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Influence of Anti-Metabolites of Essential Vitamins on Growth and Acid Production of *Lactobacillus acidophilus* (Hadley).*

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The findings of Hill and Kniesner[1] and of Weisberger and Johnson[2] indicate that pantothenic acid, nicotinic acid and thiamine are essential for the maximum growth and acid production of the oral *Lactobacillus acidophilus* recovered from the saliva of caries active patients. In addition to being necessary for the optimum growth and acid production of the oral lactobacilli, thiamine and nicotinic acid in the role of coenzymes take part in the degradation of glucose to lactic acid.[3] The production of acids from fermentable foodstuffs about the sheltered areas of the teeth by the acidogenic and aciduric organisms of the oral cavity in concentrations sufficient to cause a decalcification of tooth substance is the basis of the widely accepted chemico-parasitic theory of the etiology of dental caries. Since it has been shown that pyridine-3-sulfonic acid, pantoyltaurine and pyrithiamine are metabolic antagonists‡ of nicotinic acid, pantothenic acid and thiamine respectively for some species of bacteria and laboratory animals,[5,6,7] this study was undertaken to determine whether these compounds exerted an inhibitory effect on the growth and acid production of the oral *Lactobacillus acidophilus*, the organism intimately associated with the caries process.

Materials and Methods. A pure culture of the *Lactobacillus acidophilus* (Hadley) re-

TABLE I.
Composition of Test Medium.*

Constituent	Quantity
Casein hydrolysate	2.5 ml
Salt solution A†	0.5 "
" " B‡	0.5 "
Glucose	1 g
Sodium acetate	600 mg
Asparagine	25 "
Tryptophane	10 "
Cystine	10 "
Guanine hydrochloride	500 μg
Adenine sulfate	500 "
Xanthine	500 "
Uracil	500 "
Pyridoxine hydrochloride	40 "
Riboflavin	20 "
Calcium pantothenate	20 "
Nicotinic acid	20 "
Thiamine hydrochloride	10 "
Folic acid	1 "
Biotin	0.5 "
Distilled water to 50 ml.	

* Double strength, Landy and Dicken medium adjusted to pH 6.8 with NaOH.

† Salt solution A:

K_2HPO_4	5 g
KH_2PO_4	5 g
Distilled water to 50 ml.	

‡ Salt solution B:

$MgSO_4 \cdot 7H_2O$	10 g
NaCl	0.5 g
$FeSO_4 \cdot 7H_2O$	0.5 g
$MnSO_4 \cdot 2H_2O$	0.337 g
Distilled water to 250 ml.	

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† We wish to thank Dr. Hans Molitor of Merck and Company for providing us with the anti-metabolites used in these studies.

1 Hill, T. J., and Kniesner, A. H., *J. D. Res.*, 1942, **21**, 467.

2 Weisberger, D., and Johnson, F. G., *J. D. Res.*, 1946, **25**, 35.

3 Tauber, H., *Enzyme Chemistry*, John Wiley and Sons, Inc., New York, 1937.

‡ Metabolic antagonists or competitive inhibitors have been defined as "the mutually antagonistic components of a system in which the role of either the metabolite or the anti-metabolite is reversibly and competitively interfered with by its antagonist, the relative amounts of the two substances in combination with the enzymic or other cellular components being dependent upon their relative affinities for them and upon their concentration-ratio."[4]

4 Welch, Arnold D., *Phys. Rev.*, 1945, **25**, 687.

5 McIlwain, H., *Brit. J. Exp. Path.*, 1940, **21**, 136.

6 Snell, E. E., Chan, L., Spiridanoff, S., Way, E. L., and Leake, C. D., *Science*, 1943, **97**, 168.

7 Woolley, D. W., and White, A. G. C., *J. Exp. Med.*, 1943, **78**, 409.

TABLE II.
Effect of Pantoyltaurine, Pyridine-3-Sulfonic Acid, and Pyrithiamine on Growth and Acid Production of the *Lactobacillus acidophilus* (Hadley)*.

Tube	Metabolite-anti-metabolite ratio	Growth (packed cell volume) †	% decrease in acid production‡
Pantoyltaurine (μg)			
1,000	1:250	+++	00.0
2,000	1:500	+++	24.2
4,000	1:1000	+++	43.3
7,500	1:1875	±	92.2
10,000	1:2500	—	100.0
Pyridine-3-Sulfonic Acid (μg)			
1,000	1:250	+++	00.0
2,000	1:500	+++	00.0
3,000	1:750	+++	00.0
4,000	1:1000	+++	27.7
5,000	1:1250	+++	35.9
7,500	1:1875	+++	55.9
10,000	1:2500	++	71.8
Pyrithiamine (μg)			
470	1:235	+++	00.0
1,880	1:940	+++	00.0
2,820	1:1410	+++	30.6
3,350	1:1675	+++	41.5
5,000	1:2500	++	73.7
6,500	1:3250	+	87.4

* 72-hr reading.
† 0.0 -0.1 cc, —
0.1 -0.25 cc, ±
0.25-0.5 cc, +
0.5 -1.0 cc, ++
1.0 -2.0 cc, +++
‡ Acid production in control tube = 100%.

covered from a carious lesion was obtained from the American Type Culture Collection (ATCC 4646) and used as the test organism in these studies. The special medium consisted of that described by Landy and Dicken.[8] Ten cc, the amount present in each tube, contained 4 μg of nicotinic acid, 4 μg of calcium pantothenate, and 2 μg of thiamine hydrochloride (Table I). Pyridine-3-sulfonic acid, pyrithiamine and pantoyltaurine were added to 3 different series of tubes in amounts ranging from 1,000 through 10,000 μg, 470 through 6,500 μg, and 1,000 through 10,000 μg respectively. The tubes were autoclaved and inoculated with one drop of inoculum, the preparation of which has been described in detail in a previous publication.[9] They were shaken and incubated at 37.5°C for 72 hours. Growth was determined by centrifuging the cultures in Hopkins vaccine tubes for 10 minutes at high speed and then immediately measuring the packed cell volume. Acid production was determined by titrating the centrifugates with 0.1N NaOH to the pH of the uninoculated control tubes. A Beckman pH meter was employed for this purpose.

Results. The effect of the antimetabolites on the growth and acid production of the test organism at the end of the experimental period is shown in Table II. Of the amounts used in this study only pantoyltaurine in a metabolite-antimetabolite ratio of 1:2500 was completely effective as an inhibitor of the growth and acid production of the test organism. The lowest metabolite-antimetabolite ratios, which produced a significant partial reduction of acid formation, were calcium pantothenate: pantoyltaurine 1:500; nicotinic acid: pyridine-3-sulfonic acid 1:1000; and thiamine hydrochloride: pyrithiamine 1:1410. In each instance the degree of inhibition was directly related to the amount of the particular antimetabolite present in the medium.

Conclusions. The findings reveal that pan-

[8] Landy, M., and Dicken, D. M., *J. Lab. and Clin. Med.*, 1942, **27**, 1086.

[9] Dreizen, S., and Spies, T. D., *J. D. Res.*, 1947, **26**, 409.

toyltaurine, pyridine-3-sulfonic acid and pyrithiamine will inhibit the growth and acid production of the *Lactobacillus acidophilus* (Hadley) when present in sufficient concentrations in a medium containing all the known growth essentials of this organism. These metabolic analogues of pantothenic acid, nicotinic acid and thiamine have an affinity for enzymes or for the protein component of enzymes, substrates or the prosthetic groups which they resemble structurally.[10] They tend to replace their corresponding essential nutrients in the metabolism of the *Lactobacillus acidophilus* (Hadley) and thereby interfere with its ability to carry on its normal physiological functions. The findings also confirm the previously reported observations that pantothenic acid, thiamine and nicotinic acid are essential for the maximum growth and acid production of the oral *Lactobacillus acidophilus*.

[10] Welch, Arnold D., and Bueding, E., in Green, D. E., *Currents in Biochemical Research*, Interscience Publishers, New York, 1946.

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Virus of Eastern Equine Encephalomyelitis Isolated from Chicken Mites (*Dermanyssus gallinae*) and Chicken Lice (*Eomenacanthus stramineus*).*

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Within recent years several neurotropic viruses have been recovered from the chicken mite, *Dermanyssus gallinae* (De Geer), and it has been shown not only to harbor the viruses in nature but to maintain at least one of them by transovarian passage. Smith, Blattner and Heys[1] isolated the virus of St. Louis encephalitis from chicken mites taken near St. Louis during a non-epidemic period, and were able to recover this virus a second time[2] from mites taken in the same chicken house and from nearby areas. They demonstrated[3] the transovarian passage of the St. Louis virus in experimentally infected mites and found[4] that normal chickens could be infected when bitten by naturally infected *Dermanyssus* and that the virus could then be transmitted to uninfected mites after feeding upon these same chickens. Therefore, the cycle for the St. Louis virus of mite-bird-mite seems to be established. Sulkin[5] recovered the virus of western equine encephalomyelitis from *Dermanyssus gallinae* obtained on a farm in Dallas County, Texas, where cases of equine encephalomyelitis had occurred. Reeves, Hammon and co-workers[6] isolated the virus of western equine encephalomyelitis from wild bird mites, *Liponyssus sylviarum*, removed from the nest of a yellow-headed blackbird, *Xanthocephalus xanthocephalus* (Bonaparte), found in Kern County, California. More recently[7] they have reported on the isolation of another neurotropic virus from mites in this same nest. This strain seems to combine the characteristics of both the western equine and the St. Louis type of encephalitic viruses. Sulkin

[1] Smith, M. G., Blattner, R. J., and Heys, F. M., *Science*, 1944, **100**, 362.

[2] Smith, M. G., Blattner, R. J., and Heys, F. M., PROC. SOC. EXP. BIOL. AND MED., 1945, **59**, 136.

[3] Smith, M. G., Blattner, R. J., and Heys, F. M., *J. Exp. Med.*, 1946, **84**, 1.

[4] Smith, M. G., Blattner, R. J., and Heys, F. M., *J. Exp. Med.*, 1947, **86**, 229.

[5] Sulkin, S. E., *Science*, 1945, **101**, 381.

[6] Reeves, W. C., Hammon, W. McD., Furman, D. P., McClure, H. E., and Brookman, B., *Science*, 1947, **105**, 411.

[7] Hammon, W. McD., Reeves, W. C., Cunha, R., Espana, C., and Sather, G., *Science*, 1948, **107**, 77.