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MUTATIONS IN *ESCHERICHIA COLI* THAT AFFECT URIDINE DIPHOSPHATE GLUCOSE PYROPHOSPHORYLASE ACTIVITY AND GALACTOSE FERMENTATION

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SUMMARY

The metabolism of galactose was studied in two galactose-non-fermenting mutants which were isolated from *Escherichia coli* strain K12 and classified genetically as Group E by Drs. J. AND E. M. LEDERBERG. One of them, W4597, was found to have a single defect in UDPG pyrophosphorylase (UTP: α-D-glucose-1-phosphate uridylyltransferase, EC 2.7.7.9) and to be normal in galactokinase (ATP: D-galactose 1-phosphotransferase, EC 2.7.1.6), Gal-1-*P* uridyl transferase (UDP glucose: α-D-galactose-1-phosphate uridylyl transferase, EC 2.7.7.12), UDP galactose 4-epimerase (UDP glucose 4-epimerase, EC 5.1.3.2), phosphoglucomutase (D-glucose-1,6-diphosphate: D-glucose-1-phosphate phosphotransferase, EC 2.7.5.1), and also in galactose permease. Non-fermentation of galactose in this strain was, therefore, ascribed to the defect of UDPG pyrophosphorylase. The other strain, W3142, was shown to have a secondary defect in galactokinase in addition to a defect in UDPG pyrophosphorylase. When grown in the presence of galactose, W4597 accumulated intracellularly a large amount of Gal-1-*P*.

The intracellular levels of UDPG and uridine diphosphate galactose were shown to be much lower in both of these strains than in wild-type strains, irrespective of the presence or absence of galactose in the growth media. The sugars of their cell walls were composed only of an aldoheptose, hexosamines, and a smaller amount of glucose; any traces of galactose, which is present in wild-type cell walls, were not detected in their cell walls.

INTRODUCTION

The metabolism of galactose in man and microorganisms has been shown by LELOIR, KALCKAR, and their associates to proceed through the following pathway[1,2]:

Galactokinase (kinase):	Galactose + ATP $\longrightarrow$ Gal-1-*P* + ADP	(1)
Gal-1-*P* uridyl transferase (transferase):	Gal-1-*P* + UDPG $\rightleftharpoons$ UDPGal + Glc-1-*P*	(2)
UDPGal-4-epimerase (epimerase):	UDPGal $\rightleftharpoons$ UDPG	(3)
In sum:	Galactose + ATP $\longrightarrow$ Glc-1-*P* + ADP	

Abbreviations: UDPGal, uridine diphosphate galactose; DAP, α,ε-diaminopimelic acid; Gal$^+$, galactose fermenting; Gal$^-$, galactose non-fermenting; Me$^+$, methionine independent; Me$^-$, methionine requiring.

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As shown in these equations, it is necessary for the three enzymes in Steps 1, 2, and 3 to operate in order to convert free galactose to a glycolytic intermediate. The catalytic amount of UDPG needed for this conversion can be supplied by another enzyme, UDPG pyrophosphorylase (EC 2.7.7.9), as follows[3]:

$$UTP + Glc\text{-}1\text{-}P \rightleftharpoons UDPG + PP_i \quad (4)$$

As reported in a previous paper[4], we found that two mutants of the galactose-non-fermenting strains of *E. coli* K12 isolated by LEDERBERG and his associates have diminished activity of UDPG pyrophosphorylase. It was also reported that these two mutants had no detectable amounts of hexoses in their cell walls, possibly because of their inability of synthesizing a normal level of UDPG (see ref. 4).

This report deals with the quantitative analysis of the cell wall and the implications of these findings from the genetic point of view. Some experimental conditions in the preceding paper will be described in detail.

MATERIALS AND METHODS

Bacterial strains

Bacterial strains used were derivatives of *E. coli* strain K12, originated from Dr. J. LEDERBERG's laboratory at Stanford University. Table 1 summarizes the relevant properties of these strains. For preparation of UDPGal, a UDPGal-4-epimerase-less strain of *E. coli*, called C7M (ref. 5), was used. This was the kind gift of Dr. I. FUKUTOME of Showa Medical College.

TABLE I

PROPERTIES OF BACTERIAL STRAINS

The designations are according to E. M. LEDERBERG. All strains except W4520 are F^-, lambda sensitive, and prototroph. W4520 is F'-Gal^+, lysogenic for lambda, and methionine requiring.

Strain	*Mutational site*	*Group*
W3110	Gal^+	Wild type
W3102	Gal-2	A
W4242	Gal-15	B
W3780	Gal-3	C
W3109	Gal-9	C
W3805	Gal-22	D
W3350	Gal-1,2	A, B
W4597	Gal-23	E
W3142	Gal-19	E
W4520	Gal^+	

Culture media

Nutrient broth (Kyokuto meat extract, 10 g; Daigo polypeptone, 10 g; NaCl, 2 g; water to 1000 ml; pH 7) was used routinely. For the determination of intracellular levels of UDPG, UDPGal, and Gal-1-*P*, tryptone broth (Bacto tryptone, 10 g; NaCl, 5 g; water to 1000 ml) was used. BTB–galactose medium (nutrient agar containing bromthymol blue and 2 % D-galactose (Difco)) containing 1000 μg/ml streptomycine and EMS*-galactose agar[6] were used for detecting F-ductants.

* Eosine methyleneblue synthetic.

Chemicals

D-Galactose (Difco) was recrystallized twice from 70 % ethanol for biochemical experiments. Gal-1-*P* (barium salt), ATP (sodium salt), DPN, TPN, and UDPG (sodium salt) were purchased from Sigma Chemical Co. UDPGal was isolated from C7M cells grown in tryptone broth containing 0.2 % galactose according to a modified procedure of WIESMEYER AND JORDAN[7]. Glc-1,6-P_2 (barium salt) was the kind gift of Dr. L. F. LELOIR. All the solutions were neutralized before use, and when they were barium salts, barium was removed as $BaSO_4$. DAP, glucosamine·HCl, trypsin, (EC 3.4.4.4), and RNAase (EC 2.7.7.16) were purchased from Mann Research Laboratories, Ishizu Seiyaku Co., Sigma Chemical Co., and Worthington Chemical Co., respectively.

Indicator enzymes

Indicator enzymes, Gal-1-*P* uridyl transferase (EC 2.7.7.12), UDPG dehydrogenase (EC 1.1.1.22), phosphoglucomutase (EC 2.7.5.1), and Glc-6-*P* dehydrogenase (EC 1.1.1.49) are the same preparations as described by KALCKAR *et al.*[5]. Galactokinase (EC 2.7.1.6) and UDPGal-4-epimerase (EC 5.1.3.2) are partially purified from W3094 (ref. 5) and W3350 cells, respectively (unpublished methods by K. KURAHASHI and K. JOKURA). Hexokinase (EC 2.7.1.1) (Type III) was purchased from Sigma Chemical Co.

Preparation of extracts for the assay of enzymes

Cells grown overnight in 50 ml nutrient broth were diluted 10-fold with fresh broth and were agitated on a rotary shaker at 37°. The growth was followed by absorbancy readings at 660 mμ, and during the exponential phase of growth, D-galactose (final concentration 0.1 %) was added in order to prepare the induced cells. After 2 h, the bacterial culture was rapidly chilled in ice water, and the cells were resuspended in 10–15 ml of 0.05 M potassium phosphate buffer (pH 7.4). The suspension was treated with a sonicator (Kubota Co., Ltd.) at 10 kcycles for 5–10 min in the cold. The clear supernatant, obtained by centrifugation at 15000 × *g* for 15 min in the cold, was used for the assay. Extracts from non-induced cells were prepared in the same way, but without addition of galactose. Protein was determined according to the method of LOWRY *et al.*[8].

F-duction experiment

Transfer of the autonomously replicating F-factor carrying host chromosomal fragments is referred to as F-duction[9]. An F-factor carrying galactose genes (F'-Gal) was transferred from F'-Gal$^+$ strains to F$^-$ strains by incubating together at 37° for 1 h without shaking equal amounts of exponentially growing broth cultures of both strains. The mixed culture was plated on proper selective media which can differentiate donor and recipient cells.

Assay of the enzymes in LELOIR's *pathway*

This was carried out according to the specific enzymic method described by KALCKAR *et al.*[5].

Assay of UDPG pyrophosphorylase

This was performed essentially according to the procedure of MUNCH-PETERSEN *et al.*[3].

The reaction mixture for the assay consisted of cysteine·HCl solution (33 mg/ml, pH 7.5), 30 μl; $MgCl_2$ (1 M), 5 μl; Tris buffer (0.5 M, pH 7.5), 60 μl; UDPG (10 μmoles per ml), 25 μl; pyrophosphate (0.1 M, pH 7.5), 10 μl; Glc-1,6-P_2 (0.15 μmole/ml), 10 μl; TPN (30 μmoles/ml), 10 μl; phosphoglucomutase (7 units/ml), 10 μl; Glc-6-*P* dehydrogenase (7 units/ml), 10 μl; crude extract to be determined or its appropriate dilution with water solution of bovine albumin (20 mg/ml), 10 μl; water, 440 μl. The total volume was 620 μl. The reaction was started by the addition of pyrophosphate. Controls having either UDPG or pyrophosphate omitted from the incubation were always used. Reoxidation rate of TPNH in the extracts was much slower than the rate of TPN reduction, so that the initial rate of formation of TPNH could be used as a direct measure for UDPG pyrophosphorylase activity of the disrupted bacterial cell.

Assay of phosphoglucomutase

The reaction mixture for assay contained: cysteine·HCl solution (33 mg/ml, pH 7.5), 100 μl; $MgCl_2$ (1 M), 10 μl; Tris buffer (0.5 M, pH 7.5), 100 μl; Glc-1,6-P_2 (0.15 μmole/ml), 10 μl; Glc-1-*P* (0.1 M), 30 μl; TPN (30 μmoles/ml), 10 μl; Glc-6-*P* dehydrogenase (7 units/ml), 10 μl; crude extract to be determined or its appropriate dilution with water solution of bovine albumin (20 mg/ml), 10 μl; water to bring the total volume to 1.0 ml. The initial rate of formation of TPNH was used as a direct measure for phosphoglucomutase activity.

Enzymic determination of UDPG, UDPGal, and Gal-1-P

Cells were grown in tryptone broth. Exponentially growing cells, in absence, or presence of D-galactose for 2 h, were harvested and extracted with 70% ethanol (approx. 10 ml/100 mg dry wt. cells) at 70° for 5 min. After removal of ethanol by addition of ethyl ether, samples were lyophilized and dissolved in a small volume of water.

UDPG was determined by the method of KALCKAR *et al.*[10]. UDPGal was determined by the method of MAXWELL[11], utilizing UDPGal-4-epimerase partially purified from W3350. Determination of Gal-1-*P* was carried out according to the procedure of KURAHASHI AND ANDERSON[12].

Isolation of cell walls

This was accomplished essentially according to the procedure as described by SALTON AND HORNE[13]. The harvested and washed cells in post-exponential phase of growth were suspended in distilled water and were treated with a sonicator at 10 kcycles for 3 min. Then, undisrupted cells were separated by centrifugation at 3500 × *g* for 10 min, resuspended in distilled water, and again sonicated under the same conditions as above. The suspensions of disrupted cells were collected and centrifuged at 20000 × *g* for 20 min. The pellet was suspended in 0.05 M phosphate buffer (pH 7.7) and treated with crystallized trypsin (1 mg/ml) at 37° for several hours. Then the digestion was continued overnight after the addition of a few drops of chloroform. The disrupted cells were harvested, washed with water and then treated

with RNAase (0.1 mg/ml) in 0.05 M phosphate buffer (pH 7.5) at 37° for 5 h. After centrifugation, the pellet was resuspended in water and centrifuged again for 10 min at 3500 × *g*. The pellet, composed of undisrupted cells was discarded. The supernatant was again centrifuged at 20000 × *g* for 20 min. The pellet was freed from remaining intact cells by repeating three times the cycles of high- and low-speed centrifugation as described above. The cell walls thus obtained were suspended in water with a few drops of chloroform and were stored in a refrigerator. They were examined under the electron microscope* with chrome shadowing. With the strains used, the yields of the cell walls were 2–4 % of the dry weight of cells.

Quantitative determination of amino sugars

Cell walls were hydrolyzed with 1 N HCl at 105° for 18 h in a sealed tube. Quantitative determination was performed as described by BOAS[14] with glucosamine·HCl as standard.

Quantitative determination of DAP

Washed cell walls (10–15 mg) were hydrolyzed in a sealed tube with 6 N HCl at 105° for 15 h. The amount of DAP in hydrolyzates was determined according to the method devised by NIKAIDO[15].

Analysis of neutral sugars

The cell wall (5–10 mg dry wt. cell wall in total volume of 2 ml) was hydrolyzed by 2 N H_2SO_4 in a sealed tube at 105° for 10 h. The hydrolyzate was neutralized with $Ba(OH)_2$, filtered and washed with distilled water. Combined filtrate and washings were passed through columns of Dowex-50 (H^+ form, 200–400 mesh, 10 mm dia. and 30 mm length) and then Dowex-1 (HCO_3^- form, 200–400 mesh in the same size as above). The deionized sample was concentrated to a small volume by blowing air, and was then subjected to the following analysis:

(1) *Paper chromatography*. This was carried out with the following solvents[16]: (1) phenol – water (80:20, v/v); (2) *n*-butanol – ethanol – water (5:1:4, v/v); (3) ethyl acetate – acetic acid – water (14:3:3, v/v); (4) ethyl acetate – pyridine – water (12:5:4, v/v); (5) lower layer of benzyl alcohol – acetic acid – water (3:1:3, v/v). With Solvents 1, 2, and 5, ascending chromatography was carried out for 18–20 h at room temperature, while with Solvents 3 and 4, descending chromatography was usually used. With Solvent 4, in addition, multiple ascending chromatography, *i.e.*, usually three times of developments, was carried out to obtain the best separation of the sugars. The sugars were located with $AgNO_3$ or *p*-anisidine·HCl. Throughout the experiments, papers No. 5B of Toyoroshi (a product of Toyo Roshi Co., Ltd.) and Whatman No. 1 were used.

(2) *Reducing sugar*. This was determined by NELSON's method, using glucose as standard[17].

(3) DISCHE's *cysteine–H_2SO_4 reaction*[18]. This was carried out according to DISCHE. 1 ml of hydrolyzate containing about 100 μg reducing sugar (determined by the method of NELSON as glucose) was used for this reaction. Absorption spectra from

* Electron microscopy was carried out by Dr. T. TERADA of this institute to whom our thanks are due.

350 mμ to 650 mμ were followed by a spectrophotometer with automatic recorder (Shimazu Co., Ltd.).

(4) *Enzymic determination of glucose and galactose.* Glucose was measured as the absolute amount of TPNH formed in the following reaction mixture: the deionized hydrolyzate, 20–100 μl; glycylglycine buffer (0.5 M, pH 7.4), 60 μl; $MgCl_2$ (1 M), 5 μl; ATP (0.1 M) 20 μl; TPN (30 μmoles/ml), 10 μl; water to bring the total volume to 600 μl, and finally 20 μl of the enzyme mixture of hexokinase (EC 2.7.1.1) (0.04 Kunitz McDonald unit) and Glc-6-*P* dehydrogenase (0.07 unit). In a preliminary experiment, addition of 0.1 μmole galactose to 0.1 μmole glucose did not affect the result obtained with 0.1 μmole glucose under the conditions described above.

Galactose was determined in the hydrolyzates from which glucose had been removed by treatment with hexokinase, followed by passage through Dowex-50 (H^+ form) and then Dowex-1 (HCO_3^- form). The deionized samples thus obtained were subjected to the enzymic determination of galactose in the following reaction mixture: sample, 100–450 μl; phosphate buffer (0.5 M, pH 7.4), 60 μl; $MgCl_2$ (0.1 M), 10 μl; ATP (0.1 M), 10 μl; TPN (30 μmoles/ml), 10 μl; UDPG (10 μmoles/ml), 10 μl; Glc-1,6-P_2 ($6.2 \cdot 10^{-5}$ M), 10 μl; cysteine·HCl solution (33 mg/ml, pH 7.5), 10 μl; Glc-6-*P* dehydrogenase (0.07 unit), phosphoglucomutase (0.07 unit), Gal-1-*P* uridyl transferase (0.1 unit) from W3092, galactokinase (0.1 unit) from W3094 and water to bring the total volume to 620 μl. Through the coupled reactions of galactokinase, Gal-1-*P* uridyl transferase, phosphoglucomutase and Glc-6-*P* dehydrogenase, galactose was measured as the absolute amount of TPNH formed in the above reaction mixture.

RESULTS

F-duction experiment

Most of the galactose-non-fermenting mutants isolated by LEDERBERG and his associates have been classified into five categories A, B, C, D, and E by means of transductional analysis with the use of the temperate phage lambda[19]. Practically all the mutants of Groups A–D can be transformed to galactose fermenters by transducing lambda particles. On the contrary, the last Group E contains mutants which are susceptible to lambda but are not transduced to produce galactose fermenters. We confirmed that both strains of W3142 and W4597 of Group E were not transformed to galactose fermenters by lambda lyzates from wild-type strains, which is in agreement with the results of LEDERBERG. We have also studied the F-duction of Gal-genes[9,19] by transferring F'-Gal* carried by W4520 (Me^-) to $F^-(Me^+)$-Gal^- strains and by selection on EMS–galactose plates. If W3102, W4242, W3109, and W3805 (Groups A, B, C, and D, respectively) were used as recipients, F-ductants were detected as Me^+Gal^+ colonies with frequencies of 10–50 % per introduced donor cell. If, however, W4597 was used as recipient, Gal^+ colonies** were found much less frequently than in the above mentioned cases (at most 10^{-6} per introduced donor cell). The following evidences indicate that this is not due to the failure in the introduction of F'-Gal into the recipient. (1) Among the Me^+-recipient cells, F^+ colonies (detected by their sensitivity to phage f2 (ref. 20) were found with frequency of

* This type of F'-Gal, designated F8, contains no other known chromosomal markers than galactose (personal communication of Dr. Y. HIROTA of Osaka University).

** Those galactose-positive progenies have been demonstrated to originate from chromosomal recombination and not from F-duction.

approx. 30% per introduced donor cell. (2) Furthermore, by mixing the cultures of these F+-Gal- strains with that of W3350 Sm^r, many Gal+Sm^r F-ductants were obtained at the frequency usually observed in F-duction; this demonstrates that the original F+-Gal- cells were actually carrying intact F'-Gal.

The above findings suggest that there exist some other loci of galactose fermentation which cannot be incorporated in a single episome, either lambda or F-factor. Recent studies have established Groups A, B, and D to correspond to kinase, transferase, and epimerase, respectively[19,21]. It is expected, thus, that Group-E mutants may be defective in some other enzyme of galactose metabolism than the above three enzymes.

Activities of enzymes required for galactose fermentation

Table II summarizes the activities of five enzymes, kinase, transferase, epimerase, pyrophosphorylase, and mutase. As reported previously[4], both strains of W3142 and W4597 were shown to have diminished activities of pyrophosphorylase. Since W4597 had practically normal activities of kinase, transferase, epimerase, mutase (Table II), and also galactose permease[4], it was concluded that non-fermentation of galactose in this strain is due to a defect in pyrophosphorylase.

W3142 had been reported to be a kinase-less mutant[22]. In the preceding report, it was further found that this strain also had a defect in pyrophosphorylase. The defect of kinase in W3142, as will be discussed later, may be due to a secondary mutation occurred in an original strain which would have been singly defective in pyrophosphorylase. Recently, SUNDARARAJAN *et al.*[23] reported that the level of UDPG pyrophosphorylase was greatly diminished in the strains of Group E.

As is seen in this table, W3109 (Gal-9) and W3780 (Gal-3) were found to be defective in all three enzymes of the LELOIR pathway, which is in agreement with the results of KALCKAR *et al.*[5] on the strains W3099 (Gal-9) and W3264 (Gal-3) which have the same genetic defects. Pyrophosphorylase and mutase activities were found here to be normal.

W3102, W4242, and W3805 were representatives of Groups A, B, and D and had defects in kinase, transferase, and epimerase, respectively in agreement with the results of KALCKAR *et al.*[5] and JORDAN *et al.*[21]. In the present result, they were further found to have normal activities of pyrophosphorylase and also of mutase.

On the basis of these findings, it may be suggested that Group-E mutants are characterized as UDPG pyrophosphorylase-less.

Accumulation of Gal-1-P

It has already been reported[4] that the cells of strain W4597, singly defective in pyrophosphorylase, accumulated significant amounts of Gal-1-*P* intracellularly during their growth in the presence of galactose, while the cells of W3110, wild-type strain, and W3142, kinase-less and pyrophosphorylase-less strain did not (Table III). This accumulation of Gal-1-*P* in the cells of W4597 may be the reflection of the very low level of UDPG which is required for the efficient conversion of Gal-1-*P* to UDPGal. It has been demonstrated that accumulation of Gal-1-*P* is responsible for growth inhibition of galactose in transferase-less mutants of K12 (ref. 24). KALCKAR *et al.*[5] have described the isolation of a mutant, which has a defect in kinase, from a transferase-less strain grown on galactose medium. These two findings indicate that

TABLE II

ACTIVITIES OF ENZYMES REQUIRED FOR GALACTOSE FERMENTATION IN *E. coli* K12 STRAINS

Assay conditions are described in the METHOD section of the text. Unit of enzyme activities is expressed as μmoles TPNH formed per mg protein per h at 25° except for UDPGal-4-epimerase activity, which is expressed as μmoles UDPG per mg protein per h at 30°. i, induced cells; ni, non-induced cells; n.d., non-detectable.

Strain	*Group*	*Galactokinase*		*Gal-1-P uridyl transferase*		*UDPG-4-epimerase*		*UDPG pyrophosphorylase*		*Phosphoglucomutase*	
		ni	*i*	*ni*	*i*	*ni*	*i*	*ni*	*i*	*ni*	*i*
W3110	Wild type	3.3	27.8	3.0	30.5	7.8	62.4	2.4	2.5	—	4.5
W3102	A	n.d.	n.d.	18.9	31.5	28.1	25.0	1.6	2.1	3.6	3.5
W4242	B	0.6	5.1	0.2	0.3	2.7	21.0	0.8	1.6	6.4	7.9
W3109	C	0.1	0.1	0.6	0.4	n.d.	n.d.	3.3	2.5	4.4	4.0
W3780	C	—	0.2	—	0.4	—	0.1	—	1.6	—	5.0
W3805	D	—	8.0	—	7.0	—	0.2	—	1.5	—	6.3
W3142	E	n.d.	n.d.	12.2	22.5	18.3	13.7	0.2	0.2	4.3	3.7
W4597	E	1.5	6.0	1.7	10.0	0.6	19.0	0.2	0.3	—	3.8

TABLE III

INTRACELLULAR LEVEL OF UDPG, UDPGal AND Gal-1-*P* IN K12 STRAINS

Values are expressed as mμmoles/100 mg dry wt. cells. Methods of determination are described in the METHOD section of the text.

	Strain					
	W3110		*W3142*		*W4597*	
Galactose in growth medium	—	+	—	+	—	+
UDPG	32	11.4	1.2	1.4	1.9	1.6
UDPGal	5.2	17.4	<0.1	<0.1	<0.1	<0.1
Gal-1-*P*	1.3	1.5	0.7	1.6	1.4	182

TABLE IV

COMPOSITION OF CELL WALLS OF K12 STRAINS

Values are expressed as mg/100 mg dry wt. cell walls. Methods of determination are described in the METHOD section of the text.

	Strain					
	W3110		*W3142*		*W4597*	
	Expt. I	*II*	*I*	*II*	*I*	*II*
Hexosamines (as glucosamine)	5.6	3.1	3.9	3.2	3.9	3.1
DAP	1.3	—	1.3	—	1.2	—
Reducing sugars (as glucose)	4.0	3.2	1.2	1.7	1.2	1.2

in a population of cells (pyrophosphorylase-less or transferase-less), accumulating Gal-1-*P* under the presence of galactose, there may be a strong selective pressure for secondary mutants with a defect in kinase. From these considerations, we suggest that W3142 may be derived from such a secondary mutation, after which selection occurred during culture on galactose medium. In fact, galactose has a strong inhibitory effect on the growth of W4597, while growth of W3142 was much less affected by the addition of this sugar. Most recently, SUNDARARAJAN *et al.*[23] have isolated a galactose-resistant strain from W4597, which was found to have diminished activity of kinase, supporting the above argumentation.

Intracellular level of UDPG and UDPGal

We have reported previously that the level of UDPG in the cells of W4597 and W3142 was significantly lower than in W3110, reflecting the activity of pyrophosphorylase in these strains. In the present study, the amount of UDPG and UDPGal was determined on an alcohol extract from cells of each strain grown with or without galactose (Table III). In agreement with the previous result, the intracellular level of UDPG in W4597 and W3142 was much lower than that in W3110. Furthermore, UDPGal was non-detectable in both mutant strains irrespective of presence or absence of galactose.

Quantitative analysis of cell walls

It has been well established that bacterial polysaccharide is located in the lipopolysaccharide layer as integral part of the cell-wall structure. UDPG has been demonstrated[25] to be an important glucosyl donor in the biosynthesis of polysaccharides. Since there were mutants defective in the synthesis of UDPG, it was considered to be of importance to study the chemical properties of the cell walls isolated from the mutant cells.

As is seen in Table IV, the cell walls isolated from W4597 and W3142 contained nearly the same amount of DAP and hexosamines as did the cell walls of W3110. Morphologically, no differences were observed under the electron microscope between the cell walls of wild-type and of mutant cells.

However, as shown in this table, both mutant strains contained significantly smaller amounts of neutral sugars in their cell walls than did the wild-type strain. The composition in the neutral sugars, therefore, was analyzed on the hydrolyzates as described in the following section.

Compositions in neutral sugars

It was found through paper chromatography and the cysteine–H_2SO_4 reaction of DISCHE (Fig. 1) that the cell walls of W3142 and W4597 contained no detectable amount of hexoses, while the cell walls of W3110 contained glucose and galactose as hexose constituents. Heptose was found as a common component both in wild-type

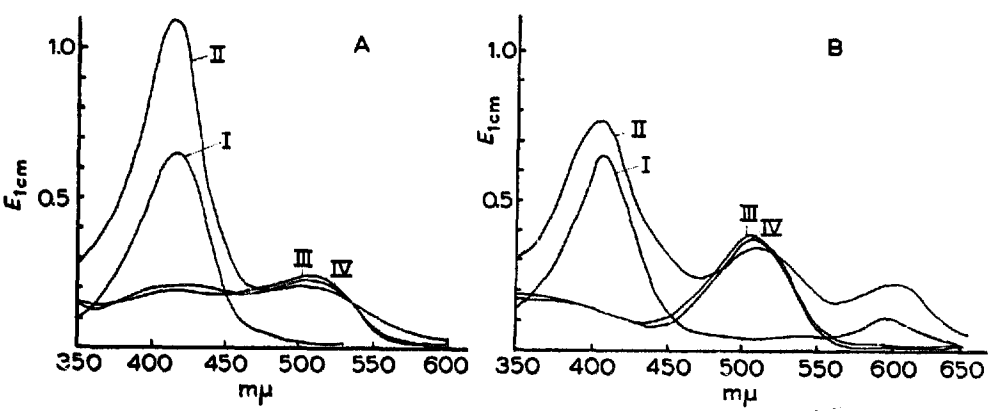

Fig. 1. Absorption spectra of cell-wall hydrolyzates of *E. coli* K12 strains. A, primary cysteine reaction; B, secondary cysteine reaction. In each case, Curve I represents absorption spectrum of 100 μg of glucose; Curves II, III, and IV represent absorption spectra for W3110, W4597, and W3142, respectively.

TABLE V

DETERMINATION OF HEXOSES IN CELL WALLS OF *E. coli* K12 STRAINS

Values are expressed as mg/100 mg dry wt. cell walls. Total reducing sugar was determined according to NELSON. Glucose and galactose were determined by specific enzymic methods described in the METHOD section of the text.

Strain	*Total reducing sugar as glucose*	*Glucose*	*Galactose*
W4597	1.2	0.19	< 0.01
W3142	1.7	0.15	< 0.01
W3110	3.2	1.1	0.18

and in mutant strains. On paper chromatogram stained with *p*-anisidine·HCl, the heptose was detected as a characteristic brown-colored spot, indicating the heptose to be an aldoheptose. By the use of the solvent systems described in the METHOD section, the heptose was shown to behave like D-glycero-L-manno heptose[26].

By the specific enzymic method mentioned earlier, glucose and galactose were determined on hydrolyzates of cell walls in these strains. As shown in Table V, the cell walls of the mutant strains contained only traces of galactose and 10–20 % of the amount of glucose present in the cell walls of wild type.

DISCUSSION

As was described in the result section, the collaborative studies of J. AND E. M. LEDERBERG and of KALCKAR have elucidated the simple correspondence between genetic alterations as grouped in cistrons and enzymic defects so far as Group A–D are concerned. The members of the last Group E, however, had not yet been well-characterized* biochemically before the present study. That two mutants of Group E are shown conclusively to be defective in UDPG pyrophosphorylase may support again such a simple correspondence between a genetic alteration and an enzymic defect in this group as was found in Group A–D. On the basis of our present results, it might be concluded that Group-E mutants represent the results of alterations in the structural gene for UDPG pyrophosphorylase, and that this gene does not belong to the gal-operon[27], even though this enzyme participates in galactose fermentation.

It was found that the intracellular level of UDPG in these mutants was 10–20 % of that in the wild-type cells. The level of UDPGal was found to be undetectable even in the cells grown in the presence of galactose, where a large accumulation of Gal-1-*P* took place. This can be taken as an evidence that UDPGal pyrophosphorylase which catalyzes the reaction, UTP + Gal-1-*P* $\rightleftharpoons$ UDPGal + PP_i, was not present in these strains of *E. coli*. The presence of such an enzyme has recently been reported for a mammalian system[28]. An experiment carried out in our laboratory with the epimerase-less strains (M mutants[30]) of *E. coli* and Salmonella also ruled out** the presence of UDPGal pyrophosphorylase in these microorganisms. Thus, together with the fact that M mutants of Salmonella do not contain any trace of galactose in their cell walls, the above findings exclude the possibility that UDPGal can be formed from UTP and Gal-1-*P* in these organisms. Therefore, the absence of UDPGal in the cells of the present mutants (even in the presence of galactose in the growth medium) can be attributed to the low level of UDPG in these mutant cells.

It was shown that cell walls from Group-E mutants contained only traces of galactose and 10–20 % the amount of glucose present in the cell walls from wild type. The correlations of the amount of glucose and galactose found in the mutant and wild-type cell walls are favorably compared with the correlations of the amount of UDPG and UDPGal found in the respective cells. These are in agreement with the concept that UDPG and UDPGal are usual or normal glucosyl and galactosyl donors in the biosynthesis of polysaccharides, so far as cell-wall lipopolysaccharides of *E. coli* are concerned (*cf.* refs. 31, 34).

* SOFFER reported W4597 to be transferase-less[29], which turned out to be pyrophosphorylase-less by the present study.

** During the incubation of UDPGal and pyrophosphate with crude extract of M mutant cells adapted to galactose, consumption of UDPGal was not observed.

With regard to the biological aspect of those chemical alterations in the structur of cell-wall polysaccharides, it seems noteworthy that these mutants cannot adsorl phage P1*. This probably indicates that the part of the polysaccharide involvin glucose and/or galactose is active as the receptor site for P1.

Recently, the chemical structure of bacterial lipopolysaccharide has been studie extensively in Salmonella[32]. In the course of these studies, particular types of mutant with modified lipopolysaccharides, such as rough mutants[32] or M mutants[33,3 (UDPGal-4-epimerase-less mutants) have furnished experimental evidence supportin the existence of a "central core" as a common substructure of the lipopolysaccharide of Salmonella. At present, the polysaccharide produced by M mutant has been inter preted[34] as representing a common "core" consisting of polyglucose–heptose. In the present study, the cell walls of the UDPG pyrophosphorylase-less mutants were shown to contain virtually only hexosamines and heptose as monosaccharide units. Also it seems worthwhile to mention here that the heptose content of the cell-wall polysaccharides seemed not to be affected by the presence or absence of UDPG pyrophosphorylase. From these considerations, we may suggest the possible presence of a "polyheptose core" as a common substructure in cell walls of *E. coli* and Salmonella and that this might form a smaller, inner "core" inside the polyglucose–heptose core of M mutants. In this sense, it would be rewarding to study more intensively the chemical properties of the lipopolysaccharides of the mutant cells. Recently, SUNDARARAJAN *et al.*[23] carried out qualitative analysis on lipopolysaccharides extracted from W4597 by the warm phenol–water procedure of WESTPHAL. They confirmed our previous finding of the lack of "detectable" glucose and galactose, and further reported the absence of detectable amount of 6-deoxyhexose (rhamnose or 6-deoxyglucose) from these mutant cells. This might indicate that just as in case of M mutants, rhamnose is usually linked to galactose or glucose, and cannot be attached directly to the heptose-core.

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* Unpublished observation of Dr. J. ADLER of Wisconsin University.

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