AN ANALYSIS OF ARGININE REQUIRING MUTANTS IN *PSEUDOMONAS AERUGINOSA*

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GENETIC analyses of auxotrophic mutants in *Salmonella typhimurium* have resulted in the finding that the majority of genes which belong to phenotypically related groups are arranged in clusters of two or more on the bacterial chromosome, and may be arranged in the same order as are the sequence of biochemical steps with which they are concerned (DEMEREK and HARTMAN 1959; DEMEREK 1964). It has been demonstrated that the genes controlling enzymes for tryptophan biosynthesis (BAUERLE and MARGOLIN 1965), for histidine biosynthesis (AMES and HARTMAN 1963), and for leucine biosynthesis (BURNS *et al.* 1966) are clustered and do not function autonomously but constitute distinct units of regulation known as operons (JACOB and MONOD 1961). An interesting exception to these findings in the enterobacteria concerns the biosynthetic pathway for arginine. It has been shown that while four of the eight genes that code for enzymes of the arginine pathway are clustered, the other four are distributed widely over the bacterial chromosome (GORINI, GUNDERSEN and BURGER 1961). The order of genes within the four-gene cluster has been found not to correspond to the sequence of the biosynthetic steps with which they are concerned (GLANSDORFF 1965), and it has been demonstrated that repression of the enzymes specified by this gene cluster is noncoordinate (BAUMBERG, BACON and VOGEL 1965).

In contrast to the clustering of phenotypically related genes found in the enterobacteria, the results of recent genetic analyses of auxotrophic mutants in the genus *Pseudomonas* indicate that genes controlling related steps in a metabolic pathway are generally unlinked (HOLLOWAY, HODGINS and FARGIE 1963; FARGIE and HOLLOWAY 1965). In *Pseudomonas putida* it has been shown that the genes coding for the six enzymes of the tryptophan biosynthetic pathway are arranged in three separate linkage groups (CHAKRABARTY, GUNSALUS and GUNSALUS 1968), while in *Pseudomonas aeruginosa*, it has been demonstrated that genes coding for histidine biosynthetic enzymes are arranged in a minimum of five groups which are not linked to one another (MEE and LEE 1967). Biochemical analyses of the patterns of the induction of enzymes involved in the catabolism of tryptophan in *Pseudomonas fluorescens* (TREMBLAY, GOTTLIEB and KNOX 1967) or of the repression of tryptophan biosynthetic enzymes in *Pseudomonas putida* (CRAWFORD and GUNSALUS 1966) have revealed mechanisms of regulation not consistent with the operon model. An exception to these findings in *Pseudomonas* is the report of clustering of the genes coding for enzymes of the isoleucine-valine pathway in *Pseudomonas aeruginosa* (PEARCE and LOUITT

1965) and the recent isolation of mutants which exert a pleiotropic effect on the synthesis of the enzymes of this pathway (MARINUS and LOUTIT 1968).

On the basis of the differences between the enterobacteria and *Pseudomonas* with regard to the arrangement of phenotypically related genes on the chromosome, it appears likely that the mode of regulation of enzyme synthesis in *Pseudomonas* would be sufficiently different from that described for the enterobacteria to justify further investigation of additional pathways in this organism. As a prelude to the investigation of the mode of regulation of arginine biosynthesis in *Pseudomonas*, this paper is a report of the transduction analysis of arginine-requiring mutants of two strains of *Pseudomonas aeruginosa* in an attempt to determine the relationship of the genes coding for arginine biosynthetic enzymes in these microorganisms to that found in the enterobacteria. Fifty one arginine mutants of *P. aeruginosa* strain 1C (HOLLOWAY 1955) were classified into six transduction groups, while 52 arginine mutants of *P. aeruginosa* strain 78 (PEARCE and LOUTIT 1965) were also classified into six transduction groups. On the basis of growth responses to intermediates of the arginine pathway, the one hundred and three arginine mutants represent a minimum of seven transduction groups which are probably not linked.

MATERIALS AND METHODS

Media: Nutrient Broth Yeast Extract (NBYE): Difco nutrient broth 0.8%, Difco yeast extract 0.5%, NaCl 0.5%. Nutrient Broth Yeast Extract Agar (NBYEA): NBYE solidified with 1.5% Difco Agar. Minimal Medium (MM): The minimal medium of VOGEL and BONNER (1956) was used and solidified with 1.5% Difco agar where necessary. Enriched Minimal Medium (EMM): Minimal medium supplemented with 0.02% Difco nutrient broth and 0.05% Difco yeast extract. Phage Agar (PA): Bacto Tryptone 1%, NaCl 0.5%. Difco Agar 1.2%. Top Agar (TA) for phage assays: Difco Agar 0.5%, NaCl 0.5%. TM buffer: Tris 0.01M, NaCl 0.15M, MgSO₄ 0.01M, pH 7.0.

Bacterial and bacteriophage Strains: *P. aeruginosa* strain 1C and transducing phage F116 (FARGIE and HOLLOWAY 1965) were obtained from B. W. HOLLOWAY. The transduction analysis of strain 1C arginine mutants reported here was performed with phage F116; all arginine donor F116 lysates were assayed for plaque-forming units on the wild-type strain 1C. *P. aeruginosa* strain 78 and transducing phage B (LOUTIT 1958) were obtained from J. S. LOUTIT. The phage B preparation received forms clear plaques on strain 78 and plates with low efficiency (10^{-5}) on strain 1C. The transduction analysis of strain 78 arginine mutants reported in this paper was accomplished with phage B; arginine donor lysates of phage B were assayed for plaque forming units on the wild-type strain 78. Cultural procedures were the same as reported previously (HOLLOWAY 1955; FARGIE and HOLLOWAY 1965), while propagation of phages F116 and B on their respective hosts was performed by the agar layer technique. Phage assays were carried out by methods described by ADAMS (1959). All phage preparations were centrifuged at $10,000 \times g$ for 30 min to remove bits of agar and bacterial debris and sterilized by passage through millipore filters.

Isolation of mutants: Auxotrophic mutants of both strain 1C and strain 78 were isolated after treatment of exponential phase cells with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) using the procedures described by ADELBERG, MANDEL and CHEN (1965). Appropriate dilutions of MNNG treated cells in TM buffer were plated directly on EMM plates, and following 18–24 hr incubation at 37°C, small colonies and those exhibiting diffuse growth were picked to NBYE agar. Colonies on NBYE agar which failed to grow when replica-plated to MM agar were transferred to a second set of NBYE plates which in turn were replica-plated on a series of 12 nutrient pools prepared as described by HOLLIDAY (1956) in order to determine primary growth require-

ments. Among several hundred auxotrophic mutants of both strain 1C and 78 obtained as the result of several isolation experiments, 51 arginine mutants of strain 1C, and 52 arginine mutants of strain 78 were selected for this study. The mutant numbers given in Tables 1-4 refer to the acquisition numbers given to the mutants when isolated.

Utilization of arginine precursors: All arginine-requiring mutants were tested for their ability to grow on arginine precursors by inoculation of MM agar plates supplemented with 20 $\mu\text{g}/\text{ml}$ of either argininosuccinate, citrulline, ornithine, N-acetylornithine, or N-acetylglutamate; mutants were also tested for their ability to utilize glutamate or proline. Supplemented plates were inoculated with drops, each containing about 2×10^6 organisms from TM buffer dilution of washed organisms from overnight NBYE cultures of each mutant. Growth responses were read at 24 hr intervals for five days at 37°C.

Syntrophism: All strain 1C arginine mutants were tested with one another as a group, while all strain 78 arginine mutants were tested with one another as a second group. Melted MM agar was seeded with washed cells of each mutant and dispensed in 15 ml aliquots to sterile Petri dishes. After hardening, the plates were inoculated with drops, each containing about 5×10^8 organisms from TM buffer dilutions of washed organisms from overnight NFBY cultures of each mutant. Growth responses were scored at 24 hr intervals for five days at 37°C.

Transduction experiments: The same procedures were used for the transduction analyses of strain 1C arginine mutants with phage F116 and strain 78 arginine mutants with phage B. Transducing phage lysates prepared on mutant donors were assayed for plaque-forming units on the respective wild-type strain and in all transduction experiments, phage was used at a titer of 2×10^{10} plaque-forming units/ml. The recipient strains were grown overnight in 20 ml NBYE broth shaken in a Warner-Chilcott water bath shaker at 37°C. Prior to transduction experiments, the recipient bacteria were washed twice in TM buffer by centrifugation and resuspended to give an approximate final concentration of 2×10^9 viable cells/ml. For qualitative transduction experiments, 0.2 ml of the washed recipient bacteria were spread over the surface of EMM plates which had been prewarmed at 37°C for 18 hrs. After drying, 0.01 ml aliquots of 12 different phage preparations, including one which was the wild-type donor, were transferred to each plate. Recombinants were scored in the areas where transduction had occurred following 48 hrs incubation at 37°C. For quantitative transduction experiments, equal volumes of recipient cells and phage were mixed (multiplicity of infection = 10), incubated at 37°C for 20 min, and appropriate aliquots of the mixtures plated on EMM. The number of recombinant colonies resulting from each cross were counted after 48 hr incubation at 37°C. For each transduction experiment, controls were plated to measure the reversion frequency of each recipient used and to confirm the absence of viable bacteria in the phage preparations. Crosses between each recipient and the wild-type donor were also included in each transduction experiment.

RESULTS

Utilization of arginine precursors: None of the arginine mutants of either strain 1C or strain 78 responded to proline, glutamate, N-acetylglutamate or N-acetylornithine during five days of incubation at 37°C. A slight growth response to argininosuccinate was exhibited by all of the mutants on the third day of incubation, no increase in this response was obtained upon further incubation. A strong growth response, similar to that shown by wild type on unsupplemented MM, was exhibited within 24 hrs at 37°C to arginine, arginine or citrulline, or to arginine, citrulline or ornithine by all of the mutants tested. The classification of strain 1C and strain 78 mutants into three phenotypic groups on the basis of their growth responses to these three compounds is presented in Table 1.

Syntrophism: Although no cross-feeding was observed among mutants which responded to arginine only, this group was found to cross-feed phenotypic groups

TABLE 1

Growth responses of 103 P. aeruginosa arginine auxotrophs to intermediates of the arginine pathway

Phenotype			Strain 1C (wt) mutants	Strain 78 (wt) mutants
Arg	Cit	Orn		
+	+	+	8, 32, 34, 84, 96, 100, 103, 109, 147, 163, 196, 170, 261, 273, 288, 293, 419, 420, 425, 430, 435, 436.	2, 109, 132, 139, 149, 181, 184, 185, 186, 194, 195, 197, 198, 203, 204.
+	+	—	..., 235, 246, 421, 428.	43, 70, 142, 187, 188, 191, 202, 205.
+	—	—	13, 26, 37, 39, 46, 54, 69, 75, 159, 177, 287, 191, 195, 218, 219, 230, 242, 260, 268, 275, 276, 422, 423, 424.	1, 6, 34, 47, 51, 67, 77, 82, 96, 106, 108, 110, 119, 125, 133, 140, 143, 144, 172, 179, 182, 183, 192, 193, 196, 199, 207.

which responded to arginine or citrulline; or, to arginine, citrulline or ornithine. The mutants which responded to arginine or citrulline cross-fed those capable of growth on arginine, citrulline or ornithine. No cross-feeding was observed among different strains of the latter group, nor was this group capable of cross-feeding the other groups. Thus, although syntrophism was observed in a manner similar to that reported for arginine mutants of *E. coli* (DAVIS 1950), this technique was not helpful in further delineation of phenotypic groups among the arginine mutants tested.

Transduction experiments: From each of the three phenotypic groups of strain 1C and strain 78 arginine mutants shown in Table 1, four or five strains were picked at random for the propagation of phage F116 or phage B respectively. These phage preparations were then utilized in qualitative transduction tests against all 51 strain 1C mutants and all 52 strain 78 mutants. The results of these experiments suggested that the arginine mutants of each strain could be classified into six groups. The strains which responded to arginine only, appeared to form a single group for the strain 1C mutants, while for strain 78 mutants of this class, two groups were observed. Both strain 1C and strain 78 mutants which responded to arginine or citrulline formed a single group with the respective phages F116 or B. Four groups were observed among 1C mutants which responded to arginine, citrulline or ornithine with phage F116, while the strain 78 mutants of this class appeared to fall into three groups with phage B.

Twenty one strain 1C mutants and 17 strain 78 mutants, which had low reversion rates, were chosen so as to include representatives of each of the six groups for quantitative transduction analysis. Phages F116 and B were grown on these selected mutants and on the respective wild-type strains from which these mutants were derived. Each mutant was used both as a recipient and as a donor, and in addition, each was treated with phage grown on the respective wild-type strains. Examples of the results obtained from these experiments are shown in Table 2A for the strain 1C-phage F116 system, and in Table 2B for the strain 78-phage B system.

TABLE 2A
Numbers of transductants obtained in crosses between strain 1C arginine-requiring mutants with phage F116

Recipient strains	Donor strains															1Cwt
	26	39	423	2	426	235	420	435	147	169	170	8	100	261	293	288
26	0	1	0	136	306	118	244	215	96	363	126	317	137	255	141	260
39	3	3	0	171	148	143	224	128	201	177	93	103	151	195	138	118
423	0	1	4	249	244	86	268	156	146	247	171	218	127	111	146	310
46	4	0	3	192	179	206	275	199	192	206	224	196	69	87	179	167
69	1	3	2	213	409	97	143	117	114	141	199	124	122	149	88	142
159	1	1	1	216	269	186	341	267	87	219	156	176	162	121	94	151
219	2	0	0	122	209	121	250	143	125	158	183	113	145	98	108	111
268	0	2	3	181	182	184	296	301	162	133	261	142	216	162	137	186
2	56	76	160	1	11	4	314	193	107	161	187	119	165	129	123	236
426	72	122	179	0	0	0	189	233	110	83	261	169	170	133	132	114
235	63	101	177	0	0	0	165	152	99	83	136	149	70	187	120	197
420	85	89	114	168	193	142	0	0	0	0	0	91	120	153	86	121
435	69	111	99	141	270	173	1	0	3	2	7	158	156	107	177	237
147	91	106	80	92	393	118	0	2	0	0	1	122	184	95	163	162
169	55	74	185	201	112	98	3	0	0	2	4	145	90	136	96	138
170	88	81	142	188	159	146	0	0	3	0	0	218	146	112	142	246
8	82	107	168	109	197	170	279	151	119	79	222	0	3	83	211	191
100	71	92	131	230	216	138	226	242	85	120	162	2	0	118	103	270
261	97	70	157	187	281	111	333	232	132	112	383	116	209	0	1	303
293	59	110	192	284	124	121	243	311	146	99	209	151	142	2	3	178
283	77	92	133	116	219	178	274	306	127	147	308	162	212	180	164	0

Each entry represents the number of transductants obtained per 10^9 bacteriophage on a single plate. The donor and recipient strains have been grouped according to their proposed linkage arrangement. The first group of 8 strains respond to arginine only; the second group of 3 strains respond to arginine or citrulline, while the third group of 10 strains respond to arginine, citrulline or ornithine.

In these experiments, a single phage preparation was made from each bacterial strain, each phage preparation was subsequently used for at least two crosses. For both the phage F116- and phage B-systems, the numbers of transductants obtained from a given cross using a single phage preparation were relatively consistent; for example, for the cross 26×147 (Table 2A); 112, 91 and

TABLE 2B
Numbers of transductants obtained in crosses between strain 78 arginine-requiring mutants with phage B

Recipient strains	Donor strains														78 wt
	67	82	182	189	43	188	109	186	139	181	195	197	195	197	78 wt
67	0	0	222	121	385	192	237	193	303	328	165	212	165	212	345
82	0	1	334	132	340	174	346	246	296	246	180	375	180	375	371
172	2	1	192	118	396	184	268	96	372	198	203	173	203	173	296
183	1	0	321	139	266	304	189	151	281	217	192	221	192	221	209
125	323	194	7	2	367	203	277	231	332	321	266	262	266	262	300
182	184	116	0	0	148	97	184	297	227	227	206	232	206	232	422
189	163	118	4	3	166	229	131	147	361	176	265	240	265	240	396
43	368	85	310	168	0	8	250	269	408	216	163	134	163	134	158
188	146	99	134	114	3	0	256	144	376	241	156	121	156	121	125
191	199	152	225	146	6	1	323	211	291	257	311	246	311	246	371
109	120	138	137	110	207	180	0	0	130	301	155	169	155	169	112
186	209	97	171	123	394	213	0	0	176	229	155	238	155	238	280
139	113	170	180	187	165	223	241	117	0	2	209	167	209	167	229
181	155	130	181	98	186	203	237	232	1	0	119	146	119	146	103
195	309	129	249	119	117	161	118	176	158	124	0	0	0	0	120
197	123	132	178	126	197	330	275	190	263	237	1	0	1	0	106
198	114	186	252	137	246	153	275	112	238	221	2	1	2	1	233

Each entry represents the number of transductants obtained per 10^8 bacteriophage on a single plate. The donor and recipient strains have been grouped according to their proposed linkage arrangement. The first group of 7 strains respond to arginine only; the second group of 3 strains respond to arginine or citrulline, while the third group of 7 strains respond to arginine, citrulline or ornithine.

96, transductants were obtained in three separate experiments, while for the cross 109×67 (Table 2B), 198, 237 and 218 transductants were obtained in three separate experiments. On the other hand, a great deal of variation was noted in the strain 1C-phage F116 system when different phage preparations were used

to infect a given recipient. For example, from the data presented in Table 2A, when strain 2 was used as a recipient in crosses with strains 26, 420 and 147; 56, 314 and 107 transductants were obtained, respectively. Examination of the data presented in Table 2B suggests that phage B is slightly more efficient than phage F116 in that the numbers of transductants obtained per 10^9 phage B particles are significantly higher than those obtained with phage F116 under the same conditions. In addition, there also appears to be less variation in the ability of different phage B preparations to transduce a given recipient strain.

In order to correct for variance in donor efficiencies, 14 of the phage F116 lysates prepared on strain 1C arginine mutants and 12 of the phage B lysates prepared on strain 78 arginine mutants were used in qualitative transduction tests against a variety of strain 1C and strain 78 auxotrophs that had growth requirements other than arginine. In each case, three of the auxotrophs tested were selected for quantitative transduction tests. The results of these experiments are depicted in Table 3 which shows the number of transductants obtained in crosses between each of the arginine donors and the three unrelated recipients. The donor efficiency of each lysate was obtained from the ratio of the average number of transductants obtained from wild-type phage to the average number of transductants obtained from phage grown on an arginine donor. It was found that the donor efficiencies of the 14 phage F116 lysates varied from 0.54 to 2.70 that of the wild-type phage, while the 12 phage B lysates exhibited donor efficiencies which closely corresponded to that of the wild-type lysate. It should be pointed out that when the phage F116 and phage B arginine lysates were used in qualitative transduction tests against other unrelated auxotrophs of strain 1C and strain 78, respectively, we were unable to detect any evidence of linkage between an unrelated marker and any of the arginine markers.

The transductional analyses of the 14 selected strain 1C arginine mutants and the 12 selected strain 78 mutants are presented in Tables 4A and 4B, respectively. The numbers in these tables represent the expression of prototrophic yields as a percentage obtained by the ratio of mutant to wild-type transductants, multiplied by 100 (CLOWES 1958); and corrected for differences in donor efficiency by a multiplication of this expression by the donor efficiency factor (SMITH 1961) of each arginine donor tabulated in Table 3. A value of approximately 100% would indicate that the two arginine markers involved in a given cross are not linked, while low values would be interpreted to mean that the two markers involved in the cross are relatively close to one another. The data depicted in Table 4A shows that the 14 selected strain 1C arginine mutants fall into six transduction groups, and that the six groups are probably not linked to each other. The high frequency of numbers significantly greater than 100 shown in Table 4A most probably is a reflection of the sources of variability inherent in transduction systems in general (HAYES 1964) and may also be a reflection of additional variation in this particular system. From the results depicted in Table 4B it can be seen that the 12 selected strain 78 arginine mutants also fall into six distinct transduction groups. With this system, the frequencies of prototroph formation show fewer numbers significantly greater than 100. Since the numbers within

TABLE 3

Donor efficiency of arginine mutants for unlinked alleles

Donor	Recipient			Average number of transductants per 10 ⁹ phage	Donor efficiency*
	<i>try</i> (21)	<i>leu</i> (24)	<i>his</i> (65)		
1C (wt)	322	168	171	220	1.00
26	68	97	149	105	2.10
39	59	125	84	89	2.70
423	215	197	234	215	1.02
2	265	215	201	227	0.97
426	369	212	315	298	0.74
235	193	104	174	157	1.40
420	482	339	403	408	0.54
435	287	355	266	303	0.73
147	154	106	171	144	1.53
8	158	123	184	155	1.42
100	242	159	301	234	0.94
261	170	128	161	153	1.44
293	119	90	200	136	1.61
288	212	348	187	249	0.89
78 (wt)	<i>leu</i> (4)	<i>try</i> (44)	<i>pro</i> (40)		
	204	179	227	203	1.00
	67	171	168	174	1.16
	82	147	192	167	1.21
	182	239	308	274	0.74
	189	152	183	172	1.18
	43	274	231	248	0.82
	188	296	257	283	0.72
	109	252	198	225	0.90
	186	219	225	236	0.86
	139	210	199	217	0.93
	181	230	224	215	0.95
	195	221	184	207	0.98
	197	201	170	186	1.09

* The donor efficiency for each phage lysate prepared on an arginine-requiring mutant was obtained from the ratio $wt \times \gamma/arg \times \gamma$ where γ = average number of transductants per 10⁹ phage for the unlinked alleles *try*(21), *leu*(24) and *his*(65) for phage F116; or *leu*(4), *try*(44) and *pro*(40) for phage B.

the boxes are significantly lower than those outside the boxes, the six groups are probably not linked to one another. Taken together, the data presented in Tables 4A and 4B suggest a minimum of seven unlinked transduction groups for the arginine pathway in *P. aeruginosa*: two unlinked groups among mutants which respond to arginine only, one group represented by those mutants which respond to arginine or citrulline, and a minimum of four unlinked groups among mutants capable of growth on arginine, citrulline or ornithine.

DISCUSSION

There are two pathways of arginine biosynthesis in bacteria, the one characteristic of *E. coli* as depicted in Figure 1 and the other, characteristic of *Micro-*

TABLE 4A
Transductional analysis of 14 arginine-requiring mutants of P. aeruginosa strain 1C

Recipient strains	Donor strains													
	26	39	423	2	426	235	420	435	147	8	100	261	293	288
26	0	0	0	88	188	76	41	50	45	136	40	109	69	71
39	1	1	0	89	57	105	64	50	152	76	74	143	115	54
423	0	0	2	134	100	57	80	62	120	179	66	85	96	101
2	84	146	114	0	5	2	122	100	114	119	110	127	148	151
426	156	324	181	0	0	0	105	139	168	244	162	241	218	103
235	74	135	100	0	0	0	50	62	83	116	78	146	107	97
420	143	196	93	131	116	162	0	0	0	104	69	175	112	89
435	65	135	48	62	90	109	0	0	1	99	67	67	128	95
147	158	235	65	74	237	136	0	1	0	140	141	109	213	119
8	97	162	95	60	82	133	85	62	99	0	1	66	189	106
100	128	210	112	190	136	165	104	141	107	1	0	140	139	205
261	63	57	49	56	64	48	56	53	60	50	62	0	0	83
293	59	135	90	128	42	78	61	116	102	97	67	1	1	73
288	53	78	45	36	51	78	48	63	59	70	64	78	82	0

The frequencies of prototroph formation, corrected for donor efficiency, are presented as percentages for crosses between arginine-requiring mutants. Each entry represents the result of a single experiment and is derived from data similar to that presented in Tables 2A and 3.

coccus glutamicus which differs in that a transacetylase is involved in the formation of N-acetylglutamate (UDAKA and KINOSHITA 1958). Although certain arginine mutants of yeasts and fungi respond to proline (FINCHAM 1953), the failure of the 103 *P. aeruginosa* arginine mutants tested in this study to respond to

TABLE 4B

Transductional analysis of 12 arginine-requiring mutants of P. aeruginosa strain 78

Recipient strains	Donor strains											
	67	82	182	189	43	188	109	186	139	181	195	197
67	0	0	49	43	91	40	64	48	83	91	46	68
82	0	0	67	43	75	33	62	54	75	64	47	103
182	53	33	0	0	19	18	40	60	50	51	47	57
189	53	36	1	1	48	46	30	33	85	43	64	63
43	280	65	143	128	0	4	140	146	143	126	98	87
188	140	95	79	99	2	0	180	99	270	178	90	95
109	128	146	90	117	131	116	0	0	107	263	129	154
186	90	42	46	53	110	55	0	0	58	78	55	89
139	60	89	59	98	59	72	95	47	0	1	88	80
181	180	151	128	114	147	140	197	190	1	0	109	150
195	309	130	152	120	81	96	89	126	121	97	0	0
197	139	149	95	201	152	194	231	144	138	185	1	0

The frequencies of prototroph formation, corrected for donor efficiency, are presented as percentages for crosses between arginine-requiring mutants. Each entry represents the result of a single experiment and is derived from data similar to those presented in Tables 2B and 3.

this compound suggests that the biosynthesis of arginine in this organism proceeds in a manner similar to that described for other bacteria. The failure of any of the *P. aeruginosa* mutants tested to respond to acetylornithine or acetylglutamate is in accordance with similar findings in *E. coli* (VOGEL 1960) and *Proteus mirabilis* (PROZESKY 1967), and suggests the absence of the respective permeases in this organism. The data presented here do not distinguish between the two known pathways of arginine biosynthesis; however, it will be of interest from an evolutionary point of view to determine which pathway is present in *Pseudomonas*.

The letters A–H in Figure 1 represent the known enzymic steps in the biosynthesis of arginine in *E. coli*. Assuming a similar biosynthetic pathway in *P. aeruginosa*, it would be expected that a mutant responding only to arginine could arise as the result of a mutation in one of two genes specifying the two enzymic steps required for the conversion of citrulline to arginine. While all such strain 1C mutants tested fell into a single transduction group, the strain 78 mutants tested were found to fall into two distinct transduction groups. Since it is unlikely that different strains of the same species would exhibit significant chromosome differences, it could be expected that a second transduction group among this

FIGURE 1.—The biosynthesis of arginine in *E. coli*. The capital letters refer to the known enzymic steps (GLANSDORFF 1965).

class of strain 1C arginine mutants would be found as additional mutants are acquired in our laboratory. In support of this conclusion, among the eight strain 2 arginine mutants tested by FARGIE and HOLLOWAY (1965), seven responding to arginine, but not to citrulline, fell into two transduction groups. Arginine mutants which respond to citrulline but not ornithine could be expected to result following mutation at a single site specifying the enzyme required to convert ornithine to citrulline; the transductional analysis of both strain 1C and strain 78 mutants of this class revealed only a single transduction group. Finally, arginine mutants responding to citrulline and ornithine would be expected to be deficient in one of the five enzymes required to convert glutamate to ornithine. The strain 1C mutants of this class were found to fall into four distinct transduction groups, while similar mutants of strain 78 were found to fall into three distinct transduction groups. The acquisition of additional mutants and further genetic analysis could be expected to yield a fourth transduction group among strain 78 mutants of this class. The results of these analyses suggest that in contrast to *E. coli* where the arginine structural genes specifying enzymes B, C, E and H in Figure 1 are clustered (GLANSDORFF 1965), the corresponding arginine structural genes in *P. aeruginosa* are unlinked to one another. Biochemical analyses of wild-type and various arginine mutant classes for enzyme activities and identification of accumulated arginine intermediates are necessary in order to specifically identify the genetic blocks present in different arginine mutants of *P. aeruginosa* which would then allow comparison of specific sites between the enterobacteria and *Pseudomonas*.

The conclusions that can be drawn from this work are that arginine mutants of *P. aeruginosa* fall into seven distinct transduction groups and that probably these groups are not linked to one another. Since these conclusions are based on transduction analyses, it is pertinent to mention that the number of transductants formed in a given experiment is subject to several variables including multiplicity of infection, transducing efficiency of different phage preparations, plating conditions, physiological state of the recipient bacteria, etc. (ADAMS 1959; CLOWES 1958; HAYES 1964). With respect to strain 1C it has been recently demonstrated that both wild-type cells and mutants derived from the wild-type strain are not only extremely sensitive to the lethal effects of dilution in nutrient broth and certain buffers but in addition, produce lethal factors which apparently exert their effect under conditions in which growing cells come in intimate contact with one another (HERMAN and GRIFFIN 1968). This variation in the viability of different strain 1C mutants could exert an additional influence on the results of transductional analyses with strain 1C and phage F116.

Further support for the conclusion that the seven arginine transduction groups are unlinked may be obtained by demonstrating linkage of the mutations of one arginine transduction group to an unrelated marker while finding no linkage with mutations of the other groups (FARGIE and HOLLOWAY 1965; MEE and LEE 1967), or by the inability to select for donor phenotypes in crosses between the appropriate mutants of different transduction groups (CLOWES 1958). On the other hand, what is clearly needed is a method which will allow the determination

of marker positions over the entire *Pseudomonas* chromosome. Although methods have been developed to map genes in *Pseudomonas* by time of entry (FEARY and WILLIAMS 1966; ROLFE and HOLLOWAY 1966; LOUITT and MARINUS 1968), no efficient *Pseudomonas* Hfr donor strains have as yet been isolated and such experiments have been consequently restricted to relatively small regions of chromosome.

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SUMMARY

The genetics of arginine biosynthesis in *Pseudomonas aeruginosa* was investigated in order to determine the relationship of genes coding for arginine biosynthetic enzymes to one another in this microorganism and to that found in the enterobacteria. Evidence was obtained which suggests that the biosynthesis of arginine in *Pseudomonas* proceeds in a manner similar to that described in other bacteria. All of the 103 arginine mutants examined could be placed in one of seven transduction groups. The distribution of these seven loci as determined by transductional analyses suggests that unlike the arginine genes in the enterobacteria, which are partly clustered, the seven arginine loci in *Pseudomonas* are probably unlinked.

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