TABLE I

DECOMPOSITION TEMPERATURES (RELATIVE THERMAL STABILITY) OF CHROMIUM TRIALKYLENEDIAMINE SALTS

Salt	Triethylene, temp., °C.	Tripropylene, temp., °C.
Thiocyanate	130	110
Nitrite	135	...
Nitrate	140	...
Cyanate	150	...
Chloride	160	175
Iodide	200	...
Sulfate	210	...
Bromide	210	195
Cyanide	230	...
Oxalate	280	...

time of heating so that no definite product could be identified. The nitrite and nitrate at slightly higher temperatures, namely, 135 and 140°, respectively, decomposed completely into chromium oxide.

Tripropylenediamine bromide and iodide also decomposed into a dark brown substance indistinguishable from the corresponding product formed by the triethylenediamine salts. The chloride, however, was converted completely after five hours at 175° to the purple *cis*-dichloropropylenediamine chromic chloride. Prolonged heating or the use of higher temperatures should be avoided because these conditions cause a pronounced darkening of the purple salt. After darkening, the salt turns black and then chars. Recrystallization of the purple salt is difficult because of its high solubility.

Anal. Calcd. for $[Cr\ pn_2Cl_2]Cl$: Cr, 16.94. Found: Cr, 16.75.

Similarly the thiocyanate gave a red-orange product at 110°. The product was difficultly soluble in cold water and quite soluble in hot, so was easily crystallized.

Anal. Calcd. for $[Cr\ pn_2(NCS)_2](NCS)$: Cr, 12.88. Found: Cr, 12.98.

The stabilities of the tripropylenediamine salts are in the same order as, and in general appear to be slightly less than, the corresponding triethylenediamine salts.

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The Biosynthesis of Pantothenic Acid[1]

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The biosynthesis of pantothenic acid has been thought to involve a coupling of β-alanine with α-hydroxy-β,β-dimethyl-γ-butyrolactone (pantoyl lactone).[2,3] Organisms requiring pantothenic acid for growth may not be able to synthesize it for one of several reasons. Some, such as lactic acid bacteria, are unable to carry out the coupling. They fail to grow on a mixture of β-alanine and pantoyl lactone, but require the intact pantothenic acid molecule.[4] In the pantothenicless mutant of *Neurospora* (5531), which has this same requirement, the coupling is under genetic control.[5] Other organisms like certain yeasts[6] and a diphtheria bacillus,[2] will grow if pantothenic acid or β-alanine is supplied but cannot use the pantoyl moiety alone. Apparently the pantoyl moiety is manufactured in these organisms and coupled with the added β-alanine to form pantothenic acid. Only the synthesis of β-alanine has been impaired. In still other organisms like *Acetobacter*,[7] a strain of *Proteus morganii*[8] and a hemolytic streptococcus,[2] the synthesis of β-alanine presumably is carried out, but the pantoyl moiety is not made. Again the capacity for coupling has not been lost since pantoyl lactone is able, even if incompletely, to replace pantothenic acid as a growth factor in the medium.

In the course of studies on the nutrition of *Clostridium septicum* we have observed that pantoyl lactone will completely replace pantothenic acid. For these experiments a single cell isolate (strain 59 Li A) was used. The clostridia were grown on a chemically defined medium which is a modification of that proposed by Bernheimer.[9] Growth was measured turbidometrically, the extinction being proportional to the milligrams of bacterial nitrogen as determined by a micro Kjeldahl on washed cultures. At 37° growth was complete in about twelve hours.

The presence of adequate amounts of calcium *d*-pantothenate, sodium *dl*-pantoate or *dl*-pantoyl lactone in the medium resulted in a maximum yield of 16–17 mg. of bacterial nitrogen per 100 cc. (Table I). Each of these substances also supported the maximum rate of growth on this medium. During the logarithmic growth phase one cell generation was produced every sixty to sixty-five minutes. However, on a molar basis calcium *d*-pantothenate is the more active. The molar concentration required to yield 8 mg. of bacterial nitrogen per 100 cc. was 0.2×10^{-6}, while *ca.* 4×10^{-6} *M* *d*-pantoyl lactone or sodium *d*-pantoate was required for the same crop (Fig. 1). When the pantoate concentration is calculated in terms of undissociated *d*-pantoic acid (pK_a = 4.0), its activity per mole is still less than that of undissociated *d*-pantothenic acid (pK_a = 4.4) but

(1) This work was supported in part by a grant from the Josiah Macy, Jr., Foundation.

(2) R. J. Williams, "Advances in Enzymol.," **3**, 253 (1943).

(3) H. R. Rosenberg, "Chemistry and Physiology of the Vitamins," Interscience Publishers, Inc., New York, N. Y., 1942, p. 263.

(4) V. H. Cheldelin, E. H. Hoag and H. P. Sarett, *J. Bact.*, **49**, 41 (1945).

(5) E. L. Tatum and G. W. Beadle, *Growth*, **6**, 27 (1942).

(6) v. N. Nielson and V. Hartelius, *Naturwissenschaften*, **45/46**, 550 (1943); H. P. Sarett and V. H. Cheldelin, *J. Bact.*, **49**, 31 (1945).

(7) L. A. Underkofler and A. C. Banty, *ibid.*, **45**, 183 (1943).

(8) G. Ivanovics, *Z. physiol. Chem.*, **276**, 33 (1942).

(9) A. W. Bernheimer, *J. Exp. Med.*, **80**, 321 (1944). Our medium differs essentially from that of Bernheimer in that the casamino acids were Norite treated, 0.2% of neutralized cysteine hydrochloride replaced the thioglycolic acid and the final medium was sterilized by autoclaving.

TABLE I

CHARACTERISTICS OF GROWTH IN THE PRESENCE OF PANTOTHENATE, PANTOYL LACTONE AND PANTOATE

	10 γ Calcium *d*-pantothenate per cc.	50 γ *dl*-Pantoyl lactone per cc.	50 γ Sodium *d*-pantoate per cc.
Fifty hr. bacterial crop, mg. bacterial nitrogen per 100 cc.	17	16	17
Av. generation time, min.	61	63	62
Av. time in min. required for the production of 2 mg. of bacterial nitrogen per 100 cc.	560[a]	...	580[a]
	810[b]	760[b]	...

[a] Experiment 1. [b] Experiment 2.

far greater than that of *d*-pantoyl lactone. Nevertheless, as Table I and Fig. 1 show, all three compounds permit the same rate of growth, show similar concentration curves, and afford the same yield of bacteria.

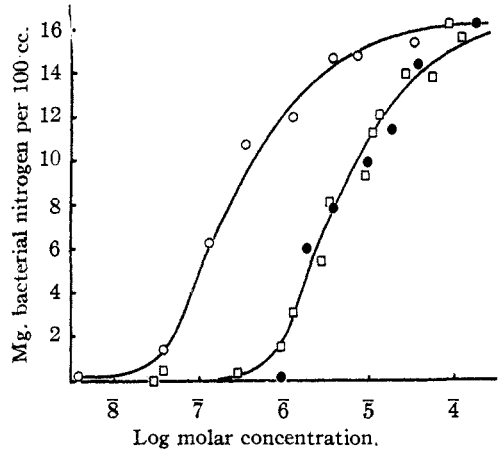

Fig. 1.—The effect of varying concentrations of calcium *d*-pantothenate, *d*-pantoyl lactone and sodium *d*-pantoate on the growth of *Clostridium septicum:* O, calcium *d*-pantothenate; □, sodium *d*-pantoate; ●, *d*-pantoyl lactone.

Growth on pantoyl lactone or pantoate may be due to one of three reasons. In the first place, the bacteria could adapt to grow somehow in the absence of pantothenic acid. Second, they could use the pantoyl moiety directly, or finally they might convert it into pantothenic acid.

In the absence of pantothenate and the pantoyl moiety no growth was obtained in seventy attempts. Concentrations of β-alanine ranging from 1 to 100 γ per cc. failed to support growth by themselves or to augment the bacterial crop in the presence of pantoate (Table II). Cultures grown in the presence of pantoate likewise failed to grow after transfer to media lacking pantothenate and the pantoyl moiety, although they grew when these were supplied. Consequently clostridia grown in the presence of pantoate or its lactone did not adapt to grow in the absence of pantothenate. Therefore, we attempted to determine whether pantothenate was produced during growth on pantoate medium.

TABLE II

THE INFLUENCE OF β-ALANINE

	Thirty-six hrs. bacterial crop, mg. bacterial nitrogen per 100 cc.	
γ,β-Alanine per cc.	O-Pantoate	0.35 γ Sodium *d*-pantoate per cc.
0	0	10
1.4	0	10
10.0	0	9
100.0	0	10

Washed cultures of *Clostridium septicum* were digested with mylase P,[10] and assayed for pantothenic acid by the use of *Lactobacillus casei.* (The latter requires the intact pantothenate molecule for growth,[4] a fact we have confirmed.) Such washed cultures supported the growth of *L. casei* which was measured turbidometrically and by titration of the lactic acid produced. The assay was not completely specific for with increasing quantities of digest larger yields of pantothenic acid were obtained. Nevertheless, the yield was independent of whether the digest was made of *Cl. septicum* cells grown upon pantoate or pantothenate. For example, in the lower range of the assay, 4.1 γ of pantothenate was obtained per 100 mg. of nitrogen of *Cl. septicum* grown on 50 γ of sodium *dl*-pantoate per cc. This approximately equals the 4.4 γ of pantothenic acid which was obtained from the same amount of *Cl. septicum* grown in the presence of 10 γ of calcium *d*-pantothenate per cc. and handled in the same way. Since pantothenic acid is synthesized when pantoate or its lactone is supplied in the medium and since there are some organisms which require the intact pantothenic acid molecule[4,5] an exclusive direct utilization of pantoate is inadequate as an explanation of growth. At the present time it is impossible to decide from the available data whether the biosynthetic coupling involves the lactone or the free acid form of the pantoyl moiety. However, analogy with the organic synthesis and consideration of the probable energetics, make the pantoyl lactone seem the more likely intermediate.

We conclude that *Clostridium septicum* is unable to synthesize the pantoyl moiety of the pantothenate molecule. However, it does synthesize β-alanine and is able to couple this with the pantoyl moiety secured from the medium to form pantothenic acid which is necessary for growth.

While this note was in press it was discovered that T. Wieland and E. F. Möller [*Z. physiol. Chem.*, **269**, 227 (1941)] had performed a complementary study on yeasts which were unable to synthesize β-alanine. They showed that resting as well as injured cells could synthesize pantothenic acid from β-alanine and pantoyl lactone.

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(10) A. L. Neal and F. M. Strong, *Ind. Eng. Chem., Anal. Ed.*, **15**, 654 (1943).