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2-Keto-3-Deoxygluconate 6-Phosphate Aldolase Mutants of *Escherichia coli*

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A new mutation in *Escherichia coli*, giving inability to grow on gluconic, glucuronic, or galacturonic acids, has been identified as complete deficiency of 2-keto-3-deoxygluconate 6-phosphate (KDGP) aldolase activity. The genetic map position of the locus, *eda*, is about 35 min. The inability to grow on the uronic acids was expected, because the aldolase is on the sole known pathway of their metabolism. However, inability to grow on gluconate was less expected, because the hexose monophosphate shunt might be used, as happens in mutants blocked in the previous step, *edd*, of the Entner-Doudoroff pathway. The likely explanation of gluconate negativity is inhibition by accumulated KDGP, because gluconate is inhibitory to growth on other substances, and one type of gluconate revertant is *eda*$^-$, *edd*$^-$. KDGP is probably the inducer of KDGP aldolase.

Pathways of gluconate metabolism in *Enterobacteriaceae* are shown in Fig. 1. Gluconate is phosphorylated by an inducible kinase (4; EC 2.7.1.12). The gluconate 6-phosphate is an intermediate in the hexose monophosphate shunt and is used by gluconate 6-phosphate dehydrogenase, a constitutive enzyme (EC 1.1.1.44; genetic abbreviation *gnd*). The gluconate 6-phosphate is also used by the Entner-Doudoroff (6) pathway. The first enzyme of this pathway is a dehydrase (EC 4.2.1.12; genetic abbreviation *edd*) induced by growth in gluconate. The product, 2-keto-3-deoxygluconate 6-phosphate (KDGP) is cleaved by an aldolase (EC 4.2.1.14; genetic abbreviation *eda*) giving pyruvate and glyceraldehyde 3-phosphate.

We have previously described mutations in two of these enzymes. *Edd* mutants grow slower than the wild type on gluconate, with their gluconate metabolism initially restricted to the hexose monophosphate shunt (20). An additional mutation in *gnd* yields a strain unable to grow at all on gluconate. A single-gene *gnd* mutant does grow on gluconate (8). Earlier we looked for, but failed to find, single-gene mutants unable to grow on gluconate but otherwise normal (*unpublished data*). One such mutant would have lacked gluconokinase; however, it was recently shown that there are two gluconokinase activities in *Escherichia coli* (Hung et al., Bacteriol. Proc., p. 146, 1970; N. Zwaig, *personal communication*). In this paper, we report a new mutation which causes gluconate negativity; the new mutants lack KDGP aldolase (*eda*). Besides not growing on gluconate, they also fail to grow on glucuronate or galacturonate, and gluconate inhibits growth on other substances.

MATERIALS AND METHODS

Organisms and media. The *eda* mutants, strains DF70 (*fdp-1*, *eda-1*) and DF75 (*fdp-1*, *eda-2*) were derived from DF1100 (formerly strain Q11; reference 10), an Hfr H prototroph carrying the *fdp-1* mutation. DF1101 (*fdp*$^+$) is a transductant of DF1100 selected on glycerol-minimal medium and is therefore like the parent of DF1100, strain 3300 (Hfr H, *fdp*$^+$; reference 10). DF71 (*fdp*$^+$, *eda-1*) is a glycerol-positive transductant of DF70, and DF71T (*fdp*$^+$, *eda*$^+$) is a glucuronate-positive transductant of DF71 (and therefore equivalent to strain DF1101).

All gene symbols (except *eda*) are described in reference 19. The ones of particular interest in this report (see also Fig. 1) and their map positions are: *fdp*, fructose diphosphatase, 84 min; *edd*, gluconate 6-phosphate dehydrase, 35 min; *gnd*, gluconate 6-phosphate dehydrogenase, 39 min; *zwf*, glucose 6-phosphate dehydrogenase, 35 min; *pgi*, phosphoglucose isomerase, 79 min; *pps*, phosphopyruvate synthetase, 32 min; and *his*, histidine operon, 38 min.

Strain K10 is a prototrophic Hfr C. Derivatives of it are DF10 (*edd-1*, reference 20), and DF1070 (*edd-1*, *gnd-1*, reference 8).

The strains used in Table 4 for mapping *eda* were made as follows. The male, DF44zwf27, is a derivative of Hfr H carrying *zwf-27*. [Previous *zwf* mutants were selected as derivatives of Hfr C (8).] Strain CA8000 (Hfr H, *thiA*$^-$, *pgi*$^+$, *zwf*$^+$), from J. R. Beckwith, was transduced to *thiA*$^+$ by using phage P1 grown on DF41 (DF40 *pgi-2*, cured of lambda prophage; reference 11), and a transductant was found which had inherited *pgi-2*. That strain, although *zwf*$^+$, grew slower on glucose

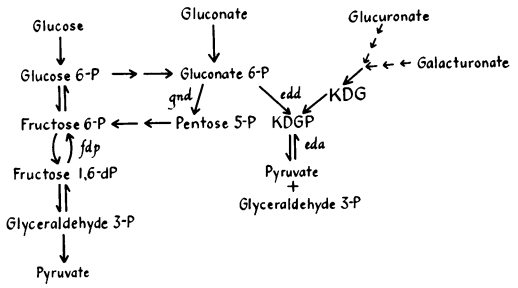

FIG. 1. *Pathways of gluconate metabolism. Gene abbreviations are: gnd, gluconate 6-phosphate dehydrogenase; edd, gluconate 6-phosphate dehydrase; eda, KDGP aldolase; and fdp, fructose diphosphatase. KDG is 2-keto-3-deoxygluconate, and KDGP is 2-keto-3-deoxygluconate 6-phosphate.*

than *pgi*$^-$ derivatives of Hfr C, and a spontaneous mutant, DF44, was selected from it which grew faster on glucose but was still *pgi*$^-$. Glucose-negative derivatives were selected from DF44 after ultraviolet mutagenesis, and DF44zwf27 is one of them (*pgi-1, zwf-27*).

The female strains were derived from a strain kindly supplied by H. L. Kornberg, K2-lt (F$^-$, *arg*$^-$, *thr*$^-$, *leu*$^-$, *pps*$^-$, *his*$^-$, *str*^{r}). It was mated with DF70 (Hfr H, *eda-1*, *str*^{s}), and a histidine-independent recombinant was selected which carried *eda-1*. That strain was then mated with DF40 (Hfr C, *pgi-1*, *str*^{s}), and an arginine-independent recombinant was found which carried *pgi-1:* strain DF1670 (F$^-$, *pgi-1*, *thr*$^-$, *leu*$^-$, *pps*$^-$, *eda-1*, *str*^{r}). DF1671 was selected in a similar way, the first step being introduction of *pgi-1* from DF40 into K2-1t (arginine selection), and the second step being introduction of *eda-1* from DF70 by lactate-selection (for *pps*$^+$ recombinants). DF1671 is F$^-$, *pgi-1*, *eda-1*, *his*$^-$, *str*^{r}. In the crosses of Table 4, since both male and female strains carried a *pgi* mutation, the *zwf* allele (*zwf*$^+$ or *zwf*$^-$) could be scored on glucose-minimal plates (16).

Minimal medium 63, tetrazolium indicator plates, and growth conditions were described earlier (11). The broth was medium 63 supplemented with 1% tryptone (Difco) and 0.4% yeast extract (Difco). Conjugation and transduction methods were also described earlier (7).

Enzyme assays. Cell-free extracts were prepared as described previously (11), either from 250-ml cultures (concentrated 100-fold before sonic treatment) or from 20-ml cultures (concentrated 10-fold before sonic treatment). For KDGP aldolase assay 2, the extracts were clarified by 2 hr of centrifugation in a Spinco rotor 50Ti at 50,000 rev/min to remove reduced nicotinamide adenine dinucleotide (NADH) oxidase. Incubation mixtures for all assays contained 0.05 M tris(hydroxymethyl)aminomethane (Tris)-chloride and 0.01 M $MgCl_2$, *p*H 7.6. Gluconokinase and gluconate 6-phosphate dehydrogenase assays were described previously (11). The Entner-Doudoroff pathway (formation of pyruvate from gluconate 6-phosphate) was assayed by a slight modification of the method of Kovachevich and Wood (12). Incubation mixtures (0.25 ml) contained buffer, 8 mM gluconate 6-phosphate, 2.4 mM reduced glutathione, and extract. At 0, 3, and 7 min of incubation, 0.02-ml samples were added to 0.1 ml of 2,4-dinitrophenylhydrazine (1 mg/ml in 2 N HCl) plus 0.08 ml of water. Five minutes later, 5 ml of 2.5 N NaOH was added, and the optical density was read in a Lumetron colorimeter with a 420 filter. Pyruvic acid was used as standard. Gluconate 6-phosphate dehydrase, the first enzyme of the Entner-Doudoroff pathway, was assayed as above, with the modification that the incubation also included 0.24 mg of protein of an extract of strain DF1070 (*gnd*$^-$, *edd*$^-$, *eda*$^+$; reference 8) to supply KDGP aldolase in excess. KDGP aldolase assay 1 was a two-step assay, the first incubation containing buffer, 0.5 mM KDGP, and extract, with samples removed periodically for pyruvate assay by the dinitrophenylhydrazine reaction (12). KDGP aldolase assay 2 was a linked spectrophotometric assay, with 0.2-ml incubation mixtures containing buffer, 0.25 mM KDGP, 0.1 mM NADH, 5 μg of lactic dehydrogenase, and extract (centrifuged at high speed) (11).

Protein was measured by the Folin method (14) with bovine plasma albumin as standard, as previously described (11). Enzyme specific activities are all given as nanomoles per minute per milligram of protein.

Chemicals. KDGP was a generous gift from W A. Wood; it was converted from the barium salt to the free acid by treatment with Dowex 50, hydrogen form. Gluconic acid was the Eastman product, glucuronic acid was from Sigma Chemical Co., and galacturonic acid was from Calbiochem. Other biochemicals were from the usual suppliers. All sugars were of the D-configuration, except where noted.

RESULTS

Isolation of the mutants. The mutants were found by chance in a selection designed for mutants in the pentose pathway. The starting strain was DF1100, a prototrophic strain lacking fructose diphosphatase (*fdp;* reference 10), and we looked for mutants which had lost the ability to grow on either gluconate or pentoses. The strain was treated in medium 63 with 0.035 volume of ethyl methane sulfonate for 30 min at 37 C (13) and grown out in minimal medium with mannitol as carbon source. In one case, the cells were then subcultured in gluconate-minimal medium to logarithmic phase, treated with 2,000 units of penicillin per ml, and plated on mannitol-minimal plates after 3 hr (99% killing); in another experiment, the penicillin selection was done in medium containing 0.2% gluconate, 0.2% xylose, and 0.2% L-arabinose. Survivors were tested by patching for the phenotype of being unable to grow on either gluconate or pentoses. No such mutants were found. However, some gluconate-negative strains which were able to grow on pentoses were recovered: 7/800 survivors in the first experiment (including strain DF70), and 5/500 in the second experiment (including strain DF75).

Enzyme activities. The activities of some en-

zymes of gluconate metabolism in the parent and two mutants are given in Table 1. DF70 contained gluconokinase. [Its level is somewhat lower than in the parent, but a similar effect on gluconokinase level has been found in an *edd* mutant (20).] It also contained gluconate 6-phosphate dehydrogenase. In an assay for the complete Entner-Doudoroff pathway, formation of pyruvate from gluconate 6-phosphate, DF70 was found to be markedly defective; individual assay of gluconate 6-phosphate dehydrase and KDGP aldolase showed that the deficiency was in the aldolase. The final two lines of Table 1 show that the aldolase deficiency was equally severe in minimal medium, with both basal and induced levels being absent in the mutant. Strain DF75, which has the same growth phenotype as DF70, also lacked the aldolase activity. However, it was also notably deficient in gluconokinase.

We assign the new lesion the abbreviation *eda* (Entner-Doudoroff aldolase) with DF70 carrying *eda-1* and DF75 carrying *eda-2*.

Growth. In view of the facts that single-gene gluconate-negative strains had not been isolated previously and that it was not expected that mutants lacking KDGP aldolase would actually be completely unable to grow on gluconate, it was necessary to determine whether the fructose diphosphatase deficiency of the strains somehow accounted for the gluconate negativity. Parent strain DF1100 and mutant DF70 were transduced to *fdp*$^+$ by using phage P1 grown on K10 (*fdp*$^+$) and selection on glycerol-minimal plates. Table 2 shows the growth pattern of the strains. The *fdp*$^+$ transductant of DF70, strain DF71, was as unable to grow on gluconate as DF70. Therefore, the fructose diphosphatase lesion does not account for the gluconate negativity, which must then be ascribed to the *eda* lesion alone (*see below*). Table 2 shows that the single-gene *eda* mutant, DF71, was also unable to grow on

TABLE 1. *Enzymes of gluconate metabolism*[a]

Enzyme	Medium	Enzyme assay[b]		
		Parent (DF1100)	Mutant 1 (DF70)	Mutant 2 (DF75)
Gluconokinase	Gluconate broth	162	69	4
Gluconate 6-P dehydrogenase	Gluconate broth	148	172	70
Entner-Doudoroff pathway	Gluconate broth	84	0	0
Gluconate 6-P dehydrase	Gluconate broth	102	80	n.d.[c]
KDGP aldolase (assay 1)	Gluconate broth	141	2	n.d.[c]
KDGP aldolase (assay 2)	Mannitol-minimal	68	0	0
KDGP aldolase (assay 2)	Mannitol-minimal + gluconate	540	1	14

[a] Cultures (250 ml) were harvested in logarithmic phase in the indicated media, extracts were prepared, and the activities were assayed as described in the text. Entner-Doudoroff pathway assay is for formation of pyruvate from gluconate 6-phosphate and depends on both the dehydrase and aldolase. Gluconate 6-P dehydrase assay also measures pyruvate formation from gluconate 6-phosphate, but the incubation includes some extract from strain DF1070 (*edd*$^-$, *eda*$^+$) to supply aldolase.

[b] Specific activities are nanomoles per minute per milligram of protein.

[c] Not done.

TABLE 2. *Growth of mutants*

Medium[a]	Colony size[b]					
	DF1100 (*fdp-1*, *eda*$^+$)	DF1101 (*fdp*$^+$, *eda*$^+$)	DF70 (*fdp-1*, *eda-1*)	DF75 (*fdp-1*, *eda-2*)	DF71 (*fdp*$^+$, *eda-1*)	DF71T (*fdp*$^+$, *eda*$^+$)
Glucose	1.6	1.6	1.5	1.6	1.4	1.6
Mannitol	1.5	1.5	1.4	1.5	1.4	1.5
Gluconate	1.5	1.5	0.0	0.0	0.0	1.5
L-Arabinose	1.4	1.3	1.5	1.4	1.3	1.4
Glycerol	0.0	0.8	0.0	0.0	0.7	0.8
Succinate	0.0	0.6	0.0	0.0	0.5	0.5
Glucuronate	0.0	1.3	0.0	0.0	0.0	1.3
Galacturonate	0.0	1.3	0.0	0.0	0.0	1.3

[a] The strains were grown to stationary phase in broth, and dilutions were spread on minimal plates with the indicated carbon sources so as to give ca. 200 colonies per plate.

[b] Average estimated size, in millimeters, attained after 48 hr of incubation at 37 C.

glucuronate or galacturonate, an expected result, because the known pathways (1) of uronic acid metabolism in *E. coli* proceed by way of KDGP (Fig. 1). The original parental strain, DF1100, was unable to grow on uronic acids because, being an *fdp* mutant, it has no pathway to hexose monophosphate (Fig. 1); DF1101, the *fdp*$^+$ transductant, grew on the uronic acids (Table 2). The *eda* mutants grew normally on minimal glucose, mannitol, and arabinose plates.

When the ability to grow on glucuronate was returned to DF71 by transduction with phage P1 grown in DF1101, the transductants (e.g., DF71T) also grew normally on galacturonate and gluconate (Table 2) and contained normal levels of KDGP aldolase (Table 3). Thus, there probably is a single KDGP aldolase in *E. coli* necessary for growth on gluconate, glucuronate, and galacturonate.

Why is an eda mutant unable to grow on gluconate? As mentioned above, a mutant lacking gluconate 6-phosphate dehydrase grows on gluconate, somewhat slower than the wild-type strain, with gluconate metabolism initially restricted to the hexose monophosphate shunt (20). Therefore, it was surprising that the *eda* mutant did not grow on gluconate at all, since it contained the enzymes of the shunt (e.g., gluconate 6-phosphate dehydrogenase; Table 1) and grew on pentoses (Table 2). One explanation of the gluconate negativity would be that accumulated KDGP is inhibitory to growth. This suggestion is supported by several experimental results. First, the growth of DF71 (*eda*$^-$) on glucose, mannitol, glycerol, succinate, or arabinose was completely prevented if gluconate, 1 mg/ml, was also included in the minimal plates. The *eda*$^+$ strain, DF1101, was not inhibited by gluconate. On rich plates with gluconate, such as gluconate tetrazolium, growth was also markedly inhibited, and in broth containing gluconate, growth was slower than in broth without gluconate. The expected growth of the mutant on gluconate alone would be equivalent to growth on a mixture of gluconate and pentose; but gluconate inhibits growth on pentoses.

Secondly, reversion of the *eda* mutants to growth on gluconate was very frequent; growth in broth (without added gluconate) from a single colony sometimes yielded 10^{-4} gluconate-positive revertants. (This high reversion frequency might explain our previous failure to find single-step gluconate-negative strains.) Reversion to growth on the uronic acids, on the other hand, was very infrequent (ca. 10^{-8}). Most of the gluconate-positive revertants were not back mutants of *eda*, but rather strains now also carrying a mutation in gluconate 6-phosphate dehydrase (*edd*). For example, strain DF1671 (*eda-1*) contained gluconate 6-phosphate dehydrase activity of 180 nmoles per min per mg, and a revertant selected on gluconate contained neither aldolase nor dehydrase activity. Such *edd*$^-$ revertants of *eda* mutants were still unable to grow on the uronic acids, because they were still *eda*$^-$. Thus, there seems to be a strong selective pressure in *eda* mutants for *edd* mutation, perhaps to prevent even that amount of KDGP accumulation which would occur because of the basal level of gluconate 6-phosphate dehydrase.

Genetic map position of eda. *Eda* is closely linked to *edd*. This was first seen in a cross between DF70 (Hfr H, *eda-1*, *str*^{s}) and DF1647 (F$^-$, *pyrD*$^-$, *edd*$^-$, *his*$^-$, *tyr*$^-$, *str*^{r}). When histidine-independent recombinants were selected on appropriately supplemented glucose-minimal plates, only 1 in 93 had the wild-type gluconate phenotype; the others were either red on gluconate tetrazolium plates (*edd*$^-$), or inhibited (*eda*$^-$). When *eda-1* was introduced by conjugation into a strain carrying *pps*$^-$ (at 34 min) and *his*$^-$ (at 39 min) [*edd* maps between these two markers (16)], recombinational analysis showed that *eda* also was between *pps* and *his*, and this was confirmed by interrupted mating experiments (J. E. Fradkin, undergraduate thesis, Harvard Univ., 1971). We have previously shown (16) that *edd* and *zwf* (glucose 6-phosphate dehydrogenase) are 95% linked in transduction. The order of *eda* with respect of *zwf*, *pps*, and *his* was determined in crosses with two new strains: DF1670 (F$^-$, *pgi*$^-$, *thr*$^-$, *leu*$^-$, *pps*$^-$, *eda-1*, str^r) and DF1671 (F$^-$, *pgi*$^-$, *eda-1*, *his*$^-$, *str*^{r}). DF44*zwf27* (*pgi*$^-$, *zwf*$^-$) was crossed with each; in the first case, DF1670, selection was for ability to grow on lactate (*pps*$^+$) and in the second, DF1671, for histidine independence (*his*$^+$).

TABLE 3. *KDGP aldolase activities*[a]

Strain	Enzyme activity[b]	
	In broth	In broth + gluconate (0.4%)
DF1101 (*eda*$^+$)	162	450
DF71 (*eda-1*)	0.2	0.0
DF71T (*eda*$^+$)	212	565
	218	492
	252	488

[a] Strains were harvested from stationary phase in the media indicated, and KDGP aldolase was measured by assay 2 as described in the text.

[b] Expressed as nanomoles per minute per milligram of protein.

Recombinants were purified and tested for *eda* and *zwf* inheritance. Results are shown in Table 4. In the first cross, the order most consistent with the data is *pps ... eda ... zwf;* in the second, the order is *eda...zwf...his*. The overall gene order thus is *pps...eda...zwf...his*, with *eda* and *zwf* 98% or more linked in conjugation. The mapping of *eda* with respect to *edd* and *zwf* will be reported separately.

Induction of KDGP aldolase. It was shown earlier that the induction of KDGP aldolase is different from the induction of gluconate 6-phosphate dehydrase. The dehydrase had a low basal level on glucose and fructose and a high level on gluconate, whereas the aldolase had a moderate level in cells grown on glucose or fructose and a higher level in cells grown on gluconate (5, 11). These data are now extended to include the uronic acids (Table 5). The dehydrase was not induced at all by growth on the uronic acids, and thus its inducer seems to be gluconate (or a derivative). The aldolase, on the other hand, had a high "basal" level even on glucose but was markedly induced by gluconate and also by the uronic acids; these latter results confirm those of Pouyssegur and Stoeber (17). The simplest explanation for induction by growth on three different carbon sources would be that KDGP itself (or a derivative) is the inducer. If that were the case, then an *edd* mutant, which cannot make KDGP from gluconate 6-phosphate, ought not to induce the aldolase on gluconate, but still would induce it on the uronic acids. The data of Table 5 confirm that prediction. KDGP induction of the aldolase might also explain the high "basal" level. It could reflect endogenous induction by the small amount of KDGP expected to be produced from reaction of the normal metabolites, glyceraldehyde 3-phosphate and pyruvate, catalyzed by the true basal level of aldolase.

DISCUSSION

KDGP aldolase is an enzyme on the pathway of glucuronic acid, galacturonic acid, and gluconic acid metabolism. Mutants lacking this enzyme, as expected, fail to grow on the uronic acids. The group at Lyon, which has published several reports on the physiology and mutant analysis of the uronic acid pathway in *E. coli* (2,

TABLE 4. *Mapping of eda*[a]

Expt	Donor	Recipient	Selected marker	No. scored	Unselected markers			
					zwf$^+$, *eda*$^+$	*zwf*$^+$, *eda*$^-$	*zwf*$^-$, *eda*$^+$	*zwf*$^-$, *eda*$^-$
1	DF44zwf27 (HfrH, *pgi*$^-$, *zwf*$^-$, *str*^{s})	DF1670 (F$^-$, *pgi*$^-$, *pps*$^-$, *eda-1*, *str*^{r})	*pps*$^+$	406	18	139	248	1
2	DF44zwf27 (HfrH, *pgi*$^-$, *zwf*$^-$, *str*^{s})	DF1671 (F$^-$, *pgi*$^-$, *eda-1*, *his*$^-$, *str*^{r})	*his*$^-$	402	7	27	347	21

[a] In both crosses, recombinants were selected on minimal-lactate plates containing threonine, leucine, and streptomycin (e.g., for *pps*$^+$, *his*$^+$ recombinants). After purification of recombinants on the same medium, the inheritance of *zwf* was scored by patching to appropriately supplemented glucose-minimal plates (because both donor and recipient were *pgi*$^-$), and inheritance of *eda* was scored on appropriately supplemented gluconate-minimal plates.

TABLE 5. *Induction of the Entner-Doudoroff enzymes*[a]

Carbon source	Enzyme activity			
	Gluconate 6-P dehydrase		KDGP aldolase	
	Wild type	*edd* Mutant	Wild type	*edd* Mutant
Glucose	8	1	300 ± 37 (7)	354 ± 48 (6)
Gluconate	98	0	1096 ± 197 (7)	326 ± 66 (6)
Glucuronate	6	1	582 ± 126 (7)	634 ± 80 (6)
Galacturonate	n.d.[b]	n.d.[b]	676 ± 137 (6)	652 ± 169 (5)

[a] The wild-type strain was K-10, and the *edd* mutant was DF10 (20). Gluconate 6-phosphate dehydrase was assayed in extracts prepared from 250-ml cultures in growth on minimal medium with the indicated carbon source. KDGP aldolase was usually assayed in extracts prepared from 20-ml cultures in logarithmic growth; standard deviations and number of independent cultures are also shown. Specific activities are nanomoles per minute per milligram of protein.

[b] Not done.

15, 17, 18), also cities the isolation of a KDGP aldolase mutant, negative on glucuronate, galacturonate, and gluconate (experiments to be reported, cited in reference 17).

The property of gluconate negativity of the *eda* mutants is more difficult to explain than the uronate negativity, because on gluconate such mutants might be expected to grow by using the hexose monophosphate shunt, as do gluconate 6-phosphate dehydrase mutants (20). Our proposed explanation of the complete inability of the *eda* mutants to grow on gluconate depends on an additional assumption: that KDGP, the substrate of the missing reaction, accumulates and is toxic. The phenomenon of toxicity of phosphorylated metabolic intermediates accumulated by mutants in sugar metabolism is familiar (3, 9). The evidence for KDGP toxicity in the *eda* mutants is still indirect: gluconate prevents growth on other substances, and reversion to gluconate positivity occurs by loss of the previous enzyme in the gluconate pathway, gluconate 6-phosphate dehydrase. That KDGP actually accumulates from gluconate and the site of its toxicity remain to be demonstrated.

The simplest interpretation of the results is that *eda* is the structural gene for a single KDGP aldolase in *E. coli*. We have not ruled out, however, a more complicated explanation according to which the *eda* mutation is a regulatory one affecting induction of more than one KDGP aldolase. This seems unlikely, in view of the rather strong indirect evidence for KDGP induction of the aldolase (*see below*). In addition, the French group cites (17) experiments (to be published) in which the aldolases isolated from growth in the three different carbon sources are found to be chromatographically identical.

On the matter of induction of the aldolase, our speculations accord with and extend those of the French group. As we confirm, they showed that the aldolase is induced by growth in glucuronate and galacturonate, as well as gluconate. They also showed that the early enzymes in the uronic acid pathways are regulated differently from the aldolase or the enzyme immediately preceeding it, 2-keto-3-deoxygluconate kinase; an inducer exists for the early enzymes which does not induce kinase or aldolase (2). In addition, in mutants blocked in an early step in glucuronic acid pathway, kinase and aldolase were not induced by glucuronate but were induced by galacturonate, and vice versa (17). These workers therefore suggested (17) that 2-keto-3-deoxygluconate, or a derivative, induces kinase and aldolase. The finding (Table 5) that in an *edd* mutant gluconate did not induce the aldolase above the high uninduced level similarly supports the idea of KDGP induction of a single aldolase. Moreover, if KDGP were the inducer, the high "basal" level of the aldolase might actually reflect partial induction above a true basal level because of formation of KDGP in the reverse reaction.

Finally, we have reported the genetic map position of *eda;* it is close to *edd* and *zwf* at about 35 min on the *E. coli* chromosome as usually drawn (19). We will discuss that linkage further in a separate report, and we note here only that, although closely linked genetically, the three enzymes seem to be regulated differently, *zwf* being constitutive, *edd* being induced by growth on gluconate, and *eda* probably being induced by KDGP.

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