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Location of Three Genes Concerned with the Conversion of 2,3-Dihydroxybenzoate into Enterochelin in *Escherichia coli* K-12

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Mutants of *Escherichia coli* K-12 unable to synthesize the iron-sequestering compound, enterochelin, from 2,3-dihydroxybenzoate have been isolated and divided into three classes on the basis of tests for enzymatic complementation. The genes affected (designated *entD*, *entE*, and *entF*) have been mapped by cotransduction and are located at about minute 14 on the *E. coli* genome. They were found to be closely linked to other genes (*entA*, *entB*, and *entC*) concerned with enterochelin biosynthesis and a gene (*fep*) concerned with the uptake of the iron-enterochelin complex. No detectable diffusible intermediate in the formation of enterochelin from 2,3-dihydroxybenzoate was formed by cell extracts of mutants carrying mutations in the *entD*, *entE*, or *entF* genes.

Enterochelin has been shown to be involved in the transport of iron in *Escherichia coli* (3, 20). It is a cyclic trimer of *N*-2,3-dihydroxybenzoylserine and is hydrolyzed by cell extracts to give the corresponding linear trimer, dimer, and monomer (13, 14). Enterochelin esterase activity, which probably plays an important role in iron utilization, is repressed by iron and appears to be a single protein which may be separated into two inactive components (O'Brien, Cox, and Gibson, *in press*). Incorporation experiments with radioactively labeled serine and *N*-2,3-dihydroxybenzoylserine indicate that the latter compound is not a precursor of enterochelin (O'Brien, Cox, and Gibson, *in press*), although at relatively high concentrations it does support the growth of a mutant unable to form enterochelin (13). Thus, synthesis of enterochelin from 2,3-dihydroxybenzoate does not proceed by reversal of esterase activity.

The pathway of enterochelin biosynthesis, as far as it is known, is outlined in Fig. 1, and the present communication describes the isolation of a number of mutants which are unable to convert 2,3-dihydroxybenzoate into enterochelin. The separation of these mutants into three classes by biochemical tests and the mapping of three genes concerned in the conversion of 2,3-dihydroxybenzoate into enterochelin are described.

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MATERIALS AND METHODS

Bacterial strains. The strains used were derived from *E. coli* K-12 and are described in Table 1. Stock cultures were maintained on nutrient agar at 4 C and were subcultured monthly.

Chemicals. Wherever possible, chemicals of analytical reagent quality were used. Amino acids were obtained from commercial sources and were not further purified. 2,3-Dihydroxybenzoic acid was obtained from Aldrich Chemical Co. *N*-2,3-dihydroxybenzoylserine and enterochelin were prepared as described previously (12, 14) and were kindly supplied by I. G. O'Brien.

Media. The mineral salts mixture used in all minimal media (and as buffer A) was the medium 56 described by Monod et al. (11) except that no ferrous sulfate was added. Thiamine-hydrochloride (1 μM) was included in all media, and glucose (30 mM) was used as carbon source. When necessary, other supplements were added to the basal salts mixture to give the following final concentrations: 2,2'-dipyridyl, 0.1 mM; 2,3-dihydroxybenzoic acid, 0.01 mM; *N*-2,3-dihydroxybenzoylserine, 0.01 mM; enterochelin 0.01 mM; L-methionine, 0.2 mM; L-histidine-hydrochloride, 0.21 mM; L-proline, 1.45 mM; L-arginine-hydrochloride, 0.7 mM; L-phenylalanine, 0.2 mM; L-tyrosine, 0.2 mM; L-tryptophan, 0.1 mM; L-isoleucine, 0.32 mM; L-leucine, 0.32 mM; L-valine, 0.36 mM; adenine, 0.15 mM; streptomycin sulfate, 0.2 mg/ml.

Agar used in solid media was first washed by repeatedly suspending Difco agar in distilled water until the decanted water no longer appeared discolored. The washed agar was dehydrated with 95% ethanol and redistilled acetone and then dried at room temperature. Amino acids, adenine, and 2,2'-dipyridyl were autoclaved with the medium 56, the other ingredients being

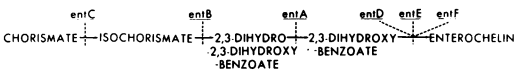

FIG. 1. *Outline of the pathway of biosynthesis of enterochelin (see reference 24) and the structural genes coding for the biosynthetic enzymes. EntC, entB, and entA code for isochorismate synthetase, 2,3-dihydro-2,3-dihydroxybenzoate synthetase, and 2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase, respectively, and have been described previously (24); entD, entE, and entF have been mapped during the present work.*

sterilized separately. Ferrous sulfate, added to media during the isolation of mutants, was sterilized by filtration and added after autoclaving the basal salts mixture. The nutrient broth used for genetic experiments was that described by Luria and Burrous (9).

Growth tests. Cells were tested for their ability to grow in the absence of 2,3-dihydroxybenzoate, *N*-2,3-dihydroxybenzoylserine or enterochelin as follows. Single colonies were suspended in distilled water to give about 10^6 cells/ml, and cells from this suspension were streaked onto solid media containing appropriate growth factors and 2,2'-dipyridyl. To reduce cross-feeding of *ent*$^-$ cells (those requiring enterochelin for growth) by nearby *ent*$^+$ cells, only four streaks were applied to each plate. All plates were incubated at 37 C.

Isolation of mutants. *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) was used as mutagen under the conditions suggested by Adelberg, Mandel, and Chen (1). After treatment with mutagen, cells were grown for about two generations in nutrient broth and washed twice in buffer A. Mutants were then selected by means of the delayed enrichment technique described by Adelberg and Myers (2) except that the penicillin treatment was omitted. After 48 hr of incubation at 37 C, an enrichment layer containing ferrous sulfate was added to each plate to give a final concentration of 0.1 mM, and the plates were reincubated for a further 2 days. All colonies which appeared after 24 hr of the initial incubation period were screened in an attempt to isolate any mutants which were able to grow before enrichment as a result of cross-feeding from nearby *ent*$^+$ cells.

Genetic techniques. Conjugation experiments were performed as described previously (4). When selections were made on solid media for *ent*$^+$ strains, mating mixtures were diluted 1:10 in buffer A before plating to reduce carry-over of growth factors. P1kc bacteriophage lysates were prepared by the method of Swanstrom and Adams (17), and transductions were performed as described by Pittard (15).

Preparation of cell-free extracts. Cells were grown in appropriately supplemented minimal medium containing 2,2'-dipyridyl in 1-liter quantities held in 2-liter flasks shaken at 37 C. Cultures were harvested in late logarithmic or in stationary phase. Cells were washed once in 0.9% NaCl, suspended in 2 ml of tris(hydroxymethyl)aminomethane (Tris)-hydrochloride buffer (0.1 M, *p*H 8) per g (wet weight), and disintegrated in a French pressure cell at 20,000 psi. The disintegrated cells were centrifuged at 20,000 × *g* for 30 min at 4 C, and the resulting supernatant, containing about 30 mg of protein/ml, was used as the cell-free enzyme preparation (cell-free extract).

Test for the enzymatic conversion of 2,3-dihydroxybenzoate to enterochelin and related compounds. Reaction mixtures contained Tris-hydrochloride buffer (*p*H 8), 50 μmoles; $MgCl_2$, 5 μmoles; 2,3-dihydroxybenzoate 1 μmole; L-serine, 1 μmole; adenosine triphosphate (ATP), 10 μmoles; and 0.2 to 0.5 ml of cell-free extract in a total volume of 1 ml. After incubation at 37 C for 60 min, the reaction mixture was acidified to about *p*H 1 with 0.2 ml of 1 M HCl and extracted with 4 ml of ethyl acetate. The organic phase was removed, dried over anhydrous sodium sulfate, and applied to a sheet of thin-layer cellulose (Eastman Chromatogram Sheet 6064). Chromatograms were generally developed in benzene-acetic acid-water (125:72:3, v/v), unless detailed information was required about the nature of the 2,3-dihydroxybenzoate-containing compounds present.

TABLE 1. *Strains of E. coli K-12 used*

Strain no.	Sex	Relevant genetic loci[a]	Other information
AN34	F$^-$	*his-4, proA2, argE3, pheA1, tyrA4, trp-401, str-754*	Derived from AN1 (7)
AN41	F$^-$	*entE405, his-4, proA2, argE3, pheA1, tyrA4, trp-401, str-754*	Isolated from AN34 after treatment with MNNG[b]
AN42	F$^-$	*entD406, his-4, proA2, argE3, pheA1, tyrA4, trp-401, str-754*	
AN49	F$^-$	*entF407, his-4, proA2, argE3, pheA1, tyrA4, trp-401, str-754*	
AN37	F$^-$	*argE3, pheA1, tyrA4, trp-401, str-754*	Derived from AN1
AB259	Hfr		Hfr Hayes
AB1515	F$^-$	*purE*$^-$, *proC*$^-$, *trp*$^-$, *leu*$^-$	Obtained from J. Pittard
KA6[c]	F$^-$	*met*$^-$	Obtained from J. Pittard
AB2332	Hfr	*met-1*	Obtained from J. Pittard
AN102	F$^-$	*proC*$^-$, *trp*$^-$, *leu*$^-$, *fep-401*	Cox et al. (3)
HfrHlip22	Hfr	*lip-22*	Obtained from J. R. Guest

[a] Genetic nomenclature is that used by Taylor (18).
[b] *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.
[c] This strain carries the F-merogenote F8 (6).

In such cases the two-dimensional system described previously (13) was used. Compounds were detected on chromatograms by their fluorescence under ultraviolet light and after spraying with *p*-nitraniline or ferric chloride (13).

Estimation of protein. Protein was estimated by the method of Lowry et al. (8) with bovine serum albumin as standard.

RESULTS

Isolation of mutants requiring increased levels of iron for growth. Mutants of *E. coli* K-12 requiring higher than normal levels of iron for growth were sought. Such a mutant of *E. coli* B has recently been described (20). The parent strain chosen for the present work was strain AN34, a triple mutant of *E. coli* unable to metabolize chorismate along the pathways to phenylalanine, tyrosine, and tryptophan. The use of such a strain greatly facilitates study of the metabolism of chorismate towards the aromatic "vitamins." When strain AN34 was treated with MNNG, nine strains were isolated which failed to grow on a medium containing 2,2'-dipyridyl and 2,3-dihydroxybenzoate, but which were able to grow when 0.1 mM ferrous sulfate was added to this medium. When these strains were streaked onto solid media in close proximity to the *ent*$^+$ strain AN37, they grew. This cross-feeding occurred whether or not 2,3-dihydroxybenzoate was included in the medium. When supplied with either enterochelin or *N*-2,3-dihydroxybenzoylserine, all strains were able to grow in the absence of added iron. This growth occurred whether or not 2,3-dihydroxybenzoate was added to the medium. It therefore seemed likely that these mutants were unable to convert 2,3-dihydroxybenzoate to enterochelin.

Metabolism of 2,3-dihydroxybenzoate by cell-free extracts prepared from ent$^+$ and ent$^-$ cells. When a cell-free extract prepared from the *ent*$^+$ strain AN34 was tested for its ability to metabolize a mixture of 2,3-dihydroxybenzoate, L-serine, and ATP, chromatography of the reaction mixture (Fig. 2) showed that more than one compound was being formed. These compounds were found to correspond in their chromatographic properties, and in their reactions with ferric chloride and *p*-nitraniline, to enterochelin, *N*-2,3-dihydroxybenzoylserine, and the linear trimer of *N*-2,3-dihydroxybenzoylserine (see reference 14).

When cell-free extracts prepared from each of the nine mutant strains were tested under similar conditions, no formation of any of the above three compounds was detected, as shown in the typical results illustrated in Fig. 2. All extracts were, however, able to convert chorismate to 2,3-dihydroxybenzoate and were also able to metabolize enterochelin to *N*-2,3-dihydroxybenzoylserine (I. G. O'Brien, *unpublished data*). These observations, together with the growth responses to enterochelin, indicated that the mutant strains were unable to convert 2,3-dihydroxybenzoate to enterochelin.

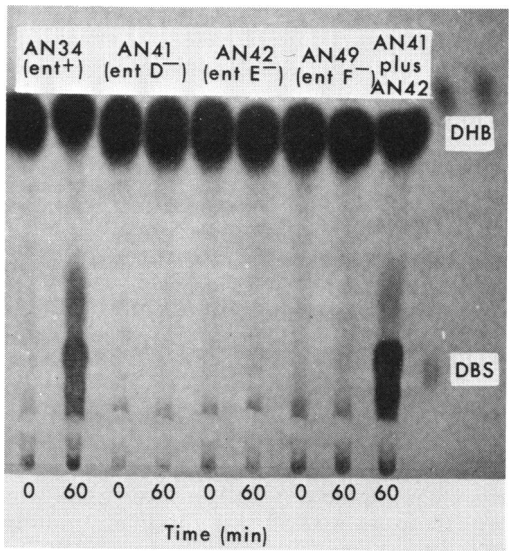

FIG. 2. *Formation of N-2,3-dihydroxybenzoylserine conjugates from 2,3-dihydroxybenzoate, L-serine, and ATP by cell extracts of a strain (AN34) wild type with respect to enterochelin biosynthesis, the three ent$^-$ strains (AN41, AN42, and AN49), and a mixture of cell extracts from two ent$^-$ strains. After incubation of the reaction mixtures for the times indicated, they were acidified and extracted with ethyl acetate, and the extracts were chromatographed in benzene-acetic acid-water on cellulose thin-layer plates. The developed plates were sprayed with ferric chloride spray. DHB and DHS are reference markers of synthetic dihydroxybenzoic acid and N-2,3-dihydroxybenzoylserine, respectively. The former compound in the reaction mixtures is held back by lipid in the cell extracts, and on rechromatography, it ran with authentic 2,3-dihydroxybenzoic acid.*

To determine whether all *ent*$^-$ strains lacked the same enzyme, cell-free extracts prepared from each of the nine mutants were mixed in pairs, and each mixture was tested for its ability to convert 2,3-dihydroxybenzoate into enterochelin. Certain of the mixtures were able to form enterochelin and its metabolism products, whereas others were not. Three distinct classes of mutants were distinguished, and complementation was observed (i.e., enterochelin was formed) when extracts of cells from any two classes were mixed and incubated with 2,3-dihydroxybenzoate, serine, and ATP (Fig. 2).

Genetic analysis of ent$^-$ strains. On the basis of the complementation experiments described

above, it was assumed that each class of mutants was affected in a different gene. The mutation in a strain from each class was then mapped. The genes concerned were denoted as follows—that present in strain AN41 as *entE405*, that in strain AN42 as *entD406*, and that present in strain AN49 as *entF407*.

The approximate locations of the *entD*, *entE*, and *entF* genes were determined from the results of uninterrupted matings between the Hfr strain, AB259, and strains AN41, AN42, and AN49. All three genes were found to be situated in the *proA* region of the chromosome. An interrupted mating between strains AB259 and AN42 showed the *entD* gene to be situated approximately 7 min from *proA* (Fig. 3). A mating between the F male strain KA6 and strain AN42 indicated that the *entE*$^+$ allele was not carried on the F8 merogenote. Similarly, matings between the Hfr strain AB2332 (the direction of transfer of which is opposite to that of strain AB259) and strains AN41, AN42, and AN49 indicated that the alleles *entD*$^+$, *entE*$^+$, and *entF*$^+$ were not transferred as early markers. Since *purE* is transferred as an early marker by strain AB2332 (reference 5; confirmed by a mating between strains AB2332 and AB1515) these results indicated that the *entD*, *entE*, and *entF* genes were situated between the *purE* locus at 13 min (18) and the 16–17 min region of the chromosome carried by the F8 merogenote (10, 16).

Recent work (3, 24) has shown that three

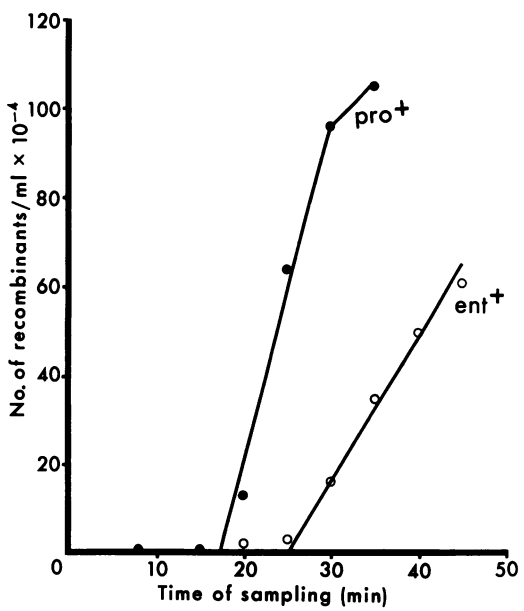

FIG. 3. *Kinetics of recombinant formation during a mating between the Hfr strain AB259 and the F$^-$ strain AN42.*

other genes concerned with the biosynthesis of enterochelin (*entA*, *entB*, and *entC*) and a gene (*fep*) concerned with the uptake system for the iron-enterochelin complex are situated in this same region of the chromosome near minute 14. Strains which carry mutations in the *entA*, *entB*, or *entC* genes are unable to convert chorismate to 2,3-dihydroxybenzoate. All four genes have been shown to be cotransducible with *purE* and with *lip*.

To determine the location of *entD*, *entE*, and *entF* relative to *purE* and *lip*, P1kc lysates prepared on strains AN42, AN41, and AN49 were used to infect the recipient strains AB1515 (*purE*$^-$) and HfrHlip22 (*lip*$^-$) and AN102 (*fep*$^-$). Transductant strains able to grow in the abscence of added adenine (*purE*$^+$) or lipoic acid (*lip*$^+$) or citrate (*fep*$^+$) were selected in the presence of enterochelin and tested to see whether they were *ent*$^+$ or *ent*$^-$. The results obtained are summarized in Table 2. The transduction frequencies obtained indicate that the *entD*, *entE*, and *entF* genes are closely linked and are closer to the *fep* gene than to the *purE* or *lip* genes. These results locate the *entD*, *entE*, and *entF* genes at about minute 14 on the *E. coli* genome.

Attempts to demonstrate the presence of a diffusible intermediate. The isolation of three classes of mutants unable to convert 2,3-dihydroxybenzoate into enterochelin and the successful enzymatic complementation tests suggested that there are at least three reactions concerned in the overall conversion, and that in each mutant two of the enzymes concerned would be unaffected. Experiments were carried out to determine whether diffusible intermediates in enter-

TABLE 2. *Transduction data for the entD, entE, and entF genes*

Donor strain	Recipient strain	Marker selected	No. of transductants carrying unselected marker
AN42 (*entD406*)	AB1515 (*purE*$^-$)	*purE*$^+$	5/191 *entD* (3%)
AN41 (*entE405*)	AB1515 (*purE*$^-$)	*purE*$^+$	2/127 *entE* (2%)
AN49 (*entF407*)	AB1515 (*purE*$^-$)	*purE*$^+$	3/80 *entF* (4%)
AN42 (*entD406*)	AN102 (*fep*$^-$)	*fep*$^+$	68/95 *entD* (72%)
AN41 (*entE405*)	AN102 (*fep*$^-$)	*fep*$^+$	66/92 *entE* (72%)
AN49 (*entF407*)	AN102 (*fep*$^-$)	*fep*$^+$	91/96 *entF* (95%)
AN42 (*entD406*)	HfrHlip22 (*lip-22*)	*lip*$^+$	25/99 *entD* (25%)
AN41 (*entE405*)	HfrHlip22 (*lip-22*)	*lip*$^+$	23/95 *entE* (24%)
AN49 (*entF407*)	HfrHlip22 (*lip-22*)	*lip*$^+$	8/76 *entF* (11%)

ochelin synthesis were formed from 2,3-dihydroxybenzoate by cell extracts of mutants carrying the *entD*, *entE*, or *entF* mutant alleles.

Reaction mixtures following incubation of cell extracts in the enterochelin synthesis system were filtered through a Diaflo ultrafiltration cell fitted with a UM-10 membrane, and the filtrate was incubated with cell extracts of mutants of each of the other two classes with or without fresh substrates. After incubation of the reaction mixtures, no formation of enterochelin or other *N*-2,3-dihydroxybenzoylserine conjugates was detected.

Attempts were made to obtain complementation of activity by using cell extracts of mutants separated by a dialysis membrane. Cell extract from one mutant strain was mixed with the buffer and substrates for enterochelin synthesis in a dialysis tube. This tube was immersed in a narrow tube holding a similar mixture but containing the cell extract of a second strain. No detectable enterochelin or related *N*-2,3-dihydroxybenzoylserine conjugates could be detected after incubation of any such pair of reaction mixtures. Control experiments showed that enterochelin diffused rapidly across the membrane under the conditions used. It seems, therefore, that none of the mutants form a readily diffusible intermediate concerned in the formation of enterochelin from 2,3-dihydroxybenzoate.

DISCUSSION

The results presented suggest that the conversion of 2,3-dihydroxybenzoate to enterochelin involves at least three different polypeptides. Further evidence to this effect has been obtained from preliminary fractionation of cell extract derived from strain AN37 (*ent*$^+$). When cell extract from this strain was passed through an agarose (Bio-GelA-0.5m, 100 to 200 mesh) column and fractions were assayed for their ability to convert 2,3-dihydroxybenzoate to enterochelin, no activity was detected. However, when cell extract derived from one of the mutant strains AN41, AN42, or AN49 was included in reaction mixtures, peaks of activity in different column fractions were observed (R. K. J. Luke, Ph.D. Thesis, Australian National University, Canberra City, 1970). It is not yet possible to order the polypeptides coded for by the *entD*, *entE*, and *entF* genes with regard to their position in the reaction sequence between 2,3-dihydroxybenzoate and enterochelin since it appears that no diffusible intermediate is formed by the cell extract from any of the mutant strains.

Figure 4 shows the location of the *ent* genes and the *fep* gene in relation to the *lip*, *gal*, and *purE* genes. The cotransduction frequency of

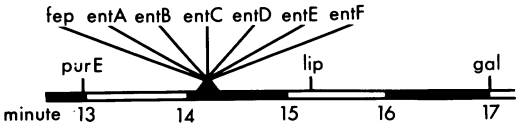

FIG. 4. *Genetic map showing the approximate positions of the entD, entE, and entF genes in relation to the entA, entB, entC, fep, lip, gal, and purE genes (see also 3, 18, 24). The order of genes in the cluster and their exact locations are not known.*

11% for the *entD* and *purE* genes given previously (3) has, as a result of the present experiments, been amended to 3% which is consistent with the *entD* gene being close to the other *ent* genes. Further work is needed to determine the order of the seven clustered genes shown in Fig. 3 and to determine whether any of them are contiguous.

The close linkage of *entD*, *entE*, and *entF* and the close proximity of these genes to *fep*, *entA*, *entB*, and *entC* are of considerable interest, particularly in view of the control exerted by iron over the enzymes concerned in the biosynthesis of enterochelin (21). At least two other enzymes in *E. coli*, namely enterochelin esterase [which probably consists of two polypeptides (O'Brien, Cox, and Gibson, *in press*)] and the enzyme(s) converting chorismate into 3,4-dihydro-3,4-dihydroxybenzoate (23; I. G. Young, Ph.D. thesis, Australian National University, Canberra City, 1969), are repressible by iron. It will be interesting to determine whether the structural genes for these enzymes map in the region of minute 14 on the *E. coli* genome and whether they, together with the 6 *ent* genes and the *fep* gene, form part of an operon regulated by iron.

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LITERATURE CITED

1. Adelberg, E. A., M. Mandel, and G. C. C. Chen. 1965. Optimal conditions for mutagensis by N-methyl-N'-nitro-N-nitrosoguanidine in *E. coli* K12. Biochem. Biophys. Res. Commun. **18**:788–795.
2. Adelberg, E. A., and J. W. Myers. 1953. Modification of the penicillin technique for the selection of auxotrophic bacteria. J. Bacteriol. **65**:348–353.
3. Cox, G. B., F. Gibson, R. K. J. Luke, N. A. Newton, I. G. O'Brien, and H. Rosenberg. 1970. Mutations affecting iron transport in *Escherichia coli*. J. Bacteriol. **104**:219–226.
4. Cox, G. B., F. Gibson, and J. Pittard. 1968. Mutant strains of *Escherichia coli* K-12 unable to form ubiquinone. J. Bacteriol. **95**:1591–1598.
5. Fulton, C. 1965. Continuous chromosome transfer in *Escherichia coli*. Genetics **52**:55–74.
6. Hirota, Y., and P. H. A. Sneeth. 1961. F' and F-mediated transduction in *Escherichia coli* K12. Jap. J. Genet. **36**: 307–318.

7. Huang, M., and F. Gibson. 1970. Biosynthesis of 4-aminobenzoate in *Escherichia coli*. J. Bacteriol. **102**:767–773.
8. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
9. Luria, S. E., and J. W. Burrous. 1957. Hybridization between *Escherichia coli* and Shigella. J. Bacteriol. **74**:461–476.
10. Matney, T. S., E. P. Goldschmidt, N. S. Erwin, and R. A. Scroggs. 1964. A preliminary map of genomic sites for F-attachment in *Escherichia coli* K12. Biochem. Biophys. Res. Commun. **17**:278–281.
11. Monod, J., G. Cohen-Bazire, and M. Cohn. 1951. Sur la biosynthèse de la β-galactosidase (lactase) chez Escherichia coli. La spécificité de l'induction. Biochim. Biophys. Acta **7**:585–599.
12. O'Brien, I. G., G. B. Cox, and F. Gibson. 1969. 2,3-Dihydroxy-N-benzoylserine: chemical synthesis and comparison with the natural product. Biochim. Biophys. Acta **177**:321–328.
13. O'Brien, I. G., G. B. Cox, and F. Gibson. 1970. Biologically active compounds containing 2,3-dihydroxybenzoic acid and serine formed by *Escherichia coli*. Biochim. Biophys. Acta **201**:453–460.
14. O'Brien, I. G., and F. Gibson. 1970. The structure of enterochelin and related 2,3-dihydroxy-N-benzoylserine conjugates from *Escherichia coli*. Biochim. Biophys. Acta **215**:393–402.
15. Pittard, J. 1965. Effect of integrated sex factor on transduction of chromosomal genes in *Escherichia coli*. J. Bacteriol. **89**:680–686.
16. Signer, E. R., J. R. Beckwith, and S. Brenner. 1965. Mapping of suppressor loci in *Escherichia coli*. J. Mol. Biol. **14**:153–166.
17. Swanstrom, M., and M. H. Adams. 1951. Agar layer method for production of high titer phage stocks. Proc. Soc. Exp. Biol. Med. **78**:372–375.
18. Taylor, A. L. 1970. Current linkage map of *Escherichia coli*. Bacteriol. Rev. **34**:155–175.
19. Taylor, A. L., and M. S. Thoman. 1964. The genetic map of *Escherichia coli* K-12. Genetics **50**:659–677.
20. Wang, C. C., and A. Newton. 1969. Iron transport in *Escherichia coli:* relationships between chromium sensitivity and high iron requirement in mutants of *Escherichia coli*. J. Bacteriol. **98**:1135–1141.
21. Young, I. G., G. B. Cox, and F. Gibson. 1967. 2,3-Dihydroxybenzoate as a bacterial growth factor and its route of biosynthesis. Biochim. Biophys. Acta **141**:319–331.
22. Young, I. G., and F. Gibson. 1969. Regulation of the enzymes involved in the biosynthesis of 2,3-dihydroxybenzoic acid in *Aerobacter aerogenes* and *Escherichia coli*. Biochim. Biophys. Acta **177**:401–411.
23. Young, I. G., and F. Gibson. 1969. The enzymic and chemical formation of 3,4-dihydro-3,4-dihydroxybenzoic acid: a new compound derived from chorismic acid. Biochim. Biophys. Acta **177**:182–183.
24. Young, I. G., L. Langman, R. K. J. Luke, and F. Gibson. 1971. Biosynthesis of the iron-transport compound, enterochelin: mutants of *Escherichia coli* unable to synthesize 2,3-dihydroxybenzoate. J. Bacteriol. **106**:51–57.