Journal of General Microbiology (1979), **111**, 193–200. *Printed in Great Britain*

Mutants of *Escherichia coli* K12 Accumulating Porphobilinogen: a New Locus, *hemC*

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(*Received* 28 *July* 1978)

Mutants of *Escherichia coli* K12 which accumulated the haem precursor porphobilinogen are described. The mutants grew very slowly on carbon and energy sources which K12 uses only oxidatively, and they had low catalase activities, suggesting that they were deficient in haem. Extracts had one-tenth of the parental activity of the enzyme porphobilinogen deaminase. In transduction, the mutation mapped close to genes *ilvD* and *metE* at minute 84. The gene was tentatively identified as *hemC*, coding for porphobilinogen deaminase. The gene symbol *hemC* replaces the earlier and temporary symbol *popE*.

INTRODUCTION

Escherichia coli K12 is impermeable to haemin and so the genetics of haem biosynthesis has not been open to ordinary methods of analysis. To obtain mutants with defects in intermediate reactions of porphyrin biosynthesis, Cox & Charles (1973) used a strain carrying mutation *popA1*. The strain gave reddish-brown colonies because the bacteria accumulated protoporphyrin IX. From the mutant, secondary mutants were isolated which grew as small white or pink colonies. The new mutations were given the temporary symbol *sec* and an isolation number, e.g. *sec-20*. Secondary mutants contained less cytochromes and catalase than the parent strain and they grew very slowly on non-fermentable carbon and energy sources. Their growth was not stimulated by haemin and porphyrins. Some mutants accumulated large quantities of haem precursors; thus a strain carrying mutations *popA1 sec-20* accumulated coproporphyrin III and possessed one-fifth of the coproporphyrin oxidase activity of strain *popA1* (Cox, 1973). A strain carrying mutations *popA1 sec-130* accumulated 5-aminolaevulinate and lacked detectable activity of 5-aminolaevulinate dehydratase (Powell, 1975; Powell *et al.*, 1973). Mutants accumulating porphobilinogen or uroporphyrin were not obtained, probably because too few mutants were examined. In the experiments described in this paper a successful search was made for mutants which accumulated porphobilinogen. One of the mutants, carrying mutation *sec-200*, was briefly mentioned by Powell *et al.* (1973). The symbol *sec-200* is now replaced by the symbol *hemC200*, in accordance with the suggestion of Săsărman *et al.* (1976).

METHODS

The materials, media, cultures and methods were, in general, as described by Cox & Charles (1973). Glucose minimal medium (GM) was the medium of Vogel & Bonner (1956) and contained (g l^{-1}): glucose, 5; $MgSO_4.7H_2O$, 0·25; citric acid.H_2O, 2·5; K_2HPO_4, 12·5; $NH_4NaHPO_4.4H_2O$, 4·37. Thiamin.HCl (0·8 mg l^{-1}) was also added because many strains carried a *thi* mutation. Glucose minimal agar (GMA) was GM containing Difco Bacto-agar (13 g l^{-1}). Complete medium contained (g l^{-1}): Tryptone (Oxoid), 10; yeast extract (Difco), 5; K_2HPO_4, 3; KH_2PO_4, 1; glucose, 5. Phage P1kc was used in transduction experiments; the adsorption time was 12 min. The bacterial strains are shown in Table 1. Gene symbols and references to the linkage map follow Bachmann *et al.* (1976).

0022-1287/79/0000-8291 $02.00

Table 1. *Strains of Escherichia coli* K12

Strain		Genotype or description	Reference or source
AB1621	F⁻	*ara-14 tfrA lacY1 tsx-57 galK2 rpsL xyl-5 mtl-1 thi-1* λ^-	Adelberg *et al.* (1965)
POPA1	F⁻	The original *popA1* mutant of AB1621; allele *popA1* is now designated *hemG1* (Säsärman *et al.*, 1975)	Cox & Charles (1973)
SEC200	F⁻	POPA1 carrying mutation *sec-200*; allele *sec-200* is now designated *hemC200*	Powell *et al.* (1973)
AB3509	F⁻	*proA2 tsx-3 galK2 trp-3 his-4 rel-1 malA1 mtl-1 ilvD144 metE46 argH1 thi-1* $\lambda^R\lambda^-$ *sup-16? sup-38* or *sup-48*	Wechsler & Adelberg (1969)
MLM200		*hemG1 xyl*$^+$ *hemC200*: a *xyl*$^+$ transconjugant from conjugation between AB2575 and SEC200; other markers not tested	
MLM509		*trp*$^+$*ilvD metE*: a *trp*$^+$ transconjugant from conjugation between AB2575 and AB3509; other markers not tested	
AB312	Hfr	*thr leu rpsL*	Taylor & Adelberg (1960)
AB2575	Hfr	*ilv thiA tsx* (Hfr Hayes type)	E. A. Adelberg
AT716	Hfr	*rel-1 rbsP thi-1* λ^-	B. J. Bachmann

Assay of 5-aminolaevulinate synthase (EC 2.3.1.37). The method of Burnham & Lascelles (1963) was used. The reaction mixture contained, in 2 ml: glycine, 100 μmol; sodium succinate, 100 μmol; coenzyme A, 0·58 μmol; pyridoxal phosphate, 0·25 μmol; ATP, 7·5 μmol; $MgCl_2$, 10 μmol; EDTA, 1·0 μmol; glutathione, 1·5 μmol; Tris (pH 7·8), 50 μmol; bacterial extract, 1 ml (15 to 20 mg protein). The reaction was stopped by adding 1 ml trichloroacetic acid (10 %, w/v). The product of the reaction, 5-aminolaevulinic acid (5-ALA), was assayed by the method of Gibson *et al.* (1958).

Assay of 5-aminolaevulinate dehydratase (EC 4.2.1.24). The method of Burnham & Lascelles (1963) and Cox (1973) was used. The reaction mixture contained, in 2 ml: 5-ALA, 10 μmol; $MgCl_2$, 5 μmol; L-cysteine, 25 μmol; potassium phosphate buffer (pH 7·5), 125 μmol; bacterial extract, 1 ml (15 to 20 mg protein). The reaction was started by adding 5-ALA. It was stopped in one tube immediately, and in a second after 1 h at 37 °C by adding 1 ml trichloroacetic acid (10 %, w/v). Controls were (a) reaction mixture without extract and (b) reaction mixture without 5-ALA. Precipitated protein was removed and the supernatant liquid was diluted and assayed for porphobilinogen by the method of Mauzerall & Granick (1956), using modified Ehrlich's reagent and measuring the absorbance at 555 nm.

Assay of porphobilinogen deaminase (EC 4.3.1.8). The assay was based on the method of Bogorad (1958). The reaction mixture contained, in 2 ml: porphobilinogen (PBG; Sigma), 400 nmol; Tris buffer (pH 8·2), 125 μmol; extract, 1 ml (15 to 20 mg protein). The reaction was started by adding PBG. It was stopped in one tube immediately, and in a second after 1 h at 37 °C by adding trichloroacetic acid (10 %, w/v). Protein was removed and the supernatant medium was assayed for PBG. Controls were (a) reaction mixture without extract and (b) reaction mixture without PBG.

Assay of ability to form porphyrins from PBG. Cox & Charles (1973) and Powell (1975) showed that crude extracts gave good conversion of 5-ALA to coproporphyrin III. Because pure substrates of some intermediate reactions were not available, the overall formation of porphyrins from 5-ALA or PBG offered a convenient method of testing for gross defects in intermediate reactions. The assay mixture contained, in 2 ml: PBG, 400 nmol; phosphate buffer (pH 7·5), 100 μmol; extract, 1 ml (15 to 20 mg protein). The mixture was incubated in the dark at 37 °C with shaking. The controls were (a) buffer plus PBG, (b) buffer plus extract and (c) buffer plus PBG plus extract heated to 100 °C for 5 min. The reaction was stopped after 200 min by adding 1 ml trichloroacetic acid (10 %, w/v) and the mixtures were aerated in daylight with a vortex mixer to convert porphyrinogens to porphyrins. Porphyrins were extracted and esterified by the method of Falk (1964), and were then separated as methyl esters on silica gel plates (Doss & Philipp-Dormston, 1971) and assayed spectrophotometrically using the millimolar absorption coefficients of Falk (1964).

Catalase activity. This was measured by the method of Herbert (1955). Volumes (5 ml) of a solution of hydrogen peroxide (0·01 M) in phosphate buffer (0·01 M, pH 6·8) were pipetted into five test tubes incubated at 25 °C; 1 ml bacterial suspension was added to each of four tubes and the reactions were stopped after 15, 30, 45 and 60 s, respectively, by adding 1 ml sulphuric acid (2·0 M); sulphuric acid was added to the fifth tube before the bacterial suspension. The amount of peroxide remaining in each tube was assayed by adding 0·5 ml potassium iodide (10 %, w/v) and one drop of ammonium molybdate (1 %, w/v) and, after 3 min, titrating the liberated iodine with sodium thiosulphate (0·02 M) in the presence of starch solution as

indicator. The velocity constant, K_{obs}, for each of the four reaction times was calculated using the formula $K_{obs} = (1/t) \log_e (S_0/S)$ (in s^{-1}), where S_0 and S were the concentrations of peroxide at times 0 and t, respectively. The specific catalase activity, K, of the intact bacteria was calculated from $K = K_{obs}/c$, where K_{obs} was the mean of the experimental values obtained over the four time intervals and c was the dry weight (in mg) of the bacterial suspension divided by the volume of the assay mixture (6 ml). For catalase assay, bacteria were grown for 17 h in GM, washed and resuspended in phosphate buffer (0·01 M, pH 6·8) to an approximate concentration of 200 mg wet wt ml^{-1}.

Preparation of pure samples of the pyrrole (PBG) accumulated by the mutants and assay of PBG. The method of Cookson & Rimington (1954) was used, which makes use of the fact that PBG forms a mercuric salt which is insoluble in water and decomposed by H_2S. Bacteria were grown in 800 ml GM, with shaking for 24 h. The pH value of the supernatant medium was adjusted to 4·0 with glacial acetic acid, and then a solution of mercuric acetate (15 %, w/v) was added until the medium ceased to give a pink colour with Ehrlich's reagent. The precipitate was washed with mercuric acetate (1 %, w/v), resuspended in 3 ml water and decomposed with H_2S. The mercuric sulphide was removed by centrifugation and H_2S was removed by passing air through the solution. The solution was then treated successively with lead acetate (10 %, w/v) and silver nitrate (20 %, w/v) to remove dipyrrolic and tetrapyrrolic substances; the precipitates were discarded. To the filtrate an excess of mercuric acetate (15 %, w/v) was added. The precipitate was collected by centrifugation and decomposed without addition of water by passing H_2S into the paste and stirring with the gassing tube. The mercuric sulphide was removed by centrifugation and air was passed through the solution to remove H_2S.

PBG was assayed by the method of Mauzerall & Granick (1956). Equal volumes of Ehrlich's reagent were added to serial dilutions of the preparations, and after 15 min the absorbance at 555 nm was measured using a Perkin Elmer Hitachi 124 automatic scanning spectrophotometer. The reference cuvette contained equal volumes of Ehrlich's reagent and water. The concentration of PBG was calculated using the absorption coefficient $\epsilon_{mM} = 61$ (Mauzerall & Granick, 1956).

RESULTS

Isolation of mutants which accumulated porphobilinogen

Strain POPA1, carrying mutation *popA1* (Cox & Charles, 1973) now called *hemG1* (Săsărman *et al.*, 1975), was treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (30 mg l^{-1}) for 20 min, washed, incubated in complete medium for 14 h and plated on GMA. Amongst the reddish-brown colonies of 5 mm diameter were occasional white colonies of 1 mm or less; 300 of these were transferred to 3 ml volumes of GM. After 48 h incubation, without shaking, the supernatant media were tested with Erhlich's reagent as modified by Mauzerall & Granick (1956). The media from three cultures gave a pink colour, indicating that they accumulated a substance having a pyrrole or indole ring. The parental strain POPA1 did not give a coloured product with the reagent. The new mutant strains were designated SEC200, SEC203 and SEC206.

Other pyrroles, as well as PBG, form pink compounds with Ehrlich's reagent, and it was necessary to test whether the substance accumulated was PBG. To obtain pure samples, supernatant media from cultures of the three mutants were treated by the method of Cookson & Rimington (1954) as described in Methods. The absorption spectra of the pink compound(s) formed on addition of modified Ehrlich's reagent were recorded between 700 and 380 nm. The substance from each mutant gave the same spectrum as that obtained on addition of the reagent to an authentic sample of PBG (Sigma), with an absorption maximum at 555 nm and a lesser maximum at 325 nm. The amounts of PBG accumulated are shown in Table 2.

More evidence that the accumulated substance was PBG came from chromatography, using the method of Westall (1952). Samples of the substances isolated from the supernatant media, and reference samples of PBG (1 μg), were applied as spots in a line across the grain of 20 × 20 cm duplicate sheets of Whatman no. 1 paper. The ascending method was used, at 18 °C, and the solvent was butanol/glacial acetic acid/water (40:10:15, by vol.). When the solvent had traversed the sheets, one sheet was sprayed with Ehrlich's reagent to detect the spots. The second sheet was heated to 100 °C for 15 min in acetic

Table 2. *Catalase activity and accumulation of porphobilinogen by mutant and parental bacteria*

For measurements of PBG accumulation, bacteria were grown for 24 h in 800 ml GM, with shaking at 200 rev. min^{-1}, and PBG was assayed by adding 2 ml of modified Ehrlich's reagent to an equal volume of supernatant culture medium and measuring the absorption at 555 nm after 15 min. For measurements of catalase activity, bacteria were grown aerobically for 17 h in GM, resuspended in phosphate buffer (0·01 M, pH 6·8) to about 200 mg wet wt ml^{-1} and assayed as described in Methods.

Strain	PBG accumulated in culture medium (μmol)	$10^3\times$ Catalase activity [l (g dry wt)$^{-1}$ s^{-1}]	Dry weight of bacteria [g (800 ml culture medium)$^{-1}$]
SEC200	2·9	0·3	0·43
SEC203	1·1	0·6	0·49
SEC206	2·7	0·5	0·44
POPA1	ND	4·4	0·92
AB1621	ND	10·1	0·98

ND, Not detectable (< 0·1 μmol).

acid vapour to convert PBG to uroporphyrin, which was then detected under ultraviolet radiation at 380 nm as red fluorescent spots. The substance obtained from the mutants had the same R_F as the reference sample of PBG and, like PBG, gave red fluorescent spots on heating in acetic acid vapour. The conversion to porphyrin by heating in acetic acid vapour is firm evidence that the substance was PBG (Falk, 1964).

Indirect evidence that the mutants were deficient in haem

The PBG-accumulating mutants grew very poorly with carbon and energy sources that K12 uses oxidatively but not fermentatively, such as lactate, succinate and acetate. The mutants grew more vigorously on fermentable substrates. Growth was not stimulated by haemin.

Beljanski & Beljanski (1957) showed that haem-deficient mutants of *E. coli* had low catalase activity. The PBG-accumulating mutants had very low catalase activities (Table 2), suggesting that they were largely but not completely defective in haem biosynthesis.

Evidence that strain SEC200 *was deficient in porphobilinogen deaminase*

Assay of 5-ALA synthase showed that this activity was absent from SEC200 and its progenitors POPA1 and AB1621. On previous occasions, activity of the enzyme had been observed in POPA1 and AB1621 and the reason for lack of activity on this occasion is unexplained (see Discussion). Extracts of SEC200 possessed as much activity of 5-ALA dehydratase as extracts of parental bacteria, but had only one-tenth of the parental activity of PBG deaminase (Table 3).

Because substrates for some of the later reactions of haem biosynthesis were not available, the ability of extracts to convert PBG to uroporphyrin, coproporphyrin and protoporphyrin was assayed (see Methods). The results (Table 4) show that extracts of SEC200 formed smaller amounts of porphyrins than the progenitor strains, in agreement with the evidence that the mutant was relatively deficient in PBG deaminase. Ferrochelatase was not assayed: the mutant is partly deficient in ferrochelatase because of the *hemG1*(*popA1*) mutation which causes protoporphyrin accumulation (Cox & Charles, 1973; and Table 4).

Evidence that mutation hemC200 mapped between genes ilvD and metE

Mutant allele *hemC200*, in SEC200, was mapped by conjugation and transduction. Mapping was laborious because the mutation was not conditionally lethal and it was not possible to select for *hemC* transconjugants. In two experiments, Hfr strains AB2575 and AB312 were used in uninterrupted conjugation with recipient SEC200 *ara hemG1 gal rpsL*

Table 3. *5-Aminolaevulinate dehydratase activity and porphobilinogen deaminase activity in crude extracts of* SEC200 *and its progenitors*

The same extracts were used for assays of both enzymes; assays were made in duplicate using different extracts. For details, see Methods. 5-ALA dehydratase activities are expressed as nmol PBG produced h^{-1} (mg protein)$^{-1}$, and PBG deaminase activities as nmol PBG consumed h^{-1} (mg protein)$^{-1}$.

Strain	Description of strain	Activity of 5-ALA dehydratase	Activity of PBG deaminase
AB1621		9·5	7·6
		8·0	5·3
POPA1	Mutant of AB1621 accumulating	8·8	5·8
	protoporphyrin IX	8·3	5·1
SEC200	Mutant of POPA1 accumulating PBG	8·7	0·3
		7·9	0·2

Table 4. *Conversion of porphobilinogen to porphyrins by crude extracts of* SEC200 *and its progenitors*

Extracts were assayed for the ability to form porphyrins from PBG as described in Methods. Results show the amounts of different porphyrins in the reaction mixture after 200 min incubation.

Source of extract	Amount of PBG added (nmol)	Uroporphyrin (nmol)	Coproporphyrin (nmol)	Protoporphyrin (nmol)	Protein (mg)
AB1621	400	8·4	14·0	6·6	22·1
	0	0	0	0	21·1
POPA1	400	7·0	18·0	24·0	18·0
	0	0·7	1·6	14·0	18·9
SEC200	400	2·0	3·5	1·7	17·6
	0	1·1	2·3	1·2	17·6
No extract	400	0·4	0	0	0

xyl hemC200. Selection was made for *ara*$^+$, *gal*$^+$ and *xyl*$^+$ transconjugants with the intention of classifying them as *hemC200* and *hemC*$^+$ according to colony size and colour. Colony size proved to be unsatisfactory for this purpose because the colonies were often slimy and diffuse, especially on galactose minimal medium. One hundred colonies of each selected type, from each conjugation, were therefore restreaked and cultured separately in liquid minimal medium (3 ml) of the same constitution as the selective medium from which they were picked, and the supernatant media were tested for PBG with Ehrlich's reagent. All the *ara*$^+$ and *gal*$^+$ recombinants accumulated PBG, but amongst the *xyl*$^+$ recombinants 68% (donor AB2575) and 97% (donor AB312) did not accumulate PBG, showing that *hemC* was near *xyl* at minute 80.

Strains carrying *hemC200* were used as donors in transduction, and selection was made for transduction of *xyl*$^+$, *rbs*$^+$, *ilv*$^+$ and *metE*$^+$ into appropriate recipients; 920 transductants were cultured separately and their supernatant media were tested for PBG. To check that transductants which were scored as *hemC200* (Table 5) did indeed accumulate PBG and not other compounds which reacted with Ehrlich's reagent, one transductant of each of the classes *ilvD*$^+$ *hemC200*, *metE*$^+$ *hemC200* and *rbs*$^+$ *hemC200* was grown separately in 800 ml GM containing the amino acids required for growth, and the compound accumulated was extracted and analysed as described above. All samples from the transductants were identical with a reference sample of PBG. A positive reaction with Ehrlich's reagent was therefore a satisfactory indicator of the *hemC200* genotype. Table 5 shows that *hemC200* was 6%, 24% and 40% cotransducible with *rbs*$^+$, *ilvD*$^+$ and *metE*$^+$, respectively. The frequency of cotransduction of *ilv* and *metE* depended on which marker was selected, as discussed by Bachmann *et al.* (1976). The data do not clearly point to a particular gene order.

Table 5. *Cotransduction of hemC200 with genes rbsP*$^+$, *ilvD*$^+$ *and metE*$^+$

The donors used were MLM200 (*xyl*$^+$ *hemG1 hemC200*) and SEC200 (*hemG1 hemC200*).

Donor	Recipient and relevant markers	Selected allele	Transductants per 10^7 phage	No. scored	Unselected donor allele present	Cotransduction frequency (%)
MLM200	AB1621 *xyl*	*xyl*$^+$	11	100	*hemC*	0
MLM200	MLM509 *metE*	*met*$^+$	21	64	*hemC*	39
SEC200	AT716 *rbsP*	*rbs*$^+$	6	112	*hemC*	6
SEC200	AB2575 *ilv*	*ilv*$^+$	14	48	*hemC*	25
SEC200	MLM509 *ilvD metE*	*ilv*$^+$	18	312	*hemC*	23
		met$^+$	20	284	*hemC*	38
SEC200	MLM509 *ilvD metE*	*ilv*$^+$	18	200	*hemC*	17
					met$^+$	4
					hemC met$^+$	8
		met$^+$	20	200	*hemC*	10
					ilv$^+$	16
					hemC ilv$^+$	32
		ilv$^+$*met*$^+$	8	80	*hemC*	78

At the start of the transduction experiments it was found that the supernatant media of AB3509 transductants always gave a pink product with Ehrlich's reagent, having an absorption maximum at 564 nm compared with 555 nm for the product of Ehrlich's reagent and PBG. Strain AB3509 required tryptophan, which apparently caused accumulation of a substance which reacted with Ehrlich's reagent. Tryptophan did not give a pink reaction with Ehrlich's reagent at the concentration present in the growth medium (40 mg l^{-1}). The reactive substance may have been indole. To avoid the problem a *trp*$^+$ transconjugant, MLM509 *ilvD metE*, of AB3509 was prepared and used in the transduction experiments.

DISCUSSION

Mapping showed that one of the mutations represented a new gene at minute 84 on the linkage map of Bachman *et al.* (1976). The gene was briefly mentioned by Powell *et al.* (1973) and given the temporary symbol *popE*, now superceded by the symbol *hemC*, which Săsărman *et al.* (1976) allotted to the gene for PBG deaminase. Haemin and porphyrins do not penetrate *E. coli* K12 bacteria under normal cultural conditions (Săsărman *et al.*, 1968), and mutants which are fully defective in an enzyme of haem biosynthesis, such as PBG deaminase, are therefore likely to be inviable. Exceptions are mutants defective in the synthesis of 5-ALA, the first intermediate of the haem biosynthetic sequence; mutants responding to 5-ALA are well known, and they do not respond to haemin. The *hemC* mutants had about one-tenth of the PBG deaminase activity of their parental bacteria. This may explain why the mutants were able to grow slowly on glucose minimal medium and accumulate PBG. Mutants which accumulate PBG and have low or undetectable PBG deaminase have been described by Hatch & Lascelles (1972) in *Rhodopseudomonas*, by Berek *et al.* (1974) in *Bacillus* and by Săsărman *et al.* (1976) in *Salmonella*.

A problem in the enzymological analysis was the failure to detect 5-ALA synthase activity in parental and mutant strains, even though it should have been present in all. On other occasions, activity of 5-ALA synthase has been detected in the parental strain AB1621 and in some haem-deficient mutants of K12 (Powell *et al.*, 1973). The synthase is a difficult enzyme to assay, even in favourable organisms, and is especially difficult in *E. coli*; a better assay is needed than was available to us. The enzyme may be labile as in *Rhodopseudomonas* (Warnick & Burnham, 1971) and may require a trisulphide activator (Davies *et al.*, 1973). Presumably all the strains possessed 5-ALA synthase, because they did not require 5-ALA for growth. Taken together, the facts that *hemC* mutants accumulated PBG and had only

10% of the PBG deaminase activity of the parental bacteria provide evidence that the gene specifies PBG deaminase.

The gene *hemC* is typical of genes for haem biosynthesis in *E. coli* in mapping apart from other *hem* genes. In *Bacillus subtilis* (Anderson & Ivánovics, 1967; Kiss *et al.*, 1971; Berek *et al.*, 1974) and *Staphylococcus aureus* (Tien & White, 1968) the genes for haem biosynthesis tend to be closely grouped.

Selection for *ilvD*$^+$ *metE*$^+$ gave transductants of which 78% were *hemC*, suggesting that the gene order may be *ilvD hemC metE*, but better data are required to establish the order. In *Salmonella typhimurium*, mutations in a gene *hemC* similarly cause accumulation of PBG and map between genes *ilv* and *metE* at minute 122 (Săsărman *et al.*, 1976). The linkage map of K12 (Bachmann *et al.*, 1976) shows 10 genes between *ilv* and *metE*, five of which are of uncertain position. Finding the position of *hemC* amongst the other genes would be time-consuming using the methods described here. The isolation of mutants of K12 which are permeable to haemin (McConville & Charles, 1975) has now greatly simplified genetical experiments with *hem* mutants and may simplify the mapping of the *hemC* gene. However, no mutants deficient in PBG deaminase were present amongst 55 haemin-requiring mutants isolated in a haemin-permeable strain (McConville, 1977).

This work was greatly aided by a Medical Research Council project grant to H. P. Charles, and by technical assistance from David L. Morris. The data in this paper are from the Ph.D. thesis of M. L. McConville (1977).

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