ENZYME REPRESSIBILITY AND REPRESSOR EFFECTIVENESS IN PHENOTYPIC REVERTANTS OF ARGININE AUXOTROPHS¹

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AS part of a study of enzyme repression in the arginine system of *Escherichia coli*, we have isolated a number of phenotypic revertants from various mutants blocked in acetylornithinase (VOGEL 1953; VOGEL and BONNER 1956), which is a biosynthetic enzyme of this system and is specified by gene *argE*. The mutants were derived from strain W, for which the enzymes of arginine synthesis are known and the corresponding genes have been mapped; additional relevant genes, including the pathway-specific regulatory gene (*argR*), have also been studied (see Table 1 and Figure 1).

TABLE 1

*Enzymes of arginine synthesis in Escherichia coli**

Step	Common name	Enzyme	Systematic name
1	N-Acetylglutamate synthetase	Acetyl-CoA:L-glutamate N-acetyltransferase (EC 2.3.1.1)	
2	N-Acetyl-γ-glutamokinase	ATP:N-acetyl-L-glutamate 5-phospho- transferase	
3	N-Acetylglutamic γ-semi- aldehyde dehydrogenase	N-Acetyl-L-glutamate γ-semialdehyde: NADP oxidoreductase (phosphorylating)	
4	Acetylornithine δ-transaminase	α-N-Acetyl-L-ornithine:2-oxoglutarate aminotransferase (EC 2.6.1.11)	
5	Acetylornithinase	α-N-Acetyl-L-ornithine amidohydrolase	
6	Ornithine transcarbamylase	Carbamoyl phosphate:L-ornithine carbamoyltransferase (EC 2.1.3.3)	
7	Argininosuccinate synthetase	L-Citrulline:L-aspartate ligase (AMP) (EC 6.3.4.5)	
8	Argininosuccinase	L-Argininosuccinate arginine-lyase (EC 4.3.2.1)	

* The eight steps correspond to the following biosynthetic sequence: L-glutamate, N-acetyl-L-glutamate, N-acetyl-γ-L-glutamyl phosphate, N-acetyl-L-glutamic γ-semialdehyde, N^α-acetyl-L-ornithine, L-ornithine, L-citrulline, L-argininosuccinate, L-arginine (see VOGEL, BACON, and BAICH 1963; ALBRECHT and VOGEL 1964).

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FIGURE 1.—Genes of arginine system in *Escherichia coli*, strain W, and reference markers. The arginine genes are labeled inside the schematic circular chromosome; the reference markers are labeled on the outside. Arginine genes *argA* through *argH* correspond to steps 1 through 8 (see Table 1), respectively. See the text regarding gene symbols. Gene *argR* is the system-specific regulatory gene. Gene *argM* corresponds to an arginine-inducible transaminase (VOGEL and BACON 1966).

In addition to acetylornithinase, which catalyzes the cleavage of N^{α} -acetylornithine to ornithine, two other arginine enzymes were examined in the present investigation: acetylornithine δ -transaminase (VOGEL 1953; ALBRECHT and VOGEL 1964; VOGEL *et al.* 1967) and argininosuccinase (BAUMBERG, BACON, and VOGEL 1965; ORTIGOZA-FERADO, JONES, and VOGEL in preparation), which mediate the transamination of N -acetylglutamic γ -semialdehyde to N^{α} -acetylornithine and the splitting of argininosuccinate to yield arginine, respectively.

In this paper, evidence is presented that, although in the revertants the level of acetylornithinase activity varies over a broad range, the repression-derepression behavior is essentially unchanged, and that the repressor effectiveness in the regulation of the formation of the three enzymes examined is not uniform.

MATERIALS AND METHODS

Mutants and phenotypic revertants: All strains used are derivatives of *E. coli*, strain W (ATCC 9637). Acetylornithinase mutants and other auxotrophic mutants were isolated by penicillin screening, after ultraviolet (UV) irradiation. Phenotypic revertants of acetylornithinase mutants were selected on (arginine-free) solid glucose-salts medium, following exposure to UV radiation.

Strains for testing linkage relationships: For the introduction of the Hfr character into *pro*⁻

derivatives of phenotypic revertants of acetylornithinase mutants, we employed the Hfr strain G-34-14 (*argE*⁻, *strA*-*r*, *phe*⁻, *trp*⁻), which injects markers in the order *leu*—*argE*—*met*—*strA*. The F⁻ strain D2-18-0 (*leu*⁻, *pro*⁻, *strA*-*r*) was used to confirm the presence of the Hfr character in the (Str-s) revertants. The F⁻ strain D2-175-1-8 (*argE*⁻, *met*⁻, *pro*⁻, *strA*-*r*) was the recipient in crosses with Hfr revertants, carried out to determine the linkage relation between the revertant mutation and *met*. The gene symbols used here for *E. coli* (see TAYLOR and TROTTER 1967) differ, in the case of the arginine genes, from the homologous symbols for *Salmonella* (SANDERSON 1967). (In previous publications, the *Salmonella* nomenclature has been applied to *E. coli*.) The *met*⁻ and *pro*⁻ markers correspond to *metB* or *metF* and *proA* or *proB*. For crosses, the procedure was essentially that of BACON and TREFFERS (1961), with mating for 90 or 120 min at 37°C, without shaking.

Strain for introducing mutant regulatory gene: The Hfr strain 57-7-1 (*argR*⁻, *strA*-*r*, *his*⁻), which injects markers in the order *argG*—*argR*—*str*—*met*, served as source of an *argR*⁻ allele, in crosses with the original (*argR*⁺) revertants; with respect to *argR*⁻, strain 57-7-1 was isolated as a canavanine-resistant mutant. Selection was made for Str-*r* recombinants which were screened for the presence of *argR*⁻ by (a) canavanine resistance on solid Difco Arginine Assay medium and (b) cross-feeding of an *argG* auxotroph in the presence of L-ornithine.

Enzyme methods: For enzyme experiments, organisms were cultivated anaerobically (in a nitrogen atmosphere) at 37°C in 300 ml of liquid glucose-salts medium (in a 1-liter flask), either supplemented with L-arginine hydrochloride (100 µg per ml) or unsupplemented, as indicated. As inocula, organisms grown on correspondingly supplemented or unsupplemented medium were used, to give a Klett-Summerson reading (red filter) of 10 in the experimental flask; growth was allowed to proceed to a reading of 50, and suitable portions of the cultures were harvested, treated, and disrupted, as described earlier (VOGEL 1960). The resulting extracts served for the determination of enzyme activities. For acetylornithinase, the assay and unit of VOGEL and BONNER (1956) was used, and for acetylornithine δ-transaminase, the assay and unit of ALBRECHT and VOGEL (1964). For argininosuccinase, the assay was that of RATNER *et al.* (1953), in which ornithine (VOGEL and BONNER 1956) rather than urea was determined. One unit of argininosuccinase is that amount of enzyme which will catalyze the formation of 0.1 µmole of ornithine, with a 15-min incubation period. Protein was determined by the method of LOWRY *et al.* (1951).

RESULTS

The general experimental design was as follows. A number of acetylornithinase mutants of strain W were isolated, and from them a variety of revertants was obtained. The revertants were screened for acetylornithinase activity levels achieved upon cultivation with added arginine, and organisms representing a range of such levels were chosen for further study.

The organisms to be tested were examined as to the approximate location of their revertant mutations. For this purpose, an appropriate Hfr character was introduced by recombination, the Hfr revertant was crossed with a suitably marked F⁻ strain, and the linkage of the revertant mutation to a reference marker (*met*) was determined.

Into each original (*argR*⁺) revertant, a particular *argR*⁻ allele was introduced, again in suitable crosses. The original revertants and their *argR*⁻ derivatives were then compared in repression-derepression experiments, with respect to acetylornithinase and (in some cases) to two other arginine enzymes, acetylornithine δ-transaminase and argininosuccinase.

Linkage relationships of revertant mutations: For the introduction of the desired Hfr character, a *pro*⁻ (see MATERIALS AND METHODS) mutation was in-

duced in each revertant, and the derivative thus obtained was crossed with the Hfr strain G-34-14. Pro⁺, Str-s recombinants were tested (a) for the Hfr character by screening for ability to transfer *leu*⁺, at high frequency, to strain D2-18-0 and (b) for acetylornithinase level.

Revertants having the Hfr character were crossed with strain D2-175-1-8 for the determination of linkage of the revertant mutation to *met*. Selection was made for Met⁺, Str-r, Pro⁻ recombinants, which were screened for growth in the

TABLE 2

*Phenotypic revertants of acetylornithinase mutants: linkage relationships and repression characteristics**

Revertant strain	Linkage of revertant mutation to <i>metB</i> , <i>F</i> (percent)	Specific activity of acetylornithinase (units/mg protein)		Ratio A	Ratio B
		+ arg	— arg		
74-20-R	90	10	36	9.1	3.6
74-20-D		91	85		
51-2-R	85	9	34	8.8	3.8
51-2-D		79	86		
44-1-R	0	8	30	9.6	3.8
44-1-D		77	71		
38-2-R	80	7	24	8.4	3.4
38-2-D		59	52		
40-2-R	0	6	21	8.2	3.5
40-2-D		49	41		
40-6-R	0	1.6	5.1	7.6	3.2
40-6-D		12.1	12.9		
21-1-R	0	0.6	2.6	7.5	4.3
21-1-D		4.5	4.5		
81-1-R	86	0.5	2.7	9.2	5.4
81-1-D		4.6	4.4		
21-2-R	0	0.3	2.6	8.3	8.7
21-2-D		2.5	2.7		
51-5-R	0	0.1	0.9	9.0	9.0
51-5-D		0.9	1.0		

* The suffixes R and D in strain numbers indicate *argR*⁺ and *argR*⁻ strains, respectively. Strain numbers having identical first two digits refer to revertants of the same acetylornithinase mutant. See the text regarding enzyme experiments with the streptomycin-responsive strains 40-6-R and 40-6-D. A linkage value of 0 means that none out of 100 Met⁺ recombinants tested is Orn⁺. The symbols +arg and —arg indicate cultivation in the presence or absence of L-arginine hydrochloride (100 µg per ml), respectively. Ratio A is computed by dividing the enzyme value for a D strain by that for the corresponding R strain, both strains having been grown with added arginine. Ratio B pertains to the R strains and is computed by dividing the acetylornithinase value obtained for cultivation without added arginine by that obtained with added arginine. The specific activity values for the parental strain W and for a typical prototrophic *argR*⁻ derivative of strain W are indistinguishable, within the accuracy of the methods, from those for strains 74-20-R and 74-20-D, respectively.

absence of ornithine. Since, in control crosses, the *argE* locus shows approximately 86% linkage to *met*, a linkage value of 80–90% in experimental crosses was considered presumptive evidence that the revertant mutation maps at or near the *argE* locus.

As shown in Table 2 (in the first two columns), the revertant mutations exhibit either 80–90% linkage or no detectable linkage. The mutations thus appear either to be in the general region of *argE* or to be suppressor mutations occurring at a significant distance from this locus.

The suppressor-carrying strains 40–6–R and 40–6–D also carry a marker for streptomycin (dihydrostreptomycin) resistance (UNGER, BACON, and VOGEL 1965). These strains, on solid minimal medium (but not in liquid, in these tests) with or without added arginine, proved to be streptomycin-dependent. Both strains, on cultivation in liquid medium plus or minus arginine, give levels of acetylornithinase that can be increased by a factor of 2 to 3 when adequate amounts of dihydrostreptomycin are provided in the medium. For the enzyme experiments with strains 40–6–R and 40–6–D, cultivation was, therefore, carried out in the presence of dihydrostreptomycin (100 $\mu\text{g/ml}$). The levels of acetylornithine δ -transaminase or argininosuccinase are not appreciably affected by growth with dihydrostreptomycin. Strain 40–6–R and a corresponding streptomycin-sensitive strain, grown in minimal medium, behave alike with respect to levels of the transaminase and argininosuccinase; in the case of acetylornithinase, the level for strain 40–6–R grown on dihydrostreptomycin is approximately the same as that obtained for the streptomycin-sensitive strain grown without dihydrostreptomycin (UNGER, BACON, and VOGEL 1965).

Repression characteristics of revertants: The specific activity of acetylornithinase was determined for the *argR*⁺ revertants and the corresponding *argR*[−] derivatives, grown with or without added arginine (see Table 2). The *argR*[−] derivatives were obtained as described under MATERIALS AND METHODS. Ratio A (Table 2) is the ratio of the derepressed enzyme level to the repressed level for each pair of *argR*⁺ and *argR*[−] strains. The ratio is seen to be relatively constant, ranging from 7.5 to 9.6. Thus, although the levels of enzyme activity vary over a broad range, the repressibility values obtained are approximately the same throughout. With respect to the *argR*⁺ strains, Table 2 lists the ratios of enzyme activity value obtained without added arginine to that obtained with added arginine (Ratio B). The ratios for the first six *argR*⁺ strains are seen to range from 3.2 to 3.8; they then rise and, for the last two strains, Ratio B falls within the range of Ratio A. The values obtained for cultivation with added arginine are taken to represent full repression for all the *argR*⁺ strains. The values for cultivation without added arginine, in the case of the first six *argR*⁺ strains, are considered to reflect the usual steady-state intracellular arginine concentration that these strains, as well as their parent (strain W), achieve. In the case of the seventh and eighth *argR*⁺ strains (21–1–R and 81–1–R), the acetylornithinase activity level presumably has been lowered enough to reduce the steady-state arginine concentration, with a resulting shift of regulatory conditions in the direction of derepression. For the last two *argR*⁺ strains, the arginine concentration is so

restrictive as to lead to complete derepression, i.e., to enzyme levels like those of the corresponding *argR*⁻ strains.

Nonuniform repressor effectiveness: It then became of interest to compare the repression behavior of acetylornithinase with that of other arginine enzymes, particularly in the revertants with relatively low acetylornithinase activity. For this comparison, argininosuccinase (whose gene is in the same cluster as the gene for acetylornithinase) and acetylornithine δ -transaminase (whose gene is not closely linked to any of the other arginine genes) were chosen. Table 3 shows the results obtained with five pairs of *argR*⁺ and *argR*⁻ strains. Data for the wild-type strain W and an *argR*⁻ strain derived from it are included. The pairs of strains are listed in order of decreasing acetylornithinase level of the *argR*⁻ strains. With respect to argininosuccinase and the transaminase, it can be seen that, for the *argR*⁻ strains, the levels of each of these enzymes are relatively constant throughout. Thus, the mutations at the acetylornithinase locus and the various suppressor mutations, including that in strain 40-6-D, do not appear to affect the formation of argininosuccinase and the transaminase. The levels of these two enzymes are also approximately constant for the *argR*⁺ strains (grown without added arginine), except for the last strain listed. In this case (strain 51-5-R), conditions have clearly become derepressive, as indicated by the Ratio C values which approach unity. It is noteworthy that, for strain 21-2-R, the Ratio C values are within the usual (i. e., not fully derepressed) range as regards both of these en-

TABLE 3

*Nonuniform repressor effectiveness in the regulation of enzyme formation in the arginine system**

Strain	Acetylornithinase		Acetylornithine δ -transaminase		Argininosuccinase	
	Specific activity	Ratio C	Specific activity	Ratio C	Specific activity	Ratio C
W	36	2.5	7.5	2.3	3.5	3.5
W-D-13	89		17.2		12.1	
40-6-R	5.1	2.5	7.3	2.0	3.4	3.0
40-6-D	12.9		14.8		10.3	
21-1-R	2.6	1.7	6.2	2.1	3.3	2.8
21-1-D	4.5		13.0		9.1	
81-1-R	2.7	1.6	7.6	1.9	3.5	2.9
81-1-D	4.4		14.3		10.2	
21-2-R	2.6	1.0	6.2	2.0	2.8	3.2
21-2-D	2.7		12.3		8.9	
51-5-R	0.9	1.1	10.9	1.2	9.5	1.2
51-5-D	1.0		13.4		11.7	

* The suffixes R and D in strain numbers indicate *argR*⁺ and *argR*⁻ strains, respectively. Strain W-D-13 is an *argR*⁻ derivative of the (*argR*⁺) wild-type strain W. See the text regarding the streptomycin-responsive strains 40-6-R and 40-6-D. Specific activity is expressed in units per mg protein. The specific activity values given are those found upon cultivation of the strains without added arginine. Ratio C is computed for each enzyme by dividing the specific activity found for a D strain by that found for the corresponding R strain.

zymes. In contrast, acetylornithinase is indicated to be at the fully derepressed level in this strain. The effectiveness of the functional repressor, therefore, can vary from enzyme to enzyme.

DISCUSSION

The pairs of revertant strains listed in Table 2 can be regarded as falling into two groups, on the basis of the fully repressed acetylornithinase levels of the *argR*⁺ strains: The first five pairs achieve 60–100% of the normal enzyme level, strain 74–20–R essentially having wild-type character; the last five pairs are in the range of 1–16% of the normal level. The first group contains two suppressor-bearing strains, in which the suppression is remarkably efficient. The second group, with low-level enzyme activity, predominantly contains suppressed strains, only one strain having the revertant mutation at or near the acetylornithinase locus.

As for the repression-derepression behavior, this study has revealed no appreciable differences between those strains in which the revertant mutation is in the region of the acetylornithinase locus and those in which the mutation is at a considerable distance from this locus. Thus, the pair of 51–2 strains and the pair of 44–1 strains give rather similar results, as do the 21–1 strains compared with the 81–1 strains (see Table 2). Even the streptomycin-responsive, suppressor-bearing 40–6 strains show the usual repressibility behavior. (Discussions of suppressor action in relation to sensitivity to, resistance to, and dependence on streptomycin have been presented by GORINI and BECKWITH 1966, and by DAVIES 1966.)

In general, the conclusion can be drawn that, although in the phenotypic revertants the level of acetylornithinase activity varies over a 100-fold range on the basis of repressed levels, the repression-derepression behavior is essentially unchanged, regardless of the type of the revertant mutation. The level of enzyme activity and the regulation of enzyme synthesis are, therefore, indicated to be separable phenomena.

One possibility that had been kept in mind during our study was that some of the revertants might show an *enzyme-specific* alteration of repressibility behavior, which however, was not observed. Interestingly enough, in a parallel study with phenotypic revertants of acetylornithine δ -transaminase, a species of transaminase was encountered which is arginine-inducible instead of arginine-repressible (VOGEL, BACON, and BAICH 1963). More recently, an unusual mutant has been isolated and studied, in which both enzyme level and repression behavior are altered (BAUMBERG, BACON, and VOGEL 1965; VOGEL *et al.* 1967). The mutation involved maps in the region of the four-gene cluster of the arginine system, and affects three out of the four enzymes specified by the clustered genes but not acetylornithinase. This pleiotropic mutation is thought to have brought about an alteration in repressor recognition which, in this system, is believed to take place at the ribosome, with the possible involvement of tRNA's (VOGEL 1967; VOGEL and VOGEL 1967). On the other hand, the mutations of the present study that cause loss of acetylornithinase activity presumably affect that part of the

corresponding gene which does not carry the information for repressor recognition.

The data with the 21-2 strain pair shown in Table 3 indicate that there are conditions under which acetylornithinase can be fully derepressed while the other two arginine enzymes are still at levels that are characteristic of the wild type growing in glucose-salts medium. We would infer that strain 21-2-R maintains a steady-state level of arginine which in turn gives rise to a level of functional repressor that is too low to repress acetylornithinase but high enough to produce partial repression of the other two enzymes. It would thus seem that the enzyme-forming systems involved are not equally susceptible to the functional repressor.

Recently, nonuniform repressor effectiveness was found under conditions of adjustable arginine restriction in batch cultures (A. BOLLON, T. LEISINGER, and H. J. VOGEL, unpublished observations). In these experiments, an arginine double auxotroph (*argB*⁻ *argG*⁻), derived from *E. coli* W, was used. The double auxotroph can grow in liquid glucose-salts medium supplemented with N-acetyl-L-arginine. Addition of increasing amounts of N^α-acetyl-L-ornithine (which does not yield arginine in this organism but acts as an analogue of N-acetyl-L-arginine) leads to progressively lower growth rates and a shift in regulatory conditions in the direction of derepression. Various manifestations of differential repressor effectiveness were thus noted. For example, at 0.2 mM N-acetyl-L-arginine plus 3 mM N^α-acetyl-L-ornithine, acetylornithinase is derepressed, whereas argininosuccinase remains repressed.

SUMMARY

Arginine-repressed acetylornithinase activity, restored in phenotypic revertants of acetylornithinase mutants of *E. coli*, strain W, ranges from 100% to 1% of the (arginine-repressed) wild-type level. The phenotypic revertants, isolated after UV irradiation, arise either by mutations that map in the region of the acetylornithinase locus or by suppressor mutations that are not closely linked to this locus. For all revertants, the ratio of derepressed acetylornithinase levels in *argR*⁻ derivatives to repressed acetylornithinase levels in the corresponding *argR*⁺ strains remains constant. Acetylornithinase levels in the phenotypic revertants and the repressibility characteristics of this enzyme thus are separable phenomena, whether the revertant mutations occur at the acetylornithinase locus or not. For the (*argR*⁺) revertants grown without added arginine, the steady-state intracellular arginine concentration appears to be independent of the acetylornithinase level until this level drops to approximately 10% of that of the wild type. For a revertant showing a 3% level, the intracellular arginine concentration, and hence the concentration of functional repressor, is indicated to be fully derepressive for acetylornithinase but partially repressive for two other arginine enzymes. Susceptibility to functional repressor, therefore, seems to vary among the enzyme-forming systems involved.

LITERATURE CITED

- ALBRECHT, A. M., and H. J. VOGEL, 1964 Acetylornithine δ -transaminase: partial purification and repression behavior. *J. Biol. Chem.* **239**: 1872-1876.

- BACON, D. F., and H. P. TREFFERS, 1961 Spontaneous and mutator-induced reversions of an *Escherichia coli* auxotroph. I. Prototrophic types and their growth characteristics. *J. Bacteriol.* **81**: 786-793.
- BAUMBERG, S., D. F. BACON, and H. J. VOGEL, 1965 Individually repressible enzymes specified by clustered genes of arginine synthesis. *Proc. Natl. Acad. Sci. U. S.* **53**: 1029-1032.
- DAVIES, J., 1966 Streptomycin and the genetic code. *Cold Spring Harbor Symp. Quant. Biol.* **31**: 665-670.
- GORINI, L., and J. R. BECKWITH, 1966 Suppression. *Ann. Rev. Microbiol.* **20**: 401-422.
- LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, and R. Y. RANDALL, 1951 Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265-275.
- RATNER, S., W. P. ANSLOW, JR., and B. PETRACK, 1953 Biosynthesis of urea. VI. Enzymatic cleavage of argininosuccinic acid to arginine and fumaric acid. *J. Biol. Chem.* **204**: 115-125.
- SANDERSON, K. E., 1967 Revised linkage map of *Salmonella typhimurium*. *Bacteriol. Rev.* **31**: 354-372.
- TAYLOR, A. L., and C. D. TROTTER, 1967 Revised linkage map of *Escherichia coli*. *Bacteriol. Rev.* **31**: 332-353.
- UNGER, L., D. F. BACON, and H. J. VOGEL, 1965 Enzyme-specific action of streptomycin on suppressor-restored acetylornithinase formation. *Bacteriol. Proc.* p. 23.
- VOGEL, H. J., 1953 Path of ornithine synthesis in *Escherichia coli*. *Proc. Natl. Acad. Sci. U. S.* **39**: 578-583. — 1960 A "pace-setting" phenomenon in derepressed enzyme formation. *Biochem. Biophys. Res. Comm.* **3**: 373-376. — 1967 A common molecular basis for enzyme repression and induction. *Proc. 7th Canad. Cancer Congr.*: 133-138.
- VOGEL, H. J., and D. F. BACON, 1966 Gene aggregation: evidence for a coming together of functionally related, not closely linked genes. *Proc. Natl. Acad. Sci. U. S.* **55**: 1456-1459.
- VOGEL, H. J., D. F. BACON, and A. BAICH, 1963 Induction of acetylornithine δ -transaminase during pathway-wide repression. pp. 293-300. In: *Informational Macromolecules*. Edited by H. J. VOGEL, V. BRYSON, and J. O. LAMPEN. Academic Press, New York.
- VOGEL, H. J., S. BAUMBERG, D. F. BACON, E. E. JONES, L. UNGER, and R. H. VOGEL, 1967 Gene-ribosome-enzyme organization in the arginine system of *Escherichia coli*. pp. 223-234. In: *Organizational Biosynthesis*. Edited by H. J. VOGEL, J. O. LAMPEN, and V. BRYSON. Academic Press, New York.
- VOGEL, H. J., and D. M. BONNER, 1956 Acetylornithinase of *Escherichia coli*: partial purification and some properties. *J. Biol. Chem.* **218**: 97-106.
- VOGEL, H. J., and R. H. VOGEL, 1967 Regulation of protein synthesis. *Ann. Rev. Biochem.* **36**: 519-538.