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Mapping of purine markers in *Escherichia coli* K 12

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1. INTRODUCTION

The present knowledge of purine biosynthesis is based on information obtained from experimental observations on avian, mammalian and microbial systems. The description of the mechanism of purine biosynthesis in higher organisms, especially in pigeon liver, is rather complete (for reviews see Buchanan & Hartman, 1959; Schulman, 1961). The scheme for the biosynthesis of purines in pigeon liver is given in Fig. 1. Our knowledge of purine biosynthesis in micro-organisms is more limited, but the facts indicate an overall similarity between purine biosynthesis in micro-organisms and in higher organisms. A number of mutants with known biochemical blocks have been isolated in *Escherichia coli* (Gotts, 1950; Love & Gotts, 1955; Gotts & Gollub, 1957; Gollub & Gotts, 1959), in *Salmonella typhimurium* (Yura, 1956), and in *Aerobacter aerogenes* (Magasanik & Brooke, 1954; Balis, Brooke, Brown & Magasanik, 1956; Magasanik & Karibian, 1960), and a number of intermediates have been isolated from bacteria as well as yeasts and moulds. Genetic analysis of the various mutations has been done by crosses in Neurospora (Giles, Partridge & Nelson, 1957; Ishikawa, 1960), with the aid of transduction in *Salmonella* (Yura, 1956) and by conjugation in *E. coli* K 12 (Jacob & Wollman, 1961). Only a few mutations have been mapped in the last system but the corresponding biochemical blocks are unknown. The attempt of the present work was to map the purine markers on the *E. coli* K 12 chromosome.

2. MATERIALS AND METHODS

(i) *Strains*

The donor strains employed were:
Hfr R_4 (Reeves, 1960) and its derivative: Hfr R_4 118 *argura*$^-$.
Hfr. H (Hayes, 1953). A λ^- derivative was used.
Hfr AB 313 (Taylor & Adelberg, 1960) and its derivative: Hfr AB 313 *tyr*$^-$.
5833 (*F lac*$^+$ *ade*$^+$) (Hirota & Sneath, 1961).

The acceptor strains employed were:
thi$^-$ *gal*$^-$ *lac*$^-$ (Stouthamer, de Haan & Bulten, 1963) and its derivatives *thi*$^-$ *his*$^-$ *try*$^-$ *gal*$^-$ *lac*$^-$, *thi*$^-$ *tyr*$^-$ *his*$^-$ *gal*$^-$ *lac*$^-$, *thi*$^-$ *arg*$^-$ *gal*$^-$ *lac*$^-$ and *thi*$^-$ *tyr*$^-$ *his*$^-$ *try*$^-$ *ura*$^-$ *gal*$^-$ *lac*$^-$. All acceptor strains were streptomycin resistant, the donor strains with the exception of Hfr AB 313 were sensitive to this drug.

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The symbols of the markers represent:

ade, adenine; *thi*, thiamine; *adth*, adenine + thiamine; *arg*, arginine; *argura*, arginine + uracil; *gua*, guanine; *his*, histidine; *leu*, leucine; *met*, methionine; *pur*, purine; *pyr*, pyrimidine; *thr*, threonine; *try*, tryptophane; *tyr*, tyrosine; *gal*, galactose; *lac*, lactose; *str*, streptomycin.

(ii) *Isolation of mutant strains*

Most of the amino acid and purine-deficient mutants were obtained with the aid of nitrous acid, followed by penicillin enrichment as described earlier (de Haan, Stouthamer, Felix & Mol, 1963). In the latter part of this work N-methyl-N-nitroso-N′-nitroguanidine was used as mutagen. One ml. of an overnight culture was inoculated into 9 ml. of prewarmed broth and incubated for 4 hours at 37°C. on an inclined turntable. The culture was then centrifuged and the sediment was washed once with 0·1 M acetate buffer, pH 5·0. The sediment of 10 ml. culture was finaly suspended in 0·5 ml. of acetate buffer and 0·1 ml. of a N-methyl-N-nitroso-N′-nitroguanidine solution (4 mg./ml. in acetate buffer) was added. After 2 hours' incubation at 37°C. 8 ml. of broth were added. The suspension was centrifuged and the sediment resuspended in 10 ml. broth. Afterwards the culture was incubated overnight.

Dr Brenner kindly supplied the N-methyl-N-nitroso-N′-nitroguanidine and the directions for its use.

(iii) *Methods of crossing*

In liquid medium

Overnight cultures of donor and acceptor strains were made by inoculating 5 ml. of nutrient broth and incubating at 37°C. without aeration. Fresh cultures were prepared by diluting 1 ml. of the overnight cultures into 9 ml. of prewarmed broth in screw-capped bottles. The cultures were then incubated for 90 min. on an inclined turntable. Mating mixtures were obtained by mixing 1 vol. of donor with 10 vol. of acceptor cells. Five min. after mixing, the non-rotated suspension was diluted into prewarmed minimal medium to prevent further contact formation.

Transfer was interrupted at the desired moment by violently shaking for 30 sec. on a Microid flash shaker (when Hfr R_4 and Hfr H were used) or by adding a suspension of ultra-violet killed phage T_6 (when Hfr AB 313 was the donor strain)

On solid medium

Fresh cultures of donor and acceptor cells were centrifuged, washed twice with saline and resuspended in ten times the original volume of saline. About 2×10^6 cells of both types were plated on selective medium.

(iv) *Determination of biochemical block*

The biochemical block in the purine mutants was determined as follows: The mutants were first divided into three groups, adenine specific, guanine specific and

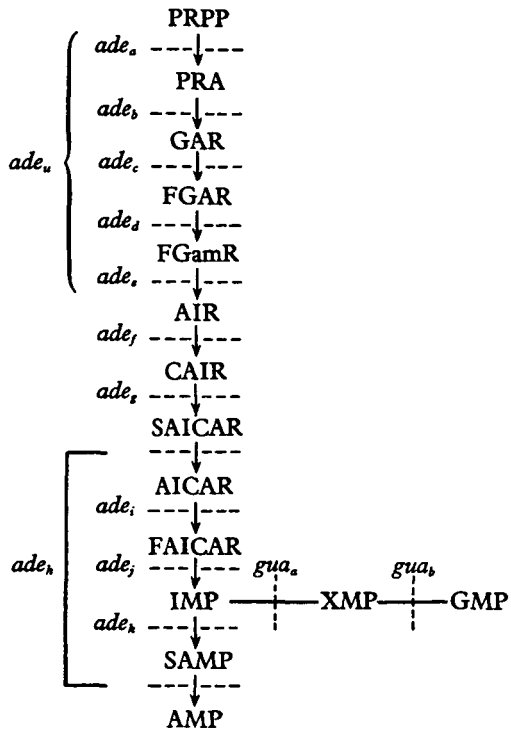

Fig. 1. Pathway of purine biosynthesis. PRPP, 5-phosphoribosyl-1-pyrophosphate; PRA, 5-phosphoribosylamine; GAR, glycinamide ribonucleotide; FGAR, formyl glycinamide ribonucleotide; FGamR, formyl glycinamidine ribonucleotide; AIR, 5-aminoimidazol ribonucleotide; CAIR, 5-amino-4-imidazolcarboxylic acid ribonucleotide; AICAR, 5-amino-4-imidazolcarboxamide ribonucleotide; SAICAR, 5-amino-4-imidazol-N-succinocarboxamide ribonucleotide; FAICAR, 5-formamido-4-imidazolcarboxamide ribonucleotide; IMP, inosinic acid; AMP, adenylic acid; SAMP, adenylosuccinic acid; XMP, xanthylic acid; GMP, guanylic acid.

non-specific strains. The non-specific strains grew on adenine, guanine or hypoxanthine, showing that the mutants were deficient in the synthesis of IMP (cf. Fig. 1). The guanine specific strains were divided into two groups: IMP dehydrogenase negative mutants with a positive growth response on xanthine (gua_a^-) and xanthosine aminase negative mutants (gua_b^-) which grew only on guanine.

The adenine specific and the non-specific mutants were tested for accumulation of imidazol derivatives during growth. The mutants were grown in minimal medium with a suboptimal amount of adenine (10 μg./ml.) for a period of 24–40 hours under aerobic conditions (shaking). After centrifugation, the culture fluid was diazotized and the diazo compound coupled with N(naphthyl) ethylenediamine according to the method of Bratton & Marshall (1939). The absorption maxima of the chromophores were: 502 mμ for AIR and 540 mμ for AICAR and SAICAR. It is known that imidazol derivative-accumulating mutants accumulate a mixture of ribotides and ribosides (Gollub & Gotts, 1959). This however does not effect the absorption maxima in the Bratton-Marshall reaction.

Mutants deficient in the enzyme, which catalyses the formation of SAICAR from CAIR also accumulate AIR in their culture fluids, as CAIR is unstable and decarboxylates spontaneously to AIR (Gotts, personal communication). The presence of SAICAR was shown by treating the culture fluid with HNO_2 for 30 sec. (Gotts & Gollub, 1957) instead of 8 min. Mutants deficient in the formation of adenylosuccinase (ade_h^-) accumulate SAICAR. The enzyme also catalyses the formation of AMP from SAMP. The SAICAR-accumulating strains are therefore adenine specific (cf. Fig. 1) and the adenine specific strains, which do not accumulate SAICAR are consequently deficient for the enzyme adenylosuccinate synthetase (ade_k^-).

Mutants which did not accumulate Bratton-Marshall positive material were tested for the accumulation of F-AICAR by acid hydrolysis of the culture fluid (10′ at 100°C. in 0·2 N acid) followed by a Bratton-Marshall reaction on AICAR.

A number of non-specific strains were isolated which did not accumulate detectable amounts of Bratton-Marshall positive material (steps $ade_{a,b,c,d \text{ and } e}$). No attempts were made to elucidate the position of the biochemical blocks in this group. The mutants are designated ade_u (unknown block).

A number of *adth*$^-$ mutants which need thiamine and adenine for growth were isolated. These mutants have been described before by Yura (1956). All *adth*$^-$ mutants grew on hypoxanthine instead of adenine and did not accumulate Bratton-Marshall positive material. A number of *adth*$^-$ mutants were isolated from $\times$*thi*$^-$ acceptor strains. The *adth*$^-$ mutants were detected in these strains by plating 10^8 cells on minimal medium supplemented with the appropriate growth factors (purine, tyrosine, histidine and tryptophane) with the omission of thiamine. The *thi*$^-$–*adth*$^-$ mutants gave no back mutants to thi$^+$ under this condition, whereas all *thi*$^-$–*ade*$^-$ mutants did.

(v) *Calculation of recombination frequencies*

The recombination frequencies between selected and unselected markers were calculated from the frequencies of the four recombinant classes with the method used by Bailey (Bailey, 1951*a*, *b*, 1961) for four-point crosses. Let us consider a four-point cross of the type:

	Y_1		Y_2		Y_3		
a^+		b^+		c^+		d^-	O
a^-		b^-		c^-		d^+	

in which a^+d^+ recombinants are selected. O represents the origin of the Hfr fragment.

The recombination frequencies over the three segments are Y_1, Y_2 and Y_3 and interference is supposed to be absent. We may then write down the expectations and observations for this cross as in Table 1.

There are three degrees of freedom in this cross and three unknowns to estimate.

By equating the observations to their expectations we obtain:

$$\frac{Y_1}{1-Y_1} = \left(\frac{ad}{bc}\right)^{1/2}; \qquad \left(\frac{Y_2}{1-Y_2}\right) = \left(\frac{bd}{ac}\right)^{1/2}; \qquad \left(\frac{Y_3}{1-Y_3}\right) = \left(\frac{cd}{ab}\right)^{1/2}. \tag{1}$$

The recombination frequencies are calculated with the aid of equation (1).

In some crosses the F^- counterselection marker d^+ was not available. The recombination frequencies were calculated with the same method on the assumption that counterselection for the part of the F^- chromosome corresponding to the origin of the Hfr chromosome takes place (Verhoef, personal communication).

Table 1. *Expectations and observations in a four-point cross of the type given in the text*

Recombination in segment	Expected frequency	Observed frequency
1	$n\ Y_1\ (1-Y_2)\ (1-Y_3)/R$	a
2	$n\ (1-Y_2)\ Y_2\ (1-Y_3)\ R$	b
3	$n(1-Y_1)\ (1-Y_2)\ Y_3/R$	c
123	$n\ Y_1\ Y_2\ Y_3\ /R$	d
Total	n	n

n is the total number of recombinants; R is the total recombination over the whole segment.

3. RESULTS

(i) *Preliminary mapping*

For preliminary mapping experiments mutants deficient in the biosynthesis of adenine or guanine were isolated from the acceptor strains *thi*$^-$ and *thi*$^-$ *tyr*$^-$ *his*$^-$ *try*$^-$ and the biochemical blocks were determined as described under Materials and Methods.

All mutants were screened for *pur*$^+$ recombinants in crosses with the Hfr strains R_4, H and AB 313 (mating time 40 min.). The AIR accumulating strains were also crossed with 5833 (mating time 15 min.). About half of the AIR accumulating strains gave high yields of *ade*$^+$ cells when crossed with strain 5833 and gave also positive results in crosses with Hfr H. The positive result with 5833 showed that these strains lacked the enzyme AIR carboxylase (*ade*$_f^-$) (Gotts, personal communication). This marker is identical with the *pur*$_2$ marker on the chromosome map of Jacob & Wollman (1961). The remainder of the AIR accumulating mutants (*ade*$_g^-$) gave *ade*$^+$ recombinants when crossed with Hfr AB 313. One of the 13 *ade*$_u^-$ mutants gave positive results when crossed with Hfr R_4 or Hfr H; the remaining *ade*$_u^-$ mutants gave positive results when crossed with Hfr AB 313. In this way the *ade*$_u^-$ mutants could be divided into two groups *ade*$_{u,a}$ and *ade*$_{u,b}$. (Note: *a* and *b* do not reflect the reactions *a* and *b* in Fig. 1.) The *ade*$_h$ marker was transferred by Hfr H; three loci (*adth*$_a$, *ade*$_i$ and *ade*$_k$) were transferred by Hfr R_4 whereas Hfr AB 313 transferred the remaining purine markers (*ade*$_{u,b}$, *ade*$_g$, *adth*$_b$, *gua*$_a$ and gua$_b$). The *adth*$^+$ recombinants were selected on thiamine supplemented medium when *thi*$^-$ *adth*$^-$ strains were used as acceptor.

(ii) *Interrupted mating experiments*

A more exact position of the purine markers on the chromosome map was obtained by following the penetration of the markers into the recipient cell in interrupted mating experiments.

The $adth_a$, ade_i and ade_k loci were transferred by Hfr R_4 about 1–2 min. before the *thi* markers and the five loci transferred by AB 313 ($adth_b$, $ade_{u,b}$, ade_g, gua_a and gua_b) entered the recipient cell from 0 to 5 min. after the *tyr* marker.

The ade_h marker was located between *gal* and *try* and the $ade_{u,a}$ marker was transferred at the same time as *thr*.

The differences found in the penetration times of the three *ade* loci transferred by Hfr R_4 or the five loci by Hfr AB 313 were too small to determine the exact order of the loci, and some of the loci were so closely linked to the reference locus that differences in penetration times between marker and reference locus (*thi*, or *tyr*) could hardly be detected.

As it was also realized that (unknown) unfavourable conditions for one kind of recombinant (differential viability) could suggest a false order of penetration of closely linked markers, another type of experiment was tried to verify the order of penetration of reference locus and purine marker. In this type of experiment early and late recombinants for reference marker or purine marker were selected and the fraction of the unselected marker was then determined. Early selection, by interrupted mating, of cells recombinant for the marker nearer to the origin prevents the entrance of the unselected marker, thus destroying the linkage between the two markers. It was expected that early selection for the distal marker had less influence on the linkage between the two markers as every recombinant had received both markers.

In Table 2 a typical experiment (Hfr $R_4 \times thi^-$ ade_i^-) is given. The ade_i marker is transferred just prior to the *thi* marker. In these experiments thi^+ and ade^+ recombinants were selected during an interrupted mating experiment and the fraction of recombinants with the Hfr allele of the unselected marker among these recombinants was determined.

It may be seen from Table 2 that early interruption of contact prevents the penetration of the distal marker (*thi*) when recombinants for the proximal marker (ade_i) are selected. Selection for thi^+ recombinants at early times also prevents the incorporation of the *ade* marker into the recombinant. This unexpected result is probably due to the fact that the early interruption allows only small fragments beyond the *thi* marker to be transferred to the zygote. Thus all the early thi^+ recombinants result from a recombinational event behind and close to the *thi* marker. High negative interference might then separate the *thi* and ade_i marker. At later times the high negative interference, taking place at a point far beyond the *thi* marker, has no influence on the linkage between *thi* and ade_i. Similar results were obtained with entrance curves of the *tyr* and the *tyr*-linked purine markers.

It was concluded that the order of closely linked markers could not be determined by early interruption of mating. Therefore a method giving a higher

Table 2. *Interrupted mating experiment with Hfr R_4 as donor and a* thi$^-$ ade$_i^-$ *acceptor strain*

Time (min.)	Number of *ade$_i$* recomb.	Percentage *thi*$^+$	Number of *thi*$^+$ recomb.	Percentage *ade*$^+$
5	0		0	
20	15	69·5	10	70·0
25	20		20	
29	99	80·0	91	88·0
35	432		423	
40	780		640	
55	1960	98·0	1820	98·0

For mating procedure see Materials and Methods (liquid medium). The mating mixture was diluted 1:100 5 min. after mixing. Chromosome transfer was interrupted at intervals by blending and samples of 0·1 ml. were plated. Recombinants prototrophic for *ade* or *thi* were selected and the fraction with the Hfr allele of the unselected marker was determined.

resolution was used. This method, selecting for a distal Hfr and a proximal F^- marker, is described in the next part of this paper.

(iii) *The linkage group* thi–adth$_a$–ade$_i$–ade$_k$

The preliminary mapping results showed that three markers, *adth$_a$*, *ade$_i$* and ade$_k$ were transferred by Hfr R_4 and from interrupted mating experiments it was concluded that the purine markers entered the zygote before the *thi* marker. The order of the four loci was determined in crosses between Hfr R_4 118 (*argura*$^-$) and *thi*$^-$ *arg*$^-$ strains into which the purine mutations were introduced. The *argura*$^-$ marker is located between *thr* and *leu* (Beckwith, Pardee, Austrian & Jacob, 1962). The *thi*$^-$ *arg*$^-$ mutant, isolated from the *thi*$^-$ acceptor strain did not grow on citrulline or ornithine, indicating that the marker was identical with either *arg 1*, *2*, *3* or *4*. The marker is transferred by Hfr R_4 about 3 min. after *thi* and the *arg* marker is thus identical with *arg$_2$* or *arg$_4$* (Gorini, Gundersen & Burger, 1961; Maas, 1961). The results of the crosses between Hfr R_4 and the *arg*$^-$ *thi*$^-$ strain are summarized in Table 3. In all crosses *arg*$^+$ *argura*$^+$ recombinants were selected and the *thi* and purine markers were scored as unselected markers. The counter

Table 3. *The fraction of donor alleles among* arg$^+$ argura$^+$ *recombinants in crosses between Hfr R_4* argura$^-$ *and* arg$^-$ thi$^-$ pur$^-$ *strains*

Donor	Acceptor	No. of *arg*$^+$ *argura*$^+$ recombinants tested	Percentage of donor allele	
			thi	Purine marker
R_4 118	*arg*$^-$ *thi*$^-$ *adth$_a$*$^-$	700	—	73·6
R_4 118	*arg*$^-$ *thi*$^-$ *ade$_i$*$^-$	966	76·8	71·9
R_4 118	*arg*$^-$ *thi*$^-$ *ade$_k$*	462	86·8	40·0

Mating conditions in liquid medium were as described in Materials and Methods. Recombinants, prototrophic for the donor marker *arg*$^+$ and the acceptor marker *argura*$^+$ were selected.

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selection for *argura*$^+$ selects recombinants with crossovers in the *thi* region and avoids the necessity of scoring large numbers of recombinants which had received all donor alleles.

It may be seen from Table 3 that the order of the loci is *origin–argura–ade*$_k$–*ade*$_i$–*adth*$_a$–*thi–arg* (see Fig. 2). The order of the purine markers was verified by reciprocal crosses.

A considerable variation in the transfer frequency of the *thi* marker in the three experiments is observed Similar variations of the *thi* marker were also observed when experiments with the same donor and acceptor strains were repeated. The variations were sometimes significant when tested with the chi-square test. The variation is probably due to a variable viability of one of the recombinants, the viability differing from experiment to experiment. The recombination frequencies between the *thi* and the purine markers are given in Fig. 2. They should not be taken too literally, as the effect of differential viability could not be taken into account.

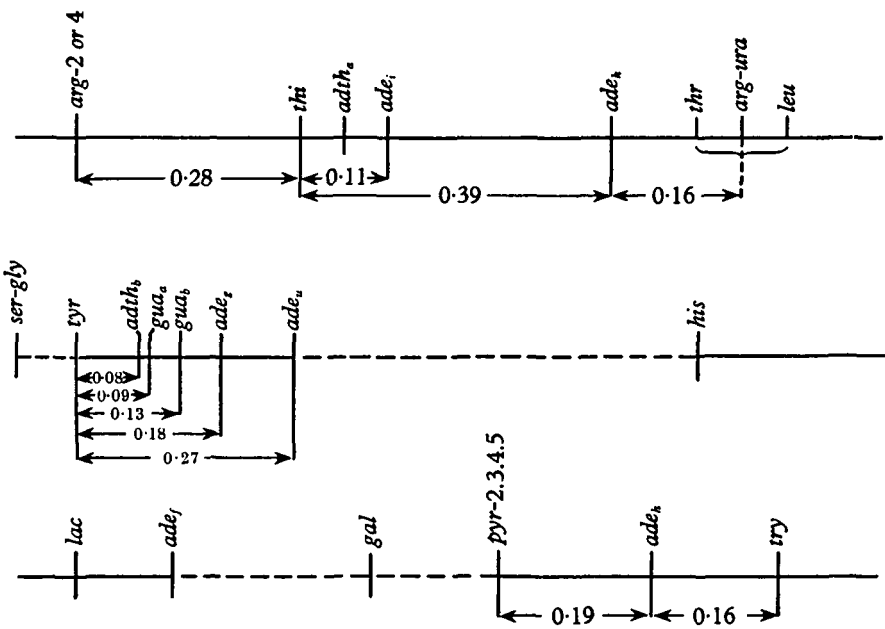

Fig. 2. Recombination frequencies between purine and reference markers. A recombination frequency of f.i. 0·39 for the segment *thi–ade*$_k$ means that the two markers are separated with a frequency of 39%.

The recombination frequency between the *thi* and *adth* marker is not given as only three phenotypes are scored in this type of cross. The *thi*$^+$ *adth*$^-$ and *thi*$^-$ *adth*$^-$ recombinants are both scored as thiamine and adenine deficient.

(iv) *The linkage group* adth$_b$ gua$_a$–gua$_b$–ade$_g$–ade$_u$

The purine markers transferred by Hfr AB 313 entered the zygotes at about the same time as the *tyr* marker and the *tyr* marker is transferred about 10 min.

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before the *his* marker. The order of the purine markers was determined in crosses of the type Hfr AB 313 × *F*⁻*thi*⁻*his*⁻*tyr*⁻*pur*⁻; *his*⁺ recombinants were selected and *tyr*⁺ and *pur*⁺ frequencies were scored. The results are given in Table 4. It is evident that all markers are located between *tyr* and *his* and that only the $adth_b$, gua_a, and gua_b markers are closely linked. The recombination frequencies between *tyr* and purine markers were calculated from the frequencies of the four recombinant classes with the method given under Materials and Methods. The results of the calculations are given in Fig. 2. The most probable order of the loci is *origin*–$adth_b$–gua_a–gua_b–ade_g–ade_u–*his*. The order gua_a–gua_b–was confirmed by crosses between Hfr AB 313 *tyr*⁻ gua_b⁻ × *thi*⁻ *tyr*⁺ *tryp*⁻ *his*⁻ gua_b⁻ and Hfr AB 313 *tyr*⁻ gua_b⁻ × *thi*⁻ *tyr*⁺ *tryp*⁻ *his*⁻ gua_a⁻. The first cross gave the largest percentage *gua*⁺ among the *tyr*⁺ *his*⁺ recombinants. In the same way the order ade_g–ade_u was confirmed.

Table 4. *The fraction of donor alleles among* his⁺ *recombinants in crosses between Hfr AB 313 and* thi⁻ tyr⁻ pur⁻ his⁻ *strains*

Pur marker in acceptor	Percentage of donor allele	
	tyr⁺	*pur*⁺
$adth_b$	46·2	49·1
gua_a	46·4	49·0
gua_b	45·1	48·5
ade_g	48·0	53·3
$ade_{u,b}$	42·9	53·6

For mating procedure see Materials and Methods (solid medium).

(v) *The location of* ade_h

The position of ade_h was determined by crossing Hfr H with a *try*⁻ *gal*⁻ *ura*⁻ ade_h⁻ acceptor strain. The *ura* marker was identical with the *pyr 2, 3, 4, 5* marker (Beckwith, Pardee, Austrian & Jacob, 1962).

Tryptophane auxotrophic recombinants were selected and the unselected markers *gal*, *ura* and ade_h were scored. The result: 77% ade_h, 61% *ura*⁺ and 28% *gal*⁺ indicated a relative order *origin*–*gal*–*ura*–ade_h–*try* (Fig. 2). The position of the ade_h marker is in accordance with its time of penetration.

4. DISCUSSION

Eleven different mutations, each representing a step in purine biosynthesis were mapped. From the five steps leading to AIR only two non-identical loci were found and mapped (ade_u, *a* and *b*). No mutant accumulating FAICAR was found. This is in accordance with the suggestion that the transformations AICAR→FAICAR→IMP are mediated by a single enzyme (cf. Flaks, Erwin & Buchanan, 1957). The position of the purine markers on the chromosome of *E. coli* K 12 is shown in Fig. 3. The purine markers are scattered all over the chromosome and

they do not form a cluster of linked genes as for instance the gene for lactose fermentation (Jacob & Wollman, 1961). Thus the genes controlling purine biosynthesis do not form a single operon. Most probably $adth_a$ and ade_i are adjacent genes and the same applies to $adth_b$, gua_a and gua_b. The situation with the purine markers is thus more or less the same as with the arginine markers, some of which form a cluster and some of which are found on different places on the chromosome (Gorini, Gundersen & Burger, 1961; Maas, 1961). Demerec (1964) has found that

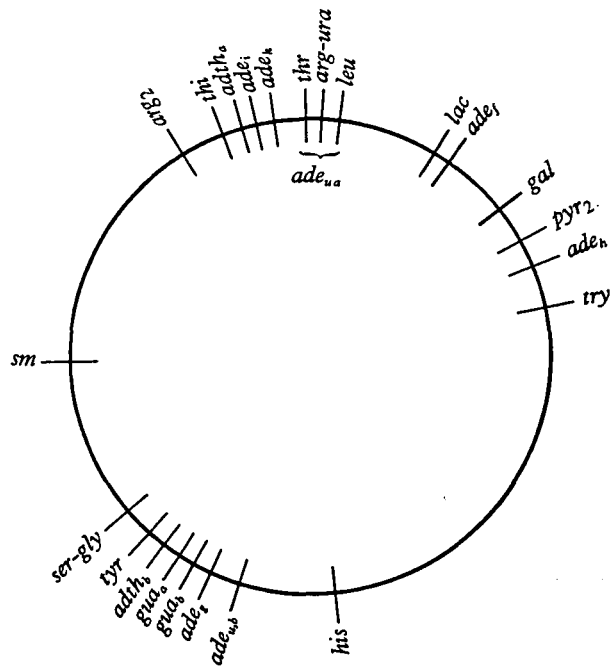

Fig. 3. Relative position of purine markers and reference markers on the chromosome of *E. coli* K 12.

in *Salmonella typhimurium* ten different purine markers occurred as one group of three loci, one group of two loci and five single loci. The situation in *S. typhimurium* is thus the same as in *E. coli*.

The recombination frequencies between a number of markers were determined with the method of Bailey (see Materials and Methods). As differential viabilities are not taken into account, the recombination frequencies should not be taken too literally. In a number of crosses the recombination frequencies between the F^- point corresponding with the origin and the first marker was also calculated. In a cross of the type Hfr AB 313$^-$ × F$^-$ *tyr*$^-$ *his*$^-$ *pur*$^-$, recombination frequencies for the first segment *origin–tyr* of about 0·5 were found. This value is in accordance with the observed frequency of incorporation of markers which are close to the origin of the Hfr chromosome (Pittard & Adelberg, 1964).

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SUMMARY

A number of mutants of *E. coli* K 12, deficient in purine biosynthesis, have been isolated and the biochemical blocks have been determined. The mutations were mapped in conjugation experiments. In some cases the differences in penetration times were too small to determine the exact order of the loci by interrupted mating experiments. In these cases the recombination frequencies were determined in four factor crosses. In this way the location of eleven different purine markers has been determined. The loci are scattered over the chromosome. Only two groups of linked genes were found.

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