MUTATIONS AFFECTING THE BRANCHED-CHAIN-AMINO-ACID AMINOTRANSFERASE IN *SALMONELLA TYPHIMURIUM*

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The last step in the synthesis of isoleucine, valine and leucine in bacteria is catalyzed by a common enzyme, the branched-chain-amino-acid aminotransferase (transaminase B) EC 2.6.1.6 (Rudman and Meister 1953) (Fig. 1). Genetic and biochemical studies on *ilvE* strains of *Salmonella typhimurium* deficient in transaminase B have been carried out by Glanville and Demerec (1960), Wagner and Bergquist (1960), Armstrong and Wagner (1964), and Roth and Sanderson (1966). Recently, additional isoleucine-valine-leucine requiring mutants were isolated in this laboratory. The biochemical blocks in these strains have been shown to involve the α , β -dihydroxyacid dehydratase, and transaminase B or permeation of valine. In this communication, we describe the genetic and biochemical characteristics of these mutants.

MATERIALS AND METHODS

Strains used in this study were *Salmonella typhimurium* LT-2, and its derivatives. Strains, SU452 (*purC7*, *HfrA*) and SB1332 (*ilvE401 metE338 ara-9*) were supplied by Dr. P. E. Hartman, and SJ1784 (*ara-9 gal-205 leu-728*) by Dr. T. Iino.

KA strains that require isoleucine, valine and leucine for growth were isolated by

Fig. 1. The biosynthetic pathways of isoleucine, valine, leucine and pantothenate.

treatment of the wild-type strain (LT-2) with N-methyl-N'-nitro-N-nitrosoguanidine (Adelberg, Mandel and Chein-Ching-Chen 1965), followed by penicillin screening and replica plating. The replica plating was carried out from a master nutrient plate onto minimal medium agar plates supplemented with; LIV, IV and L (L: *L*-leucine 10 $\mu\text{g/ml}$, I: *L*-isoleucine 10 $\mu\text{g/ml}$, V: *L*-valine 10 $\mu\text{g/ml}$). KA strains were able to form colonies only on LIV.

Composition of nutrient and minimal media, as well as the methods for auxanography and measurement of bacterial generation time, were described previously (Kiritani, Matsuno and Ikeda 1965).

For preparation of cell-free extracts, strains were grown in minimal medium or minimal medium supplemented with LIVP (L: *L*-leucine 10 $\mu\text{g/ml}$, I: *L*-isoleucine 10 $\mu\text{g/ml}$, V: *L*-valine 10 $\mu\text{g/ml}$, P: Ca-pantothenate 1 $\mu\text{g/ml}$), respectively. Thirty ml of an over-night culture was added to 300 ml of fresh medium and growth was continued for 3 hours at 37° on a reciprocal shaker. The cells were harvested by centrifugation for 10 minutes at $1,700 \times g$, washed with 100 ml saline, recentrifuged, and then resuspended in 10 ml of glycerin-phosphate buffer (0.05 M phosphate buffer plus 30% glycerin v/v), pH 8.0. The cells were subjected to 3 minutes of sonic disruption (Tominaga Sonicator, 20 KC), then centrifuged for 30 minutes at $60,000 \times g$. The resultant supernatant fraction served as the cell-free extract. These procedures were carried out in the cold.

Activity of threonine dehydratase was measured by the method of Umbarger and Brown (1957), α -acetohydroxyacid synthetase at pH 8.0 by the method of Radhakrishnan and Snell (1960), reductoisomerase by the method of Kiritani, Narise and Wagner (1966b), dihydroxyacid dehydratase (EC 4.2.1.9) by the method of Kiritani, Narise and Wagner (1966a). In these reaction mixtures, 0.1 M phosphate buffer at the described pH was used. For assay of transaminase B activity, the method described by Taylor and Jenkins (1966) was used. The reaction mixture contains 20 μmoles of a substrate (*L*-isoleucine, *L*-valine, or *L*-leucine), 20 μmoles of α -ketoglutarate (KG), 100 μmoles of sodium pyrophosphate, 0.5 μmoles of pyridoxal-phosphate (PLP), 0.05 to 0.3 ml of cell-free extract, and 0.05 M Tris-HCl buffer, in 2 ml, pH 8.0. The reaction mixture was incubated at 37° for 10 minutes. To determine the amount of α -keto acid produced, the 440 m μ absorbance of the hydrazone was measured with a Bausch and Lomb Spectronic 20 colorimeter. The standard curve of α -ketoisocaproate (KL) was used for measurement of the hydrazone of KL, and the curve of α -ketoisovalerate (KV) for KV and α -keto- β -methylvalerate (KI).

Heat treatment of transaminase B was carried out in 1 ml of 0.05 M Tris-HCl buffer, pH 8.0. The buffer was prewarmed before 0.05 or 0.1 ml of cell-free extract (about 5 mg protein/ml) was added. The enzyme was heated for 5 minutes then immediately cooled in ice.

Specific activities are expressed as μmoles per mg protein per hour. Protein was determined by the method of Lowry, Rosebrough, Farr and Randall (1951).

For purification of transaminase B, bacteria were grown in 9 l minimal medium or LIVP, harvested by centrifugation at $1,700 \times g$ for 10 minutes, washed with 1 l saline, recentrifuged and then suspended in 150 ml of 0.05 M phosphate buffer containing 1 mmole α -ketoglutarate (A buffer), pH 6.0. The procedure used to prepare cell-free extract was similar to the method mentioned above. Sonic disruption was carried out for

30 minutes. Fifteen ml of 10% streptomycin was added to the cell-free extract and the precipitate was removed by centrifugation at $1,200 \times g$ for 10 minutes. Sufficient solid ammonium sulfate was added to the supernatant fraction to attain 40% saturation. The solution was stirred for 10 minutes and the precipitate removed by centrifugation at $1,200 \times g$ for 10 minutes. The ammonium sulfate concentration of the remaining supernatant fraction was then increased to 50% saturation. The precipitate was collected by centrifugation and dissolved in 16 ml of 0.05 M phosphate buffer containing 5 mmoles α -ketoglutarate (B buffer), pH 6.5. The total enzyme solution was added to a Sephadex G-100 column (3×40 cm) equilibrated with A buffer at pH 6.0 and eluted with the same buffer. Five ml fractions were collected. The Sephadex G-100 fractions with the highest activity were passed through a CM-Sephadex column equilibrated with A buffer, pH 5.5, and eluted with the same buffer in 5 ml fractions. The eluted fractions containing the active enzyme were added to a DEAE-Sephadex column (1×16 cm) prepared with A buffer, pH 7.0. Protein was then eluted with A buffer, pH 7.0, by linear gradient elution using NaCl, the concentration of which was 0.1 to 0.3 M. The volume of each fraction collected was 2 ml. The active enzyme was found in the fractions containing about 0.25 M NaCl. These procedures were carried out in the cold. The final fractions possessing the highest activity were stored at -20° , and served as purified enzyme preparation.

Conjugation experiments were carried out following the method described by Pittard, Loutit and Adelberg (1963). After bacteria were brought to log phase ($OD_{560\text{ m}\mu}=0.25$, about 10^8 cells/ml) in Penassay broth (Difco), 2 ml of SU452 culture was mixed with 5 ml of a recipient at 37° . After 5 minutes, the mixture was then diluted 10^3 -fold with warm minimal medium and incubation continued. The mating was interrupted at intervals by vigorous agitation, and 0.1 ml aliquots of the mixture were plated on duplicate IV and L plates. Colonies on the plates were scored after 48 hours incubation at 37° .

Thiamine pyrophosphate, NADPH (Sigma Type V), Pyridoxal-5'-phosphate, α -keto- β -methylvalerate and α -ketoisovalerate were obtained from Sigma Chemical Company, Ca-pantothenate from Wako Pure Chemical Company, and DEAE-Sephadex A-50 and CM-Sephadex C-50 from Pharmacia, Inc.. The α -acetohydroxybutyrate (AHB) and α -acetolactate (AL) were synthesized by the method of Krampitz (1948), and the α , β -dihydroxyisovalerate (DHV) and α , β -dihydroxy- β -methylvalerate (DHI) by the method of Sjolander, Folkers, Adelberg and Tatum (1954).

RESULTS

Biological characteristics

All KA strains are genetically stable and no spontaneous reversion to Ilv^+Leu^+ , Ilv^-Leu^+ or Ilv^+Leu^- phenotypes have been detected when 10^9 cells were spread on selective agar media. KA strains form colonies on LIV-supplemented agar medium after 48 hours incubation at 37° . When 10^8 cells of a KA strain are seeded in IV agar and incubated, slightly turbid confluent growth is visible after 48 hours. Minimal media supplemented with $10\text{ }\mu\text{g}$ L-isoleucine and $20\text{ }\mu\text{g}$ L-valine per ml support growth of KA19

and about 5% of the cells in a population of KA6 after 96 hours incubation. The percentage of colony formers of KA6 increased to 50% when the amount of valine is increased to 50 μg in the supplemented medium. The KA6 and 19 strains also formed colonies on minimal agar medium supplemented with 10 μg *L*-isoleucine and 40 μg glycyl-*L*-valine after 48 hours incubation. Thus, it appears that the mutations in KA6 and KA19 strains affect valine permeability. The other KA strains could not form colonies under these conditions.

Auxanographic studies showed that all KA strains could grow on a combination of isoleucine and KV. However, combinations such as isoleucine and leucine, isoleucine and KL, valine and leucine, or any single amino acid or α -keto acid do not support growth of the KA strains. As presented in Table 1, many KA strains grow slowly in

Table 1. Growth stimulation by pantothenate in shaking culture

Strain	Generation time in minutes	
	LIV*	LIVP*
KA1	120	60
KA2	180	80
KA6	540	80
KA12	80	80
KA13	420	80
KA19	>540	50
KA21	>540	90
wild-type	50	50

*: L=10 μg *L*-leucine/ml, I=10 μg *L*-isoleucine/ml,
V=10 μg *L*-valine/ml, and P=1 μg Ca-pantothenate/ml.

LIV liquid medium; however, growth is markedly stimulated by the addition of pantothenate to the LIV medium. Addition of other vitamins, amino acids or bases of nucleic acid to the LIV medium does not stimulate bacterial growth.

Enzyme activities

Activities of five enzymes on the isoleucine-valine pathway in wild-type and KA mutant strains are presented in Table 2. In general, levels of the α -acetoxyacid synthetase and reductoisomerase activities in cell-free extracts of KA strains except KA21 are similar to or higher than those in wild-type. The reductoisomerase activity of KA21 when AHB is used as the substrate is 1/20 of the level found in the wild-type strain. The threonine dehydratase activity in KA2, 12, and 13 is less than 1/6 of that in wild-type. All the KA strains lack dihydroxyacid dehydratase activity. The transaminase B of the wild-type strain is about equally active with *L*-isoleucine, *L*-valine or *L*-leucine. Transaminase B activity in KA1, 2, 12 and 13 is lacking, and the activity for valine in KA21 is low. As presented in Table 3, the rate of the transaminase B reaction is reduced when PLP is omitted from the reaction mixture. The reaction rate of wild-type and KA6 is reduced by about 20%; whereas, the rate of KA19 and 21 is halved. As shown in Fig. 2a, the formation of α -keto acid by a cell-free extract from

Table 2. Enzyme activities in cell-free extracts of KA mutant strains

Strain	Specific activities								
	α -Acetohydroxy acid synthetase	Reducto-isomerase		Threonine dehydratase	Dihydroxyacid dehydratase		Transaminase B		
	Pyr	AHB	AL	Thr	DHI	DHV	I	V	L
wild-type	9.1	4.4	0.8	25.6	3.0	4.3	5.0	5.0	4.9
KA1	10.5	6.0	0.9	18.0	0.0	0.0	0.0	0.0	0.0
KA2	11.5	4.6	0.8	1.4	0.0	0.0	0.0	0.0	0.0
KA6	9.0	6.4	1.2	24.3	0.0	0.0	8.3	8.2	6.4
KA12	11.3	10.6	1.7	3.9	0.0	0.0	0.0	0.0	0.0
KA13	13.9	3.9	0.9	2.0	0.0	0.0	0.0	0.0	0.0
KA19	7.8	8.5	1.3	25.0	0.0	0.0	6.1	8.4	4.0
KA21	18.6	0.2	1.0	16.2	0.0	0.0	6.6	1.8	6.6

Numbers given as μ moles of indicated substrate show average values from separate triplicate experiments.

Pyr=Na-pyruvate, AHB= α -aceto- β -hydroxybutyrate, AL= α -acetolactate, Thr=*L*-threonine
 DHI= α , β -dihydroxy- β -methylvalerate, DHV= α , β -dihydroxyisovalerate, I=*L*-isoleucine
 V=*L*-valine, L=*L*-leucine.

Table 3. Effect of PLP on activity of transaminase B

Strain	Specific activity*		
	Reaction Complete	mixture -PLP	-PLP/Complete
Wild-type	4.4	3.4	0.77
KA6	7.8	6.2	0.79
KA19	4.2	2.2	0.52
KA21	8.4	3.7	0.44

*: Specific activity of the transaminase B was measured with *L*-leucine as substrate.

the wild-type strain occurs at a constant rate over a 30 minute time period with or without added PLP. As shown in Fig. 2b, the transaminase B of KA21 is almost equal to the wild-type enzyme in activity for isoleucine, but has very low activity for valine and a significant lag period for attaining the maximal rate with leucine. When an assay of α -keto acid production by the extract was carried out without adding PLP, the reaction rate decreased rapidly with time and leveled off after 20 minutes. These results suggest that the transaminase B of KA21 differs in structure from the wild-type enzyme. It appears that the transaminase B of KA19 is also different from the wild-type enzyme. The pattern of α -keto acid formation by the enzyme of KA19 is similar to that of KA21, although the enzyme activity for valine in the presence of PLP is normal (Fig. 2c). As illustrated in Fig. 3, the transaminase B of KA19 unlike that of wild-type or other KA strains, is heat-labile.

Freundlich and Umbarger (1963), and Elliot and Armstrong (1968), reported that

Fig. 2. The formation of α -keto acid from indicated substrate.

Average value of μ moles from duplicated samples is plotted against time intervals. A reaction mixture contained 0.1 ml of indicated cell-free extract, and mg protein per ml of the extract was as follows; wild-type 6.8, KA19 4.7, and KA21 5.8.

Fig. 3. Effect of heating on transaminase B of wild-type and KA strains.

Ordinate shows percent of remaining activity with leucine as substrate after heating at indicated temperature, and the values are average of duplicated samples. (○) wild-type, (□) KA6, (●) KA19, (▲) KA21.

genes controlling threonine dehydratase and dihydroxyacid dehydratase are contained in the same operon. As KA2, 12 and 13 strains are found to possess low activity of threonine dehydratase in addition to the defect in dihydroxyacid dehydratase activity, they may be classified as polarity mutants.

Properties of partially purified transaminase B

The results presented in Fig. 2 prompted an investigation of partially purified transaminase B of KA strains. The purity of transaminase B at each step of the purification was variable depending on the strain used. In case of wild-type, the enzyme was purified 2- to 4-fold at the ammonium-sulfate-fractionation step, 10- to 20-fold at Sephadex G-100, 15- to 35-fold at CM-Sephadex, and 30- to 90-fold at DEAE-Sephadex, respectively, over the activity of the cell-free extract. The recovery was generally about 20%. Purification of the heat-labile enzyme of KA19 was unsuccessful beyond the Sephadex G-100 step. The degree of purification achieved is presented in Table 4. Partially purified transaminase B of each strain has a pH optimum between 7.5 and 8.0. Omission of the enzyme or substrate from the reaction mixture completely diminished

Table 4. Km values of partially purified transaminase B

Strain	Isoleucine	Valine	Leucine	α -Ketoglutarate	Purification
wild-type	5.0×10^{-4}	4.4×10^{-3}	5.6×10^{-4}	6.4×10^{-4}	37×
KA6	6.1×10^{-4}	3.0×10^{-3}	6.6×10^{-4}	6.3×10^{-4}	92×
KA19	5.9×10^{-4}	2.3×10^{-3}	1.6×10^{-3}	9.0×10^{-4}	5.6*×
KA21	3.4×10^{-3}	3.8×10^{-2}	5.4×10^{-3}	1.2×10^{-3}	19×

*: Purity at step of ammonium sulfate fractionation as described in the text.

Fig. 4. Effect of substrate concentration on production of α -keto acid by partially purified transaminase B. Lineweaver-Burk plot.

Fig. 5. Effect of pyridoxal-5'-phosphate on activity of partially purified transaminase B of the indicated strains. Specific activity given as μ moles α -keto acid/mg protein/hour.

the formation of α -keto acid; omission of α -ketoglutarate resulted in loss of more than 90% of the enzyme activity. Figs. 4a and b show the effect of changing substrate concentrations on transaminase B activity of wild-type and KA21, respectively. K_m values of transaminase B for substrates calculated from the Lineweaver-Burk plots are illustrated in Table 4. The K_m value of each transaminase B for valine is ten times higher than those for the other substrates, hence, affinity of the enzyme is 1/10 lower. Transaminase B of KA21 has 1/10 the affinity for three amino acids as that of wild-type. Fig. 5 shows the effect of changing PLP concentration. In the absence of PLP, purified transaminase B has activity less than 10% of maximal level. For half maximal activity, transaminase B of wild-type, KA6 and 19 required about $5 \mu\text{g/ml}$ PLP, and KA21 $12 \mu\text{g/ml}$.

Interrupted mating experiments

Crosses between SU452 and KA strains were carried out to see if KA strains harbor mutations in regions other than the *ilv* cluster. To standardize the time of entry of the *ilv*⁺ and *leu*⁺ loci, SU452 was crossed to recipients with previously mapped *ilv* and *leu* markers, SB1332 and SJ1784. The *ilvE*⁺ and *leu*⁺ recombinants appeared at 6 and 30 minutes, respectively, after mixing and increased in numbers for about 15 minutes. The time of entry of the *ilvE*⁺ marker corresponds with the results reported by Roth and Sanderson (1966). Fig. 6 depicts results of interrupted mating experiments between SU452 and KA6, SU452 and KA19 as representatives. Maximum numbers of recombinants obtained in each cross with a KA strain ranged from 250 to 700. Although *Ilv*⁺ and *Leu*⁺ recombinants were selected, no colony other than *Ilv*⁺*Leu*⁺ was detected among 600 isolated colonies from each cross. It may be concluded that mutational sites of all KA mutants are confined to the *ilv* cluster.

Fig. 6. Time of entry of *ilv*⁺ loci.
Average recombinant numbers on duplicated plates,
IV (O, ●) and L (Δ, ▲), were plotted.

DISCUSSION

It was reported earlier that three mutants, deficient in the transaminase B, of *Escherichia coli* K-12 and *Salmonella typhimurium* were isoleucine requirers (Rudman and Meister 1953; Wagner and Bergquist 1960). This means that deficiency in transaminase B does not result in a requirement for isoleucine, valine and leucine, although the enzyme is common to the last steps of the biosyntheses. In these mutants, valine can partially be synthesized by alanine aminotransferase (EC 2.6.1.2) and leucine by aromatic amino acid transaminase, the activities of which are 1/100 and 1/10 of the transaminase B, respectively (Rudman and Meister 1953). Contrary to this concept, Ramakrishnan and Adelberg (1965) described that an isoleucine, valine and leucine requiring mutant of *E. coli* was indeed deficient in the transaminase B, though biochemical data on the mutant were not presented. KA mutant strains, which have been isolated as requiring all three amino acids, can be classified into two groups. The first group consisting of KA1, 2, 12, 13 and 21, has a deficiency in activity of α , β -dihydroxyacid dehydratase, and deficiency or alteration in activity of transaminase B. This group is further divided into two subgroups defined by degree of the threonine dehydratase activity; polarity mutants, KA2, 12 and 13, and nonpolar, KA1 and 21. Lack of transduction data does not allow us to determine whether the *ilvE* locus in *S. typhimurium* is also contained in the *ilvA ilvD* operon (which controls the threonine dehydratase and the α , β -dihydroxyacid dehydratase), as in the case in *E. coli* (Ramakrishnan and Adelberg 1965). The second group, consisting of KA6 and 19, is deficient in α , β -

dihydroxyacid dehydratase activity and displays a low permeability for valine, although transaminase B of KA19 is also altered in structure. As in the conclusion reported by Rudman and Meister (1953), and Wagner and Bergquist (1960), one can assume that alteration of transaminase B found in the first group of KA strains may result in the mutant requiring isoleucine. However, an additional genetic block on the isoleucine-valine biosynthetic pathway may result in shortage of *in vivo* α -ketoisovalerate, and as a consequence limited syntheses of leucine and pantothenate cause the bacteria to require the multiple growth factors (Fig. 1). With regard to KA6 and 19 strains, low permeability for valine, together with the deficiency of α , β -dihydroxyacid dehydratase activity, restricts α -ketoisovalerate synthesis. Thus, one can predict that mutations affecting transaminase B will be, with high probability, found among isoleucine-valine-leucine-pantothenate requiring mutants.

Although purified transaminase B requires pyridoxal-5'-phosphate as a cofactor, about 80% of the enzyme activity in cell-free extract of wild-type is measurable without adding pyridoxal-5'-phosphate in the reaction mixture. On the other hand, transaminase B in cell-free extract of KA19 and 21 requires pyridoxal-5'-phosphate as an essential component for the catalytic action. In the case of KA21, this phenomenon may be due to the low affinity of the enzyme for pyridoxal-5'-phosphate. It should be noted that pyridoxal-5'-phosphate does not stimulate growth of KA21 in minimal medium supplemented with isoleucine and valine. Although the enzyme of KA19 strain has normal affinity for pyridoxal-5'-phosphate, the significance is doubtful because of low purity of the enzyme.

SUMMARY

Seven isoleucine, valine and leucine requiring mutants were isolated after treatment of *Salmonella typhimurium* LT-2 with nitrosoguanidine. Growth of six strains is stimulated by pantothenate. The mutant strains are classified into two groups. Mutants of group 1 have deficiency in activity of the α , β -dihydroxyacid dehydratase, and deficiency or alteration in the branched-chain-amino-acid aminotransferase. Group 1 is divided into polarity mutants, KA2, KA12 and KA13, and nonpolar mutants, KA1 and KA21, as judged by activities of the threonine dehydratase and the α , β -dihydroxyacid dehydratase. Activity of the reductoisomerase in the KA21 strain is also low. Mutants of group 2, KA6 and KA19, are deficient in activity of the α , β -dihydroxyacid dehydratase and show low valine permeability. The branched-chain-amino-acid aminotransferase of KA19 is also altered. The mutational site in each of the mutants is located in the *ilv* cluster. The evidence indicates that limited synthesis of α -ketoisovalerate is responsible for the multiple requirements of bacteria.

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LITERATURE CITED

- Adelberg, E. A., M. Mandel, and G. Chein-Ching-Chen, 1965 Optimal conditions for mutagenesis by N-methyl-N'-nitro-N-nitrosoguanidine in *Escherichia coli* K12. *Biochem. Biophys. Res. Commun.* **18**: 788-795.
- Armstrong, F. B., and R. P. Wagner, 1964 Isoleucine-valine requiring mutants of *Salmonella typhimurium*. *Genetics* **50**: 957-965.
- Elliott, C. J., and F. B. Armstrong, 1968 Isoleucine-valine requiring mutants of *Salmonella typhimurium*. II. Strains deficient in dihydroxyacid dehydratase activity. *Genetics* **58**: 171-179.
- Freundlich, M., and H. E. Umbarger, 1963 Control of isoleucine-valine biosynthesis in *S. typhimurium* and *E. coli* (Abstr.). *Bacteriol. Proc.*, p. 126.
- Glanville, E. V., and M. Demerec, 1960 Threonine, isoleucine, and isoleucine-valine mutants of *Salmonella typhimurium*. *Genetics* **45**: 1359-1374.
- Kiritani, K., T. Matsuno, and Y. Ikeda, 1965 Genetic and biochemical studies on isoleucine and valine requiring mutants of *Escherichia coli*. *Genetics* **51**: 341-349.
- Kiritani, K., S. Narise, and R. P. Wagner, 1966a The dihydroxyacid dehydratase of *Neurospora crassa*. *J. Biol. Chem.* **241**: 2042-2046.
- Kiritani, K., S. Narise, and R. P. Wagner, 1966b The reductoisomerase of *Neurospora crassa*. *J. Biol. Chem.* **241**: 2047-2051.
- Krampitz, L. O., 1948 Synthesis of α -acetolactic acid. *Arch. Biochem.* **17**: 81-85.
- Lowry, O., N. J. Rosebrough, A. L. Farr, and R. J. Randall, 1951 Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265-275.
- Pittard, J., J. S. Loutit, and E. A. Adelberg, 1963 Gene transfer by F' strains of *Escherichia coli* K-12. I. Delay in initiation of chromosome transfer. *J. Bacteriol.* **85**: 1394-1401.
- Radhakrishnan, A. N., and E. E. Snell, 1960 Biosynthesis of valine and isoleucine. II. Formation of α -acetolactate and α -aceto- α -hydroxybutyrate in *Neurospora crassa* and *Escherichia coli*. *J. Biol. Chem.* **235**: 2316-2321.
- Ramakrishnan, T., and E. A. Adelberg, 1965 Regulatory mechanisms in the biosynthesis of isoleucine and valine. II. Identification of two operator genes. *J. Bacteriol.* **89**: 654-660.
- Roth, J. R., and K. E. Sanderson, 1966 Orientation of the isoleucine-valine genes in the *Salmonella typhimurium* linkage map. *Genetics* **53**: 971-976.
- Rudman, D., and A. Meister, 1953 Transamination in *Escherichia coli*. *J. Biol. Chem.* **200**: 591-604.
- Sjolander, J. R., K. Folkers, E. A. Adelberg, and E. L. Tatum, 1954 α , β -Dihydroxyisovaleric acid and α , β -dihydroxy- β -methylvaleric acid, precursors of valine and isoleucine. *J. Am. Chem. Soc.* **76**: 1085-1087.
- Taylor, R. T., and W. T. Jenkins, 1966 Leucine aminotransferase. *J. Biol. Chem.* **241**: 4391-4395.
- Umbarger, H. E., and B. Brown, 1957 Threonine deamination in *Escherichia coli*. II. Evidence for two L-threonine deaminases. *J. Bacteriol.* **73**: 105-112.
- Wagner, R. P., and A. Bergquist, 1960 Nature of genetic blocks in the isoleucine-valine mutants of *Salmonella*. *Genetics* **45**: 1375-1386.