The Effect of Some Pantothenate Derivatives on Growth and Coenzyme-A Synthesis in Bacteria

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As shown previously (Pierpoint & Hughes, 1952, 1954) the synthesis of coenzyme A (CoA) by washed suspensions of *Lactobacillus arabinosus* is greatly stimulated by the addition of cystine. Because pantotheine replaces both pantothenate and cystine it seems probable that cystine gives rise to the β -mercaptoethylamine residue of pantotheine and hence of CoA. In order to study further the role of cystine in CoA synthesis a number of pantothenyl peptides related to pantotheine have been tested for their ability to replace pantothenate or pantothenate and cystine for the growth of some bacteria and for CoA synthesis in washed suspensions of *Lb. arabinosus*.

MATERIALS AND METHODS

Organisms. *Lactobacillus arabinosus* 17-5 (National Collection of Industrial Bacteria no. 8014), *Leuconostoc mesenteroides* P 60 (N.C.I.B. no. 6990) and *Proteus morganii* (National Collection of Type Cultures, nos. 2818, K 653 and a locally isolated strain, KI) were maintained by daily subculture on the casein hydrolysate-glucose medium of Nossal (1951). *Lactobacillus helveticus* Strain 80 was a culture obtained from Dr E. E. Snell; it was maintained on the above medium enriched by the addition of pantotheine (10^{-4} M).

Growth tests. The ability of the various pantothenate derivatives to replace pantothenate for the growth of *Lb. arabinosus* was tested on the pantothenate assay medium of Barton-Wright (1952). The medium (4 ml.) was placed in 6 in. $\times$ $\frac{1}{2}$ in. test tubes, covered with aluminium caps (Oxoid Ltd.). Growth was measured turbidimetrically in

an EEL nephelometer (Evans Electroselenium Ltd., Harlow, Essex) after incubation at 37° for 16-18 hr. In some experiments the lactic acid formed was also measured by titration with 0.02N-NaOH in a Beckman automatic titrator. The results obtained by the two methods agreed satisfactorily. The substances to be tested were either sterilized by filtration through a Knight & Fildes (1936) type Seitz filter and added to the medium aseptically, or autoclaved at 10 lb./in.² for 10 min. after addition to the medium. Growth tests with *Lb. helveticus* were carried out as above except that the test medium was that of Craig & Snell (1951), to which was added Tween 80 (Honeywell and Stein Ltd., 21 St James's Square, London, S.W. 1) in the place of Tween 40 and oleic acid. The medium for *P. morganii* was that of Barton-Wright (1952) for cystine assay, in which the salts A and the sodium acetate were replaced by 4.5 g. KH_2PO_4 and the pH was adjusted to 7.6. Tests were carried out in 50 ml. conical flasks to which side-arm test tubes had been sealed. This was to allow an adequate supply of oxygen while the medium was incubated in the flasks; growth could then be measured by tipping the medium into the side arm which fitted the nephelometer.

The effects of pantothenate and pantotheine on the cystine requirements of *Lb. arabinosus* and *Ln. mesenteroides* were tested on the cystine-assay medium (Barton-Wright, 1952).

The synthesis of CoA by washed suspensions. The ability of compounds to replace pantothenate, or, in compounds containing sulphur, to replace pantothenate and cystine, was tested with washed suspensions of pantothenate-deficient cells as previously described (Pierpoint & Hughes, 1954). Washed cells (approx. 10 mg.) were suspended in saline to which was added 0.05M phosphate buffer (pH 7.0) and glucose (0.05M). Pantothenate and cystine when added

Compound	R
Pantotheine	$-\text{NH} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{SH}$
Pantothenylcysteine	$-\text{NH} \cdot \text{CH}(\text{CO}_2\text{H}) \cdot \text{CH}_2 \cdot \text{SH}$
Pantothenylserine	$-\text{NH} \cdot \text{CH}(\text{CO}_2\text{H}) \cdot \text{CH}_2 \cdot \text{OH}$
Pantothenyl-2-hydroxyethylamine	$-\text{NH} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{OH}$
Pantothenylaminoacetaldehyde	$-\text{NH} \cdot \text{CH}_2 \cdot \text{CHO}$
Pantothenylglycine	$-\text{NH} \cdot \text{CH}_2 \cdot \text{CO}_2\text{H}$
Pantothenyl- α -alanine	$-\text{NH} \cdot \text{CHMe} \cdot \text{CO}_2\text{H}$
Pantothenyl- β -alanine	$-\text{NH} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CO}_2\text{H}$
Pantothenamide	$-\text{NH} \cdot \text{H}$
Pantothenylglutamic acid	$-\text{NH} \cdot \text{CH}(\text{CO}_2\text{H}) \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CO}_2\text{H}$

Fig. 1. Structures of pantothenyl derivatives.

were at a concentration of $2 \times 10^{-4} \text{M}$ and the various pantothenate derivatives were added over a range of 10^{-5} to 10^{-3}M . The suspensions were incubated at 37° for 60 min., after which the cells were collected and washed by centrifuging. Their CoA content was estimated by the method of Handschumacher, Mueller & Strong (1950). The results are expressed as Lipmann units CoA. Where weights of organisms are given these refer to dry weights and were estimated by drying a sample of the washed suspension at 110° and subtracting the dry weight of an equal volume of saline.

Materials. The derivatives of D(+)-pantothenate (Fig. 1) were synthesized by Baddiley & Mathias (1954). The amino acids were commercial samples and, with the exception of L(+)-glutamic acid, were not purified further. The glutamic acid was contaminated with cystine and histidine, from which it was freed by passage through Zeo-Karb 215, elution with HCl and crystallization (Partridge & Westall, 1949). No cystine was detected in the product by either microbiological or chromatographic tests.

RESULTS

Effect of pantothenate derivatives on the synthesis of CoA by washed suspensions

Pantetheine-4'-phosphate, which supports CoA synthesis in liver (Levintow & Novelli, 1952), did not support synthesis in washed suspensions of *Lb. arabinosus*. Pantothenyl-L-cysteine, pantothenyl-cysteine-L-4'-phosphate and dipantothenyl-L-cystine gave rise to 0–10% of the amount of CoA given by equivalent amounts of pantothenate and cystine, but this effect was variable and may have been due to traces of impurities in the samples.

The following pantothenyl peptides, which do not contain sulphur (Fig. 1), were found to be without appreciable activity in replacing pantothenate for synthesis when cystine was also present: *N*-pantothenyl-2-hydroxyethylamine, pantothenyl-DL- α -alanine, pantothenyl-DL-serine, pantothenyl- β -alanine, pantothenyl-DL-glutamic acid, *N*-pantothenylaminoacetaldehyde and pantothenamide. In contrast to this pantothenylglycine replaced pantothenate to some extent (Fig. 2), but was never as active as pantothenate itself. The effectiveness of different samples of the compound was the same in any single batch of organisms but varied widely in different batches of cells (Table 1). Although the

Fig. 2. The effect of varying concentrations of pantothenylglycine and pantothenate on CoA synthesis. Deficient cells (6.4 mg.) were suspended in glucose-phosphate-saline containing cystine ($2 \times 10^{-4} \text{M}$) and either pantothenic acid (x) or pantothenylglycine (o). They were incubated at 37° for 60 min. and their CoA contents estimated.

Fig. 3. Time curve of CoA synthesis from pantothenylglycine and pantothenate. Washed deficient cells (9.3 mg.) were suspended in 1.9 ml. phosphate saline containing glucose, $1.6 \times 10^{-4} \text{M}$ cystine and either 10^{-4}M pantothenylglycine (o) or 10^{-4}M pantothenic acid (x).

Table 1. CoA synthesis from pantothenylglycine and pantothenate

The organisms were suspended in the phosphate saline containing glucose, $2 \times 10^{-4} \text{M}$ cystine, and either 10^{-4}M pantothenylglycine or 10^{-4}M pantothenate. The suspensions were incubated at 37° for 60 min. and the CoA was then estimated.

Expt.	Organism (mg./tube)	CoA synthesized from pantothenylglycine (units/tube)			Synthesis (percentage of that from pantothenate)
		Sample 1	Sample 2	Sample 3*	
1	10	1.7	1.8	—	53
2	9	0.6	0.6	—	18
3	6.4	—	2.15	—	77
4	5.5	—	1.3	1.4	54

* Sample 3 was the dimethylamine salt.

initial rate of CoA synthesis from pantothenylglycine was often almost the same as that from pantothenate, the synthesis came to a stop sooner and before the cells were saturated with CoA (Fig. 3). The lack of activity of pantothenylglutamate is of interest, since King, Fels & Cheldelin (1949) reported the formation of a very active pantothenate compound by yeasts on incubation with pantothenate and glutamic acid. In view of the present findings it is possible that their glutamic acid was contaminated by cystine, which commonly occurs in most samples of glutamic acid prepared from natural sources, and that the active pantothenate was pantetheine or one of its derivatives.

Effect of pantothenate derivatives on the growth of Lb. arabinosus

Of the pantothenate peptides not containing sulphur, only pantothenylglycine had marked activity in replacing pantothenate for the growth of *Lb. arabinosus* (Table 2). The results of growth

Table 2. *Effect of pantothenyl derivatives in replacing pantothenate as a growth factor for Lb. arabinosus*

The compounds were tested in concentrations from 10^{-7} to 2×10^{-5} M. Growth was measured turbidimetrically at intervals up to 100 hr.

Compound	Growth-promoting activity relative to pantothenate (%)
N-Pantothenyl-2-hydroxyethylamine	5
Pantothenyl-L-cysteine	1
Dipantothenyl-L-cystine	3
Pantothenyl-DL- α -alanine	1
Pantothenyl- β -alanine	1
Pantothenyl-DL-serine	1
Pantothenamide	2
N-Pantothenylaminoacetaldehyde	1
Pantothenylglycine	80-100
Pantetheine-4'-phosphate	1
Pantothenyl-L-glutamic acid	6

Pantothenylaminoacetaldehyde increased some 28% in activity when autoclaved in the medium. None of the other compounds showed a similar change in activity.

tests thus agree with the experiments on washed suspensions. Although after about 16 hr. growth promoted by pantothenylglycine was considerably less than that promoted by equivalent amounts of pantothenate, at 60 hr. it was the same (Fig. 4). This lag before maximum growth is attained is similar to the effect of pantetheine described by Craig & Snell (1951). Under the present conditions, however, pantetheine would only support 10-25% of the growth produced by pantothenate (Fig. 5); similar findings have also been reported with this organism by McRorie & Williams (1951).

Fig. 4. Replacement of pantothenate by pantothenylglycine for the growth of *Lb. arabinosus* 17-5. Growth in the presence of pantothenylglycine was measured at 17 hr. (Δ), 42 hr. ($\bullet$) and 66 hr. ($\bigcirc$ --- $\bigcirc$). In the presence of pantothenate, it was measured at 17 hr. ($\times$), 42 and 66 hr. ($\blacksquare$).

Fig. 5. Replacement of pantothenate by pantetheine for the growth of *Lb. arabinosus*. Growth supported by pantothenic acid was measured at 16 hr. ($\bigcirc$) and 130 hr. (Δ), and by pantetheine at 16 hr. ($\times$) and 130 hr. ($\square$). The results were not affected by the method used to sterilize the pantetheine.

Effect of pantothenate derivatives on the growth of Lb. helveticus

In this organism, pantetheine is approximately 100 times more active in promoting growth than is pantothenate (Fig. 6) (cf. Craig & Snell, 1951). When tested in concentrations up to 10^{-5} M the following compounds were without marked effect in

replacing pantetheine: pantothenyl-L-cysteine, di-pantothenyl-L-cystine, pantothenyl-DL-serine, pantothenyl-L-cysteine-4'-phosphate, pantetheine-4'-phosphate, pantothenyl-β-alanine, pantothenyl-DL-α-alanine, pantothenylglycine, pantothenamide.

Effect of pantothenate derivatives on the growth of *P. morganii*

Pantetheine had approximately the same activity as pantothenate in supporting the growth of *P. morganii* (Table 3). The compounds listed above were tested with one strain (K 653) and only panto-

thenyl-β-alanine had any marked growth-promoting activity, amounting to 20–30 % of the equivalent effect of pantothenate after 3 days' growth.

Effect of pantoyltaurine on the utilization of pantoylglycine

The activity of pantothenylglycine in supporting growth and CoA synthesis in *Lb. arabinosus* suggests either that it is an intermediate in the synthesis or that it is hydrolysed and thus forms a source of pantothenate. Attempts were made to detect the formation of glycine upon incubation of the peptide with washed suspensions. These were unsuccessful owing to release by the cells into the medium of large amounts of amino acids, including glycine. The effect of pantoyltaurine, which is known to inhibit pantothenate utilization (McIlwain & Hughes, 1944), was therefore tested. If pantothenylglycine were indeed an intermediate in CoA synthesis it would be expected to be much more active than pantothenate in overcoming inhibition by pantoyltaurine. However, both in growth tests (Table 4), and in experiments on CoA synthesis by washed suspensions (Table 5),

Fig. 6. The effect of pantetheine (O) and pantothenic acid (x) on the growth of *Lb. helveticus*.

Table 3. Effect of pantetheine and pantothenate on the growth of *P. morganii* (strain K 653)

Conditions were as described in the text, and growth was measured after 15 hr.

Concn. of growth factor (10 ⁻⁶ M)	Nephelometer reading	
	Pantothenate present	Pantetheine present
0	4	6
5	46	52
25	59	61
250	108	109

Table 4. Effect of pantoyltaurine on the growth of *Lb. arabinosus*

The organism was grown on the pantothenic acid assay medium (Barton-Wright, 1952) with the addition of the growth factors listed below. The Anti-Bacterial Index, or the ratio of the molar concentration of inhibitor to the molar concentration of growth factor necessary to produce complete inhibition of growth, was measured after 4 days' incubation at 37°.

Expt.	Growth factor	Concn. of pantoyl-taurine (10 ⁻⁴ M)	Anti-Bacterial Index
1	Pantothenate	1.3	6000
	Pantothenylglycine	1.7	1000
	Pantetheine	9	1000
2	Pantothenate	1.7	6000–10 000
	Pantothenylglycine	1.7	1000
	Pantetheine	8.6	1000

Table 5. Effect of pantoyltaurine on the synthesis of CoA from pantoylglycine and pantothenate in *Lb. arabinosus*

Pantothenate-deficient cells were washed and suspended in phosphate saline containing glucose and 0.28M pantoyltaurine. They were incubated for 15 min. at 37°, and then cystine and either pantothenate or pantothenylglycine were added to final concentrations of 5 × 10⁻³M. The suspensions were further incubated for 60 min. and the CoA synthesized (units/tube) estimated.

Organisms (mg./tube)			Expt. 1	Expt. 2
...			10	11
CoA synthesized	{ From pantothenate	...	3.9	2.5
	{ From pantothenylglycine	...	0.9	0.5
CoA synthesized in presence of pantoyltaurine	{ From pantothenate	...	3.2	1.4
	{ From pantothenylglycine	...	0.5	0.0
Inhibition (%)	{ From pantothenate	...	17	44
	{ From pantothenylglycine	...	45	100

pantothenylglycine was less effective in overcoming inhibitions by pantooyltaurine than was pantothenate. This result suggests that pantothenylglycine is hydrolysed to pantothenate before utilization for CoA synthesis in *Lb. arabinosus*. However, the correctness of this interpretation is doubtful since it was found that pantetheine too was less effective in overcoming inhibition by pantooyltaurine than was pantothenate. This is in contrast with its effect in some other organisms where it is more effective (McRorie & Williams, 1951).

Effect of pantothenate derivatives on the cystine requirements of Lb. arabinosus

In order to follow the uptake of cystine from the medium by washed suspensions of *Lb. arabinosus*, estimations were made using the microbiological assay method of Barton-Wright with *Lb. arabinosus* as the test organism. It was found, however, that impossibly high recoveries of cystine were obtained when the mother-liquor in which cells had been incubated was tested. The addition of such a mother-liquor to the standards indicated that the error was due to a sparing effect at low concentrations of cystine; in the higher concentrations no effect on growth was found. Besides being needed for protein synthesis, cystine in this organism is also needed for CoA synthesis. It seemed possible therefore that some intermediate in the synthesis of CoA from pantothenate and cystine might have a sparing effect. As shown in Fig. 7 pantetheine had such an effect on the cystine requirements of *Lb. arabinosus*: that is, growth in the lower concentra-

tions of cystine was increased several-fold by the addition of pantetheine, but no effect was found in the higher concentrations. No sparing effect was found with dipantothenyl-L-cystine, pantetheine-4'-phosphate and pantothenylglycine. Pantothenyl-L-cysteine had a small effect amounting to about 5% of that of equivalent amounts of pantetheine. High concentrations of pantothenate ($5 \times 10^{-4}M$) often delayed growth on the low concentrations of cystine but had no such effect in the higher concentrations (Fig. 7).

Pantetheine had no sparing effect on the cystine requirements of *Ln. mesenteroides* (Table 6) and this may account for the greater specificity of the assay using this organism which has recently been described by Barton-Wright (1952).

Table 6. *Effect of pantetheine on the cystine-limited growth of Ln. mesenteroides*

The medium was that described by Barton-Wright (1952) and the growth conditions were the same as those used for *Lb. arabinosus*. When pantetheine was added, its final concentration was $2.5 \times 10^{-5}M$.

Cystine concn. ($10^{-5}M$)	Nephelometer reading pantetheine absent		Nephelometer reading pantetheine present	
	17 hr.	36 hr.	17 hr.	36 hr.
0.12	11	19	11	23
0.25	22	38	23	43
1.25	58	105	61	102
2.5	76	114	73	112

Uptake of cystine by washed suspensions of Lb. arabinosus

Washed suspensions were incubated under the conditions described for CoA synthesis and the uptake of cystine was measured by microbiological assay using *Ln. mesenteroides* as the test organism. No interference with the assay was found with the incubation mother-liquor and satisfactory recoveries of cystine were obtained. The washed suspensions took up cystine from the incubation medium in both the presence and absence of pantothenate (Table 7). Cells which had been grown on the casein hydrolysate medium and in the presence of adequate pantothenate took up cystine but did not take up pantothenate. The cystine that was taken up in the absence of pantothenate could not be extracted from the dried cells by boiling with water for 10 min. There is thus no indication of the conversion of the cystine into a form which could be used for the synthesis of CoA on the addition of pantothenate; nor does this appear to occur during growth, because cells grown with adequate cystine, but with limiting amounts of pantothenate, still need the further addition of cystine for CoA synthesis (Pierpoint & Hughes, 1954).

Fig. 7. The effect of pantetheine on the cystine requirement of *Lb. arabinosus* 17.5. Growth was measured after incubation for 16 hr. (●) and 72 hr. (□) in the presence of $2.5 \times 10^{-5}M$ pantetheine. The control tubes were also measured after 16 hr. (○) and 72 hr. (×). Additional pantothenate was added ($5 \times 10^{-4}M$) and the growth read after 16 hr. (▽) and 72 hr. (▼).

Table 7. Cystine uptake by washed cell suspensions

Washed cells were incubated for 60 min. at 37° in the glucose-saline solution, with pantothenate and/or cystine in a final volume of 1.7 ml. After incubation the cells were collected and their CoA content was assayed. The pantothenate and cystine remaining in the supernatant liquid were then estimated.

Expt.	Concn. of pantothenate in growth medium (10 ⁻⁷ M)	Organisms (mg./tube)	Cystine (μg.)			Pantothenate (μg.)			CoA (Lipmann units/tube)	
			Added	Remaining	Uptake	Added	Remaining	Uptake	Before incubation	After incubation
1	1	12	—	—	—	2.93	1.22	1.7	0.55	1.17
			5.84	2.1	3.74	2.93	1.54	1.39	0.55	3.85
2	1	9.8	5.84	2.1	3.74	—	—	—	0.55	0.67
			9.6	4.55	5.05	6.9	3.55	3.35	0.1	4.55
3	10	10	9.6	4.45	5.15	—	—	—	0.1	0.2
			—	—	—	8.1	7.9	0.2	—	5.25
			9.7	7.8	1.9	—	—	—	5.4	5.4
			9.7	7.8	1.9	8.1	8.1	0	5.4	5.4

DISCUSSION

The following provisional scheme for the synthesis of CoA from pantothenate has been suggested (Hughes, 1953; Novelli, 1953) from microbiological and enzymic studies:

- (1) Pantothenate + cysteine → Pantothencycysteine
- (2) Pantothencycysteine → Pantetheine + CO₂
- (3) Pantetheine + ATP → Pantetheine-4'-phosphate + ADP
- (4) Pantetheine-4'-phosphate + ATP → Dephospho-CoA + pyrophosphate
- (5) Dephospho-CoA + ATP → CoA + ADP.

Steps (3), (4) and (5), by which pantetheine is converted into CoA, have been well characterized by isolation from liver and partial purification of the enzymes concerned (Levintow & Novelli, 1954; Hoagland & Novelli, 1954; Wang & Kaplan, 1954). The steps leading to the synthesis of pantetheine from pantothenate are less firmly established. Previously it seemed reasonably clear that cystine was in some way involved in the synthesis (Pierpoint & Hughes, 1954), and in the present paper additional but still rather indirect evidence supports this view. The additional evidence is that pantetheine has a sparing effect on the cystine requirements of *Lb. arabinosus*. This sparing effect is likely to be due to competition for cystine between processes using it for protein synthesis and processes using it for CoA synthesis when excess of pantothenate is added. When pantetheine replaces pantothenate all the cystine becomes available for protein synthesis, and growth in the presence of limiting cystine is greatly increased. Such a marked sparing effect of pantetheine is, however, somewhat surprising in view of its low activity as a growth factor for *Lb. arabinosus*. Recently, Brown & Snell (1954) have shown that the reduced forms of pantetheine and its derivatives are generally much more active as bacterial growth-factors than are the oxidized forms. It seems unlikely, however, that this is the explanation of the low activity of pantetheine as a growth factor in the present experiments because it was added to the growth medium as the SH compound and was autoclaved in the medium to ensure that it remained reduced at least until the medium was inoculated (also discussed by Cheldelin & King, personal communication).

The inactivity of pantothencycysteine and pantetheine-4'-phosphate in supporting the growth of lactobacilli and in supporting CoA synthesis in *Lb. arabinosus* is in agreement with the findings of Brown & Snell (1954). Since these compounds are active as growth factors for *Acetobacter suboxydans* the simplest explanation of their inactivity in the

other organisms is that they cannot enter the cells. This is a well-known limitation of microbiological methods for the study of synthetic sequences. An additional limitation, especially with growth tests and experiments with intact cells, is that compounds which are not normal intermediates may be converted into them by metabolic reactions. This is illustrated in the present work by the activity of pantothenylglycine, which may equal or even surpass that of pantetheine, although it is probably first converted into pantothenate. In view of such limitations in the microbiological approach to CoA synthesis the inclusion of pantothenylcysteine as an intermediate as shown in steps (1) and (2) is still hypothetical.

The main support for the inclusion of pantothenylcysteine in the scheme is that it will serve as a growth factor for *Acet. suboxydans* and that dried cells of this organism will convert it into a substance which serves as a growth factor for *Lb. helveticus*, an organism which normally needs pantetheine for growth as shown by Brown & Snell (1953). In addition, these authors have shown that *P. morganii* converts pantothenate and cystine into a product that is without activity for *Lb. helveticus* until it is treated with dried cells of *Acet. suboxydans*. That this product is in fact pantothenylcysteine, as suggested by the authors, cannot be accepted without further characterization, especially since both pantetheine-4'-phosphate and dephospho-CoA would behave in a similar fashion to pantothenylcysteine in these microbiological tests (Brown & Snell, 1953). The formation of such phosphorylated compounds in bacteria is extremely likely because pantothenate itself is readily phosphorylated both in intact cells and cell-free extracts of *Lb. arabinosus* and yeasts (Baddiley, Hughes, Mathias & Pierpoint, 1954).

In animal tissues the status of pantothenylcysteine in the synthesis of CoA is likewise still uncertain. Hoagland & Novelli (1954) have shown that pantetheine may be formed by rat-liver preparations from pantothenylcysteine. In contrast to this, pantothenylcysteine cannot replace pantothenate for the growth of deficient chicks and rats (Thompson & Bird, 1954).

SUMMARY

1. The following compounds have been tested for ability to replace pantothenic acid or pantetheine in growth and CoA synthesis by various bacteria: pantothenyl-L-cysteine; dipantothenyl-L-cystine; pantothenyl-L-cysteine-4'-phosphate; pantothenyl- β -alanine; pantothenyl-DL- α -alanine; pantothenyl-DL-serine; pantothenylglycine; *N*-pantothenylaminoacetaldehyde; *N*-pantothenyl-2-hydroxyethylamine; pantothenamide; pantothenyl-L-glutamic acid; pantetheine-4'-phosphate.

2. Of these substances only pantothenylglycine is an effective substitute for pantothenic acid in the growth of *Lb. arabinosus* and in CoA synthesis by suspensions of this organism. Evidence was obtained, by the use of pantoyletaurine as an inhibitor, that pantothenylglycine is effective only after its hydrolysis to pantothenic acid.

3. None of these compounds (pantothenyl-L-glutamic acid and *N*-pantothenyl-2-hydroxyethylamine were not tested) replace pantetheine in supporting the growth of *Lb. helveticus*.

4. With the exception of pantothenyl- β -alanine (pantothenyl-L-glutamic acid and pantothenyl-2-hydroxyethylamine were not tested), none of the above compounds supports the pantothenic acid-limited growth of *Proteus morganii*. This compound supports only 20–30% of the growth due to an equivalent amount of pantothenic acid.

5. Pantetheine has a sparing effect on the cystine requirements of *Lb. arabinosus* but not on those of *Leuconostoc mesenteroides*. This probably accounts, in part at least, for the greater reliability of the cystine assays in which the latter organism is used.

6. Washed suspensions of *Lb. arabinosus* took up cystine from the medium. The uptake was independent of the presence of added pantothenate or of the pantothenate concentration in the cells.

7. The mechanism of CoA synthesis is discussed in relation to these findings.

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The Reduction of Diphosphopyridine Nucleotide by Ethanol under the Influence of X-Irradiation

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Two types of mechanism have been suggested to explain dehydrogenase action. On the one hand, it has been postulated that the oxidations and reductions may be univalent in character, with free radicals as intermediates (Waters, 1948; Swallow, 1953, 1954*a*; Barron & Levine, 1952). On the other hand, an ionic mechanism has been proposed (Burton & Kaplan, 1954). Recent work on the reduction of diphosphopyridine nucleotide (DPN) by sodium dithionite (Yarmolinsky & Colowick, 1954; Colowick, 1954; Swallow, 1955) supports the ionic mechanism.

In this paper are presented some further experiments on the reduction of DPN by ethanol under the influence of ionizing radiations, a reaction which is believed to proceed through the agency of free radicals. Some of the results have been briefly reported elsewhere (Stein & Swallow, 1954; Swallow, 1954*b*).

EXPERIMENTAL

Irradiation arrangements. Two Victor Maximar X-ray sets were used. One was operated at 220 kVp and the radiation filtered through 1 mm. aluminium, while the other was operated at 190 kVp with the radiation unfiltered. Doses were determined by the FeSO_4 method (Miller & Wilkinson, 1952), the value for the yield being taken as 15.6 molecules of Fe^{3+} produced/100 eV of energy absorbed (Farmer, Rigg & Weiss, 1955). The dose rate with the 220 kVp set was 640 r/min. and with the 190 kVp set 4000–5000 r/min. The temperature of irradiations was not controlled, and was normally 20–30°. Irradiation vessels were similar to those previously employed (Swallow, 1952), two sizes of tube being used, to take 1 or 3 ml. of solution.

Deoxygenation of solutions was by the method previously described (Swallow, 1952). When 3 ml. of solution

were to be irradiated in the absence of dissolved oxygen, 1 ml. of water + volatile solute was first evaporated off by the deoxygenation procedure, and allowance made for changes in concentration. It was assumed that the rate of evaporation of each constituent was proportional to its vapour pressure and to its mole fraction in the liquid.

Chemicals. Oxidized DPN (DPN^+) was obtained from Sigma Chemical Co., U.S.A. (90% pure) and C. F. Boehringer and Soehne, Germany (100% pure). Reduced DPN (DPNH) was from Sigma Chemical Co. Pig-heart lactic dehydrogenase was kindly given by members of the Biochemistry Department, University of Cambridge. Crystalline yeast alcohol dehydrogenase was obtained from C. F. Boehringer and Soehne. Other chemicals were of A.R. purity.

Determination of hydrogen. This work was done at King's College, Newcastle upon Tyne, by kind permission of Dr J. Weiss. The author wishes to thank Dr R. G. A. Johnson, Mr G. G. Jayson and other members of the radiation chemistry group for help with the apparatus. Irradiations of 100 ml. solution (air-free) were performed with 200 kVp unfiltered X-rays at a dose rate of 3100 r/min. Gas analysis was by the modified method of Stein & Weiss (see Johnson, Scholes & Weiss, 1953) and results were accurate to within 10%.

Ultraviolet absorption curves. The curves shown in Figs. 1 and 2 were obtained by diluting the solution with 2 vol. of water and measuring optical densities with a Unicam SP. 500 spectrophotometer with distilled water as blank. Curves shown in Fig. 3 were on undiluted solutions.

RESULTS

Absorption spectra

The effect of X-irradiation on the absorption curve of oxygen-free aqueous solutions of DPN^+ (0.27 mM) containing ethanol (0.5 M) is shown in Fig. 1. This result was for solutions 0.01 M in sodium pyrophosphate, adjusted to pH 7.8 with HCl. It was found that the effect of a dose of 39 000 r was the same when the buffer was 0.01 M borax–boric acid

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