Biochemical Characterization of the Actions of Chemotherapeutic Agents

3. RELATIONSHIPS BETWEEN METABOLIC AND GROWTH INHIBITIONS BY PANTOTHENATE ANALOGUES: THEIR STRUCTURAL AND SPECIES SPECIFICITY

By H. McILWAIN (Leverhulme Research Fellow) AND D. E. HUGHES, *Department of
Bacterial Chemistry (Medical Research Council), The University, Sheffield*

(Received 4 January 1945)

It was reported in Part 2 (McIlwain & Hughes, 1944) that pantooyltaurine inhibited a process of inactivation of pantothenate which was brought about by haemolytic streptococci. Characteristics of the metabolic inhibition suggested it to be connected with the inhibition of growth of the organism, which was also caused by pantooyltaurine, and which was shown to be the basis of its chemotherapeutic effects (McIlwain & Hawking, 1943). Little is known either of the reactions undergone by pantothenate in living organisms, or of the types of interaction which may underlie the chemotherapeutic action. Further evidence bearing upon the relationship between the two inhibitory effects of pantooyltaurine has therefore been sought. The present investigation compares the actions (1) of a series of pantothenate analogues upon the two processes in haemolytic streptococci and (2) of pantooyltaurine upon the two processes in a series of organisms.

The effects of the compounds upon growth were expressed as the concentrations of them required to affect visible growth of the organisms in the presence of defined concentrations of pantothenate, which antagonized the growth-inhibitory effects of several of the compounds. Certain analogues have been examined in this respect previously (McIlwain, 1942; Barnett, 1944; Barnett, Dupre, Holloway & Robinson, 1944; Madinaveitia, Martin, Rose & Swain, 1944). Inactivation of pantothenate was measured by microbiological assay of the amount of this compound remaining, in solutions which initially contained pantothenate and the substances under examination, after exposure of these solutions to the organisms studied.

EXPERIMENTAL

Pantothenate assay

Only microbiological methods were available for assay of the quantities of pantothenate concerned in the present experiments, and of these methods Pelczar & Porter's (1941) use of *Proteus morganii* appeared most promising, as the organism is rela-

tively insensitive to inhibition by pantothenate analogues which would be present in certain of the reaction mixtures. As optimal growth of this organism has not yet been described in chemically defined media (see Pelczar & Porter, 1943), and as it was desired to assay metabolic mixtures and bacterial extracts without separation of pantothenate, a complex basal medium similar to that used in certain metabolic studies with pantothenate was employed.

Assay medium. To the stock solution (20 ml.) of McIlwain & Hughes (1944) (a hydrolysate of casein prepared in the presence of titanium salts), were added: KH_2PO_4 , 13.6 g.; $(\text{NH}_4)_2\text{SO}_4$, 0.5 g.; NH_4Cl , 0.5 g.; anhyd. MgSO_4 , 20 mg.; $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$, 10 mg.; 10^{-2}M -nicotinamide, 1 ml. The whole was boiled in about 600 ml. of water for 5 min., cooled, made to 700 ml. with water, filtered, distributed in 100 ml. portions and autoclaved to sterilize. The assay medium was prepared from this solution by adding, per 100 ml., immediately before use: a cystine-glucose supplement (10 ml.); N-NaOH (2 ml.); water (6 ml.); and an opacity-standardized suspension of *Pr. morganii* containing about 10^6 cells. The supplement was prepared by dissolving cystine (0.1 g.) and glucose (5 g.) in N-HCl (10 ml.), diluting to 50 ml., and filtering to sterilize.

Assay. A 10^{-2}M solution of Ca^{++} pantothenate in phosphate buffer of pH 6.5 was filtered to sterilize and kept in sealed tubes in a refrigerator. The following dilutions were prepared from it fortnightly in the same buffer: 10^{-4} , 10^{-6} , 10^{-7} and 1.5, 1.25, 1.00, 0.75, 0.5, $0.25 \times 10^{-8}\text{M}$. Reaction mixtures containing pantothenate were sterilized by filtration at pantothenate concentrations of 10^{-5} – 10^{-6}M and diluted aseptically for assay to a range of concentrations expected to be about 0.5 – $1.5 \times 10^{-8}\text{M}$. One ml. each of the series of standard solutions (15 – 2.5×10^{-12} g.-mol. of pantothenate) and three dilutions of each solution to be assayed were pipetted into $6 \times \frac{3}{4}$ in. tubes (cleaned with chromic-sulphuric acid, plugged and sterilized), 5 ml. of the already inoculated assay medium added, and the tubes incubated at 37° . Between 27 and 48 hr. after inoculation the density of growth was determined

in a Hilger photoelectric absorptiometer, using neutral filters and cells taking 0.5 ml. of solution.

Choice of conditions of assay

(a) *Organisms*. The growth of four strains of *Pr.morganii* with varying quantities of pantothenate was examined; the response of the two strains used in assay is shown in Fig. 1. Strain no. 2818 of the National Collection of Type Cultures (N.C.T.C.) has been obtained from the National Collection on two occasions separated by 3 years and the older specimen maintained by frequent subculture in this laboratory, but the response of the two specimens did not differ appreciably. Strain N.C.T.C. 1707 and a laboratory strain gave curves similar to that of N.C.T.C. 2818, but the laboratory strain 2 was more sensitive to pantothenate. Inocula for assays were taken from 16–18 hr. cultures on broth-agar slopes; older inocula tended to give erratic results. Prior growth of inocula in the assay medium was not advantageous. Response to pantothenate with inocula of about 10^4 cells/100 ml. was slower than with the number ($c. 10^6$) adopted. It was also less directly related to the quantity of pantothenate, and this was again the case with inocula of $c. 10^9$ cells, which, however, offered no advantage, in speed of growth, over the size of inoculum chosen.

Fig. 1. Pantothenate assay by means of *Proteus morganii*. The relation between growth of two strains at 26 (○) and 48 (●) hr. after inoculation, and the quantity of pantothenate.

Readings between 27 and 48 hr. after inoculation were found equally satisfactory, but earlier or later readings less so. A little growth only took place between those times (Fig. 1). The test organism during assays formed both a diffuse growth and a deposit which easily dispersed on shaking. While the conditions of assay were being investigated,

readings of samples (1.5 ml.) were taken at intervals while incubation of the bulk continued.

Dilution in the basal medium of a suspension of *Pr. morganii*, grown under the conditions of assay, and measurement of the optical densities of the resulting solutions, showed that, within the limits of accuracy of reading, densities were proportional to the quantity of organisms added. The readings were: original suspension, 0.295; 8 ml. diluted to 10 ml., 0.24; 6 ml. to 10 ml., 0.182; 4 ml. to 10 ml., 0.119; 2 ml. to 10 ml., 0.062; 1 ml. to 10 ml., 0.028.

(b) *Medium*. The casein hydrolysate was the only medium-constituent not chemically defined; three caseins of different origins have, however, behaved similarly. The assay medium when compared with water gave a reading of 0.056 (indicating satisfactorily little colour) which did not change after growth of the organisms in it for 2 days. The medium was highly buffered to avoid pH changes following from the active glucose metabolism of *Pr. morganii*; the change of pH over the range chosen in assay was 0.2 unit or less in 30 hr. Phosphate buffer afforded a more regular response to pantothenate than did bicarbonate; the high phosphate concentration was not deleterious to growth.

The medium was adequate to give a specific response to pantothenate. A group of nine substances which can function as growth factors for some micro-organisms (those of the group A addenda of McIlwain, 1944, not already included in the present medium) and glutathione, glutamine, and a cozymase preparation, did not increase the readings given by 10 and 20×10^{-12} g.-mol. pantothenate. Acid- and alkali-treated peptone preparations (Pelczar & Porter, 1941) and asparagine did not increase, but in some concentrations decreased, the response to pantothenate. A solution to which streptococci had been exposed under the conditions chosen for pantothenate metabolism (see below), did not affect the readings with standard pantothenate solutions. Pantoyltaurine (0.5×10^{-6} g.-mol.) did not affect the assay of 5 or 30×10^{-12} g.-mol. of pantothenate.

Pantothenate inactivation

Reactions were carried out anaerobically in Warburg vessels shaken at 37° and containing, in 3.3 ml.: organisms (of dry wt. 1–10 mg.), pantothenate ($5\text{--}20 \times 10^{-9}$ g.-mol.), MgSO_4 (0.5×10^{-6} g.-mol.), KH_2PO_4 (10×10^{-6} g.-mol.), NaHCO_3 (150×10^{-6} g.-mol.), glucose (100×10^{-6} g.-mol.), yeast adenylic acid (0.7×10^{-6} g.-mol.) and the substances whose effects were being studied. Accelerating effects of the Mg, adenylic acid and phosphate had been observed in some preliminary experiments with streptococci, and these substances were therefore added

in all cases. With these additions and with the present methods of growing and harvesting the organisms, the presence of hydrolyzed casein (cf. McIlwain & Hughes, 1944) was not essential to pantothenate metabolism. The highest concentrations of pantoyletaurine which were tested were 0.075M, and, for comparison of its effects upon different organisms, successive twofold dilutions were examined. CO₂ evolution was measured during pantothenate metabolism, but under the circumstances considered in this paper it was not found to be affected by the presence of the pantothenate analogues.

Organisms. The material used in metabolic experiments was grown aerobically from inocula of about 10⁶ cells in a mixture of: the basal medium of McIlwain & Hughes (1944) (70 ml.); M-glucose (5 ml.); 0.02M-arginine (2 ml.); 0.02M-glutamine (2 ml.); group A of McIlwain (1944) (5 ml.) (containing one-twenty-fifth of the concentration of Fe salts there described); N-NaOH (1 ml.); 10⁻⁴M-pantothenate (1 ml.); water (17 ml.).

The β -haemolytic streptococci studied included the 'Richards' strain and another group A strain; the previously employed group G strain was used in the majority of the experiments in which pantothenate analogues were compared. Two strains each of *Streptococcus faecalis*, *C. diphtheriae* (a *mitis* and a *gravis* strain) and *Pr. Morganii* were examined. Organisms were collected by centrifuging and washed twice with volumes of saline one-tenth of those of the original culture.

Growth inhibition

The medium of the previous paragraph, dispensed in 10 ml. portions and containing various concentrations of pantothenate, was employed; the general conditions followed those of McIlwain (1942). Panto-

thenate analogues were added aseptically, as solutions which had been sterilized by filtration, in concentrations up to 10⁻²M; if inhibitory they were examined over their range of action in twofold dilutions.

RESULTS

Structural specificity

Table 1 gives the formulae of the compounds used and summarizes their effects upon the growth of, and pantothenate inactivation by, *Streptococcus haemolyticus*. With optically active analogues, only the *dl*-forms were examined. Compounds were judged to be without inhibitory effect upon growth when no diminution of growth was seen by visual examination between 16 hr. and 7 days after inoculation of solutions containing 10⁻⁸M-pantothenate and the substance under investigation in concentrations up to 2 $\times$ 10⁻³M. Antagonism by pantothenate to growth inhibition by an analogue was considered absent if inhibition in the presence of 10⁻²M-pantothenate was not distinguishable from that in the presence of 5 $\times$ 10⁻⁸M-pantothenate. The characteristics of the compounds of Table 1 (numbers refer to that table) suggest their classification in the following three groups:

(i) *Compounds affecting neither pantothenate inactivation nor growth.* β -alanine, pantolactone and taurine (products of simple hydrolysis of pantothenate and pantoyletaurine) inhibited neither growth nor pantothenate inactivation when present in M concentrations up to 4000 times those of the concomitant pantothenate. A pantothenate homologue (6) and a substituted pantoyletaurine (7) were also without action (Table 2).

Table 1. *Pantothenate analogues, and their effects upon the growth of β -haemolytic streptococci*

Symbols used in formulae below:

P—=CH₂OH(CH₂)₂.C.CHOH.CO—

— β -al=—NH.CH₂.CH₂.COOH

Ts=toluene *p*-sulphonyl-

		Effect of analogues on growth	
		Inhibition	Antagonism of inhibition by pantothenate
A. Compounds not affecting pantothenate inactivation			
(1) to (3)	Pantolactone; β -alanine; taurine	0	—
(4)	CH ₂ OH.CH ₂ .CH ₂ .CO— β -al	+	0
(5)	CH ₂ .CH ₂ OH.CH ₂ .CH ₂ .CO— β -al	+	0
(6)	CH ₂ OH(CH ₂) ₂ .C.CHOH.CH ₂ .CO— β -al	0	—
(7)	CH ₂ OH(CH ₂) ₂ .C.CHOTs.CO—NH.CH ₂ .CH ₂ .SO ₃ H	0	—
B. Compounds inhibiting pantothenate inactivation			
(8) (pantamide)	P—NH ₂	+	+
(9) (panthydrazide)	P—NH.NH ₂	+	+
(10) (pantoyletaurine)	P—NH.CH ₂ .CH ₂ .SO ₃ H	+	+
(11) (pantoyletauramide)	P—NH.CH ₂ .CH ₂ .SO ₃ NH ₂	+	+
(12)	P—NH.CH ₂ .CH ₂ .SO ₂	+	+
(13)	(P—NH.CH ₂ CH ₂) ₂ SO ₂	+	+
(14)	(P—NH.CH ₂ .CH ₂) ₂ S ₂	+	+

Table 2. *Pantothenate inactivation by bacteria in the presence of pantothenate analogues*

20×10^{-6} g.-mol. of pantothenate were initially present in each reaction mixture. The quantity remaining, after a period (constant with a given batch of organisms) of about 1 hr. at 37° , was determined.

Organisms			Analogues and quantities (g.-mol. $\times 10^{-6}$) used. Numbers of analogues refer to Table 1	Panto- thenate inacti- vated (g.-mol. $\times 10^{-9}$)
Strain and batch		Dry wt. (mg.)		
<i>Strep. haemolyticus</i>				
(group G)	I	8.0	0	16.9
"	I	8.0	Pantoyltaurine, 0.4	1.1
"	I	8.0	β -Alanine, 40 or panto- lactone, 40; or tau- rine, 40 or analogue (7), 4 or 40	16.7-17.2
"	II	9.5	0	18.0
"	II	9.5	Pantoyltaurine, 0.4	1.2
"	II	9.5	Analogues (4), (5), or (6), 4 or 40	17.6-18.1
"	III	4.4	0	4.0
"	III	4.4	Pantoyltaurine 0.012	3.7
"	III	4.4	" 0.032	3.4
"	III	4.4	" 0.120	1.8
"	III	4.4	" 0.320	1.2
<i>C. diphtheriae</i>				
strain 49 (<i>gravis</i>)		3.7	0	4.1
"		3.7	Pantoyltaurine 0.5	3.5
"		3.7	" 1.0	1.9
"		3.7	" 2.0	0.7
"		3.7	" 10.0	0.3

(ii) *Substances not affecting pantothenate inactivation but inhibitory to growth.* A *bisnordeoxypantothenate* (4) and an *isonordeoxypantothenate* (5) did not affect the process of pantothenate inactivation when in M concentrations 4000 times those of pantothenate, but inhibited growth. The effect of these two compounds upon growth differed from that of

the group (iii) compounds (below), in the failure of pantothenate to antagonize the inhibition of growth.

(iii) *Both growth and pantothenate inactivation were inhibited* by relatively low concentrations of pantoyltaurine (10) and its amide (11); by the amide (8) and hydrazide (9) of pantoic acid and by *bis*(pantoyl- β -aminoethyl) sulphone (13) and the corresponding disulphide (14). The effects of these compounds upon pantothenate metabolism by the group G streptococcus were determined in each case by experiments of the type quoted in Table 2 (batch III). Studies, which will be reported later, of the kinetics of pantothenate inactivation have shown that experiments of this type, in which pantothenate usage is kept to about one-quarter of the total amount present, yield valid measures of the activities of the inhibitors. By interpolation between such results, the concentration of inhibitor corresponding to 50 % inhibition of pantothenate inactivation was determined. These concentrations are compared in Table 3 with the concentrations of the inhibitors which affect growth. Growth was not measured quantitatively, but concentrations of the inhibitors were found which were minimal for just observable inhibition, and which completely inhibited visible growth during 2 days. Where possible these concentrations were determined in the presence of the same concentration of pantothenate as was used in metabolic experiments, but where this would have necessitated a concentration of inhibitor $> 10^{-2}$ M, the pantothenate concentration used in growth was lowered. It was not necessary to decrease it below 5×10^{-8} M, which is adequate for almost optimal growth of the organism. Pantothenate antagonizes the action of pantoyltaurine and, to allow for the effects of varying pantothenate concentrations, the quantities of pantoyltaurine necessary for inhibition have been expressed in Table 3 as ratios, obtained by dividing their concentrations by that of the concomitant

Table 3. *Comparison of effects of pantothenate analogues (8)-(14) on bacterial growth and metabolism*

Compound (numbers refer to Table 1)	Organism	Concentration of compound (as multiple of the concentration of pantothenate present) necessary for		
		50% inhibition of pantothenate inactivation (a)	Just observable inhibition of growth (b)	Prevention of visible growth for 2 days (c)
Pantoyltaurine (10)	(1) <i>Strep. haemolyticus</i> (group G)	5.0	40	200
"	(2) <i>C. diphtheriae</i> strain GI (<i>mitis</i>)	13.0	100	500
"	(3) <i>C. diphtheriae</i> strain 49 (<i>gravis</i>)	45	2,000	10,000
"	(4) <i>Strep. faecalis</i> strain II	100	2,000	15,000
"	(5) <i>Proteus morganii</i> strain N.C.T.C. 2818	1,450	50,000	200,000
Sulphonamide (12)	<i>Strep. haemolyticus</i> (group G)	13	160	800
Pantoyltauramide (11)	"	25	400	2,000
Panthydrazide (9)	"	60	800	4,000
Pantamide (8)	"	270	4,000	20,000
Sulphone (13)	"	350	6,600	40,000
Disulphide (14)	"	520	8,000	40,000

pantothenate. The effect of a given ratio of pantooyltaurine and pantothenate concentrations upon growth of the present organism is only approximately constant and varies with the absolute concentrations of the compounds. The variation is, however, small in comparison with the range of values of Table 3. Approximate constancy has also

Fig. 2. Correlations between the effects of pantothenate analogues on pantothenate inactivation and growth.

(a) Effects of seven different analogues (numbered according to Table 1) on *Streptococcus haemolyticus*.

(b) The effect of pantooyltaurine on five bacterial strains (numbered according to Table 3).

Ordinates: logarithms of ratios of concentration of inhibitor to concentration of concomitant pantothenate which (○) just affect growth and (●) which inhibit growth for 2 days. *Abscissae*: logarithms of ratios of inhibitor/pantothenate which inhibits pantothenate inactivation by 50%.

been shown in the effects of a given ratio of pantooyltauramide, panthydrazide, the sulphonamide derivative (12), the sulphone (13) and disulphide (14) upon growth of the present organism and of a group A haemolytic streptococcus. Expressed as such ratios, the inhibitory powers of the compounds studied decreased from pantooyltaurine with a ratio of 40:1 needed to have an observable effect on growth, to the disulphide (14), required in a ratio of 8000:1 to produce the same effect. The seven compounds of Table 3 fell into the same order when

their activity in inhibiting pantothenate inactivation was considered. The concentrations of compounds causing the different types of inhibition are not directly proportional; the ratio of the values of column (b) to (a) of Table 3 varies, with different analogues, from 8 to 19, and of column (c) to (a), from 40 to 110. The variation is, however, progressive, increasing with the increasing concentrations required of the less active compounds, and it is evident that a close connexion exists between the quantities of inhibitor required for the two effects. This is exhibited in Fig. 2a, and is especially striking as the concentrations required for a given growth effect vary over a 200-fold range. The curves of Fig. 2a are approximately linear, with slopes just greater than unity; graphic extrapolation would give positive intercepts on the ordinate. These characters reflect the lack of direct proportionality between values affecting metabolism and growth, and the ability of the compounds to inhibit metabolism (pantothenate inactivation) by 50% at concentrations which are without observable effects on growth.

Species specificity

The pantooyltaurine resistance of bacteria which synthesize pantothenate has been discussed previously (McIlwain, 1942, 1943). The present report concerns organisms requiring added pantothenate for growth, and species were chosen which required, for inhibition of their growth, widely differing minimal concentrations of pantooyltaurine. These organisms were all found to inactivate pantothenate during various metabolic processes which will be described in detail elsewhere. The effect of pantooyltaurine upon their pantothenate inactivation was compared during glycolysis, which all the organisms carried out at rates which did not greatly differ with the different organisms. Glycolysis was also a major process during the growth experiments with which the pantothenate metabolism was being compared.

The effect of pantooyltaurine upon pantothenate inactivation by a strain of *C. diphtheriae* is shown in Table 2. From such values the ratio of pantooyltaurine to pantothenate concentrations required for 50% inhibition of pantothenate inactivation was obtained for each organism. The remarks of the previous section respecting the use of such ratios apply also to the present problem, as the effects upon growth of given ratios of inhibitor/pantothenate have been found to be approximately constant in the following cases: of pantooyltaurine, pantooyltauramide, the sulphone (13) and disulphide (14) on growth of *C. diphtheriae*; of pantooyltaurine on growth of *Strep. faecalis* and *Pr. morganii*. In Table 3 the organisms are arranged in order of the sensitivity of their growth to pantooyltaurine. This is seen also to be the order of sensitivity of their

pantothenate metabolism. Again the values for the two types of inhibition are not directly proportional, but correlation between the values is shown in Fig. 2*b* and is of the same type as that of Fig. 2*a*.

A full range of comparative experiments was performed only in the case of pantoyltaurine. The species-specificity of other analogues was not the same. The sulphone (13) and disulphide (14) inhibited two strains of *C. diphtheriae* in concentrations lower than those producing comparable effects on *Strep. haemolyticus*. This order of sensitivity reverses that shown by the same organisms to pantoyltaurine. These findings do not affect the significance of the correlations of Fig. 2, but suggest that other relationships, such as that of Fig. 2*a*, might be obtained by examination of other organisms.

DISCUSSION

The action of pantoyltaurine upon bacterial growth was initially characterized as a bacteriostasis antagonized by pantothenate, and its action upon metabolism as an inhibition of pantothenate inactivation without corresponding inhibition of an energy-yielding process such as glycolysis. A connexion between the two effects of pantoyltaurine is now supported by the following evidence.

(1) Six other compounds which, like pantoyltaurine, inhibited growth and whose inhibition was antagonized by pantothenate, also inhibited pantothenate inactivation. Seven compounds whose effects on growth were without those characteristics did not affect the pantothenate inactivation. Of these seven, two are of special interest because, although they did inhibit growth, this inhibition was not antagonized by pantothenate and thus lacked the initial criterion which grouped together compounds acting in the manner of pantoyltaurine. Thus the two compounds whose action would be suggested by growth experiments to be independent of pantothenate (though the compounds, 4 and 5 of Table 1, are structurally related to pantothenate) have also been shown to be without effect on the particular reaction of pantothenate which is studied in the present experiments. The behaviour of these two compounds thus supports the general connexion between the actions of pantoyltaurine-like compounds.

(2) The seven compounds which inhibited pantothenate inactivation were required in widely different concentrations to produce a given effect, but their activities placed them in the same order as inhibitors of growth, as of inhibitors of the metabolic effect. Moreover, the concentrations required of the different compounds to produce a given metabolic and growth effect were quantitatively correlated (Fig. 2*a*).

(3) A similar correlation existed between the concentrations of pantoyltaurine which were re-

quired to inhibit growth and pantothenate inactivation with five different organisms (Fig. 2*b*).

The correlations show metabolic and growth inhibitions to be very closely connected. Thus the process of pantothenate inactivation may itself be an essential part of growth; or it may be associated with reactions which are necessary to growth. Morel (1941) regards a process which in many ways is similar to the present one as a 'wearing out' of a coenzyme during its functioning. If such a view be taken of pantothenate inactivation, it could be considered an index of pantothenate functioning, this latter being the process essential to growth. On the other hand, the pantothenate metabolism of the present non-proliferating suspensions may be a process which itself is directly coupled with growth when the presence of other nutrients permits this to occur.

Consideration of the results as they relate to the mode of action of pantoyltaurine, shows that they are consistent with the inhibition of pantothenate inactivation (or of its associated processes) being responsible for the effects of the inhibitor on growth. In the course of establishing the present correlations, a type of action similar to that of pantoyltaurine on haemolytic streptococci has been shown to be possessed by many related compounds, and upon several other bacterial species. The process of pantothenate inactivation is also seen to be widespread.

Among the compounds examined, a connexion also exists between structure and action, in that particular types of variation in the pantothenate molecule are associated with compounds capable of inhibition of pantothenate metabolism. Compounds which can be considered as derived from pantothenate through alteration in its hydroxy acid portion do not affect pantothenate metabolism, though they may affect growth by other means. The compounds inhibiting pantothenate inactivation are altered in the amino-acid portion only. These generalizations include compound (7) which is altered in both portions and does not affect pantothenate metabolism; the tosyl substituent has in this case converted the most active inhibitor of metabolism to an inactive compound. An aromatic sulphonic acid derivative does not have this effect when attached to the amino-acid portion of the molecule in analogue (12); the substituent groups are not, however, identical.

SUMMARY

1. Strains of streptococci, of *C. diphtheriae* and of *Proteus morganii* required widely different concentrations of pantoyltaurine to inhibit growth in the presence of a given concentration of pantothenate. They all inactivated added pantothenate (destroying its growth-promoting properties for the organisms) during glycolysis; pantoyltaurine inhi-

bited this inactivation. The concentrations of pantooyltaurine required for inhibition of the pantothenate metabolism by different organisms varied over a 300-fold range but were correlated with the concentrations required for inhibition of growth of the respective organisms.

2. Of 14 compounds structurally related to pantothenate, seven, which inhibited growth of haemolytic streptococci, inhibited their inactivation of pantothenate. The activities of the compounds in inhibiting the inactivation of pantothenate were correlated with their activities in inhibiting growth. Five compounds affected neither process; two com-

pounds which inhibited growth and which were not antagonized by pantothenate did not affect pantothenate inactivation.

3. Of the compounds examined, those which inhibited pantothenate inactivation were all amides of pantoic acid; those not containing an intact and unsubstituted pantoic acid portion did not affect pantothenate inactivation.

We are greatly indebted to Drs Barnett and Robinson, and to Dr Swain, for gifts of pantothenate analogues; to the Department of Bacteriology of this University for bacterial strains, and to Miss L. Whipple for assistance during the development of the assay method.

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The Activation of Jack-bean Arginase by Cobalt, Manganese, and Iron

By A. B. ANDERSON, *Biochemistry Laboratory, Department of Pathology of the University and Royal Infirmary, Glasgow*

(Received 8 January 1945)

The hydrolysis of arginine by preparations of jack-bean 'urease' in the presence of cobaltous ions was reported by Hellerman & Perkins (1935). Stock, Perkins & Hellerman (1938) showed that an arginase present in jack-bean extracts was strongly activated by cobalt, by manganese, and to a less extent by nickel. Cobalt produced a shift in the optimal pH from 9.0 to 7.7, and at the latter reaction the metal was necessary for activity. The present paper deals with the activation of this enzyme by cobalt, manganese, and iron, and with a preliminary investigation into the nature of the substance activated.

METHODS

Enzyme preparation. Jack-bean urease powder (Peters & Van Slyke, 1932) was used unless otherwise stated. The powder formed a stable, reproducible source of arginase and urease combined. A weighed amount of the powder was dissolved in distilled water afresh for each experiment.

Buffers. Clark & Lub's phosphate buffers and Sorensen's glycine buffers were used, allowances being made for the temperature coefficient of the latter (Clark, 1928). Clark & Lub's borate buffer could not be used, as borate inactivates urease. In a few experiments the methylglyoxaline buffers

of Kirby & Neuberger (1938) were used, but their high temperature coefficient made them less suitable.

Hydrolysis. The reaction was carried out in the apparatus for the determination of urea described by Kay & Sheehan (1934). In the reaction tube were placed: 1 ml. urease solution, 5 ml. buffer, 1 ml. activating metal solution; the racks of tubes were placed in a bath at 40°; after 10 min. 1 ml. of a solution of *D*-arginine hydrochloride (2.4 g./100 ml.) was added, and the tubes containing 8 ml. total volume were stoppered and connected to the receiving tubes. The reaction mixture was stirred at intervals by drawing a gentle current of air through the apparatus. At the end of 1 or 2 hr. the tubes were cooled rapidly, and the reaction was stopped by adding 20 ml. of saturated potassium carbonate solution to each tube. The ammonia formed was then aerated and determined by titration in the usual manner.

RESULTS

The activation by cobaltous ions. Preliminary experiments confirmed the finding of Stock *et al.* (1938) that the hydrolysis of arginine by jack-bean urease in the presence of cobaltous ions is a pseudomonomolecular reaction. Two factors in the reaction were determined: first, the relationship between the concentration of cobalt ion and the concentration of