Further tests of inhibition of β-alanine synthesis by D-serine were done by incubation of washed 16-h cells in a Dubnoff shaker at 30°. Analysis for β-alanine was accomplished using cell supernatants and a boiled extract of cells (10 min using 3 ml water per 20 mg cells). Under these conditions, D-serine strongly decreases the level of intracellular β-alanine (Table II).

Although most β-alanine was found to be intracellular, the same pattern of inhibition by D-serine was observed when testing for extracellular β-alanine. Surprisingly, when the pH of the incubation medium was varied, 100 % more β-alanine was excreted at pH 6.0 as compared to pH 7.0 (0.26 *vs.* 0.13 μg β-alanine per ml supernatant per mg cells) in control flasks (no D-amino acid present). Thus it could be postulated that cellular retention of β-alanine and subsequent synthesis of pantothenic acid is, in part, pH dependent. This has been confirmed using 16-h growing cultures of Erwinia sp. Cells from a medium wherein pH at harvest time was 5.6 contained 0.66 μg pantothenic acid per unit cell mass whereas cells from a medium wherein pH at harvest was 6.9 contained 1.4 μg pantothenic acid per unit cell mass.

In conclusion, our data provide direct evidence for inhibition of synthesis of β-alanine from aspartic acid by D-serine in this species of Erwinia. Further, we have demonstrated that excretion of β-alanine and subsequent synthesis of pantothenic acid is, in part, pH dependent.

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Alterations of rat-tissue cytochrome c levels by a chronic cold exposure

Recent studies have indicated that one of the factors responsible for the improved thermogenic capacity of the cold-acclimatized animals is an alteration in the pattern and magnitude of electron transport. Thus, within the electron-transport system an increased activity or concentration of succinate dehydrogenase (EC 1.3.99.1) and

malate dehydrogenases (EC 1.1.1.37)[1], coenzyme Q (ref. 2), cytochrome oxidase (EC 1.9.3.1)[1], and TPNH (ref. 3) have been observed in various tissue preparations from cold-exposed rats. In addition, the liver of cold-acclimated hamsters was reported to have an increased DPNH– and TPNH–cytochrome *c* reductase activities[4]. In contrast, alterations in the activities of these two latter enzymes, as well as of lactate dehydrogenase (EC 1.1.1.27) and TPNH–DPN^+ oxidoreductase (EC 1.6.1.1), and in the levels of DPN^+, DPNH and TPN^+, were not found in liver tissues from

TABLE I

CONCENTRATION OF CYTOCHROME *c* IN TISSUES OF COLD-ACCLIMATED RATS

Results are given as mean ± standard error of mean (5 rats)

Tissues	*Weeks of exposure*	*Cytochrome c (μg/g tissue)*	
		25°	*5°*
Heart	0	221 ± 10	221 ± 10
	1	226 ± 15	242 ± 16
	2	223 ± 14	275 ± 21*
	3	238 ± 19	319 ± 20*
	4	215 ± 17	309 ± 16*
	20	237 ± 26	298 ± 20*
Kidney	0	211 ± 13	211 ± 13
	1	210 ± 23	229 ± 24
	2	209 ± 21	259 ± 18*
	3	225 ± 16	270 ± 20*
	4	218 ± 20	282 ± 14*
	20	230 ± 18	275 ± 23*
Liver	0	32 ± 4	32 ± 4
	1	35 ± 5	45 ± 7
	2	39 ± 4	49 ± 5
	3	29 ± 6	77 ± 9*
	4	38 ± 4	88 ± 8*
	20	24 ± 7	80 ± 5*
Lung	0	35 ± 7	35 ± 7
	1	32 ± 6	33 ± 7
	2	38 ± 8	41 ± 5
	3	40 ± 9	49 ± 6
	4	34 ± 2	55 ± 7*
	20	32 ± 4	58 ± 5*
Skeletal muscle	0	36 ± 4	36 ± 4
	1	30 ± 6	37 ± 3
	2	32 ± 5	45 ± 8
	3	38 ± 2	69 ± 10*
	4	32 ± 6	77 ± 9*
	20	39 ± 7	80 ± 9*
Spleen	0	42 ± 9	42 ± 9
	1	43 ± 5	48 ± 7
	2	45 ± 7	52 ± 9
	3	48 ± 3	50 ± 9
	4	42 ± 6	68 ± 5*
	20	45 ± 9	66 ± 8*

* Difference from warm group ($P < 0.05$).

cold-acclimatized rats[1,2,5]. In addition to these enzyme and cofactor alterations an increased activity of various oxidases and a reduced efficiency of oxidative phosphorylation has been observed[5-8]. The present report is concerned with the effect of a chronic cold exposure on an additional component of the electron-transport system, namely cytochrome *c*.

Male, Sprague–Dawley rats, ranging in weight from 200–250 g and maintained in individual wire cages, were exposed to temperatures of 5° or 25° for varying periods of time. Commercial laboratory diet (Purina Checkers) and water were available at all times. At the end of each exposure period the animals were sacrificed by decapitation, the tissues were immediately excised and frozen until analyzed. Extraction of cytochrome *c* from the tissues and its spectrophotometric determination were conducted according to the procedure of PRADER AND GONELLA[9]. A commercial preparation of cytochrome *c* (Sigma Chemical Company, St. Louis, U.S.A.) was employed as a standard.

The data (Table I) indicate that a chronic cold exposure resulted in increased cytochrome *c* levels in all tissues studied. The cytochrome *c* content in the heart and the kidney was markedly elevated by the end of the second week of the experimental period, while the other tissues required at least a 3-week cold exposure to show a significant change. Thereafter, the levels remained increased throughout the whole 5-month experimental period.

These changes would seem to reflect adaptive phenomena associated with the sustained increase in the metabolic rate and sustained high activity over the electron-transport system of chronically cold-exposed animals. It should be pointed out that the thyroid hormone is one of the factors controlling the tissue concentration of this electron carrier, since it has been demonstrated that the tissues of hyperthyroid rats contain increased concentrations of cytochrome *c*, while the tissues of the hypothyroid or thyroidectomized rats contain less than one-half the amount found in normal animals[10]. These findings are concordant with the fact that cold-adapted rats show thyroid-gland hyperplasia[11], and synthesize more thyroid hormones as measured by incorporation of radioactive iodine into circulating hormones[12], and by their increased O_2 consumption[13].

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