological patterns depend partly on the presence of "odd" sugars, epimerase deficits are apt to alter these patterns. Finally, susceptibilities to infections, especially virus infections, might be greatly altered.

These considerations should not be taken entirely as a joke. Studies on hereditary defects in galactose metabolism in microorganisms illustrate this particularly clearly.

Some Alterations of Social Patterns in Microorganisms

As I mentioned earlier, Lederberg and his co-workers isolated *Escherichia coli* strains with hereditary galactose metabolism defects (*22*) and found the three genetic loci controlling galactose metabolism bunched together and transducible through a bacteriophage "λ gal" (*22, 36*). I also mentioned that the three genetic loci correspond to the genes controlling the synthesis of the enzymes galactokinase, transferase, and epimerase (see *13*). Galactose has a cytotoxic effect on the transferase-deficient *E. coli* which is reminiscent of its effect on the tissues of transferase-deficient humans (*23, 37*). Galactose has a bacteriostatic effect on transferaseless *E. coli* (*31*), and this mutant accumulates galactose-1-phosphate (*31*).

When the galactose-negative K-12 mutants were first surveyed about 8 years ago, no simple epimerase-deficient microorganism had been isolated, and attempts were being made to select for such defects in type XIV pneumococci (see *11*) through the use of antiserum against the galactose-rich polysaccharide XIV capsules. However, events were to take another turn. During a visit to Tokyo in 1957, I had the pleasure of discussing my observations with Nikaido and Fukasawa, who for some time had been interested in studying certain types of *Salmonella typhimurium* mutants lysed in the presence of galactose in the growth medium. Fukasawa and Nikaido found that these mutants, which are unable to ferment galactose, accumulate galactose-1-phosphate and UDP-galactose if the growth medium contains galactose (*38*). After one to two generations in nutrient medium, lysis occurs (*31, 39*). In hypertonic medium, formation of protoplasts could be demonstrated (*39*). The enzyme defect was traced down to a single defect, a lack of epimerase (*13, 38*). Subsequently Nikaido and Fukasawa concentrated their efforts on making a chemical and biological study of the alterations of the cell-wall characteristics of these epimerase-deficient mutants. They discovered a number of features which were novel in the sense that they had never before been placed in a logical context. The outcome of their studies almost surpassed the science-fiction sketch just given.

It was found that epimeraseless *Salmonella* mutants grow very well indeed in the absence of galactose. Their cell wall, although fully competent, undergoes many changes. The colony morphology changes from smooth to rough. The surface antigenic pattern [Kauffmann-White O-antigen pattern (*40*)] is almost completely erased. The most revealing studies were perhaps the chemical studies of the cell wall. The classical work by Westphal and Lüderitz and by Staub (*41*), as well as later studies along these lines (*42*), has taught us that the cell wall of *Salmonella* and of various *Escherichia coli* strains contains lipopolysaccharide consisting of an inner backbone (containing the lipid and phosphate), a so-called core, containing 2-keto-3-deoxyoctonate, heptose, *N*-acetylglucosamine, glucose, and galactose, and side chains of varying composition. In *S. typhimurium* the side chains contain galactose, L-rhamnose, and some odd 3,6-dideoxyhexoses (*41, 42*). Nikaido was able to show that most of the side chains—that is, dideoxyhexoses and rhamnose as well as galactose—are absent in epimeraseless mutants. Only glucose and the inner core were preserved (*38*).

The dideoxy sugars accumulate in the form of their respective nucleotides. Nikaido isolated cytidine diphosphoabequose and cytidine diphosphotyvelose, two of the "active" 3,6-dideoxy sugars (*43*), and was also able to demonstrate the incorporation of these sugars in vitro (*44*). Perhaps the most important biological experiment by Nikaido and Fukasawa illustrating

the role of galactose and the mono- and dideoxyhexoses in the sociology of the cell was the following (*45*). An epimeraseless mutant of *Salmonella typhimurium* was incubated for 20 to 30 minutes in a growth medium containing galactose. After 30 minutes the galactose-containing medium was replaced by ordinary growth medium. During the short exposure to galactose, UDP-galactose was synthesized by the cells from the exogenous galactose and was transferred to the incomplete cell wall; this was followed by the incorporation of other sugars located more peripherally in the lipopolysaccharide. As a result, the sociological characteristics of the epimeraseless mutant changed in the direction of normalcy. The immunological patterns characteristic of the smooth wild type returned. Likewise phage P-22 was able to absorb to the host. The further development of the phage inside the cell was independent of the presence of galactose.

The alterations (or even obliterations) of immunological pattern or of phage receptor sites are the result of changes in the polysaccharides on the exterior of the cell wall. For this reason we might call these polymers "ektopolymers" (from Greek *ektos*, meaning exterior) or "ektopolysaccharides," and the biological characters so affected, "ektobiological" characters. This name is more noncommittal than expressions like "surface immunological patterns" or "social patterns" or "recognition patterns." Although it is difficult to make a clear distinction, we still do not mean to include the characteristics of the rigid basal layers of the cell wall (mucopeptides) among ektobiological characteristics. Inability to make the building blocks of the basal layers usually is incompatible with viability (in some cases, slow-growing, so-called L-forms, are developed). In contrast, inability to synthesize one or more of the units of ektobiological polymers does not jeopardize viability, but merely alters the sociological characteristics.

Cell Sociological Patterns of Strain K-12

Among the *Escherichia coli* strains, the so-called strain K-12 shows particularly interesting social characteristics in that the cells are susceptible to infection with phages, such as lambda (*36*) or P-1 (*46*), which can become integrated in the genetic apparatus. These strains are susceptible to transduction of galactose genes [through λ Gal (*36*)] and can carry sex characteristics (F+ characters). The lipopolysaccharides of *E. coli* strain K-12 have been the subject of recent investigations (*32*). Since only occasional references have been made to these studies, I shall briefly describe some of the problems.

A number of K-12 mutants unable to ferment galactose ("gal negatives" on EMB agar plates) have lately turned out to have altered sociological patterns with respect to two more or less virulent phages, P-1 and the recently discovered C-21 (*47*). Mutants lacking galactokinase or galactose-1-phosphate uridyl transferase do not show any aberrant patterns with respect to these phages as compared with "gal positives" (*32*). However, epimeraseless K-12 mutants demonstrate a different social pattern. These mutants can be attacked by a phage C-21 (*48*). Since it is now known that the epimeraseless K-12 mutants have retained 50 percent of the galactose of the lipopolysaccharide and essentially all the glucose and rhamnose (*48*), it seems apparent that even minor defects in the lipopolysaccharides render K-12 cells susceptible to C-21. Mutants unable to synthesize UDPG (*49, 32*) can likewise be infected by C-21 (*47*). The lipopolysaccharides of the UDPG-defective mutants have lost practically all their glucose, galactose, and rhamnose (*32*). However, in view of the observations on epimeraseless K-12 mutants, loss of glucose or rhamnose seems not to be a prerequisite for susceptibility to C-21. On the other hand, phage P-1, which is able to infect most K-12 strains (including most "gal negative" K-12 mutants, even the epimeraseless ones) is unable to infect the UDPG defectives (*46*).

The correlation between F characters (male or female) and the sugars of the lipopolysaccharides is probably quite different. Neither the epimeraseless mutants nor the UDPG defectives are affected (*32, 49*). It seems that either these sugars are unimportant and only the inner core may be essential for F characters or that other surface polysaccharides may play a role. It is noteworthy, for instance, that the UDPG-defective mutants are able not only to make glycogen (*50*) but also to synthesize some complex glucose containing polysaccharides different from glycogen as well as lipopolysaccharides (*51*). It is not known whether these polysaccharides which contain glucosamine as well as glucose (*51*) are surface polysaccharides and involved with the biosynthesis of F+ or F− recognition sites. In connection with this problem it might be of interest to examine a third type of mutants, which are unable to use glycerol or fructose in glucose catabolism, yet are viable in the presence of these two polyols as sole carbon sources. These mutants, which were first isolated in Stocker's laboratory at the Lister Institute, were shown to lack the enzyme phosphoglucoisomerase (*52*). The lipopolysaccharide of this type of *Salmonella* mutant lacks glucose, galactose, rhamnose, and several other sugars but retains heptose, 2-keto-3-deoxyoctonate, and glucosamine (*53*).

The molecular basis of F characters is still unknown; carbohydrates may not be involved at all. Nevertheless, the observations by Sneath and Lederberg (*54*) have shown that metaperiodate in low concentrations rapidly inactivates the "male" surface characters. This fact may suggest involvement of sugars for the F+ phenotype. It would be interesting to try to study F+ character on isomeraseless mutants in synthetic media with fructose or fructose plus glucose as carbon sources. The question arises as to whether a fructose or glycerol medium would be able to sustain the F+ phenotype in such mutants. In any event it seems worth pointing out that, if the isomeraseless mutants are not strikingly leaky, their viability in glycerol ammonia medium is fully retained.

Most recently, Brinton has demonstrated the existence of specific "F-pili" in F+ cells. Ordinary pili are found in F+ as well as in F− cells. However, the long pili which also attract "F+-specific" phages occur only in F+ ("male") *Escherichia coli* (*55*).

Alterations of Cell Social Characteristics by a Prophage

A remodeling of the ektopolysaccharide structure occurs from other causes as well as from defects due to mutations. Robbins and Uchida (*56*) have described a novel type of remodeling due to a repression of an enzyme (a galactose-O-transacetylase) brought about by a prophage which is incorporated into the host (*Salmonella anatum*) as an episome. This episome

brings with it a gene which programs the synthesis of a repressor of the host transacetylase. When the transacetylation of galactose ceases, the synthesis of the ektopolysaccharide follows a somewhat different pattern, which Robbins and Uchida could account for both chemically and immunologically. This work may furnish some novel and fruitful ideas on cell sociology in higher organisms (in areas such as differentiation and malignancy).

Cell Sociology and Abnormal Developments in Tissues

The social characteristics of the cell surface of higher animals have been investigated in numerous studies ever since the pioneering work of Holfreter and Paul Weiss. A review of this large field is beyond the scope of this article, as is a review of the studies on the ektobiology of the fertilization process (*57*). Therefore I shall attempt to summarize current thinking, in the field of microbiological surface immunology, on problems dealing with differentiation, placing special emphasis on neoplastic (malignant) growth. Regardless of whether malignancy is accompanied by a loss of organ-specific surface antigens (*58*) or by the acquisition of new surface antigens (*59*), it seems worthwhile to try to bring to bear on the subject of differentiation in higher organisms ideas generated from observations on the social characteristics of bacterial mutants.

One could draw many parallels between the cell surface patterns in microorganisms and in mammalian cells. Gottschalk's discovery of sialyl oligosaccharides and sialidase was the first biochemical approach to the subject of virus receptor sites. This venture into cell sociology was initiated by Hirst and Burnet. The exact and specific role which sialic acid plays in the operation of the virus receptor site for influenza virus is not known. Sialidase (neuraminidase), which hydrolyzes the sialyl-galactose linkage (*60*) (as well as the sialyl-*N*-acetylgalactosamine linkage), renders the receptor sites inactive. Earlier studies on phage receptor sites by Jesaitis and Goebel and by Weidel illustrated features strikingly related to those seen in the adsorption of animal viruses to animal cells. In one instance (the study by Fukasawa and Nikaido mentioned above) it could be shown that oligosaccharide side chains containing galactose and deoxyhexoses are required for an effective adsorption of phage P-22 to the *Salmonella* host.

It is known that mammalian cells contain complex galactosyl oligosaccharides tied to lipids as well as to proteins. The blood-group substances have already been mentioned. The so-called cytolipins contain lactose and ceramides. However, it has not been settled as yet whether the latter are specific cell-surface components. They have been demonstrated mainly in human tumor cells (*61*); they may, however, be as abundant, if not more so, in normal cells. No data on this point are at present available.

Membranes have been isolated recently from mammalian cells. For such studies, the most useful material has been Ehrlich ascites tumors (*62, 63*). Wallach and his co-workers (*63*) have been able to provide a rather wide characterization of the membrane as an "ekto" membrane, in contrast to the endoplasmic reticulum. Sharp separation was achieved in an inert polymer by density gradient equilibrium centrifugation (*63*). The development of these techniques may pave the way for more exact biochemical studies of the sociology of animal cells and in particular for studies of the malignant cells. The isolation of membranes from well-characterized clones such as George Klein's benign and malignant ascites sarcoma will, presumably, become a step of particular importance.

The membranes isolated contain proteins, sialic acid, and various sugars (*63*), among them galactose (*64*). The chemical basis of cell-surface-specific patterns, such as Moscona's organ-specific surface patterns or Yaffe and Feldmann's specific recognition patterns for myofibrils, is still unknown (*65*).

Loss of organ-specific surface antigens has been demonstrated in hepatomas and kidney tumors in studies with Coon's fluorescent antibody technique (*58*). Loss of blood groups, as demonstrated by Coombs's coagglutination technique, has been reported in human bladder carcinoma (*66*). It seems indeed worthwhile to extend these studies to human skin epithelium.

Since the UDP-galactose pathway presumably plays a major role in the biosynthesis of mammalian surface polysaccharides, including blood groups, a study of the UDP-galactose pathway in various types of growing cells and especially in tumor cells seemed of considerable interest. The outcome of these studies showed that a number of tumor cells were highly defective in galactose metabolism (*67*). It had been reported previously (*68*) that L-cells and HeLa cells do not contain detectable amounts of epimerase. However, we found in our own studies that extracts from L-cells as well as from HeLa cells contain appreciable amounts of epimerase. Rather, the "input" of galactose-1-phosphate as catalyzed by transferase (Table 1, step 2) was rate-limiting and very low. Nevertheless, in the intact cells, in which the transferase reaction also proceeds very slowly, the rate-limiting reaction is in fact the epimerase reaction. The capacity of the epimerase reaction is indeed so low (0.1 to 0.3 mμmole for 10^6 cells per hour at 37°C) that one may question whether it can keep up with the biosynthesis of the various galactose compounds found in mammalian cells, especially in rapidly growing cells. This problem can now be studied by means of Wallach's techniques for isolating membranes from tumor cells. These extremely low rates of transferase and epimerase reaction have been observed in C_3H mammary carcinoma, HeLa cells, Ehrlich ascites carcinoma, and L-cells. In regenerating liver the epimerase reaction proceeds at a rate which is at least 5 to 10 times that observed in Ehrlich ascites tumor cells or L-cells. A possible cellular "feedback" inhibition of the epimerase reaction and its correlation to aerobic glycolysis await study. Likewise, a possible remodeling of the cell-surface oligosaccharide because of the scanty formation of galactosyl compounds from the metabolic pool in tumor cells deserves investigation. In any event, lymphocyte studies made by means of glycosidases (*69*), along the lines of studies by Watkins and Morgan on the hydrolysis of blood-group substances, point to the importance of L-fucose and *N*-acetylgalactosamine for the existence of recognition sites on these cells. The sequence of oligosaccharides of glycoproteins (whether of the mannose type or of the sialyl galactose type) is being successfully worked out (*70*). The possibility that the oligosaccharides affect the conformation of the protein should also be seriously considered. In fact, conformational changes of glycoproteins may well be crucial for a variety of cell social characteristics.

The primary factor in growth regula-

tion may well depend on a specific contact between cells (*71*). Such a contact inhibits the protein-synthesizing apparatus by lowering the population of polysomes (*72*). Tumor cells seem to have lost an important link in this sequence of events which commences with cell contact (*71*). In tumor cells the contact itself seems disorderly (*73*), hence, ektobiological patterns may be crucial for normal cell contact and growth control. Surface charge and decrease in charge after removal of sialic acid by treatment with neuraminidase is clearly detectable in most tumor cells, whereas in normal cells under identical conditions no decrease in charge is detectable (*74, 75*). Whether the clue to the etiology of malignancy may be provided by analogies to be found in the field of microbiology is a question for the future. In an attempt to understand uncontrolled growth, aspects concerning cell surface recognition patterns and their possible role in controlled and uncontrolled growth may well pose problems of particular relevance to an understanding of cell population dynamics in higher organisms.

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Changes in Scientific Activities with Age

The life of an established scientist changes little over the years—unless he goes into administration.

Anne Roe

In the years 1947 to 1949 I studied a group of eminent research scientists (*1*). My reasons for seeking them out then were to find out if scientists differ in any consistent ways from nonscientists, or if different kinds of scientists differ consistently from each other, and to find out why they became scientists rather than something else.

In 1962 and 1963 I interviewed these same men again. I was concerned to learn what changes had taken place in the nature or amount of their scientific work, in the pattern of their lives generally, and in their opinions about such things as the nature and management of research activities (*2*).

The highlights of the earlier study can be reviewed briefly. There were 64 men in the group, 20 in the biological sciences, 22 in the physical sciences, and 22 in the social sciences. They were selected by their peers for the excellence of their scientific contributions. At that time their average age was 48, all of them were married, and most of them had children. Five were from Jewish homes, one was from a home of free-thinkers, and the rest had Protestant backgrounds. Just over half of them had fathers who were in professional occupations; none were sons of unskilled laborers, and none were from very wealthy, aristocratic families.

There were some characteristic patterns in their early histories. Most of the social scientists were socially active from an early age. Most of the others were rather shy, socially late-maturing boys, with strong hobbies and noticeable persistence in them. With the exception of some of the experimental physicists, all of them were voracious, if unselective, readers throughout their childhoods. More of the natural scientists regarded their fathers with great respect but felt somewhat distant from them. More of the social scientists had had strong conflicts in the family.

These scientists are of extraordinarily high intelligence. On a verbal test, their median was about equivalent to an IQ of 166, with the lowest about 121. That seems to be about a minimum IQ for a research scientist; higher levels are no drawback, but above that level other aspects of the person's nature or endowment may become more important. Perhaps the most influential of these other aspects is motivation—the degree to which the individual's work is important to him. All of these men are, and have always been, so immersed in their work that other considerations play much smaller roles (*3*). They give a picture of hard-working, driven, and devoted men, but they are these by choice. For the most part they spend their time doing what they want to do, and they always have. It is this and the respect of their peers that repays them, for their financial rewards are far from commensurate with the contributions they make to society.

Of the 64 men originally studied, 54 are still living; two of the biologists, three of the physicists, one of the psychologists, and four of the anthropologists have died. Some of the 54 had retired, some had moved to other institutions, but I was able to see all but two, who were in Europe at the time. From one of these I received a long report. Most of the interviews were tape-recorded.

Their present ages are from 47 to 73. There are 17 over 65, and 11 of these are biologists. The age difference is of more significance now than it was 15 years ago, because of the retirement issue.

Many scientists move about from one institution to another and stay for varying periods in various places, but, aside from wartime assignments and visiting professorships, 16 of these men (ten of them physical scientists) have stayed at one institution. Two biologists have been at seven different institutions, and one biologist and one social scientist, at six.

Honors

Many honors have come to these men, in addition to the visiting professorships and lectures, which are in themselves honorific. The most obvious are medals, prizes and awards, honor-

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