N-acyl-D-mannosamine 6-phosphate + PEP →
N-acylneuraminic acid 9-phosphate + Pi.

Whether the N-acetyl- and N-glycolylneuraminic acid 9-phosphates are formed by the same enzyme remains to be established as well as the possible relationship between these substances and the biosynthesis of nucleotides containing sialic acids.[14]

* The Rackham Arthritis Research Unit is supported by a grant from the Horace H. Rackham School of Graduate Studies of The University of Michigan. This investigation was supported in part by grants from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, and by the American Cancer Society.

† Post-doctoral Fellow of the Arthritis and Rheumatism Foundation.

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*INDUCTION BY ARGININE OF ENZYMES OF ARGININE BIOSYNTHESIS IN ESCHERICHIA COLI B**

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Communicated by George Wald, May 2, 1961

In many cases the formation of an enzyme has been observed to be induced by its substrate and repressed by the end-product of the sequence in which it participates. Thus it appeared a paradox when the discovery of the nonrepressibility by arginine of ornithine-transcarbamylase (enzyme 5)† in *Escherichia coli* strain B[1] disclosed that arginine actually stimulates the synthesis of this enzyme.[2] This stimulatory effect is modest in wild type B, which in the presence of arginine increases the enzyme level twofold. This effect has also been observed in bacteria of other species.[3] However, we have now obtained hybrids of strain B with K12

exhibiting much increased inducibility as well as mutants of B which are no longer induced by arginine. Moreover, it has been shown that induction affects at least two enzymes of the arginine sequence, and a genetic block between these enzymes and arginine does not affect induction. It is thus clear that this newly observed feedback induction, like the already well-known feedback repression, concerns several (if not all) enzymes of the sequence and is not sequential.

An attempt has been made[4] to explain this perplexing situation in terms of current concepts about end-product repression. It was speculated that arginine may compete with the repressor (say arginine—RNA) if this latter is altered and consequently has a low affinity for its site of action. This hypothesis would imply that the low enzyme level found in strain B[2] would be due to incomplete end-product repression. However, this conclusion is contradicted by the observation that the enzyme level may not be raised by growing an arginine auxotroph of strain B in a chemostat under arginine limitation. This result and genetic evidence discussed elsewhere[5, 6] support the assumption that the arginine biosynthetic pathway in strain B is under a new kind of control, different from repression by end-product. For this second control we have provisionally suggested the term "modulation."[5]

The present paper describes arginine induction in detail and considers its possible relationship to modulation.

Bacterial strains: The following strains of *E. coli* were used in the present experiments.

Wild type B.

B4S-7, a spontaneous mutant derived from B.[5] B4S-7 is a prototroph, like B, but possesses a high level of arginine enzymes.

BP8 and BB18, hybrids between strains B and K12. BP8 was obtained by crossing B with Hfr K10, BB18 by recrossing BP8 with the same donor. Since in such crosses B and BP8 are the recipient cells, BP8 and BB18 may be considered essentially B derivatives. They are both prototrophs.

Wc2, a prototroph nonrepressible by arginine isolated by I. B. Weinstein from the repressible strain W using canavanine for selection.[7]

K12 Hfr mutants were used as donors in the recombination experiments: K10 a prototroph originally from L. Cavalli, 3000 B_1^-, originally from W. Hayes, and P76 obtained from F. Jacob. These strains differ in the time of injection of the Lac marker.‡ Auxotrophic mutants were obtained by UV irradiation and selection by penicillin with an improved method.[8] Mutant BP8-I-III with two blocks in the arginine pathway, one before ornithine and a second after citrulline, was obtained and checked as previously described.[2] The Sm^R mutants were selected on nutrient agar plates containing 500 μg/ml of streptomycin. Valine as a selective marker depended on the natural sensitivity of K12 and resistance of B.

Growth conditions: The organisms were grown with aeration at 37° (when not otherwise indicated) in mineral-citrate medium A[9] supplemented with arginine or other growth factors when necessary. Glucose or other carbon sources (when indicated) were used at 0.1%. When enriched medium was used, 0.2% casein hydrolysate (NZ-Case Sheffield) and 0.2% yeast extract (Difco) were added to the minimal medium A. The chemostat experiments followed the usual procedure.[10]

Genetic recombination experiments: Exponentially growing cultures of the mates in enriched medium (bacterial density about 10^8 cells/ml) were mixed (1 to 1) and left at 37° for 150 min (5 ml total in a 125 ml flask to insure good aeration). Then 0.1 ml portions of a 10^{-2} dilution of the mating mixture were spread on appropriate selective plates. The various selective factors were used at the following concentrations: histidine, 20 μg/ml; arginine, 100 μg/ml; streptomycin, 500 μg/ml; valine, 100 μg/ml; lactose (instead of glucose), 0.1%. A first rough screen for repressibility by arginine (R_{arg}) was done by scoring the Sm marker: 98% of recombinants carrying the Sm marker of the recipient also had its R_{arg} character; 80% of those having the Sm marker of the donor also had its R_{arg} character.[5] The final score of R_{arg}, of high or low enzyme level (Md),

and of inducibility characters was done by direct enzyme determination (generally enzyme 5 when not otherwise indicated) in cells grown in minimal glucose medium with or without arginine (100 μg/ml).

Determination of enzyme activity: Enzyme 5 or 7 activity was determined on toluenized cells as previously indicated.[6, 11] One unit of enzyme 5 or 7 is the amount which synthesizes 1 μmole of citrulline or arginine respectively per hour. The figures reported in the tables are units of enzyme per ml of culture measuring optical density 1 at 490 mμ in a Beckman spectrophotometer. This optical density roughly corresponds to 1 mg bacteria (dry weight).

Strains Inducible by Arginine.—The effect of arginine on the synthesis of enzymes 5 and 7 has been tested on different strains not repressible by arginine (R_{arg}^-). Table 1 indicates that the strains with a low level of these enzymes were

TABLE 1

INDUCING EFFECT OF ARGININE ON ENZYMES 5 AND 7 IN DIFFERENT STRAINS NOT REPRESSIBLE BY ARGININE

Strains #	Origin	Specific Activity of Enzyme 5, Arginine during growth: Absent	Enzyme 5: Present	Enzyme 7, Arginine during growth: Absent	Enzyme 7: Present
B	"Low level" wild type	5.4	10.5	0.21	0.38
BB18	Hybrid B × K12	10.6	57.8	0.14	0.48
B4S-7	"High level" B mutant	178.0	180.0	1.50	1.60
Wc2	Nonrepressible W mutant	89.0	92.0	0.75	0.76

The cultures were grown at 37° with aeration to exhaustion of glucose in minimal medium A with or without addition of 100 μg/ml arginine. Specific activity: enzyme units/mg dry weight bacteria.

induced to varying degrees, whereas the strains with a high level were not further stimulated by arginine. It appears that the amount of enzyme made by B4S-7 corresponds to the maximal cell capability: inducibility has been demonstrated only in cells with a low enzyme level.

Specificity of Induction.—Various substances related to arginine or to general metabolism have been tested for their ability to induce enzyme 5 synthesis. These include some of the biosynthetic precursors of arginine (citrulline, ornithine, glutamate); the end-products of related biosynthetic pathways (uracil, proline); the other basic amino acids (lysine, histidine); the product of arginine degradation usually found in these strains (agmatine); the stereoisomer analog (D-arginine); canavanine; and finally a mixture of common amino acids, nucleic acid bases, and vitamins. It can be seen in Table 2 that L-arginine and its immediate precursors were the only inducers.

Because of the reversibility of the reaction connecting ornithine to citrulline and arginine, one cannot decide whether these three compounds are independently active or whether only one of them is able to induce, the others being converted into this one by cellular metabolism. To settle this point, we isolated an arginine auxotroph from BP8 (BP8-I-III) with two blocks in the arginine pathway, one before ornithine and another after citrulline. The effect of ornithine, citrulline, and arginine was tested by growing this organism in a chemostat under steady-state limitation either by arginine (10 μg/ml) or by glucose (with arginine 50 μg/ml, leaving a calculated residue of 40 μg/ml). To the culture growing under arginine limitation 40 μg/ml of either ornithine or citrulline was added. In contrast with Table 2, Table 3 shows that these intermediates did not induce in an organism that could not convert them into arginine. The inducing effect of arginine therefore appears to be specific.

TABLE 2

INDUCING ACTIVITY OF L-ARGININE COMPARED WITH OTHER COMPOUNDS

Additions to the medium	Specific activity of enzyme 5
None	8.8
L-arginine (100 μg/ml)	40.7
Citrulline "	37.9
Ornithine "	38.5
Glutamate "	7.2
ProUracil (20 μg/ml)	12.3
Lysline (50 μg/ml)	8.5
Hisine "	7.5
Agtidine "	9.0
D-matine (100 μg/ml)	6.8
Arginine "	12.8
Canavanine (50 μg/ml)	7.5
Difco arginine assay medium	5.5
Same + arginine (100 μg/ml)	40.2

A culture of BP8 was grown at 37° with aeration to exhaustion of glucose in minimal medium A plus the additions Difco arginine assay medium contains all growth factors for *Leuconostoc mesenteroides* with the exception of arginine (see Difco Manual, 9th edition, page 235). It was used at 0.4%. Specific activity: enzyme units/mg dry weight bacteria.

It should be noted (Table 2) that in cells grown in a rich medium deprived of arginine (Difco medium) the enzyme level is lower than in those grown in minimal medium. It has been shown that arginine prototrophs in such a rich medium grow under limitation of this amino acid, and hence are derepressed in a repressible strain.[12] Therefore this result indicates that induction by arginine also responds to the endogenous arginine concentration. A similar indication has been obtained in the chemostat experiment of Table 3. It was found that at increased growth rates (division time close to 1 hr instead of 2 hr), i.e., with higher internal levels of arginine, the level of enzyme 5 tends to increase, approximating that found with arginine in excess. Thus, it appears that enzyme formation not only is stimulated by addition of exogenous arginine, but also is decreased by lowering the level of the endogenous arginine.

Influence of the Carbon Source and Growth Temperature on Basal Level and on Extent of Induction.—It is conceivable that arginine specifically counteracts a repressor produced by the general metabolism. To test this hypothesis, cells of different strains were grown in different carbon sources with or without addition of arginine. By examining the results presented in Table 4, one may deduce the following:

TABLE 3

ABSENCE OF INDUCTION BY ORNITHINE OR CITRULLINE IN DOUBLY BLOCKED BP8-I-III

Steady-state conditions	Residual arginine (μg/ml) (calculated)	Additions to chemostat (40 μg/ml)	Enzyme 5 specific activity
Growth* controlled by arginine	0	None	11.8
	0	Ornithine	10.3
	0	Citrulline	12.0
Growth† controlled by glucose	40	None	61.6

BP8-I-III is grown in chemostat at 37° and at division time = 2 hr. The growth factors per liter of medium A and the culture density are as follows:

Condition	Glucose gr.	Arginine mg	O.d. at 490 mμ
*	1.0	10	0.244
†	0.4	50	0.274

Specific activity = enzyme units/mg dry weight bacteria.

TABLE 4

INFLUENCE OF THE CARBON SOURCE ON THE BASAL ENZYME LEVEL AND ON INDUCTION BY ARGININE

Strains			Arginine in the medium	Specific Activity of Enzyme 5 in Cells Grown in			
#	Enzyme level	Induction in glucose		Glucose	Glycerol	Lactate	Succinate
B4S-7	High	Absent	Absent	184.0	202.0	180.0	—
			Present	180.0	200.0	189.0	—
Wc2	High	Absent	Absent	120.0	145.0	104.0	—
			Present	125.0	136.0	110.0	—
BB18	Low	High	Absent	13.7	100.0	15.2	62.0
			Present	59.0	103.0	55.0	87.0
B	Low	Slight	Absent	11.0	31.0	8.0	8.7
			Present	21.0	38.0	15.0	17.0
BC39	Low	Absent	Absent	3.1	10.1	4.2	9.0
			Present	3.2	23.7	4.0	17.2

The cultures were grown at 37° with aeration to exhaustion of the carbon source in minimal medium A with or without addition of 100 μg/ml or arginine. Occasional samples were also removed during the log phase and not found to be significantly different. Specific activity: enzyme units/mg dry weight bacteria.

strains like B4S-7 and Wc2 possess a high level of enzyme irrespective of the nature of the carbon source and are insensitive to arginine. In contrast, in strains inducible by arginine, such as B or BB18, the enzyme level is more or less strongly dependent upon the nature of the carbon source. The higher the inducibility, the higher the carbon source dependency. For instance, when BB18 is grown in glucose (or lactate) the basal level is low and induction by arginine is quite evident, whereas when it is grown in glycerol (or succinate) the basal level is higher and arginine is barely effective. This behavior is consistent with the idea that the high enzyme level in B4S-7 or Wc2 is the "constitutive" level and that in the inducible strains the enzyme level is under control of a repressor which originates from the general metabolism and can be counteracted by arginine. Changing the carbon source presumably reduces the rate of repressor synthesis and thus makes arginine competition more effective. For example, strain BC39, which is a recombinant B × K12 with low enzyme level (Md^+), is not inducible by arginine when grown in glucose or lactate, but when grown in glycerol or succinate the basal level is increased and further inducibility by arginine becomes evident. These results suggest that the observed noninducibility in glucose is not due to absence of sensitivity to arginine, but rather is due to excessive formation of repressor.

The effectiveness of arginine as an inducer also varies with temperature. In all inducible strains studied, a shift of temperature from 37° to 26° changes only slightly the basal level of enzyme 5 or 7 in glucose medium but remarkably increases the extent of induction. In strain BB18, for example, as shown in Table 5, at 37° arginine increased the basal level of either enzyme only 3 times, but at 26° it increased the level 14 times for enzyme 5 and 9 times for enzyme 7. It is conceivable that at 26° repressor is formed at a rate still sufficient to saturate the repressible

TABLE 5

TEMPERATURE EFFECT ON INDUCTION

Arginine in the medium	Specific Enzyme Activity of Cells Grown at 26°		Specific Enzyme Activity of Cells Grown at 37°	
	Enzyme 5	Enzyme 7	Enzyme 5	Enzyme 7
Absent	10.8	0.10	13.0	0.14
Present	149.0	0.88	51.0	0.48

Cultures of BB18 growing exponentially with aeration in minimal-glucose medium A with or without addition of 100 μg/ml of arginine. Specific activity: enzyme units/mg dry weight bacteria.

system (the basal level is unchanged) but is more efficiently counteracted by arginine.

Genetics of Inducibility.—Recombination experiments were undertaken to study the genetic relationship of arginine induction to arginine repression and to modulation. So far, this approach has had some major limitations. One is that in crosses with K12 we were able to use strain B only as a recipient; hence, all hybrids had their cytoplasm derived from B. Furthermore, inducibility could not be selected directly, and its screening required a tedious determination of enzyme activity in clones grown under different conditions; hence, only a limited number of recombinants could be analyzed.

With respect to repressibility and modulation, the mates had the following phenotype and genotype. The arginine enzymes of the K12 donor cells are repressible by arginine and are boosted to a high level under conditions where arginine limits growth. In the recipient cells, the same enzymes are not repressible by arginine, and their level is low in B and high in B4S-7. On the basis of genetic evidence discussed elsewhere,[5] repressibility by arginine (R_{arg}) and level (Modulation = Md) are controlled by two genetic factors that can be separated from each other and from the structural genes of the enzymes. They appear to be pleiotropic regulatory genes. The donors used here were $R_{arg}{}^{+}Md^{-}$ and the recipients either $R_{arg}{}^{-}Md^{+}$ (B) or $R_{arg}{}^{-}Md^{-}$ (B4S-7).

Phenotypic expression of inducibility can occur only in $R_{arg}{}^{-}$ cells, for in $R_{arg}{}^{+}$ it would be masked by repressibility. (Accordingly, in crosses yielding a mixed population of $R_{arg}{}^{+}$ and $R_{arg}{}^{-}$ recombinants, only the $R_{arg}{}^{-}$ progeny were further analyzed.) Moreover, since it appears that the high enzyme level in Md^{-} strains corresponds to maximal use of their capacity for making that enzyme, there would be no way in Md^{-} strains, except further genetic analysis, for determining the inducibility genotype.

The results of the genetic studies establish the following points. (*a*) *Inducibility and genes concerned with enzyme structure:* To determine the relationship between inducibility and the gene for the structure of one of the enzymes affected, mutants Arg 5^{-} were obtained from B and B4S-7. (The inducibility pattern of their parents was conserved in these mutants as shown by the behavior of enzyme 7.) B Arg $5^{-}Sm^{R}$ and B4S-7 Arg $5^{-}Sm^{R}$ were crossed with Hfr K10 Arg$^{+}Sm^{S}$. Recombinants Arg^{+} were selected in the presence of Sm, and enzyme 5 level and inducibility were then determined in 50 recombinants B × K10 and 20 recombinants B4S-7 × K10. All the recombinants B4S-7 × K10 were Md^{-} and, like the original B4S-7 parent, were not further inducible. Of recombinants B × K10 96 per cent were Md^{+} and inducible like their original parent B. The remaining 4 per cent were Md^{-} and not inducible. This proves that genetic material for enzyme 5 from the donor may assume the inducibility pattern of the recipient. Moreover, by crossing B with K10 one obtains inducible recombinants with various I indexes (see Tables 6 to 8). In every case that has been analyzed, the I indexes for enzymes 5 and 7 were very similar.

These results indicate that the inducibility by arginine observed here can be separated from the genes determining the structure of the individual enzymes.

(*b*) *Inducibility and modulation:* One may analyze the experiments discussed in the preceding section from another point of view. The crosses were performed

between recipients either Md$^+$ (B) or Md$^-$ (B4S-7) and the same donor Md$^-$ (K10). It was found that inducibility was observed only with Md$^+$ recombinants. The inability of Md$^-$ recombinants to be further induced was independent of the type of their recipient parent (either B inducible or B4S-7 not inducible). The same results were obtained in other similar experiments (not involving Arg$^-$) totalling more than 100 recombinants. This pattern is consistent with the hypothesis that induction by arginine reflects a competitive interference with a type of repression (Md) peculiar to strain B. Only cells possessing this repression (Md$^+$) can be induced.

To analyze further the genetic relationship between inducibility and modulation, the inducibility of enzyme 5 was studied in the 48 Md$^+$ recombinants obtained in the cross B $\times$ K10 discussed in the preceding section. It was found that the basal enzyme level, with but two exceptions, ranged between 3.1 and 8.9 units with an average of 5.6 units. (The two exceptional clones had a level of 11.3 and 15.5 units.) The enzyme level after growth in the presence of arginine was then determined, and the inducibility index was calculated. Table 6 shows that from the same cross, where the I index of the recipient parent was 2, one may obtain recombinants with different I indexes ranging from 1 (zero inducibility) to 7. A clone representative of each group was subcultured several times and tested again for the I index of enzymes 5 and 7. In this way, it was ascertained that the I index is inheritable and similar for both enzymes. This result indicates that inducibility has a genetic control distinct from modulation and that it is a multigenic affair.

(c) *Genetic factors controlling the degree of inducibility:* Some attempts were made to analyze the genetic basis for the various degrees of inducibility observed. The recombinant with the highest I index, BP8, was crossed again with the same donor, K10. The cross was analogous to the preceding one: BP8 $Sm^RVal^RHis^-$ was the recipient and Hfr K10 $Sm^SVal^SHis^+$ was the donor. Recombinants Val^R-His^+ were selected, and R_{arg}^- clones were screened as described under methods. Finally, inducibility was determined in a number of the R_{arg}^- recombinants. The results are given in Table 7. It can be seen that a multiplicity of I indexes was again obtained, ranging from 1 to 6.

TABLE 6

TRANSMISSION OF DIFFERENT I INDEXES IN THE SAME CROSS

Number of recombinants Md$^+$ scored	Range of Inducibility Index						
	1	1.5 to 2	2 to 3	3 to 4	4 to 5	5 to 6	6 to 7
48	2	19	6	6	8	6	1

Recipient: B Arg 5$^-$$Sm^R$. Donor: Hfr K10 Arg$^+$$Sm^S$. Recombinants: Arg$^+$$Sm^R$. Scoring Md: test of level of enzyme 5 in cells grown in glucose in absence of arginine. This test gives also the basal level. Test for inducibility: determination of enzyme 5 level in cells grown in glucose in the presence of 100 μg/ml of arginine. Inducibility index: ratio of enzyme 5 in cells grown in arginine over that in cells grown in minimal.

TABLE 7

THE VARIABILITY OF INDUCIBILITY INDEX AFTER CROSSING

Recipient	Donor: K10 $Sm^SVal^SHis^+$					
	R$^-$ recombinants scored	Number of R$^-$ recombinants with I index of				
		1	1.5 to 2	2 to 3	3 to 5	6
B $\times$ K10 #8 $Sm^RVal^RHis^-$	28	1	19	4	4	1

Recombinants: Val^RHis^+. Scoring I: test of behavior of enzyme 5. Inducibility index: ratio of enzyme 5 in cells grown in arginine over that in cells grown in minimal.

A second type of experiment arose from the observation that when a strain B mutant Arg 5$^-$Lac$^-$(B90 Lac$^-$) was crossed with Lac$^+$ donors, recombinants with high I indexes were frequently found. A systematic analysis was attempted on recombinants obtained from B90 Sm^{R}Lac$^-$ crossed with different donors Arg$^+$-Lac$^+$Sm^S. The selected recombinants were Sm^{R}Arg$^+$ and they were essentially all R$^-$ (because of the use of the Sm^R character as a counterselector). They were scored for the Lac character and for inducibility. The results obtained with K10 (Table 8) suggest that some factor located in the Lac region is responsible for the appearance of I indexes higher than 2, the value found in wild type B.

These results confirm the conclusion that inducibility is controlled by several genes, one or more probably being located in the Lac region.

Discussion.—Whereas arginine represses the formation of enzymes of its biosynthetic pathway in several strains of *E. coli*, it is shown in this paper that arginine stimulated the synthesis of these enzymes in strain B and in certain B × K12 recombinants. The extent of enzyme induction varied with the strain and with growth conditions in a range from 2 to 14 times the basal activity. The ability of arginine to induce, like its ability to repress in other strains,[13] is specific for arginine: no induction was seen with other amino acids or with metabolic precursors or degradative products of arginine. Moreover, the induction is not sequential since arginine induces enzymes separated from it by a genetic block (see Table 3) just as it represses these enzymes in other strains.[13] Therefore, the induction by arginine, compared with classical induction systems, is peculiar in two ways: it is exerted by the end-product and it is not sequential.

TABLE 8

RELATION TO LAC$^+$ OF HIGH INDEX OF INDUCIBILITY

		Inducibility Index in Recombinants					
		Lac$^+$			Lac$^-$		
Donor	% Lac$^+$	Colonies analyzed	Index 2	Index 3 to 8	Colonies analyzed	Index 2	Index 3 to 8
K10	53	18	2	16	16	10	6
3000	80	33	0	33	9	1	8
P76	31	5	0	5	0	—	
		56		54	25		14
With index 3 to 8			95%			55%	

Recipient—F$^-$B = Arg 5$^-$ Lac$^-$Sm^R (B90). Donors—Hfr K12 = Arg 5$^+$Lac$^+$Sm^S (K10; 3000; P76). Recombinants = Arg 5$^+$ Sm^R. Inducibility index = ratio of enzyme 5 in cell grown in arginine over that in cells grown in minimal. Values of 2 is equal to that found in B. Values >2 are considered high I index.

The lack of repressibility by arginine characteristic of the B strain is obviously a requisite for the phenotypic manifestation of the inducibility by arginine. However, the two actions involve separate mechanisms, for R_{arg}^- mutants isolated from R_{arg}^+ strains (W or K12) have proved to be not inducible but simply indifferent to arginine. Inducibility by arginine is a feature so far peculiar to the B strain.

This feature appears to be related to another distinctive characteristic of strain B, its low level of arginine enzymes despite the absence of repression by arginine. In contrast, the R_{arg}^- mutants of W or K12, which are not inducible, possess a level of these enzymes 20 to 30 times higher than that of R_{arg}^- strain B. When strains with a high level of enzymes were obtained from B, either by mutation or by recombination with K12, they were also found not to be inducible by arginine. It

appears, therefore, that inducibility is restricted to R_{arg}^- strains with a low enzyme level.

Since both low and high enzyme levels have been found associated with either R_{arg}^- or R_{arg}^+, [5] and at least four enzymes of the arginine sequence vary their level in parallel,[6] it was postulated that the low enzyme level in B reflected the presence in this strain of another regulatory gene, like R_{arg}, with a pleiotropic effect on the enzymes of arginine biosynthesis. The arginine pathway in *E. coli* would thus be subject to two regulatory mechanisms: end-product repression (R_{arg}) and enzyme level setting or "modulation" (Md).

The facts presented in this paper help clarify the mechanism of modulation. First of all, according to our results, the high enzyme level of Md^- mutants corresponds to structural genes working at capacity. It may be designated as the "constitutive level" because it is the level of enzyme supported by a constitutive gene, i.e., one free of both R_{arg} and Md controls. The low enzyme level of strains Md^+, in contrast, may be considered a "basal level" which can be raised by arginine. This action of arginine, which in a formal sense is that of an inducer, suggests the possibility that "modulation" might indeed involve an unknown repressor which is reversed by arginine. The function of arginine would then be that of an antirepressor, as in the usual repressor-inducer systems.

This concept is supported by the fact that in Md^+ strains the low level of enzyme is strongly dependent on the nature of the carbon source. In glucose (or lactate) the basal level is the lowest, in glycerol the highest. Since the level of the induced enzyme does not vary proportionally, the net result of shifting the carbon source from glucose to glycerol is to raise the basal level so high that the inducing effect of the arginine may be practically canceled, as in Md^- mutants. In the Md^- strains, however, the constitutive level is practically independent of the carbon source. These findings are all consistent with the hypothesis that the setting of enzyme level is due to the action of a repressor whose formation is so directly related to the general metabolism that it can be influenced by factors as nonspecific as the carbon source and temperature (see Tables 4 and 5). The situation suggests that the classical "glucose effect," described for β-galactosidase[14, 15] and for other degradative enzymes,[16] can be extended in some cases to a biosynthetic pathway.

To account for the specificity of the antagonism of arginine to repression of the arginine pathway, it seems reasonable to assume that the same repressor is formed from various carbon sources, and that different amounts of repression are due to differences in its steady-state concentration. However, a correlation between amount of repression and rate of general metabolism, as found in other systems,[16] was not found in this one. In fact, on the basis of their decreasing ability to repress, the carbon sources studied in Table 4 are arranged in the following sequence: glucose and lactate > succinate > glycerol; however, on the basis of the rate of growth they are able to support, the order would be: glucose (growth index = 1) > glycerol (growth index = 0.8) > lactate and succinate (growth index = 0.5).

By comparing the enzyme level in different strains, it is seen that Md^- is an extreme point of a range covered by the class $Md.^+$ This variety of enzyme levels is shown in Table 4 and is obtained whenever Md markers are recombined.[5] This pattern is paralleled by a similar variety of degrees of inducibility obtained in the same crosses (see Tables 6, 7, and 8). This situation formally resembles the mul-

tiplicity of phenotypic stable states of induction of β-galactosidase observed with variations in carbon source and in concentration of inducer.[15, 17] However, in the arginine system it is so far unclear whether or to what extent a genetic basis exists for this phenomenon. In any case, the behavior of Md^+ recombinant BC39 (Table 4), which is insensitive to arginine in glucose or lactate but is inducible in glycerol or succinate, underlines the reciprocal relation between Md and I. These findings suggest that the noninducibility of this strain in glucose depends on its rapid production of repressor rather than on absence of a receptor sensitive to the inducer.

The competitive nature of this inducer-repressor system is further supported by the apparent competition of endogenous arginine with the postulated non-end-product repression. Evidence for this competition was provided by the finding that the enzyme 5 level in inducible cells grown in a rich medium deprived of arginine is lower than that found in the same cells grown in minimal medium, in which the intracellular arginine concentration is presumably higher (see discussion of Table 2). Indication in the same direction was obtained when an arginine auxotroph of an inducible strain was grown at different rates in a chemostat under arginine limitation. It was found that increasing internal levels of arginine were associated with increased levels of enzyme 5. This result would appear a paradox from the point of view of end-product repression, and actually with R_{arg}^+ W or K12 strains the opposite occurs.[12, 13] However, with inducible strain BB18, in which arginine is antagonizing a "non-end-product repression," these results are what one would expect.

In conclusion, what we have called "modulation" appears to be the action of an unknown repressor derived, as in the widely known "glucose effect," via the general metabolism. Accordingly, we propose the symbol R_x, which appears to be more appropriate than Md, for this "non-end-product" type of repression, in contrast to R_{arg} for arginine repression. The nonsequential nature of induction by arginine indicates that one compound (arginine itself or a derivative) is able to reverse the R_x repression of different enzymes of its pathway. This compound may interfere either with formation of R_x or with its action. Interference with action at a single site seems unlikely at the level of the chromosome because the structural genes of arginine enzymes in *E. coli* are not clustered[18] in an operon chromosomal unit.[19] Remaining possibilities include interference with the formation of R_x or with its action at multiple sites or at a single ribosomal cluster of sites making different arginine enzymes. A discrimination among these alternatives is not possible with the data presented here concerning enzymes 5 and 7 only. For, given the lack of gene clustering in the arginine pathway, the parallel behavior of enzymes 5 and 7 cannot be generalized to the other enzymes of this sequence until they are individually tested.

Summary.—Whereas arginine represses the formation of enzymes of the arginine biosynthetic pathway in several strains of *E. coli*, it is shown to stimulate the synthesis of certain of these enzymes in strain B. This strain has a low "basal" enzyme level controlled by "modulation"; mutants of strain B that have high levels of the enzymes are not further stimulated by arginine. In strains with low or moderate basal levels, a shift in carbon source can influence the formation of these biosynthetic enzymes in a manner similar to the "glucose effect" observed with

many inducible degradative enzymes.

These results suggest that modulation involves repression by a "non-end-product" formed via general metabolism, and induction by arginine involves reversal of this repression. The symbol R_x is proposed, instead of Md, for this non-end-product repression as opposed to R_{arg} for the end-product repression.

We are indebted to Dr. B. D. Davis for valuable assistance in the preparation of this manuscript. The skillful technical assistance of Mrs. Barbara Colton is gratefully acknowledged.

* This work was supported by U. S. Public Health Service Grant E-2011 C2.

† The schematic outline of arginine biosynthesis is given below. The numbers refer to the enzymes that catalyze each reaction.

glutamate $\xrightarrow{1}$ acetylglutamate $\xrightarrow{2}$ acetylglutamatesemialdehyde $\xrightarrow{3}$ acetylornithine $\xrightarrow{4}$ ornithine $\xrightarrow{5}$ citrulline $\xrightarrow{6}$ arginine-succinate $\xrightarrow{7}$ arginine

‡ The following abbreviations are used for the genetic markers: Arg = arginine; His = histidine; Lac = lactose; Sm = streptomycin; Val = valine; R_{arg} = repressibility by arginine; Md = modulation or enzyme level setting; I = inducibility; R_x = non-end-product repressibility.

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