CELL DIVISION IN A SPECIES OF *ERWINIA*

IV. Metabolic Blocks in Pantothenate Biosynthesis and their Relationship to Inhibition of Cell Division[1]

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ABSTRACT

GRULA, MARY M. (Oklahoma State University, Stillwater) AND E. A. GRULA. Cell division in a species of *Erwinia*. IV. Metabolic blocks in pantothenate biosynthesis and their relationship to inhibition of cell division. J. Bacteriol. **83**:989-997. 1962—Four compounds that inhibit cell division in an *Erwinia* sp., D-serine, D-histidine, D-phenylalanine, and penicillin, decrease the intracellular pantothenate content of *Erwinia* at culture ages of 10 and 16 hr. In the case of penicillin, it appears to be the result of excessive leakage from long cells; however, with the three D-amino acids, there is a genuine inhibition of synthesis.

Among agents tested that reverse the inhibition of division, only pantoyl lactone, and to a lesser extent, ω-methylpantoyl lactone, restore intracellular content of pantothenate. This restoration is considerably less effective with D-serine as a division-inhibiting agent than with the others. Other lactones, L-α-alanine, and ammonium chloride are ineffective, or only slightly effective, in restoring pantothenate synthesis. Effects of division-inhibiting compounds and reversing agents upon cellular coenzyme A activity in general parallel their effects on pantothenate synthesis. There is no direct correlation between ability of a compound to reverse cell-division inhibition and ability to restore synthesis of either pantothenic acid or coenzyme A.

Evidence is presented that D-serine interferes with the utilization of aspartic acid and also blocks synthesis of pantoic acid.

Compounds (including glucose) which tend to produce long cells result in the accumulation of pyruvic acid in the growth medium. Pantoic acid reduces, and pantoyl lactone abolishes completely, this accumulation of pyruvate. Other reversing agents do not abolish the pyruvate accumulation.

Several observations have been made (Grula and Grula, 1962), using a species of *Erwinia*, which indicate that certain D-amino acids, particularly D-serine, capable of inhibiting cell division may decrease pantothenate synthesis by this organism. At least two reactions in the pathway of synthesis of pantothenate have been reported to be blocked by D-serine. They are: the condensation of β-alanine and pantoic acid (Maas and Davis, 1950), and the transformylation step converting α-ketoisovaleric acid to ketopantoic acid (Altenbern and Ginoza, 1954). With *Erwinia* sp., pantothenate partially overcomes growth inhibition by D-serine (Grula and Grula, 1962), and intermediates in the pathway of pantothenate synthesis may reverse either growth inhibition, cell-division inhibition, or both. For these reasons, it was necessary to determine: whether D-serine inhibited pantothenate synthesis by *Erwinia*; location of the metabolic block(s); whether there was a correlation between inhibition of pantothenate synthesis and cell elongation; and whether a relationship existed between reversing ability and ability to stimulate pantothenate synthesis in the presence of D-serine or other division-inhibiting agents.

MATERIALS AND METHODS

In general, methods for handling *Erwinia* cultures, determination of cell size and optical density, and composition of the basal defined medium were the same as reported previously (Grula, 1960*b*; Grula and Grula, 1962).

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Cultures were pooled at the end of the growth period (10 or 16 hr), and samples were taken for cell size and optical density determinations. Use of tubes as culture vessels was continued to maintain the same surface-volume ratio as in previous work. Pooled cultures were centrifuged at 4 C, and cell sediments were mixed thoroughly in 1.5 ml of distilled water. These cells were then frozen (−20 C) and thawed (25 C) slowly four times before being assayed for pantothenate. This procedure for rupturing cells gave reasonably consistent pantothenate values on different cell preparations grown under the same conditions. Supernatant fluids to be assayed were stored frozen until used.

Pantothenate assays were carried out according to the procedures, based on the method of Skeggs and Wright (1944), outlined by the Association of Vitamin Chemists, Inc. (1951). A frozen-and-thawed cell suspension (1 ml) was subjected to digestion by both alkaline phosphatase and chicken-liver acetone powder (Worthington Biochemical Corp., Freehold, N. J.). Preliminary tests indicated that 98% or more of intracellular pantothenate was bound and released by the action of the two enzymes. Difco pantothenate assay medium and *Lactobacillus arabinosus* (*L. plantarum*) 17-5 (ATCC 8014) were used for microbiological assay. Assay cells were grown for 18 to 20 hr at 37 C (water bath) and optical density measured at 540 mμ.

In all cases where a substance possibly inhibitory for *L. arabinosus* was present (e.g., penicillin, D-serine, ω-methylpantoyl lactone), controls containing an intermediate level of pantothenate and a concentration of potential inhibitor equal to or greater than that in the test media were set up. Rarely was growth of *L. arabinosus* detectably decreased at inhibitor concentrations equal to those in the test media. Controls to test for nonspecific stimulation were also employed.

In computing intracellular pantothenate content, correction factors for amount of growth and number of tubes were applied such that all values are related to a single arbitrary standard, and can thus be directly compared with one another.

Cells for coenzyme A assays were prepared in the same manner as for pantothenate assays. Cultures were always grown for 16 hr. Considerably greater quantities of cells were necessary, since the assay for coenzyme A was relatively less sensitive than the pantothenate assay. The method of Kaplan and Lipmann (1948) for the assay of coenzyme A was used. The standard was a commercial preparation containing 300 units of coenzyme A per mg. Since it was differences in coenzyme A content rather than absolute values we were interested in, results are expressed in terms of comparison with an arbitrary standard.

α-Keto acids were estimated by conversion to their 2,4-dinitrophenylhydrazone derivatives, using the technique of Strassman, Shatton, and Weinhouse (1960). Cultures were grown under division-inhibited or division-inhibition reversed conditions for 16 hr, after which the cells were centrifuged in the cold and the supernatants analyzed for α-keto acids. Hydrazones of known α-keto acids were prepared, and their R_F values were determined in two solvent systems: isopropyl alcohol–water–ammonium hydroxide (100:10:5, v/v), and *n*-butanol–water–ethyl alcohol (50:40:10, v/v).

RESULTS AND DISCUSSION

Preliminary tests of pantothenate synthesis at a culture age of 10 hr (when, in the presence of D-amino acids, degree of elongation is small) showed that D- and DL-serine decreased both intracellular (by 75–80%) and excreted (by 97%) pantothenate. Of several reversing agents tested, only pantoyl lactone restored pantothenate synthesis to a significant degree (about 35%). DL-Alanine, L-alanine, NH_4Cl, ω-methylpantoyl lactone, and ribonolactone increased pantothenate synthesis little or none under these conditions.

At 16 hr, with D-amino acids, filaments of *Erwinia* have reached their maximal length. Tests of the effects of D-serine, D-phenylalanine, D-histidine, and penicillin on pantothenate synthesis at 16 hr revealed that pantothenate remains low (about 70% below control) in the case of D-serine. D-Phenylalanine depresses pantothenate synthesis much less than does D-serine, although it is more effective as an elongating agent. With the D-amino acids, excreted as well as intracellular pantothenate was reduced; however, with penicillin, the total pantothenate synthesis was not significantly reduced. Lowered intracellular pantothenate in the presence of penicillin is apparently the result of leakage of pantothenate from the long cells. Data illustrating these points are given in Table 1. The effects of pantoyl lactone and

TABLE 1. *Effect of division-inhibiting agents and division-inhibition reversing agents on pantothenate synthesis by Erwinia sp.*

Additions to basal medium* and M concn	Cell size		Pantothenate per ml supernatant	Pantothenate per 5 ml culture per unit mass	Change in intracellular pantothenate	Restoration of intracellular pantothenate
	μ		μg	μg	%	%
None		3–10	0.048	0.77	—	—
D-Serine, 0.021	40%	5–50				
	60%	50–300	0.027	0.22	−71	—
D-Serine + pantoyl lactone, 0.072		2–3	0.05	0.35	−55	24
D-Serine + ω-methylpantoyl lactone, 0.009		2–3	0.013	0.09	−88	None
D-Phenylalanine, 0.0084	30%	5–50;				
	70%	50–300	0.040	0.55	−29	—
D-Phenylalanine + pantoyl lactone, 0.072		3–4	0.045	0.93	+20	120
D-Phenylalanine + ω-methylpantoyl lactone, 0.009		2–3	0.030	0.76	None	100
D-Histidine, 0.017	40%	5–50;				
	60%	50–100	0.035	0.29	−62	—
D-Histidine + pantoyl lactone, 0.072		2–3	0.044	0.73	−5	92
D-Histidine + ω-methylpantoyl lactone, 0.009		2–3	0.038	0.49	−37	42
Penicillin†, 50 units.ml	50%	5–50;				
	50%	50–100	0.052	0.47	−40	—
Penicillin + pantoyl lactone, 0.072	80%	3–5;				
	20%	5–10	0.055	1.01	+31	131
Penicillin + ω-methylpantoyl lactone, 0.009	80%	3–5;				
	20%	5–10	0.038	0.69	−10	73

* All tubes contained basal medium (Grula and Grula, 1962). The M concentration of glucose was 0.017.

† Sodium penicillin G (California Corporation for Biochemical Research).

ω-methylpantoyl lactone on pantothenate synthesis in the presence of these three amino acids and penicillin are also given in Table 1. In every case except that of D-serine, restoration of pantothenate synthesis by pantoyl lactone is close to, or greater than, 100%. Surprisingly, ω-methylpantoyl lactone produced a significant restoration in every case except with D-serine. Other lactones, NH_4Cl, or L-alanine did not restore pantothenate synthesis appreciably, although the cells were very short in their presence. It is clear that a decreased pantothenate synthesis does not necessarily mean that filaments will be formed. From this, and from the lack of correlation of elongating efficiency with ability to inhibit pantothenate synthesis (Table 1), we have concluded that there is no direct relationship between ability to inhibit pantothenate synthesis and ability to inhibit cell division.

In general, the relative coenzyme A activities of cells grown with the various division-inhibiting agents and reversing agents parallel the pantothenate content of cells grown under the same conditions. Data on coenzyme A activities of cells from a number of media are given in Table 2. Most of the observations regarding coenzyme A content and cell size may be interpreted in the same way that analogous observations with pantothenate were interpreted. The essential point illustrated by the data in Table 2 is that cells may be of "normal" size, i.e., the rate of division relative to the rate of growth may be normal, and yet the coenzyme A content of these cells remains low, far below that of normal cells. A lowered coenzyme A content does not necessarily result in long cells. On the other hand, every agent tested that produced long cells did result in a lowered coenzyme A activity; but as with pantothenate, this relationship is probably fortuitous. In the case of penicillin, this is because pantothenate "leaks" out of the long cells, rather than because of an actual inhibition of synthesis.

The pathway of synthesis of pantothenate involves pyruvic acid. Pyruvate condenses with an enzyme-acetaldehyde complex (Radhakrish-

TABLE 2. *Coenzyme A activity of cells of Erwinia sp. grown in different media*

Additions to basal medium and M concn reversing agent	OD of pooled cultures	Cell size	CoA activity, units per unit cell mass	Reduction in CoA activity	Restoration of CoA activity
		μ		%	%
Basal only	0.55	2–3	3.4	—	—
Glucose*	1.10	3–5	2.2	35 (Below basal)	—
Glucose + D-serine	0.52	10–150	0.4	84†	—
Glucose, D-serine + L-alanine, 0.034	0.50	2–3	0.3	86	None
Glucose, D-serine + NH_4Cl, 0.074	1.10	2–4	0.3	86	None
Glucose, D-serine + pantoyl lactone, 0.07	0.10	1–2	1.2	45	44
Glucose, D-serine + ω-methylpantoyl lactone, 0.009	0.49	3–4	0.85	61	25
Glucose, D-serine + ribonolactone, 0.048	0.38	2–3	0.4	82	None
Glucose + D-histidine	0.17	20–50	1.1	50	—
Glucose + penicillin	0.90	20–80	0.9	59	—
Glucose, penicillin + pantoyl lactone, 0.07	0.16	2–3	2.0	9	89
Glucose, penicillin + ω-methylpantoyl lactone, 0.009	0.67	5–30	1.2	45	44

* Concentration as given in Table 1.

† This value and all below it in this column are based on the medium with glucose only. All agents inhibiting cell division are at the same concentration used in pantothenate experiments, viz., D-serine 0.021 M, D-histidine, 0.017 M, and penicillin, 50 units per ml.

nan and Snell, 1960; Singer and Pensky, 1954*a*,*b*) to form acetolactic acid, which then undergoes a rearrangement, reduction, and dehydration to form α-ketoisovaleric acid, a known precursor of pantothenate (Maas and Vogel, 1953; Purko, Nelson, and Wood, 1954). [Evidence has been presented that the rearrangement and reduction may be a single reaction, catalyzed by a single enzyme, viz., α-hydroxy-β-keto acid reductoisomerase (Radhakrishnan, Wagner, and Snell, 1960).] If agents producing long cells block a given reaction in the pathway of synthesis of pantothenate, there are several possibilities whereby an α-keto acid should accumulate in the presence of the agent. Compounds reversing division inhibition which also restore pantothenate synthesis should prevent the accumulation of the α-keto acid. Also, if pantothenate, and therefore coenzyme A, syntheses are partially blocked, there will be a shortage of coenzyme A for metabolism of pyruvic acid. In this case, the α-keto acid accumulating in largest amount should be pyruvic acid.

Experiments were done to test for the possible accumulation of specific α-keto acids (pyruvic, α-ketoisovaleric, and α-ketopantoic) in the presence of division-inhibited and division-inhibited reversing systems. No accumulation of either α-ketoisovaleric or α-ketopantoic acid could be demonstrated, either intra- or extracellularly. The only α-keto acid that accumulates appears to be pyruvic acid, and the largest amount, by far, is excreted into the medium. (Identification of the excreted product as pyruvic acid is based on cochromatography, using the two solvent systems given in Materials and Methods, of the hydrazone derivative of pyruvate with the hydrazone derivative made using the growth medium or cells after 16 hr of growth.)

Cells grown in the presence of aspartic acid (carbon and nitrogen source) alone, or with aspartic acid plus mannose, do not accumulate pyruvic acid. When glucose is used in place of mannose, a definite accumulation of pyruvic acid occurs. If D-serine is added to the aspartic acid-glucose system, a further increase in pyruvate accumulation can be demonstrated. Because D-serine causes a decrease in synthesis of pantothenic acid and coenzyme A, these results were interpreted as indicative of a decreased cellular coenzyme A content.

Some agents (pantoyl lactone or pantoic acid) which reverse cell elongation by D-serine and which partially restore pantothenate and coenzyme A levels also decrease pyruvate accumulation (Table 3). Other agents which allow cell

TABLE 3 *Accumulation of pyruvic acid in the supernatant fluids of cells grown in the presence of various reversing agents*

Reversing agent and M concn	OD	Cell size 16 hr	Pyruvic acid per ml supernatant	Total pyruvic acid per ml supernatant, corrected to OD of basal medium	Increase or decrease in pyruvic acid accumulation†
		μ	μg	μg	%
Basal only*	0.47	90% 10–80 10% >100	195	195	—
DL-α-Alanine, 0.0336	0.15	80% 3–5 20% 5–8	112	350	+80
Pantoic acid, 0.066	0.76	3–6	137	84	−57
Pantoyl lactone, 0.088	0.14	2–3	Not measurable	—	Not measurable (−100%)
Ribonolactone, 0.072	0.41	2–3	282	321	+65

* Basal medium contained salts, aspartic acid, glucose, and D-serine (Grula and Grula, 1962).
† Basal medium served as the control in these calculations.

division in the presence of D-serine, but do not restore pantothenate synthesis, actually cause an increase in the accumulation of pyruvic acid.

Differences between D-serine and other division-inhibiting D-amino acids. The ability of pantoyl lactone and pantoic acid to reverse division inhibition by D-amino acids suggested that the D-amino acids were blocking the synthesis of pantoic acid or its lactone. Evidence for the ability of D-serine to inhibit the synthesis of pantoic acid through interference with the L-serine stimulation of the hydroxymethylation of α-ketoisovaleric acid has been reported (Altenbern and Ginoza, 1954). However, in our system, the mode of action of D-serine could be different, since it is not antagonized by L-serine (Grula, 1960*b*), and since L-serine itself inhibits pantothenate synthesis. This inhibition has been observed by us and is also evident in the data reported by Altenbern and Ginoza (1954), who used *Brucella abortus*.

With D-phenylalanine and D-histidine, pantoyl lactone completely restored pantothenate synthesis. However, with D-serine, a concentration of pantoyl lactone that completely reversed cell-division inhibition restored pantothenate synthesis by only about 25%. This could mean that D-serine was blocking the condensation of pantoic acid and β-alanine, as reported for *Escherichia coli* by Maas and Davis (1950), as well as blocking the synthesis of pantoic acid or its lactone. To test this possibility, β-alanine and pantoic acid were added, with D-serine, to the basal medium. Cells from this medium showed a tenfold increase in pantothenate. Two conclusions seemed warranted from this result: β-alanine is a major limiting factor in pantothenate synthesis by *Erwinia* in the presence of D-serine under our conditions; and D-serine at a concentration sufficient to decrease growth at 16 hr by 75 to 80% inhibits the condensation reaction only slightly, if at all. If these were both true, it would indicate that D-serine was interfering with the synthesis, rather than with the utilization, of β-alanine.

TABLE 4. *Effect of D-serine on the condensation of pantoic acid and β-alanine*

Medium	D-Serine	Pantothenate per ml digest	Decrease
	M	μg	%
Basal*		0.12	—
Basal, pantoic acid, β-alanine†	None	2.19	—
Basal, pantoic acid, β-alanine	0.0084	1.91	13
Basal, pantoic acid, β-alanine	0.017	1.83	16
Basal, pantoic acid, β-alanine	0.025	1.84	16
Basal, pantoic acid, β-alanine (no cells)		Not detectable	

* Basal growth medium plus 0.017 M glucose.
† Pantoic acid, 0.046 M; β-alanine, 0.01 M.

To test the extent of inhibition of the condensation reaction by D-serine, an experiment was set up as follows. Cells were grown in the basal medium plus glucose, under the same conditions as usual. After 16 hr of growth, cells were centrifuged and resuspended in one-tenth the total culture volume of distilled water. Portions of this

cell suspension were added to tubes containing the basal medium, glucose, pantoic acid, β-alanine, and graded amounts of D-serine. These cultures were then placed on a rotary shaker at 25 C for 40 min, after which they were immediately placed in the freezer. Tubes were frozen and thawed a total of four times to release intracellular pantothenate; 1-ml samples (cells plus medium) were then subjected to digestion by alkaline phosphatase and the liver enzyme, and analyzed as usual for pantothenate.

The degree of reduction of pantothenate synthesis is not great, and it does not increase significantly upon increasing the D-serine concentration from 0.0084 to 0.025 M (Table 4). The lack of significant inhibition of the condensation reaction by increasing concentrations of D-serine is not in accord with the conclusions reached by Maas and Davis (1950). However, if D-serine interfered with the synthesis of β-alanine, then β-alanine should be a limiting factor in pantothenate synthesis by *Erwinia* in the presence of D-serine. An experiment to test this showed that β-alanine (0.01 M) added alone to the basal medium plus D-serine (0.021 M) increased the pantothenate yield per unit weight by 180%, even though growth was decreased by β-alanine below that obtained with D-serine alone. This is a much greater increase, both relative and absolute, than occurred by adding either pantoyl lactone or pantoic acid to the D-serine-containing medium. It shows that β-alanine is limiting pantothenate synthesis in the presence of D-serine, and indicates that D-serine inhibits the synthesis of β-alanine. This would be a second block to pantothenate synthesis produced by D-serine; not, however, the one responsible for division inhibition. We have interpreted these results to mean that D-serine inhibits the utilization of aspartic acid, including the formation of β-alanine. The formation of β-alanine by decarboxylation of aspartic acid has been documented in the literature, by the use of inhibition analysis (Ravel and Shive, 1946; Shive and Macow, 1946), and by the use of cell suspensions of *Rhizobium trifolii* (Billen and Lichstein, 1949). Inhibition of the utilization of aspartic acid would, to a large extent, account for the inhibition of growth by D-serine.

Further inhibition of growth by added β-alanine in the presence of D-serine is assumed to occur because β-alanine is not removed via condensation, owing to a shortage of pantoic acid, and β-alanine also inhibits the utilization of aspartic acid. β-Alanine should not exhibit toxicity in the absence of D-serine. Preliminary nutritional data have been in accord with this hypothesis.

Also, inhibition of β-alanine synthesis by D-serine would account for the fact that added pantoic acid or its lactone produced only a limited (about 25%) restoration of pantothenate synthesis in the presence of D-serine. Other cell-elongating agents do not inhibit β-alanine synthesis, or do so to a much lower degree; hence, pantoyl lactone would be expected to produce a much greater restoration with them.

Further, β-alanine added to the medium in the

TABLE 5. *Pantothenate synthesis by Erwinia sp. in the presence of* D-*serine and pantothenate precursor*

Additions to basal medium*	OD of pooled cultures	Cell size, μ per cent in each range			Intracellular pantothenate per 5 ml per unit wt	Pantothenate per 5 ml supernatant†
		3-20	20-50	50-300		
					μg	μg
None	0.31	35	30	35	0.37	0.11
β-Alanine	0.14	50	50		1.40	1.10
Pantoic acid	0.42	80	20		0.66	0.14
α-Ketoisovaleric acid	0.56	40	30	30	0.47	0.14
Ketopantoic acid	0.18	30	40	30	0.57	0.13
β-Alanine + pantoic acid	0.32	40	30	30	7.26	20.0
β-Alanine + α-ketoisovaleric acid	0.50	20	15	65	2.53	14.4
β-Alanine + ketopantoic acid	0.18	30	30	40	5.53	15.5

* All tubes contained glucose, 0.017 M; β-alanine, 0.01 M; all other precursors, 0.012 M; D-serine, 0.12 M.

† Not corrected for amount of growth (OD).

presence of D-serine exerted an elongating effect on the cells in nearly all cases. This is interpreted to mean that added β-alanine removes a large proportion of available pantoic acid (or pantoyl lactone) through condensation, thus leaving less available for triggering cell division.

Evidence for D-serine inhibition of pantoic acid synthesis. Nutritional studies indicated that the specific reaction blocked by D-serine is the reduction of α-ketopantoic acid to pantoic acid. To obtain direct evidence, we tested pantothenate synthesis with each of the three precursors of pantothenate (α-ketoisovaleric acid, α-ketopantoic acid, and pantoic acid) in the presence of D-serine, with and without added β-alanine. Synthesis of pantothenate was greatest in the presence of β-alanine and pantoic acid; with ketopantoic acid, it was 76% as great, and with α-ketoisovaleric acid it was only 35% as great (Table 5). If inhibition of specific pantothenate synthesis is a valid criterion of a block, then both reactions are blocked to a certain extent. The hydroxymethylation of α-ketoisovaleric acid is inhibited to a much greater extent than the reduction of ketopantoic acid. The partial inhibition of the reduction of ketopantoic acid to pantoic acid may be related to the fact that good aeration, as obtained on the rotary shaker, enhances long-cell formation (Grula, 1960*a*). Without adequate aeration, the reduction would be less hindered, even in the presence of D-serine, thus allowing the formation of greater amounts of pantoic acid (or pantoyl lactone), enough to stimulate a more normal rate of cell division.

Other observations regarding pantothenate synthesis and cell size. D-Serine produces essentially the same effects on cell size and pantothenate yield with pyruvate as it does with glucose. This is in accord with the proposed means by which D-serine inhibits cell division and, in part, pantothenate synthesis, i.e., by blocking the conversion of α-ketoisovaleric acid to pantoic acid. This inhibition would occur whether glucose or pyruvate was added as the carbon-energy source.

Certain metallic ions are effective reversers of D-amino acid-induced inhibition of division (Grula and Grula, 1962). Therefore, the effect of zinc ion (the most effective metallic reversing agent) and calcium ion on pantothenate synthesis in the presence of D-serine were studied. Zinc ion produced an increased inhibition of pantothenate synthesis; total pantothenate was 90% or more below the control. Calcium produced a 40% restoration.

The strong inhibition of pantothenate synthesis by zinc could mean that a specific reaction in the pathway of synthesis was blocked by zinc. If zinc blocked β-alanine synthesis or utilization, excess pantoic acid could be diverted to the cell-division pathway instead of forming pantothenate; this would account for short-cell formation in the presence of zinc. However, added β-alanine did not exert an elongating effect in the presence of zinc; this indicates that zinc does not block the synthesis of β-alanine. On the other hand, α-ketoisovalerate added to the zinc-D-serine system resulted in significantly increased growth and a marked elongation of the cells. This suggests that zinc exerts its effects on cell size and pantothenate synthesis by blocking the synthesis of α-ketoisovaleric acid. Other situations have been observed (Grula and Grula, *unpublished data*) in which added α-ketoisovaleric acid in the presence of D-serine results in very pronounced inhibition of division.

DISCUSSION

It is clear that D-serine produces at least two metabolic lesions in *Erwinia* sp. One block is the hydroxymethylation of α-ketoisovaleric acid to α-ketopantoic acid; the other involves the synthesis of β-alanine. Both blocks contribute to inhibition of growth and inhibition of pantothenate synthesis. Two considerations lead us to believe that the block resulting in a diminished content of pantoic acid is responsible for inhibition of division: (i) pantoic acid or pantoyl lactone reverses cellular elongation by D-amino acids under every situation tested; (ii) systems in which division is inhibited without added β-alanine show a decreased pantothenate content, which is reversed to varying degrees by pantoic acid or pantoyl lactone. D-Phenylalanine and D-histidine inhibit synthesis of pantoic acid and pantoyl lactone, but they do not appear to inhibit synthesis of β-alanine.

Pantoic acid and pantoyl lactone appear to have two distinct roles in the metabolism of *Erwinia* sp., one as a precursor of pantothenate, and the other a role, as yet undefined, in cell division. The relatively high concentration of

either pantoic acid or pantoyl lactone required for good reversal of division inhibition would suggest a substrate rather than a catalytic role in cell division. With pantoic acid, roughly a threefold higher concentration is required for optimal reversal of growth inhibition and division inhibition caused by D-serine than for optimal restoration of pantothenate synthesis in the presence of β-alanine; this is in accord with the postulated dual role for pantoic acid.

It seems reasonably certain that a block occurs in the transformylation step converting α-keto-isovaleric acid to ketopantoic acid. In no instance has it been possible to obtain direct evidence for the accumulation of α-ketoisovaleric acid. Probably the resultant coenzyme A deficiency prevents its formation.

A puzzling matter concerning α-ketoisovaleric acid relates to its ability to enhance cell-division inhibition in the presence of penicillin, zinc ion, and other systems to be reported elsewhere. Such results could indicate that α-ketoisovaleric acid does not accumulate because it is incorporated into some area of the cell where it might directly act to inhibit cell division.

D-Serine does not appear to inhibit cell division by being incorporated into cellular proteins in the place of L-serine. L-Serine is unable to reverse cellular elongation by D-serine, even at a concentration of from three to six times higher (Grula, 1960*b*). We believe that D-serine (as well as other division-inhibiting D-amino acids) functions as an inhibitor of certain specific enzyme reactions, rather than by replacing the L-amino acids in cellular proteins.

The elongating effect of β-alanine that occurs under most conditions has been explained by assuming that it effectively removes pantoic acid, which would have been available for cell division, via condensation to pantothenate. This would imply a very rapid and efficient conversion of pantoate to pantothenate, a relatively more efficient enzyme system than that utilizing pantoate for cell division. Using *B. abortus*, Ginoza and Altenbern (1955) found that, under the conditions they used, the enzyme functioned inefficiently. The means by which β-alanine exerts its elongating effect has an important bearing on the mechanism of inhibition of cell division by D-serine, and in all probability by other D-amino acids as well.

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