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The action of the first enzyme in the arginine pathway of *Proteus mirabilis* strain 13 is controlled by feed-back inhibition and the formation of arginine-synthesizing enzymes is influenced by the concentration of the end-product. Concentrations of arginine below 10 $\mu\text{g./ml.}$ induce their formation, but higher concentrations result in repression. The variations in enzyme synthesis caused by changes in the arginine content of the medium are markedly smaller than those observed in *Escherichia coli*. The carbon source affects the response of enzyme synthesis to arginine. Two mutants *argR-1* and *argR-2* which excrete arginine have higher enzyme levels than the wild type. In one of these the enzymes are repressible and in the other mutant the enzymes are de-repressed. The sites of these two mutants map closely in a locus *argR* which is linked to a *his* marker but not to any of the structural arginine genes. The de-repression of enzyme synthesis in these mutants is limited and less than in *argR* mutants of *E. coli*. The derepressed state of *argR-2* gives it a growth advantage over the wild type if arginine is withdrawn. The derepressed mutants also exhibit a changed response to feedback inhibition.

Arginine is synthesized in *Proteus mirabilis* strain 13 by 8 enzymes which are coded by 8 structural genes (Prozesky, 1967, 1968). The arginine pathway and its genetic topography in this organism are presented in Fig. 1. The biosynthesis of arginine in

Fig. 1. The biosynthetic pathway of arginine and its genetic topography in *Proteus mirabilis* strain 13. A to H represent enzymic steps and corresponding arginine genes in the four linkage groups (I–IV, Prozessky, 1968) defined by transductions. Reference markers are *str-r* = streptomycin resistance; *pyr* = pyrimidine; *cys* = cysteine. Map not to scale.

Escherichia coli is controlled by feed-back inhibition of the first enzyme acetylglutamate synthetase (Vyas & Maas, 1963) and by pathway-wide repression of enzyme synthesis by the end-product (Vogel, 1957). The control by repression in *E. coli* strain B differs from that in strains K-12, W and C (Gorini & Gundersen, 1961; Jacoby

& Gorini, 1967). In *E. coli* B repression is mediated by an unknown mechanism related to glucose metabolism and arginine causes induction of enzyme synthesis (Gorini, Gundersen & Burger, 1961). Induction by arginine has also been described for acetyl-ornithine- δ -transaminase in a mutant of *E. coli* strain w (Vogel, Bacon & Baich, 1963) and high-level inducibility for ornithine transcarbamylase in hybrids of *E. coli* B and K-12 (Jacoby & Gorini, 1967). In *Bacillus subtilis* the repression control is similar to that in *E. coli* K-12 (Lehrer & Jones, 1962; Vogel & Vogel, 1963). Udaka (1966) examined a number of bacterial species and found that in about half of these slight repression was exerted by arginine on ornithine transcarbamylase and that in organisms which synthesize ornithine by transacetylation (Udaka & Kinoshita, 1958) feed-back inhibition is active on the second enzyme (*N*-acetyl- γ -glutamokinase) in the pathway. Vogel, Albrecht & Cocito (1961) also found repression and non-repression of arginine-synthesizing enzymes in some enterobacteria. The control of arginine synthesis thus varies in different organisms and may have taxonomic importance (Udaka, 1966; Prozesky, 1967).

Despite differences in repression control among *Escherichia coli* strains a single genetic locus determines repression of the entire pathway. This *argR* locus (Jacoby & Gorini, 1967) is active in the *trans* position and is the regulator gene of the arginine pathway (Maas & Clark, 1964). The arginine genes in *E. coli* K-12 and w are scattered around the genome except for the *argE*, *C*, *B*, *H* genes which are closely linked (Maas, 1961; Vogel *et al.* 1963; Glansdorff, 1965). The *Proteus mirabilis* genes show a similar grouping but the *argG* gene is included in an *argE*, *C*, *B*, *G*, *H* cluster (Fig. 1). The *argE*, *C*, *B*, *H* genes in *E. coli* may constitute one or more operons (Jacob, Perrin, Sanchez & Monod, 1960). Co-ordinate repression of some of their enzymes has been demonstrated (Glansdorff & Sand, 1965) and polar mutants in or near the cluster which influence more than one gene in the group have been found (Baumberg, Bacon & Vogel, 1966; Sand & Glansdorff, 1967). *ArgE* and *argH* are not regulated co-ordinately (Glansdorff & Sand, 1965; Baumberg *et al.* 1966). The regulation of arginine synthesis in *P. mirabilis* strain 13 was investigated in an attempt to contribute to the taxonomic data available for the *Proteus*-*Providencia* group of bacteria (Coetzee, Smit & Prozesky, 1966) and possibly to obtain information about the operon status of the *argE*, *C*, *B*, *G*, *H* gene group.

METHODS

Media. The minimal medium was described by Prozesky (1967). Enriched with a mixture of growth factors (Novick & Maas, 1961; Prozesky, 1967) it was used as a complete defined arginine-free medium to which various concentrations of L-arginine were added as required. L-Canavanine sulphate (100 μ g./ml.) was substituted for L-arginine in this medium to select for de-repressed mutants (Maas, 1961). Glycerol (0.2 %, w/v) was substituted for glucose in some experiments (Gorini *et al.* 1961). Difco SS agar with streptomycin sulphate (1 mg./ml.) was used for the selection of streptomycin-resistant transductants (Prozesky, 1968).

Chemicals. Amino acids, growth factors, arginine precursors, co-enzymes and other chemicals were obtained as described (Prozesky, 1967). The L-arginine analogue L-canavanine sulphate was obtained from the Nutritional Biochemicals Corporation, Cleveland, Ohio, U.S.A., and diethyl sulphate from British Drug Houses Ltd., Poole, England.

Bacteria. *Proteus mirabilis* strain 13 (Coetzee & Sacks, 1960) and mutants of this strain (Table 1) were used. The mutants *argR-1* and *argR-2* were selected on arginine-free medium with L-canavanine. Plates were treated with 0.1 ml. diethyl sulphate (Goldberg, Keng & Thorne, 1965) and inoculated with about 5×10^8 bacteria which had been washed twice in NaCl (0.9 %, w/v). Colonies which protruded above the background growth after 48 hr incubation were picked off, purified on the same medium without diethyl sulphate and examined for excretion of arginine. This was done by streaking the mutants next to mutant *argH-1* (Maas & Clark, 1964; Prozesky, 1967) on minimal medium together with wild-type controls (Prozesky, 1967). The histidineless mutant *his-7* was selected as described (Prozesky, 1967) and has not been characterized enzymatically. Streptomycin-resistant mutants of *argR-1* and *argR-2* were obtained as described by Coetzee & Sacks (1960). Cultures were maintained on nutrient agar slants at 4° and were incubated at 37°.

Table 1. *Mutants of Proteus mirabilis* strain 13

Strain	How obtained	Mutagenic agent used	Reference
13 (wild type)	—	—	Coetzee & Sacks, 1960
Mutant			
<i>argR-1</i>	Selection	Diethyl sulphate	} See Methods
<i>argR-2</i>	Selection	Diethyl sulphate	
<i>argR-1, argB-1</i>	Transduction	—	
<i>argR-2, argB-1</i>	Transduction	—	
<i>argR-1, cysE-6</i>	Selection	MnCl ₂ + H ₂ O ₂	
<i>argR-2, cysE-7</i>	Selection	MnCl ₂ + H ₂ O ₂	} Coetzee & Sacks, 1960
<i>argR-1, str-r-17</i>	Selection	—	
<i>argR-2, str-r-18</i>	Selection	—	
<i>argB-1</i>	Selection	MnCl ₂ + H ₂ O ₂	} Prozesky, 1967
<i>argH-1</i>	Selection	MnCl ₂ + H ₂ O ₂	
<i>his-7</i>	Selection	MnCl ₂ + H ₂ O ₂	

Construction of mutants by transduction. The effect of the *argR* mutations (Table 1) on the synthesis of acetylglutamate synthetase was determined in mutants *argR-1*, *argB-1* and *argR-2, argB-1* because the *argB* (*N*-acetyl- γ -glutamokinase) enzyme block is a prerequisite in a mutant used for the determination of acetylglutamate synthetase in resting cell suspensions (Vyas & Maas, 1963; Prozesky, 1967). The linkage of the *argB* and *cysE* genes (Prozesky, 1968) made it possible to transduce the *argB-1* lesion (Prozesky, 1967) into the two *argR* mutants. Mutants *argR-1, cysE-6* and *argR-2, cysE-7* (Table 1) were selected from *argR-1* and *argR-2* respectively as described (Prozesky, 1967, 1968) and were characterized by Mr W. O. K. Grabow (Grabow & Smit, 1967). These two mutants were then transduced to cysteine independence with phage 34/13 prepared as described (Prozesky, de Klerk & Coetzee, 1965) on the arginineless mutant *argB-1* (Prozesky, 1968). Transductants were selected on minimal medium which contained L-ornithine monohydrochloride (100 μ g./ml.). Replication to minimal medium enabled the identification of ornithine-requiring colonies which were tested for the *argB-1* marker by enzyme assay (Prozesky, 1967).

Preparation of cell-free extracts and enzyme assays. Cultures were grown and cell extracts were prepared by sonication as described (Prozesky, 1967). Protein concentrations in the extracts were determined by the method of Gornall, Bardawill & David

(1949). Acetylglutamate synthetase was measured with resting suspensions of mutant *argB-1* (wild-type activity) and in constructed mutants *argR-1*, *argB-1* and *argR-2*, *argB-1* for de-repressed mutant activity. The enzymes and their assay methods (Prozesky, 1967) are given in Table 2. *N*-Acetylglutamic- γ -semialdehyde dehydrogenase could not be determined in our strain (Prozesky, 1967) and this enzyme was omitted from this investigation. Arginase was determined according to Ramaley & Bernlohr (1965) and the ornithine produced by this enzyme was determined by the method of Chinard (1952).

Feed-back inhibition of enzyme activity. This was determined by assay of acetylglutamate synthetase (the first enzyme in the pathway) and *N*-acetyl- γ -glutamokinase (the second enzyme in the pathway) in the presence of various concentrations of L-arginine hydrochloride (Vyas & Maas, 1963; Udaka, 1966).

Table 2. *Enzymes of the arginine pathway in Proteus mirabilis strain 13*

Enzyme	E.C. no.*	Position in pathway	Reaction product measured	Reference for method
Systematic name				
AcetylCoA: L-glutamate <i>N</i> -acetyltransferase	2.3.1.1	A	<i>N</i> -Acetylglutamate	Vyas & Maas (1963)
ATP: <i>N</i> -acetylglutamate 5-phosphotransferase		B	<i>N</i> -Acetyl- γ -glutamyl hydroxamate	Baich & Vogel (1962)
L-Ornithine: 2-oxoacid aminotransferase	2.6.1.11	D	<i>N</i> -Acetylglutamic- γ -semialdehyde	Albrecht & Vogel (1964)
α - <i>N</i> -Acetyl-L-ornithine amidohydrolase	3.5.1.d	E	Ornithine	Vogel & Bonner (1956)
Carbamoylphosphate: L-ornithine carbamoyltransferase	2.1.3.3	F	Citrulline	Jones (1962)
L-Citrulline: L-aspartate-ligase	6.3.4.5	G	Disappearance of citrulline	Ratner (1955)
L-Argininosuccinate-arginine lyase	4.3.2.1	H	Ornithine	Baumberg, Bacon & Vogel (1965)
L-Arginine ureohydrolase	3.5.3.1	—	Ornithine	Ramaley & Bernlohr (1965)

* = E.C. no. = Enzyme Commission number.

Repression and de-repression of enzyme synthesis. Enzyme levels were determined in cultures grown in arginine-free or minimal medium which contained different concentrations of L-arginine. In some experiments glycerol was used as carbon source in the medium to determine the effect of glucose catabolism on the effect of the end-product L-arginine (Gorini *et al.* 1961).

Adaptation from L-arginine-containing to an L-arginine-free medium. The wild-type and mutant *argR-2* were grown with aeration (Prozesky, 1967) in arginine-free medium (100 ml.) enriched with L-arginine hydrochloride (10 μ g./ml.) for 6 hr. The cultures were centrifuged at 12,000 *g* for 10 min. and washed twice with cold NaCl (0.9%, w/v). The pellets were resuspended quantitatively in prewarmed arginine-free medium (100 ml.) and grown as before. The extinctions of the cultures at 580 m μ were determined (Vyas & Maas, 1963) at 30 min. intervals.

Genetic mapping of argR mutants. Lysates of phage 34/13 were prepared and transduction experiments performed as described (Prozesky *et al.* 1965; Prozesky, 1968).

Recipients were mixed with phage at a multiplicity of input of 5, and adsorption continued for 15 min. at 37°. The bacteria were centrifuged, resuspended in NaCl (0.9%, w/v) and samples were plated. Appropriate controls were included (Prozesky & Coetzee, 1966; Prozesky, 1968). Direct selection for transfer of *argR* markers by L-canavanine-resistance (Jacoby & Gorini, 1967) was not possible with this strain because of poor differential toxicity of the L-arginine analogue for *argR* and *argR*⁺ (wild-type) bacteria and the high rate of mutation to canavanine resistance (see below). The *argR* property was scored as an unselected marker by streaking next to *argH-1* on minimal medium to detect excretion of arginine. The arginineless and other auxotrophic mutants previously used to detect linked transduction of *arg* markers (Prozesky, 1968) were recipients in transduction experiments with *argR-1* and *argR-2* as donors. Prototrophic transductants were selected on minimal medium and examined for concomitant inheritance of *argR-1* and *argR-2* markers. In initial scanning experiments with the large number of auxotrophic mutants (Prozesky, 1968) 50 colonies from each cross were tested for arginine excretion. For possible co-transduction of *argR* and streptomycin-resistance (*str-r*) the wild type was used as recipient with *argR-1*, *str-r-17* and *argR-2*, *str-r-18* as donors. Selection was for *str-r* (Prozesky, 1968) and the *argR* markers were scored as above.

Table 3. *Feedback inhibition of early enzymes in the arginine pathway*

Enzyme A = acetylglutamate synthetase. Enzyme B = *N*-acetyl- γ -glutamokinase. Results are the average of two experiments. Enzymes were determined in cultures grown for 36 hr in arginine-free medium enriched with L-arginine-HCl (10 μ g./ml.) Arginine was added to the incubation mixtures in various concentrations.

L-arginine-HCl in the incubation mixture (μ g./ml.)	Enzyme specific activity			
	Enzyme A			Enzyme B Strain 13
	13 (wild type)	<i>argR-1</i>	<i>argR-2</i>	
0	59.9*	49.6	62.8	1.1†
5	42.5	—	—	1.0
10	39.6	33.0	58.7	1.0
50	38.2	—	—	1.1
100	36.6	—	—	1.0

* 1 unit = 1 μ g./mg. dry wt bacteria/hr; † 1 unit = 1 μ mole/mg. protein/hr.

RESULTS

Feed-back inhibition of enzyme activity. L-Arginine exerts strong feed-back inhibition on the first enzyme in the pathway acetylglutamate synthetase but the second enzyme (*N*-acetyl- γ -glutamokinase) is not significantly affected by the presence of the end-product (Table 3). Similar results were described by Vyas & Maas (1963) for *Escherichia coli* K-12 and by Udaka (1966) for *Proteus vulgaris*. As in the case of *E. coli* K-12 feedback inhibition is especially active at low arginine concentrations (Table 3). *ArgR-1*, *argB-1* shows more pronounced feedback inhibition of acetylglutamate than the wild-type while in *argR-2*, *argB-1* the action of the enzyme is affected less by arginine (Table 3).

Response of synthesis of arginine enzymes to L-arginine in the wild-type organism. The effect of various concentrations of the end-product on arginine enzyme synthesis is

Table 4. *The effect of L-arginine on enzyme synthesis in the wild type and in derepressed mutants*

Enzymes were determined in bacteria grown in arginine-free medium or minimal medium (where indicated) supplemented with none or various concentrations of L-arginine. Figures are the average of two experiments.

Enzyme	Arginine concentration ($\mu\text{g./ml.}$) ...				Specific activity* of enzymes in strains							
	0		5		10		20		50		100	
	<i>arg R-1 arg R-2</i>		<i>arg R-1 arg R-2</i>		<i>arg R-1 arg R-2</i>		<i>arg R-1 arg R-2</i>		<i>arg R-1 arg R-2</i>		<i>arg R-1 arg R-2</i>	
	<i>arg R-1</i>	<i>arg R-2</i>	<i>arg R-1</i>	<i>arg R-2</i>	<i>arg R-1</i>	<i>arg R-2</i>	<i>arg R-1</i>	<i>arg R-2</i>	<i>arg R-1</i>	<i>arg R-2</i>	<i>arg R-1</i>	<i>arg R-2</i>
13 (wild type)												
Acetylglutamate synthetase	42.0†	56.2	61.4	50.5	50.8	62.0	58.4	44.4	62.6	—	—	—
N-Acetyl- γ -glutamokinase	2.0	3.6	3.4	2.8	2.7	3.5	3.5	2.1	3.6	—	—	—
N-Acetylmethionine- δ -transaminase	2.8†	3.7	3.7	2.9	3.5	3.8	3.6	3.3	3.8	—	—	—
Acetylmethioninase	18.6	27.8	29.2	21.2	25.9	29.4	23.1	23.8	29.6	—	—	—
Ornithine transcarbamylase	2.4	4.0	3.0	2.8	3.7	3.3	3.2	3.3	3.7	3.0	3.1	3.5
Ornithine transcarbamylase (minimal medium)	0.7	1.0	0.9	0.8	0.9	0.9	0.8	0.8	0.9	—	—	—
Condensing enzyme	0.3	0.6	0.5	0.4	0.5	0.5	0.5	0.4	0.6	—	—	—
Argininosuccinase	0.5	1.7	1.4	0.8	1.5	1.5	1.0	1.3	1.5	1.0	1.2	1.5
Argininosuccinase (minimal medium)	0.4	0.9	0.7	0.5	0.7	0.7	0.5	0.6	0.8	—	—	—
Arginase	0.0578	0.095	0.061	—	—	—	—	—	—	—	—	—
											0.083	0.076
												0.080

shown in Table 4. The response of two of the enzymes is illustrated in Fig. 2. L-Arginine induces the enzymes until its concentration exceeds 10 $\mu\text{g./ml.}$ Higher concentrations cause a progressive decline in enzyme levels. The seven enzymes are not induced or repressed co-ordinately (Ames & Garry, 1959). This is best seen in the responses of ornithine transcarbamylase and argininosuccinase (Fig. 2). The levels of the former enzyme vary more than those of the latter. This was also found in *Escherichia coli* (Vogel, 1961). There may be a co-ordinate response to arginine by *N*-acetyl- γ -glutamokinase, argininosuccinase and the condensing enzyme but the small variations in levels of these enzymes (Table 4) do not allow conclusions to be made. The induction

Fig. 2. The effect of arginine on the synthesis of arginino-succinase and ornithine transcarbamylase in the wild type and the *argR* mutants. Cultures were grown for 36 hr in arginine-free medium to which various concentrations of arginine were added. Enzymes were determined in extracts of disrupted cells. Solid lines: Argininosuccinase. Broken lines: Ornithine transcarbamylase. 13 (wild-type), —○—; *argR*-1, —△—; *argR*-2, —●—.

by low concentrations of arginine suggest that as in *E. coli* strain B (Gorini & Gundersen, 1961) L-arginine is an antagonist of an unknown inhibitor of the synthesis of the enzymes; possibly derived from glucose metabolism (Gorini *et al.* 1961). This was tested by repeating the assays of two of the enzymes with glycerol as carbon source (Fig. 3). The enzyme levels are slightly raised by the glycerol medium and there is less induction by arginine but also less repression at higher concentrations. Results obtained with bacteria grown in minimal medium (Table 4) show that enzyme levels are markedly lower than when arginine-free medium is used but the pattern of response to arginine is similar in the two media.

Mutants with aberrant regulation of arginine synthesis. The yield of canavanine-

resistant colonies was about 1 per 10^8 bacteria plated. Of 51 such colonies tested three excreted arginine. One of these *argR-1* showed a poor feeding response with *argH-1*. *ArgR-1* and *argR-2* were chosen for detailed investigation. Enzyme assays show (Table 4, fig. 2) that *argR-1* and *argR-2* have higher levels of arginine enzymes than the wild type, but in *argR-1* there is repression by both low and high concentrations of arginine. *ArgR-2* is less inducible and also less repressible by the end product. In glycerol medium *argR-1* and the wild type behave similarly and *argR-2* is less affected

Fig. 3. Response of synthesis of argininosuccinase and ornithine transcarbamylase to variations in arginine concentration when glycerol is substituted for glucose as the medium carbon source. Procedures and designations are as in Fig. 2.

by the different carbon source (Fig. 3). The arginase levels of the wild type and the *argR* mutants show that *argR-1* and *argR-2* do not excrete arginine because of reduced breakdown of arginine by arginase. Other possible degradation reactions of arginine were not investigated.

Adaptation to the presence or absence of arginine. When *argR-2* and the wild type are switched from an arginine-containing to an arginine-free medium the resumption of growth by the former is more prompt and growth proceeds faster than that of the wild type (Fig. 4). The derepressed state of *argR-2* confers a growth advantage to it which it does not possess in the presence of L-arginine. This is probably because maximum arginine synthesis occurs in the mutant in the presence or absence of the amino acid (Gorini *et al.* 1961).

Co-transduction argR and other markers. No co-transduction of *argR-1* and *argR-2* could be demonstrated with any of the structural arginine genes (Prozesky, 1968) or with the *str-r* marker. Of the miscellaneous auxotrophic mutants tested only a number

of *his* (histidineless) mutants were rendered prototrophic by transduction with concomitant transfer of both *argR* markers. Five of the *argR* colonies from each of the crosses *argR-1* (donor) $\times$ *his-7* (recipient) and *argR-2* (donor) $\times$ *his-7* (recipient) were

Fig. 4. Growth curves of the wild type and *argR-2* when the bacteria are switched from arginine-enriched to arginine-free medium. Cultures were grown with aeration in arginine-free medium enriched with L-arginine (10 μ g./ml.) for 6 hr, washed in the cold and inoculated quantitatively in arginine-free medium (arrow). The E_{580} of samples was determined at intervals. 13 (wild type), —○—; *argR-2*, —●—.

Table 5. Levels of ornithine transcarbamylase and argininosuccinase in *argR* transductants

Cultures were grown in arginine-free medium and enzymes were then determined in cell-free extracts. OTC = ornithine transcarbamylase; AS = argininosuccinase.

Organism	OTC	AS
13 (wild type)	2.4*	0.5*
Transductant: <i>argR-1</i> $\times$ <i>his-7</i> a	3.8	1.5
b	3.5	1.5
c	4.1	1.5
d	4.0	1.6
e	4.0	1.8
Transductant: <i>argR-2</i> $\times$ <i>his-7</i> a	3.2	1.4
b	3.2	1.4
c	3.4	1.6
d	3.0	1.2
e	3.0	1.3

* Specific activity of enzymes: 1 unit = 1 μ mole/mg. protein/hr.

picked off, purified and grown in arginine-free medium for assays of ornithine transcarbamylase and argininosuccinase. The enzyme levels in these cultures were compared with the levels obtained in the wild type (Table 5). Enzyme levels in the transductants were significantly higher. The variation in enzyme activity among different clones is unexplained.

Distance of argR-1 and argR-2 from his-7. A stable marker *his-7* (O. W. Prozesky, unpublished) was chosen as a linked reference marker for a ratio test with *argR-1* and *argR-2*. The results are presented in Table 6 and show that *argR-1* and *argR-2* are closely linked and situated in the same locus *argR* (Jacoby & Gorini, 1967).

Table 6. *A ratio test with argR mutant donors and his-7 as recipient in transduction experiments*

Donor	No. of colonies examined*	<i>argR</i>	<i>argR</i> ⁺	$\frac{\text{argR}^+}{\text{argR} + \text{argR}^+}$
<i>argR-1</i>	700	132	568	81.14
<i>argR-2</i>	700	116	584	83.43

* Selection was made for prototrophs. *ArgR* and *argR*⁺ were scored as unselected markers by their ability or inability respectively to feed *argH-1* on minimal agar.

DISCUSSION

The feed-back inhibition of acetylglutamate synthetase by arginine confirms the finding that arginine is synthesized by the 'enterobacterial' pathway in *Proteus mirabilis* (Prozesky, 1967). The feed-back inhibition is active at low arginine concentrations as was found in *Escherichia coli* (Vyas & Maas, 1963). The finding that *argR-1* is more sensitive to feed-back inhibition than the wild type and that *argR-2* is refractive parallels the repression results and supports the hypothesis that feedback inhibition and repression are reflexions of translational control (Vogel & Vogel, 1967) and basically constitute the same process (Cline & Bock, 1966). Similar results have been obtained by Somerville & Yanofsky (1965) and Ames (1965) for tryptophan and histidine biosynthesis. Acetylglutamate synthetase is measured with resting cell suspensions (Vyas & Maas, 1963) because determination of the enzyme in cell-free extracts is not sensitive enough to reflect feed-back inhibition (Vyas & Maas, 1963). Arginine added to minimal medium without a nitrogen source during the determination may constitute a nitrogen source for *P. mirabilis* strain 13 (O. W. Prozesky, unpublished) so that an amount of repression may be reflected by the results of the feed-back inhibition experiments. The difference between the repression results (Table 2) and feed-back inhibition values (Table 3) indicate that the repression concomitantly measured is small. Support for this is that the activity of acetylglutamate synthetase measured during feed-back inhibition with the wild type is not raised by low arginine concentrations while enzyme induction is observed at low arginine concentrations in repression experiments.

The presence of arginine in the growth medium has a surprisingly small effect on synthesis of arginine enzymes (Table 2). Similarly, the differences between the enzyme levels in the wild-type and in mutants with defective regulation are not as extreme as encountered in *Escherichia coli* (Jacoby & Gorini, 1967), although the slightly raised levels of enzymes in the *Proteus mirabilis argR* mutants cause excretion of arginine.

Udaka (1966) also reported small variations in the synthesis of ornithine transcarbamylase in a number of bacterial species tested (including *P. vulgaris*) for their repression response to arginine. The dramatic repression and derepression by the end-product observed in *E. coli* strains K-12 and W (Maas, 1961; Vogel, 1961; Jacoby & Gorini, 1967) may be a feature of only some bacteria. The induction of anabolic arginine enzymes by arginine is a feature of the entire arginine pathway in *E. coli* B (Gorini & Gundersen, 1961) and has been described for acetylornithine- δ -transaminase in a mutant of *E. coli* W (Vogel *et al.* 1963).

The induction of arginine enzymes by low end-product concentrations followed by repression at higher concentrations (Fig. 2) has not been described in other bacteria. Vogel (1961) and Maas (1961) noted that arginine-synthesizing enzymes are less sensitive to repression in *Escherichia coli* when growth is suboptimal. This may explain the lower enzyme levels in minimal medium and glycerol arginine-free medium with less induction and repression obtained here (Table 4; Fig. 3). When arginine-free medium is enriched with arginine it is a complete defined medium which results in optimal growth (Novick & Maas, 1961). The induction in the wild type by low arginine concentrations may only reflect better growth of the organism. *ArgR-1* and *argR-2* overproduce arginine and have a growth advantage over the wild type in arginine-free medium (Fig. 4). The latter mutants show no or little enzyme induction by arginine, probably because their growth is optimal in the absence of supplied arginine. Sercarz & Gorini (1964) identified two functional arginine compartments in *E. coli*: endogenous arginine linked to protein synthesis and growth and exogenous arginine linked to repression. It is possible that an arginine content up to 10 $\mu\text{g./ml.}$ in arginine-free medium serves to replenish the endogenous (protein-synthesizing) arginine compartment of *Proteus mirabilis* strain 13 and that higher levels cause repression. The level of 10 $\mu\text{g./ml.}$ has also been established as the concentration at which arginineless mutants grow as well as the wild type (O. W. Prozesky, unpublished). This is the level at which the L-arginine permease system of *E. coli* is saturated with arginine (Maas, 1965). The low repressibility of arginine-synthesizing enzymes can be compared to the low inducibility of *E. coli* β -galactosidase observed in *P. mirabilis* F-lac and F-lac-i organisms (Colby & Hu, 1968). These workers concluded that the anomalous regulation of the above episome is due to the different cytoplasmic environment. The two *argR* mutants of *P. mirabilis* represent the two types of regulation-deficient mutants selected by canavanine-resistance in *E. coli* B (Maas, 1961). These are derepressed (*argR-2*) and repressible by arginine (*argR-1*). The gene locus *argR* of *P. mirabilis* is not linked to any of the structural arginine genes. The corresponding gene *argR* is linked to *argG* in *E. coli* strains (Jacoby & Gorini, 1967). The *argG* gene of *P. mirabilis* maps in a different position than in *E. coli* (Prozesky, 1968). Canavanine-resistant mutants which do not excrete arginine and are not regulatory deficient may be arginine permease (*argP*) mutants (Maas, 1965) or arginine-tRNA synthetase mutants (Hirschfield & Maas, 1967) and have not been investigated.

It is not known whether the *argR* gene of *Proteus mirabilis* is the counterpart of the *argR* genes of the *Escherichia coli* strains (Jacoby & Gorini, 1967). Like the latter genes, its effect is pathway-wide and it is not closely-linked to the structural genes. Experiments to test the effect of the *P. mirabilis* *argR* gene in the *trans* position (Maas & Clark, 1964) have not proved feasible in this strain due to lack of a conjugation system. The differences in the extent of control of enzymes by arginine are properties

of the *argR* genes in *E. coli* (Jacoby & Gorini, 1967). The unique behaviour of *P. mirabilis* in this respect may be a characteristic of the *argR* gene or the structural genes. This could be determined by transfer of the genes (Jacoby & Gorini, 1967) between *P. mirabilis* and *E. coli* strains. Gemski, Wohlieter & Baron (1967) have succeeded in transferring some chromosomal genes from *E. coli* K-12 to *P. mirabilis* strain WR 11. It would be interesting to establish whether *P. morganii* has the 'Proteus type' of arginine control described here or a control mechanism similar to that of *E. coli* as it resembles *E. coli* in guanine + cytosine molar content (Hill, 1966).

The small variations in enzyme levels obtained during repression and derepression and the absence of *N*-acetyl-glutamic- γ -semialdehyde dehydrogenase (enzyme C, Fig. 1) activity (Prozesky, 1967) prevented the demonstration that control of the enzymes coded by the *argE*, *C*, *B*, *G*, *H* cluster is co-ordinate (Glansdorff & Sand, 1965). No operator or promoter-type mutants (Jacob *et al.* 1960; Jacob, Ullman & Monod, 1964) were identified nor was aberrant regulation behaviour of a single enzyme encountered (Vogel *et al.* 1963; Jacoby & Gorini, 1967).

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