Pantothenic Acid Antagonists

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One of the earliest planned antimetabolites was pantoyltaurine, reported by Snell in 1941[1] and obviously patterned after the sulfonamides, whose action had only recently been shown to be reversed by *p*-aminobenzoic acid. Pantoyltaurine was a competitive inhibitor of pantothenate in micro-organisms requiring pantothenic acid as a growth factor, and was claimed at first to produce a deficiency of this vitamin in animals, although this could not be confirmed. Subsequently a great many antimetabolites based on pantothenic acid have been made and found generally to follow this same pattern: they inhibit micro-organisms requiring pantothenate as a growth factor but are relatively inactive against others and, with one exception, none has produced a pantothenate deficiency in animals. The recent elucidation and synthesis of pantethine[2] and the availability of micro-organisms using pantethine and pantothenic acid with varying degrees of efficiency presented an opportunity to study new antimetabolites of this vitamin.

Pantoyltaurine and its amides, which correspond even more closely in pattern to the sulfonamides, have been studied extensively in several species, including animals.[3,4] This was before it was fully realized what drawbacks accompany the use in animals of antimetabolites based on vitamins. Some of these compounds successfully reduced experimental streptococcus infections in certain animals, but the required dose was impractical. The results were better in rats than in mice because the blood concentration of pantothenic acid was much higher in mice.

Since that time, many antagonists of pantothenic acid have been prepared, some of them having interesting characteristics. The majority of them have resulted from alteration in the β-alanine part of the molecule; some have involved changes in the pantoyl portion, and in a few, both parts of the molecule were altered. An interesting but not very effective antagonist, pantothenyl alcohol, was introduced by Snell in 1945.[5] It was active *in vitro* against micro-organisms requiring pantothenic acid, but on being ingested by animals was converted to pantothenic acid and had the usual vitamin activity of this compound. Pantothenone, a phenylketonic derivative of pantothenic acid, was studied by Woolley,[6] who found it to inhibit many species, but its effects were reversed by pantothenic acid only in those organisms requiring an exogenous source of this vitamin. This antagonist, as well as some of the substituted amides of pantoyltaurine,[7] have shown activity against malarial infections.

A Pantothenic Acid Antagonist

A potent antagonist of pantothenic acid, made by substituting methyl for hydrogen in the terminal group of the pantoyl part of the molecule, was studied by Drell and Dunn in 1946.[8] The compound was called ω-methylpantothenic acid and had been prepared and briefly studied by a graduate student in Henze's laboratory at the University of Texas three years before. He had noted that it lacked vitamin activity but did not detect its inhibitory action. In their first study of this compound, Drell and Dunn reported that it was a competitive inhibitor of pantothenate in many species of lactobacilli requiring this vitamin; later they reported[9] success in producing a pantothenate deficiency in mice and rats which was also competitive in nature. Shils[10] has since observed a decreased ability

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of rats to acetylate sulfanilamide when receiving ω-methylpantothenate. Recently, Bean and Hodges[11] have reported success in producing a pantothenate deficiency in four volunteer human subjects by giving them ω-methylpantothenic acid. This seems to be the first report on the intentional development of a pantothenate deficiency in humans. Since this work with humans, as well as Drell and Dunn's experiments with rats, have given results contrary to our limited experiments with rats, to be described later, it may be pertinent to comment on the Bean and Hodge report. These workers fed a purified and chiefly synthetic diet, supplemented with vitamins other than pantothenic acid, to their three volunteers, following preliminary experiments with one subject. The diet alone admittedly caused some biochemical alterations. This diet, supplemented with pantothenic acid, was given during a 12-day preliminary period. During the next 35 days the pantothenic acid was replaced with 0.5 gram ω-methylpantothenic acid daily. Then a period was started during which the antagonist was continued but it was supplemented with 4-gram daily doses of pantothenic acid. After six days on this regime the subjects were in such bad shape that the schedule was abandoned and emergency therapy of unstated nature instituted. It was assumed that the symptoms exhibited by the subjects were caused by the antagonist, although no individuals were maintained on the control diet during the experimental period; furthermore, six days of treatment with the relatively huge dose of 4 grams pantothenic acid, compared to 0.5 gram antagonist, did not counteract the effects of the latter. This would tend to cast doubt on the specificity of the symptoms which were reportedly due to the antagonist.

Experimental

The generally accepted microbiological procedures were used in our experiments with antagonists of pantothenic acid. Cultures were grown in Evelyn test tubes containing 10 ml medium, so that the amount of growth could be determined directly by reading in the colorimeter. Inocula were carefully standardized by turbidity readings. The inhibition index was determined by dividing the amount of metabolite needed for complete growth into the amount of antagonist required to produce half inhibition.

The compound studied most extensively was ω-methylpantethine. The inhibition indices obtained for several preparations of this compound are compared in Table I. It may be noted that the indices obtained after 18 hours' incubation vary, and even some of the preparations which had correct analytical figures gave low microbiological activity. As was the case with pantethine, which is also noncrystalline, the only meaningful criterion of purity available was the microbiological assay. Another characteristic which all these preparations of ω-methylpantethine had, in varying degrees, was a tendency to lose their inhibitory activity after increased incubation time.

TABLE I

Inhibition Indices for Different Lots of ω-Methylpantethine on *L. Helveticus*

Preparation Number	Origin	Inhibition index			
		18 hr	24 hr	40 hr	72 hr
03983	Snell	1400	1700	>4000	—
79-2147	Wittle[1]	700	800	>4000	—
80-2147	Wittle[2]	330	700	>4000	—
3771	Cavalla	1200	1200	>4000	—
16-2357	Wittle[3]	100	100	250	850

[1] Made with lactone from California Foundation for Biochemical Research. Analyzed correctly for C, H, N, S.

[2] Made with unreacted lactone recovered from 79-2147.

[3] Made with lactone prepared at Parke-Davis Laboratory.

In Table II are given inhibition data for our best preparation of ω-methylpantethine (16-2357) compared to data obtained with ω-methylpantothenate, acting against a variety of bacteria with different relative requirements for pantothenate and pantethine. The relative amounts of the two forms of the vitamin needed for growth of each organism are shown in the upper two lines of data. Below this are the inhibition indices produced when the antagonist was ω-methylpantothenate and the metabolite was alternately pantothenate and

pantethine for each organism. At the bottom of the table, similar data are presented for experiments in which the antagonist was ω-methylpantethine. It is difficult to find any pattern in these inhibition data. For *L. helveticus* and *S. faecalis*, ω-methylpantothenate is much more active when the metabolite is pantothenate, although for *L. arabinosus* and *L. citrovorum*, the form of the vitamin in the medium on which they grow makes little difference in the amount of inhibitor required for half maximum inhibition. When the antagonist is ω-methylpantethine, inhibition is better for all four organisms when growing in medium containing pantethine. So, in general, the results indicate that ω-methylpantothenate interferes with the utilization of pantothenate, while ω-methylpantethine interferes with the utilization of pantethine.

ω-Methylpantethine was found to be a competitive antagonist of *L. helveticus* against pantethine over the range investigated. The indices found when 0.25, 0.5, and 1 μg/10 ml amounts of pantethine were present were 320, 320, and 380, respectively. However, when *L. citrovorum* was inhibited by ω-methylpantethine (Table III), the inhibition when the metabolite was pantothenic acid was competitive but not very strong. The inhibition against pantethine was much stronger but noncompetitive. Pantethine produces the effective type of reversal in which an increase of metabolite concentration takes more than an equivalent increase in antagonist to overcome it.

Pantothenic acid is normally eight to ten times more active for *L. arabinosus* than pantethine. However pantetheine, the reduced sulfhydryl form, is several times more active (Table IV) than pantethine. This is similar to the relative growth-promoting activities of these two compounds previously reported[12] for *A. suboxydans*. The same phenomenon seems to play a part in the inhibition of *L. arabinosus*

TABLE II

Inhibition of Several Organisms with Different Relative Requirements for Pantothenate and Pantethine by ω-Methylpantothenate and ω-Methylpantethine

	L. helveticus		*S. faecalis*		*L. arabinosus*		*L. citrovorum*	
	PA*	PAN†	PA	PAN	PA	PAN	PA	PAN
Relative requirement for growth	100	1	1	1	1	10	1	100
	Inhibition index							
ω-methylpantothenate	500	52,000	500	>20,000	300	30	250	50
ω-methylpantethine	2500	100	>20,000	6000	20,000	40	9000	80

* PA = Pantothenic acid.
† PAN = Pantethine.

TABLE III

Inhibition of *L. citrovorum* by ω-Methylpantethine and Reversal by Pantothenic Acid vs. Pantethine

Ratio Inhibitor/Vitamin*	Inhibition index 18 hr	24 hr	40 hr	72 hr	Type of inhibition
μg/10 ml					
PA					
9000/1	9000	11,000	15,000	20,000	Competitive
18,000/2	9000	10,000	20,000	20,000	
PAN					
750/100	7.5	10	10	20	Noncompetitive
3500/200	17.5	40	50	80	

* Giving half inhibition.

TABLE IV

Growth Response of *L. arabinosus* to Pantetheine vs. Pantethine

	Required for ½ maximum growth	
	24 hr	40 hr
	μg/10 ml	μg/10 ml
Pantothenic acid	0.08	0.08
Pantetheine (reduced)	0.20	0.10
Pantethine (oxidized)	0 70	0.33

TABLE V.

Inhibition Index of ω-Methylpantothenic Acid and Reduced vs. Oxidized Pantethine on *L. arabinosus*

Vitamin	Ratio Inhibitor/Vitamin	Inhibition index 18 hr	40 hr	72 hr
	μg/10 ml			
PAN	40/2	20	50	50
Reduced PAN	300/2	150	150	250
PA	150/0.5	300	1200	2000

by ω-methylpantothenate (Table V). The inhibition ratio for pantetheine, the reduced form, is about eight times higher than for pantethine, possibly indicating that the reduced form of the vitamin enters the cell to a greater extent and therefore requires more of the antagonist to neutralize it.

Elion and associates[13] have proposed a

TABLE VI

Inhibitory Effect of Analogues of Pantethine Containing Modifications of One and Two Parts of the Molecule on *L. arabinosus* and *L. helveticus*

Inhibitor	Organism	Metabolite	Inhibition index 18 hr	24 hr	40 hr	72 hr
Pantothenylaminoethane	*L. helv.*	PAN	80	—	150	400
Pantothenylaminoethanol	*L. arab.*	PA	40	—	60	100
	L. arab.	PAN	8	—	8	8
	L. helv.	PAN	220	—	220	220
ω-Methylpantothenylaminoethane	*L. arab.*	PA	1400	>2000	>2000	>2000
	L. arab.	PAN	400	400	400	400
	L. helv.	PAN	70	75	270	450
ω-Methylpantothenylaminoethanol	*L. arab.*	PA	1400	>2000	>2000	>2000
	L. arab.	PAN	400	400	400	400
	L. helv.	PAN	500	500	700	800

The inhibitory activity of pantethine analogues in which the β-mercaptoethylamine part of the molecule is replaced by aminoethane and aminoethanol is shown in Table VI. Also indicated is the activity of these compounds which have been further modified by the substitution of methyl for hydrogen at the terminal carbon atom of the pantoyl part of the molecule. In the case of three of these compounds, the only ones investigated in this respect, their activity against *L. arabinosus* growing on pantethine remained constant over a long incubation period. Both ω-methyl-substituted compounds were less active than pantothenylaminoethanol. The general impression is that the hybrid analogues with double substitutions have no advantage over the first two, which have only a single alteration, and that all of them are more effective antagonists against the utilization of pantethine than against the utilization of pantothenate. Further investigation of these compounds might produce interesting results.

method for determining synergism between purine antagonists. Table VII shows data obtained on *L. citrovorum* when this method was applied to the combined action of ω-methylpantothenate and ω-methylpantethine in various concentrations. Each tube contained 1 μg pantothenate as growth factor. The results are expressed as milligrams of dried cells in each culture tube, which makes it easier to visualize the synergistic effect of these two antagonists. When the resulting data are calculated by the formula derived by Elion *et al.*, as indicated above, a value of 0.61 is obtained. This suggests a considerable degree of synergism between these two compounds, since a figure less than 1 indicates synergism and more than 1 antagonism. By this same procedure,

TABLE VII

Inhibitory Effect of Combinations of ω-Methylpantethine and ω-Methylpantothenic Acid on *L. citrovorum* with Pantothenic Acid as Growth Factor

mg ω-methylpantethine per 10 ml	μg ω-Methylpantothenate per 10 ml				
	0	75	150	225	300
	mg dry cells per 10 ml				
0	3.8	2.2	1.4	0.9	0.5
2	2.8	0.8	0.5	0.2	0.3
4	1.8	0.6	0.2	0.05	0.0
6	1.2	0.6	0.1	0.0	0.0
8	0.9	0.3	0.1	0.0	0.0
10	0.6	0.2	0.1	0 0	0.1

a combination of ω-methylpantothenate and ω-methylpantethine produced an index of 0.58, indicating synergism, when acting on *L. citrovorum* with pantethine as the growth factor, while ω-methylpantethine and pantothenylaminoethanol acting on *L. helveticus* with pantethine as growth factor were antagonistic (coefficient 1.7).

or pantethine. Amounts of inhibitor up to 200 times the pantothenate or pantethine given had no antagonistic effect. Pantothenylaminoethane and pantothenylaminoethanol were each given intraperitoneally by injection at a level of 6 mg per day to deficient rats. Both these compounds produced a growth response equal to that obtained with an equivalent amount of pantothenate, indicating that these antagonists were evidently cleaved to pantothenate by the rat under the test conditions.

As indicated in a recent publication from our laboratory,[15] pantothenylcystine failed to give significant pantothenate activity in either rats or chicks, given orally or parenterally. Nor was there any detectable inhibitory activity shown by this compound, although, of course, it was given in small amounts.

Experiments were conducted to try to find why the inhibited cultures of the micro-organisms studied grew out after long incubation. Since ω-methylpantethine contains peptide bonds, a possible reason why cultures inhibited by this compound grew out might be the gradual hydrolysis of this inhibitor down to ω-methylpantoic acid which does not inhibit *L. helveticus*. *L. helveticus* was inoculated into medium containing 1000 μg ω-methylpantethine per 10 ml. After 18 hours' incubation this preparation gave an inhibition index of 200. Continued incubation for a total of 72 hours resulted in good growth of the culture. The cells of the 72-hour culture were then centrifuged and the inhibition index of the supernatant determined by inoculating dilutions of it with fresh inoculum. The index was 240, showing that the ω-methylpantethine was still intact after the *L. helveticus* cells had grown out in its presence.

TABLE VIII

Pantothenate Antagonist Studies in Rats

Compound	Level and how given	Type of diet	Result
ω-Methylpantothenate*	200x PA or PAN, orally	Low level PA or PAN	No antagonism or activity
ω-Methylpantethine	0.05% orally	PA-deficient	No antagonism
Pantothenylaminoethane	6 mg/day IP	PA-deficient	PA activity
Pantothenylaminoethanol	6 mg/day IP	PA-deficient	PA activity
Pantothenylcyst(e)ine	68 μg/day IP	PA-deficient	Insignificant activity, no antagonism

* Obtained from California Foundation for Biochemical Research.

Since Drell and Dunn[9,14] have reported the production of a pantothenate deficiency in mice and rats fed ω-methylpantothenate, we attempted a similar experiment in which rats were given a pantothenate-deficient diet to which, after 3 weeks, 0.05 per cent ω-methylpantethine was added. The data for this and similar experiments are summarized in Table VIII. This amount of antagonist did not hasten the further development of the deficiency. The test was run in this way because it seemed the most sensitive method by which an *in vivo* effect might be shown. The calcium salt of ω-methylpantothenate, the same compound reported on by Drell and Dunn, was also studied. In this case rats were made pantothenate-deficient, then fed various ratios of inhibitor and either calcium pantothenate

Another possibility which suggested itself was that resistance develops suddenly, a situation which has been observed with tubercle bacilli inhibited by isoniazid. To test this, a culture of *L. helveticus* was incubated 40 hours in the presence of ω-methylpantethine. It was then growing rapidly. A washed inoculum from this culture was added to medium containing the same antagonist, with the thought that it might have become adapted to the antagonist and would grow out more rapidly than an inoculum of a like number of uninhibited cells in the same medium. But the antagonist-treated inoculum actually grew out more slowly. In fact, even when the inoculum from medium containing antagonist was transferred to normal medium with no antagonist, it took about 40 hours to grow out, whereas normal inoculum grew out in 10 to 12 hours.

The following experiment was then carried out. A large inoculum of *L. helveticus* cells which had grown out after 40 hours' incubation in the presence of ω-methylpantethine was transferred to normal medium. After 32 hours' incubation, when the cells were growing quite rapidly, they were transferred to medium containing the antagonist and incubated for 16 hours. A small inoculum of these cells grew out at the same rate as cells from normal medium when transferred to medium containing no antagonist, while cells which had been exposed only once to the antagonist grew out as slowly as before. We interpret this behavior as an indication that cells which grow out in the presence of this pantothenate antagonist gradually become impermeable to it and, when transferred to normal medium, are unable to grow immediately because of the concentration of antagonist still inside the cells. After another passage through normal medium, however, a subsequent exposure to the antagonist does not harm the cells and leaves them in a condition to grow at the normal rate when placed in medium containing no antagonist.

Discussion

With some exceptions, notably in the cases of pantothenylaminoethanol, ω-methylpantothenylaminoethane, and ω-methylpantothenylaminoethanol acting upon *L. arabinosus* with pantethine as a growth factor, all the microbiological inhibitions studied resulted in the organisms growing out after long incubations. This is contrary to the results reported by Drell and Dunn,[9] who obtained low inhibition indices for ω-methylpantothenate in several micro-organisms even when calculated after 72 hours' incubation.

The experimental results obtained with animals in our laboratory are also in conflict with those of Drell and Dunn, and, at least by implication, with those of Bean and Hodges[11] in humans. Drell and Dunn produced a pantothenate deficiency in mice and rats with ω-methylpantothenate which could be reversed competitively with pantothenate. In our experiments, neither ω-methylpantothenate nor ω-methylpantethine produced any antagonism which could be observed in terms of growth. Bean and Hodges produced a condition reputedly due to pantothenate deficiency. However, the small amount of antagonist required (only 0.5 g per day for an adult) and the fact that the effect was not reversed in six days by enormous doses of pantothenate, leave some doubt as to the true nature of the condition these workers observed.

The inability of rats to utilize pantothenylcystine indicates that they are unable either to utilize this compound to synthesize coenzyme A or to degrade it to pantothenic acid. This is contrary to what has been reported for both bacteria and liver supernatant.

Summary

Different preparations of ω-methylpantethine varied greatly in their inhibition indices. These differences could be detected by microbiological tests, but not by chemical analyses or physical tests.

The pantothenic acid antagonists reported here were only temporary inhibitors of the growth of most micro-organisms investigated.

ω-Methylpantothenate tends to inhibit the utilization of pantothenate, while ω-methylpantethine tends to inhibit the utilization of pantethine.

None of the antagonists investigated produced a pantothenate deficiency in rats. ω-Methylpantothenate and ω-methylpantethine

were inactive both as antagonists and as growth factors replacing pantothenic acid. Pantothenylaminoethane and pantothenylaminoethanol were good antagonists of pantothenate in micro-organisms, but had pantothenate activity in rats.

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