49. Bacterial Inhibition by Metabolite Analogues

4. Analogues of Pantothenic Acid

By **Henry McIlwain**[1], *Department of Bacterial Chemistry (Medical Research Council), The University of Sheffield*

(*Received 14 April 1942*)

The production of growth-inhibiting agents by suitably modifying the structures of growth-promoting substances has been shown to be feasible by previous investigations (McIlwain [1940; 1941*a*]: considered as parts 1 and 2 of the present series). In these cases evidence was obtained which showed that the inhibitory analogues produced their effects by limiting the organism's use of the corresponding essential substances. Pantothenic acid was chosen as subject for extending these investigations as it is essential for the growth of many disease-causing organisms: for *Streptococcus haemolyticus*, for *Diplococcus pneumoniae* [Rane & Subbarow, 1940]; for initial growth of *Staphylococcus aureus*; for *Clostridium tetani* [Mueller & Miller, 1941] and *Clostridium welchii* [Tamura, Tytell, Boyd & Logan, 1941]; for certain strains of *Corynebacterium diphtheriae* [Evans, Handley & Happold, 1939], some members of the *Pasteurella* [Berkman, Saunders & Koser, 1940] and *Brucella* [Koser, Breslove & Dorfman, 1941] groups and for *Proteus morgani* [Pelczar & Porter, 1940].

The analogues tested are related to pantothenic acid (I) in the following ways.

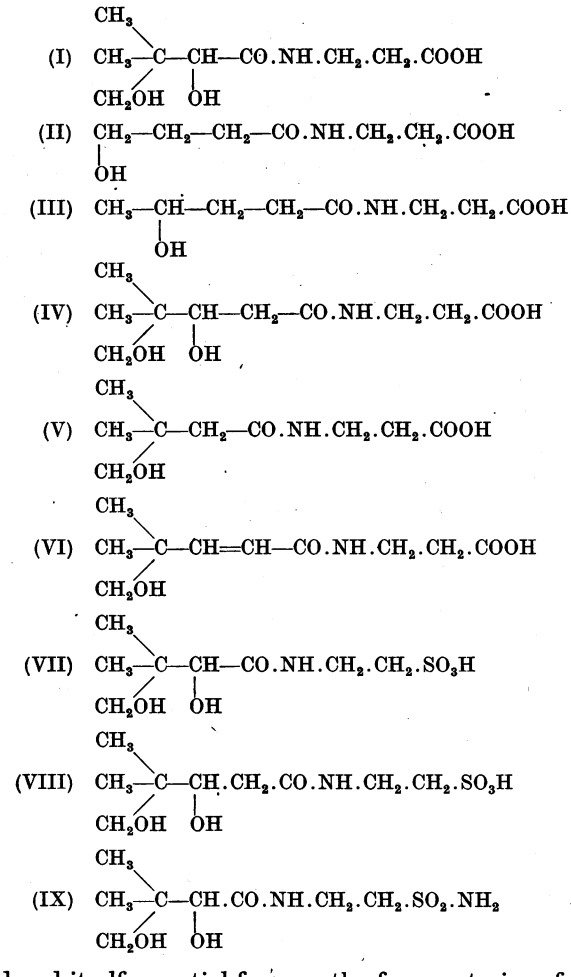
(*a*) *Homologues.* The belief that compounds so related might be inhibitory was based on the report of Robinson [1940] that a β-tri-substituted propionic acid was inhibitory to growth of *Mycobacterium tuberculosis* of which phthioic acid, a trisubstituted acetic acid, is a normal constituent. Inhibition of succinic dehydrogenase by malonate affords a further example, and more recently ethionine, $C_2H_5.S.CH_2.CH_2.CH(NH_2)COOH$, has been found to limit bacterial growth by competition with methionine [Harris & Kohn, 1941]. γ-Hydroxy-*n*-butyryl-β-alanine, (II), a *bisnor*desoxypantothenate; γ-hydroxy-*n*-valeryl-β-alanine (III), an *isonor*desoxypantothenate; homopantothenate, (IV) and a desoxypantothenate (V) were accordingly tested.

(*b*) *Related olefin.* Fildes [1941] showed that indoleacrylate inhibited bacterial growth by virtue of its relation to tryptophan. The structure of pantothenate precludes the production of an olefin by simple elimination of H_2O or NH_3, but dehydrohomopantothenate (VI), which is so related to homopantothenate, was tested.

(*c*) *Sulphonic acids and sulphonamide.* Several models are available: Woods [1940] showed sulphanilamide inhibition to be related to *p*-aminobenzoic acid; some aliphatic α-aminosulphonic acids inhibit by virtue of their relation to α-aminocarboxylic acids [McIlwain, 1941*a*], and pyridine-3-sulphonic acid and its amide by their relation to nicotinic acid and derivatives [McIlwain, 1940]. The latter instance served as model for Snell's [1941] production of an inhibitory analogue of pantothenic acid, which was published while these investigations were in progress. Analogue VII, which it is proposed to call pantoyltaurine (pantoic acid = α:γ-dihydroxy-β:β-dimethylbutyric acid) is the analogue investigated by Snell. Detailed results of its effects on pathogenic organisms have been reported elsewhere [McIlwain, 1942*b*] and are referred to here for comparison only. In addition, the corresponding sulphonamide (pantoyltauramide, IX) and a homologue (homopantoyltaurine, VIII) have now been tested. Taurine and taurine amide, which are the sulphonic acid and sulphonamide corresponding to β-alanine, a component

[1] Leverhulme Research Fellow.

Formulae



of pantothenic acid and itself essential for growth of some strains of *C. diphtheriae*, were previously examined [McIlwain, 1941*a*].

This work was undertaken in order to find therapeutically useful inhibiting agents, but opportunity was also taken to investigate the structural specificity of pantothenate for bacterial growth, and the manner in which it and the analogues act.

Experimental

The analogues were prepared by Dr J. W. Barnett & Mr F. A. Robinson [1942]. Compounds III, IV, VII, VIII and IX were racemates. (+)Pantothenate was supplied by Merck and Co., Inc. In testing, solutions were sterilized by filtration and these and other materials added aseptically to the basal media described later, in a final volume of 10 ml. The highest concentration of inhibitor usually tested was $2 \times 10^{-3} M$. The media were then inoculated lightly (*c.* 100 organisms/ml.) and incubated at 37° in 5% CO_2-air. The effects of the addenda were judged during the next 7 days by comparing the opacities of these cultures with those of controls grown at the same time. Other bacteriological details, concerning the organisms described here, have been given elsewhere [McIlwain, 1942*b*].

A. Streptococcus haemolyticus

This organism was shown to require pantothenate by fractionating a liver concentrate necessary for its growth in a casein hydrolysate medium [McIlwain, 1939] and this pantothenate-free basal medium has been used in the present work. Five streptococci representing groups A and C uniformly required the addition of $10^{-7}M$ or more pantothenate for optimal growth.

The taurine analogues VII, VIII and IX, each completely inhibited the organisms in the presence of $10^{-7}M$ pantothenate, but different quantities of the different compounds were necessary. The inhibition produced by each analogue was related to pantothenate: it could be prevented by addition of a further quantity of it. Such addition need not be made immediately; after a day's incubation with minimal optimal pantothenate and an inhibitory quantity of pantoyltaurine, further pantothenate allowed growth in the usual time. The effect of the inhibitor is thus bacteriostatic rather than bactericidal. In the presence of adequate pantothenate and excess of a taurine analogue the organisms therefore appear to be suffering from the same deficiency as in the absence of both. Reversal of the inhibition was investigated in detail in the case of pantoyltauramide and found to be specific, among known nutrients, to pantothenate. A concentration of pantoyltauramide double that just necessary for bacteriostasis was not antagonized by higher concentrations of glucose, of 14 amino-acids, nicotinamide, aneurin, riboflavin, adermin, glutamine and biotin (which substances, like pantothenate, are necessary for growth of streptococci) or by pimelate, choline and *p*-aminobenzoate. The components of pantothenate, β-alanine and pantoate, were also ineffective. A similar high degree of specificity has been found with pantoyltaurine. It therefore appears that the aim of limiting the organism's use of pantothenate has been achieved in these compounds.

The quantities of pantoyltauramide necessary for bacteriostasis in the presence of different concentrations of pantothenate showed a typical regularity which is recorded in Table 1. The presence or absence of growth was conditioned by the relative concen-

Table 1. *Interrelations between pantoyltauramide and pantothenate in growth of* Strep. haemolyticus

Substances added		Growth after			
(+)Pantothenate, $M \times 10^{-7}$	*dl*-Pantoyltauramide, $M \times 10^{-5}$	18–20 hr.	26–28 hr.	2 days	4 days
1 to 1000	0	+ + + +			
0·2	0	+ + +	+ + + +		
0·04	0	+ +	+ +	+ +	+ +
0·008 to 0	0	0	0	0	0
25	100	+ + + +			
5, 1	100	0	0	0	0
25, 5	20	+ + + +			
1, 0·2	20	0	0	0	0
1	4	+ + +	+ + + +		
0·2	4	0	0	0	0
0·2	0·8	+ + +	+ + +	+ + + +	
0·04, 0·008	0·8	0	0	0	0

trations of the two compounds, and not by the absolute amount of either present. No growth took place when the ratio M pantoyltauramide/M pantothenate was 2000 or more. Such quantitative interrelations between the concentrations (C_I) of an inhibitor just preventing growth in the presence of concentrations (C_M) of growth-promoting substances have now been frequently encountered. The term *antibacterial index* is proposed for the minimal value of the ratio C_I/C_M at which growth is prevented by such inhibitors [McIlwain, 1942*b*]. The antistreptococcal index of pantoyltauramide is thus 2000; that for homopantoyltaurine was similarly found to be 20,000 and for pantoyltaurine

was 500 (Table 4). It is not always possible to give a single number as the antibacterial index of an inhibitor, but the values are of use in comparing the effectiveness of inhibitors, and in calculating the quantity of inhibitor necessary for antibacterial action in the presence of a known amount of metabolites, e.g. in animal tissues. Some values are available for the pantothenate content of such materials [Pelczar & Porter, 1941; Wright, McMahan, Cheldelin, Taylor, Snell & Williams, 1941]; the activities of those tested in antagonizing pantoyltaurine are consistent with their pantothenate contents and with *in vivo* activity of the inhibitor.

Pantoyltauramide was equally inhibitory to anaerobic growth of streptococcus.

γ-Hydroxy-n-*butyryl-β-alanine* and the corresponding valeryl derivative were the only other analogues inhibitory to streptococcus (see Table 3). Their effects were small and not reversed by pantothenate; the compounds have been more fully studied in connexion with *Bact. coli*. The non-inhibitory analogues were examined for growth-promoting activity, by attempted growth of streptococcus on their addition to the pantothenate-deficient medium. The same small activities were found which are discussed under *P. morgani*.

B. Diplococcus pneumoniae

The nutritional requirements of this organism are not yet defined, and the most satisfactory medium found for demonstrating the effects of the present compounds was that used for *Strep. haemolyticus* with the addition of choline ($10^{-5}M$) and 2–5% of plasma. This latter contributed a small quantity of pantothenate sufficient only for little or no growth. Pantothenate was therefore added, and the organism's requirements were practically the same as is recorded in Table 1 for streptococci.

Pneumococci were slightly less susceptible than streptococci to inhibition by pantothenate analogues. No marked differences were found between different strains when representatives of Types I, II and III were examined. γ-Hydroxy-*n*-butyryl-β-alanine was not inhibitory, but the taurine derivatives were so. The inhibitions of both pantoyltaurine and pantoyltauramide were not so intense as with streptococci but showed the same competitive character; the antipneumococcal indices of the compounds were 1000 and 10,000 respectively.

C. Corynebacterium diphtheriae

This organism is of particular interest in relation to pantothenate requirements as Evans *et al.* [1939] found that only certain exacting strains require the compound. Others are less exacting in that they can synthesize it if grown in the presence of β-alanine; this compound, but not the whole pantothenate molecule, is essential for their growth. Gradations from absolute need for pantothenate to its complete replacement by β-alanine have been encountered in the present work.

The basal medium [McIlwain, 1942*b*] was similar to that used for streptococcus. Results in an amino-acid medium were comparable in the cases examined. The effects of the analogues varied markedly with different strains, and as is shown in Table 2 were correlated with their pantothenate requirements. Inhibition with pantoyltauramide was strongest with the strains (G1, G1156 and GW1) which most needed pantothenate in growth, and did not occur with those (R7, R20) which were independent of added pantothenate. Inhibition was of the same competitive character as with streptococcus and with the strain G1 was investigated in sufficient detail to establish that 2000 times the quantity of pantoyltauramide was necessary for inhibition; 400 times had little effect. Inhibition was not as permanent as with streptococcus. β-Alanine contributed to pantoyltauramide-reversal with all susceptible strains, including in some cases that for which it was not a growth factor.

The *C. diphtheriae* strains thus afford a further example of the correlation of drug-fastness in bacteria with lesser nutritional needs and greater synthetic abilities. Similar

Table 2. *Growth requirements and inhibition of* C. diphtheriae *strains*

Strain	Substances added: Promoter, $M \times 10^{-7}$	Substances added: Inhibitor, $M \times 10^{-3}$	Growth after (days): 1	2	5
G1, *mitis*	0; or, β-alanine, 10	0	0	0	0
	(+)Pantothenate, 1	0	+ + +	+ + + +	
	,, 0·2	0	+ +	+ + +	+ + +
	,, 0·04	0	+	+ +	+ + +
	,, 0·2	γ-Hydroxy-*n*-butyryl-β-alanine, 1	+ +	+ + +	+ + +
	,, 1	*dl*-Pantoyltauramide, 1, 0·2	0	0	+ + +
	,, 1	,, 0·04	0	+ + +	+ + + +
GW1, *gravis*	0	0	0	0	0
	β-Alanine, 5000	0	+	+ + +	+ + + +
	(+)Pantothenate, 1	0	+ +	+ + + +	+ + + +
	,, 1	γ-Hydroxy-*n*-butyryl-β-alanine, 1	0	+ + +	+ + + +
	,, 1	*dl*-Pantoyltauramide, 1	0	+ +	+ + + +
R7, *mitis*	0	0	0	0	0
	β-Alanine, 100	0	+	+ + + +	+ + + +
	(+)Pantothenate, 1	0	+	+ + + +	+ + + +
	,, 1, 100	γ-Hydroxy-*n*-butyryl-β-alanine, 2	0	0	0
	,, 1	,, 0·2	+	+ + + +	+ + + +
	,, 1	*dl*-Pantoyltauramide, 1	+	+ + + +	+ + + +

Strain G1156, *mitis*, grew with β-alanine in place of pantothenate more slowly than did GW1, and was affected by the analogues in a manner similar to G1. Strain R20, *gravis*, grew as rapidly with β-alanine as with pantothenate, and was not inhibited by pantoyltauramide; unlike R7, it was also not inhibited by γ-hydroxy-*n*-butyryl-β-alanine.

phenomena were encountered in normal and trained strains of *Staph. aureus* and *Bact. typhosum*: the normal strains required amino-acids and were inhibited by α-amino-sulphonic acids, while the trained strains, synthesizing their own amino-acids, were not inhibited [McIlwain, 1941*a*; 1942*a*].

γ-Hydroxy-*n*-butyryl-β-alanine was also inhibitory to the *C. diphtheriae* strains (Table 2) to varying degrees, but its action was not entirely correlated with their pantothenate needs or with their susceptibilities to the taurine derivatives. Pantothenate did not reverse its inhibitions. The hydroxyvaleryl analogue also inhibited.

D. Bacterium coli

Four strains were examined and found to differ little in rate of growth or degree of inhibition. Added pantothenate did not accelerate their growth in the ammonium lactate medium of Fildes [1938] nor with the addenda given elsewhere [McIlwain, 1941*b*] though growth was suboptimal in the former case. The effects of the analogues were examined in the ammonium lactate medium and are summarized in Table 3.

Table 3. *Summary of inhibitory effects of pantothenate analogues*

Substances added to basal media	$M \times 10^{-3}$	Duration of inhibitions (days) with: *Bact. coli*	*P. vulgaris*	*P. morgani*	*Staph. aureus*	*C. diphtheriae* Non-exacting	*C. diphtheriae* Exacting	*Pneumo-coccus*	*Strepto-coccus*
γ-Hydroxy-*n*-butyryl-β-alanine	2	>7	>7	0	3	0–5	<1	0	1–2
,, ,,	0·2	2	4–7	0	1	0	0	0	0
γ-Hydroxy-*n*-valeryl-β-alanine	2	>7	>7	0	2	— — —	— — —	— — —	1
,, ,,	0·2	1	2–4	0	1	— — —	— — —	— — —	0
dl-Homopantothenate	2 or 0·2	0	— — —	0	0	— — —	— — —	— — —	0
Desoxypantothenate	2	2–3	— — —	0	0	— — —	— — —	— — —	0
Dehydrohomopantothenate	0·4	1	— — —	0	0	— — —	— — —	— — —	0
dl-Pantoyltaurine, *dl*-Pantoyltauramide, *dl*-Homopantoyltaurine	2	0	0	0	0	0	Inhibited; see text		

γ-Hydroxy-n-*butyryl-β-alanine* and the valeryl derivative were the most effective inhibitors and the former was examined in detail. Its inhibition (10^{-3}, $2 \times 10^{-4} M$ solution) was not prevented by pantothenate concentrations of 10^{-8} to $2 \times 10^{-3} M$. Its action, however, probably bears some relation to pantothenate as γ-hydroxy-*n*-butyric acid and β-alanine were not inhibitory in concentrations sufficient for complete inhibition by their condensation product. The inhibition was shown only under the suboptimal nutritional conditions of the ammonium lactate medium and was prevented by addition of small quantities of natural materials. Such were: hydrolysed edestin or tryptic casein, of which 40μg./ml. prevented the action of $4 \times 10^{-4} M$ (*c.* 100μg./ml.) γ-hydroxy-*n*-butyryl-β-alanine; or marmite, of which 200μg./ml. were necessary. This quantity of inhibitor alone prevented growth for over 5 days; the other materials stimulated growth when added alone. This degree of inhibition was partly prevented by the addition of glucose and completely by the further addition of pure amino-acids. Pyruvate, β-alanine, asparagine and pantoate were without action. It is concluded that if inhibition of *Bact. coli* by this analogue bears any relation to pantothenate, it is a non-competitive one which secondarily affects many compounds and is not susceptible to detailed analysis by the present methods. The inhibition caused by the hydroxyvaleryl derivative was also unaffected by pantothenate; γ-valerolactone was itself found inhibitory.

The taurine analogues did not inhibit any strain of *Bact. coli* which was examined; in this the latter differs markedly from organisms previously described. This might be due to (*a*) destruction of the inhibitor during growth, (*b*) production of a high pantothenate concentration in their cultures during growth or (*c*) other inherent differences, in enzyme make-up or in permeability. That (*a*) was not the case was shown by examining the effect on streptococci, with adequate pantothenate, of different dilutions of a sterile filtrate of *Bact. coli* grown in the presence of $2 \times 10^{-3} M$ pantoyltaurine. Streptococcal growth was inhibited to almost the same degree as by similar dilutions of $2 \times 10^{-3} M$ pantoyltaurine itself. A filtrate from *Bact. coli* grown without pantoyltaurine did not inhibit streptococci in the dilutions compared. Considering (*b*), the filtrate from a normal *Bact. coli* culture was examined for pantothenate by adding different amounts to pantothenate-deficient streptococci and its growth-promoting activity was found to be equivalent to $2 \times 10^{-7} M$ pantothenate. This concentration is inadequate to antagonize the effect on streptococci of concentrations of pantoyltaurine to which *Bact. coli* is resistant. Pantoyltaurine may, however, provoke the organism to produce more pantothenate. That it does not do so to a marked extent was shown by attempting growth of streptococci in pantothenate-deficient media to which different quantities were added of a filtrate of *Bact. coli* grown in the presence of pantoyltaurine. No streptococcal growth occurred. The small difference recorded under (*a*) between the inhibiting action of such a filtrate and that of an equivalent pantoyltaurine dilution may be due to small pantothenate production or a little destruction of pantoyltaurine. (*c*) The suggestion that sensitivity differences may be due to different permeabilities of the organisms is difficult to approach experimentally but pantoyltauramide showed no difference in titre to streptococci when it, the inoculum and pantothenate were added in different orders and stood for different lengths of time, up to 2 hr., before incubation. Nor did such standing render *Bact. coli* sensitive to the inhibitor. The differences in sensitivity are probably best referred to enzyme systems concerned with pantothenate (see Discussion).

E. Proteus vulgaris *and* morgani

Table 3 shows that the effect of inhibitors on *Proteus vulgaris* (2 strains) is roughly the same as with *Bact. coli*. A partial reversal of the inhibition of the hydroxybutyryl derivative ($2 \times 10^{-4} M$) with pantothenate ($10^{-3} M$) was, however, observed. The taurine derivatives were inactive.

Proteus morgani requires pantothenate for growth [Pelczar & Porter, 1940] and limiting quantities (*c.* $2 \times 10^{-8} M$) of the compound were present during testing of the analogues. Nevertheless no analogue in concentrations up to $2 \times 10^{-3} M$ reduced the rate or mass of growth of the organism, though reduction in the quantity of added pantothenate was immediately evident. This insensitivity made it possible to test the ability of the compounds to replace pantothenate in growth. γ-Hydroxy-*n*-valeryl-β-alanine, pantoyltaurine and homopantoyltaurine caused no growth in $2 \times 10^{-3} M$ solutions, and, as the organism is sensitive to $4 \times 10^{-9} M$ pantothenate, they have $< 2 \times 10^{-6}$ of the activity of pantothenate. γ-Hydroxy-*n*-butyryl-β-alanine, homopantothenate, dehydrohomopantothenate, desoxypantothenate and pantoyltauramide gave responses corresponding to *c.* 5×10^{-6} of their concentration of pantothenate. Responses sometimes varied in different specimens of the same compounds and these figures must be regarded as maximal. The structural specificity of pantothenate is then very great. Previously recorded results with other analogues and various organisms have shown only one compound (the ω-hydroxy derivative) of the many tested to be markedly active [Mitchell, Weinstock, Snell, Stanbury & Williams, 1940; Mitchell, Snell & Williams, 1940; cf. also Reichstein & Grüssner, 1940; Subbarow & Rane, 1939; Weinstock, May, Arnold & Price, 1940; and Woolley & Hutchings, 1939].

F. Staphylococcus aureus

Two unselected strains of *Staph. aureus* have been found to require pantothenate for growth within 2–3 days in an amino-acid medium based on that of Gladstone [1937]. Growth of the organism in pantothenate-deficient media has previously been found to be unaffected by pantothenate [Krauskopf, Snell & McCoy, 1939; Porter & Pelczar, 1941] and sometimes under undefined conditions the present strains did not respond to the compound. The divergence has not been further investigated as organisms needing pantothenate for optimal but not for minimal growth were of particular interest in the present study; the strains required pantothenate throughout the present tests.

The inhibitory effects of the analogues (Table 3) were similar to, but weaker than, those with *Bact. coli* and *Proteus vulgaris*. Homopantothenate, dehydrohomopantothenate and desoxypantothenate, capable of replacing pantothenate in *P. morgani* did so also, and to the same small extent, with staphylococcus. As was the case with *Bact. coli*, staphylococcus produced pantothenate during its slow growth in media not initially containing the compound, but in small quantities only; the final culture medium contained *c.* $10^{-8} M$ pantothenate.

Discussion

The function of pantothenate in growth of micro-organisms is not yet known. The finding that it affects the respiration of cells and tissues [Pratt & Williams, 1939] has been examined with respect to bacteria by Hills [1941] but the action has not been localized to particular systems or compounds. Other growth factors are known to affect metabolism in the role of coenzymes and the hypothesis that pantothenate, or derivatives of it, play such a part would be consistent with the small quantities adequate for growth and with its extreme specificity which has been emphasized in describing results with *P. morgani*, above. The inhibitions recorded in this account afford evidence of a different type for the suggestion that pantothenate takes part in essential enzyme reactions: the actions of pantoyltaurine, pantoyltauramide and homopantoyltaurine are clearly related to pantothenate, and their quantitative interactions have the character of competitive enzyme inhibitions. Such are probably due to an analogue occupying the active centres to which pantothenate must have access for normal activity.

A. *Taurine analogues: specificity to organism.* All organisms susceptible to pantoyltaurine are affected by pantoyltauramide and homopantoyltaurine where they have

been investigated. The susceptibilities of each organism to the three compounds are in the same order, pantoyltaurine being most inhibitory, pantoyltauramide less so and homopantoyltaurine least. This may be taken as indicating that the systems affected in streptococcus, *C. diphtheriae* and pneumococcus are similar. In strains of the latter organism, susceptibility followed pantothenate needs but *P. morgani* forms an exception to a further such generalization. It is thus unlikely that the systems affected by the taurine analogues are the same in all organisms, for while the quantities of pantothenate which exacting organisms need for optimal growth are approximately equal, the effects of a given inhibitor are markedly different (Table 4); e.g. *P. morgani* requires

Table 4. *Antibacterial indices of taurine analogues of pantothenate*

Organism	*dl*-Pantoyltaurine Molar ratio, C_I/C_M, necessary for: Partial inhibition	*dl*-Pantoyltaurine Molar ratio, C_I/C_M, necessary for: Total inhibition (=antibacterial index)	*dl*-Pantoyltauramide Molar ratio, C_I/C_M, necessary for: Partial inhibition	*dl*-Pantoyltauramide Molar ratio, C_I/C_M, necessary for: Total inhibition (=antibacterial index)
Streptococcus haemolyticus	20–100	500	500	2000
*Streptococcus lactis** / *Propionibacterium pentosaceum**	800–2600	8000		
Diplococcus pneumoniae	200	1000	2000–10,000	10,000–50,000
Corynebacterium diphtheriae				
Exacting (G1)	100	500	400	2000–10,000
Less exacting (GW1, G1156)	500	5000	10,000	—
Non-exacting (R7, R20)	—	—	—	—
*Lactobacillus arabinosus**	280–300	1000–2000		
*Lactobacillus pentosus** / *Leuconostoc mesenteroides**	8000–26,000	133,000		
Bact. coli / *Proteus vulgaris* / *Proteus morgani* / *Staph. aureus*	—	—	—	—
*Shigella paradysenteriae** / *Brucella abortus**	—	—		

The sign — indicates that inhibition was not observed with the highest ratio tested.

* Calculated from the data of Snell [1941].

practically the same quantity of pantothenate as the sensitive streptococcus, which is inhibited by 1/100th of the quantity of pantoyltaurine to which *morgani* is insensitive. The interpretation favoured, and expressed in the antibacterial indices—that pantothenate and, e.g., pantoyltauramide have in different organisms different relative affinities for essential enzymes—does not in itself imply functional similarity or dissimilarity in such enzymes.

The insensitivity of organisms not needing pantothenate was discussed under *Bact. coli* and it was concluded that resistance is not due to secondary effects such as destruction of the inhibitor, but to inherent factors which were not the production of large quantities of pantothenate. Sensitivity in different organisms is not completely correlated with any morphological, biochemical or staining characteristic normally used in bacteriological classification. Indeed all gradations, from complete insensitivity to inhibition of the highest degree encountered, are exhibited by strains of the one organism, *C. diphtheriae*. A correlation so far without exception is that all sensitive organisms require added pantothenate for optimal growth. This was particularly well marked in the *C. diphtheriae* strains, though again pantothenate production by the resistant strains was insufficient to antagonize the effects of a taurine analogue on a sensitive organism. This suggests that pantothenate produced *in situ* is a more effective reversing agent than that added. It is conceivably produced in the right place at the right time, or a

higher derivative which may be the actual functioning compound is synthesized by a route not involving free pantothenate. Parallel cases are the insensitivity of trained staphylococcus and *Bact. typhosum* to α-aminosulphonic acids, while their parent strains are inhibited in the presence of quantities of amino-carboxylic acids sufficient for normal growth. The same argument would apply to the insensitivity of *Bact. coli* to pantoyltauramide, but that of *P. morgani* cannot so be explained. Among other inhibitors, pyridine-3-sulphonamide affects organisms requiring added nicotinic acid, but not one which does not need the compound. Sulphanilamide, however, inhibits organisms not needing added *p*-aminobenzoic acid, as well as those which do [Rubbo & Gillespie, 1942].

B. *Taurine analogues: structural specificity.* The different degrees of inhibition of a given organism by the taurine analogues have been taken as indicating that these compounds have different affinities for enzymes utilizing pantothenate. Estimates of these affinities may be made by supposing that, at concentrations giving partial inhibition, the ratio C_I/C_M (see p. 419) is that of their Michaelis constants (K_m). This would be exactly so at C_I values giving half the normal enzyme velocities but the present methods do not measure such velocities; they give their indirect effects on growth. Taking a pantothenate concentration giving suboptimal growth ($10^{-8}M$, Table 1) as the approximate K_m (pantothenate, streptococcus system) then the K_m (pantoyltaurine, streptococcus system) is about $10^{-6}M$ and for pantoyltauramide about $10^{-5}M$. K_m (pantoyltaurine, *P. morgani* system) is presumably $>2\times10^{-3}M$. In distinction to these computations, the antibacterial indices of the inhibitors are practical values straightforwardly determined but difficult to assess theoretically. Reactions are not stopped completely by competitive inhibition and the complete bacteriostasis represented by the antibacterial index is presumably due to the rate of essential reactions dependent on pantothenate being lowered, not necessarily greatly, but to values below critical levels necessary for growth. Pantothenate is known to affect, directly or indirectly, reactions with many compounds [Hills, 1941] and observations of the effects of substances on gross bacterial growth cannot be expected to be interpreted in terms of only the enzymes primarily affected [cf. Harris & Kohn, 1941].

That pantoyltaurine is a more effective inhibitor than its amide, pantoyltauramide, is in contrast to the greater inhibitory powers which sulphanilamide possesses over sulphanilic acid. The compound affected by sulphanilic acid, *p*-aminobenzoic acid, is, however, a slightly weaker acid (*p*K 4·9) than is pantothenic acid (*p*K 4·4), though properties of the whole molecules are probably involved [cf. Albert & Goldacre, 1942]. Inhibitions by pantoyltaurine and the amide are both of the same type, i.e. competitive, unlike those of pyridine-3-sulphonic acid and its amide, which usually differed in type [McIlwain, 1940]. In the pyridine series it is probable, however, that the acid and amide inhibit most effectively different reactions, the sulphonic acid interfering more with nicotinic acid and the sulphonamide with nicotinamide. A simple natural derivative of pantothenic acid is not known, though much of the compound is 'bound' in normal cells.

Though the values for the antibacterial indices of pantoyltaurine show it to be a very effective inhibitor in comparison with, e.g. sulphanilamide, a very large excess of pantoyltaurine is required to interfere with pantothenate. This is understandable if an enzyme system concerned is regarded as having evolved in connexion with pantothenate. Reaction with the enzyme is a necessary preliminary to functioning of a coenzyme, but all compounds so reacting would not be expected to function: this may be offered in explanation of the greater structural specificity found to be necessary for pantothenate analogues to function as growth factors, over that required for them to inhibit growth.

C. *γ-Hydroxy-*n*-butyryl-β-alanine* is the most inhibitory of the other pantothenate analogues examined. Its action appears complementary to that of, e.g., pantoyltauramide in that the two compounds most powerfully affect different groups of organisms (Table 3), though *P. morgani* is affected by neither and streptococcus by both. Its effect

is greatest on organisms not requiring pantothenate. This cannot be entirely due to the richer media used in investigating pantothenate-requiring organisms (though the effect of γ-hydroxy-*n*-butyryl-β-alanine on *Bact. coli* is antagonized by, e.g., tryptic casein), for of the several *C. diphtheriae* strains tested in the same medium, some are susceptible to the compound and others are not. Again, the strain requiring pantothenate was insusceptible and the most inhibited strain did not require the compound. One relevant metabolic difference between organisms requiring pantothenate and those which do not is that the latter synthesize the compound. The negative correlation between pantothenate needs or pantoyltaurine inhibition, and γ-hydroxy-*n*-butyryl-β-alanine-inhibition might then be explained if this compound inhibited pantothenate *formation* by virtue of its similarity to the product of an enzyme reaction. Such synthesis, in *Bact. coli* and the non-exacting *C. diphtheriae* strains, is unaffected by pantoyltaurine. Bacteriostasis due to non-competitive inhibition of pantothenate formation should be reversed by addition of pantothenate; this is only rarely the case with inhibitions due to the hydroxybutyryl derivative. There were, however, previous indications that added pantothenate was not equivalent to the functioning compound formed *in situ* (p. 424). Inhibition of the formation of such a compound provides a reasonable mechanism for the action of the hydroxybutyryl derivative.

This discussion has shown the phenomena of inhibition by pantothenate analogues to be, in general, consistent with their acting by inhibition of enzymes concerned with pantothenate but has raised many points which can be settled only by techniques different from those employed here. Reasons have been given [McIlwain, 1942*b*] for expecting pantoyltaurine to be active *in vivo* against susceptible organisms. This should apply also to pantoyltauramide in the case of streptococcus.

Summary

Pantothenate must be supplied to many bacteria before growth will take place. The hypothesis that it is necessary because it takes part in enzyme reactions whose progress is necessary to growth is supported by the present findings. Pantothenate-needing organisms cause many diseases; there is more than enough pantothenate for their growth in animal tissues. It might, however, be possible to prevent them from using it: on the above hypothesis, pantothenate, to be used by an organism, must react with a particular part of it, and by analogy with other enzyme inhibitors it was expected that other compounds structurally related to pantothenate might displace it from the essential enzymes and prevent their functioning. Three such analogues did so, though they had less affinity for the enzymes than had pantothenate and needed to be present in many times its concentration to prevent growth. The compounds are pantoyltaurine, with an antistreptococcal index of 500 (i.e. 500 times the concentration of pantothenate present was required to prevent growth of streptococcus) and an antipneumococcal index of 1000; pantoyltauramide with values of 100 and 10,000 and homopantoyltaurine which is less active. Only organisms needing pantothenate have been found susceptible to these compounds; thus, exacting strains of *C. diphtheriae* are inhibited, but not those which do not need added pantothenate. Other analogues were inhibitory to several organisms not needing added pantothenate; they also may act by interfering with pantothenate metabolism as such organisms synthesized the compound.

I am greatly indebted to Mr D. E. Hughes for assistance throughout this work.

REFERENCES

Albert, A. & Goldacre, R. [1942]. *Nature, Lond.*, **149**, 245.
Barnett, J. W. & Robinson, F. A. [1942]. *Biochem. J.* **36**, 364.
Berkman, S., Saunders, F. & Koser, S. A. [1940]. *Proc. Soc. exp. Biol., N.Y.*, **44**, 68.
Evans, W. C., Handley, W. R. C. & Happold, F. C. [1939]. *Brit. J. exp. Path.* **20**, 396.
Fildes, P. [1938]. *Brit. J. exp. Path.* **19**, 239.
—— [1941]. *Brit. J. exp. Path.* **22**, 293.
Gladstone, G. P. [1937]. *Brit. J. exp. Path.* **18**, 322.
Harris, J. S. & Kohn, H. I. [1941]. *J. Pharmacol.* **73**, 383.
Hills, G. M. [1941]. *Chem. Ind.* **60**, 220.
Koser, S. A., Breslove, B. B. & Dorfman, A. [1941]. *J. infect. Dis.* **69**, 114.
Krauskopf, E. J., Snell, E. E. & McCoy, E. [1939]. *Enzymologia*, **7**, 327.
Mitchell, H. K., Weinstock, H. H. Jr., Snell, E. E., Stanbury, S. R. & Williams, R. J. [1940]. *J. Amer. chem. Soc.* **62**, 1776.
Mitchell, H. K., Snell, E. E. & Williams, R. J. [1940]. *J. Amer. chem. Soc.* **62**, 1791.
McIlwain, H. [1939]. *Brit. J. exp. Path.* **20**, 330.
—— [1940]. *Brit. J. exp. Path.* **21**, 136.
—— [1941*a*]. *Brit. J. exp. Path.* **22**, 148.
—— [1941*b*]. *Biochem. J.* **35**, 1311.
—— [1942*a*]. *Lancet*, **1**, 412.
—— [1942*b*]. *Brit. J. exp. Path.* **23**, 95.
Mueller, J. H. & Miller, P. A. [1941]. *J. biol. Chem.* **140**, 933.
Pelczar, M. J., Jr. & Porter, J. R. [1940]. *Proc. Soc. exp. Biol., N.Y.*, **43**, 151.
—— —— [1941]. *Proc. Soc. exp. Biol., N.Y.*, **47**, 3.
Porter, J. R. & Pelczar, M. J., Jr. [1941]. *J. Bact.* **41**, 173.
Pratt, E. F. & Williams, R. J. [1939]. *J. gen. Physiol.* **22**, 637.
Rane, L. & Subbarow, Y. [1940]. *J. biol. Chem.* **134**, 455.
Reichstein, T. & Grüssner, A. [1940]. *Helv. chim. Acta*, **23**, 650.
Robinson, R. [1940]. *J. chem. Soc.* 505.
Rubbo, S. D. & Gillespie, J. M. [1942]. *Lancet*, **1**, 36.
Snell, E. E. [1941]. *J. biol. Chem.* **139**, 975; **141**, 121.
Subbarow, Y. & Rane, L. [1939]. *J. Amer. chem. Soc.* **61**, 1616.
Tamura, J. T., Tytell, A. A., Boyd, M. J. & Logan, M. A. [1941]. *J. Bact.* **42**, 148.
Weinstock, H. H., Jr., May, E. L., Arnold, A. & Price, D. [1940]. *J. biol. Chem.* **135**, 343.
Woods, D. D. [1940]. *Brit. J. exp. Path.* **21**, 74.
Woolley, D. W. & Hutchings, B. L. [1939]. *J. Bact.* **38**, 285.
Wright, L. D., McMahan, J. R., Cheldelin, V. H., Taylor, A., Snell, E. E. & Williams, R. J. [1941]. Univ. Texas Publication No. 4137, 38.