Thymidine and Vitamin B_{12}*

Marjory H. Wright

Experimental Biology and Medicine Institute, National Institutes of Health, Bethesda, Maryland

Thymidine as a growth factor for certain lactic acid bacteria has received attention recently because of a possible metabolic relationship with vitamin B_{12}. Since thymidine can replace vitamin B_{12} in the nutrition of *Lactobacillus lactis* Dorner and *L. leichmannii* 4797, Wright *et al.* (*5*, *9*) have suggested that vitamin B_{12} may be a coenzyme in the synthesis of thymidine from thymine. Shive (*4*) points out that conversely thymidine may be involved in the synthesis of vitamin B_{12}.

As crystalline vitamin B_{12} (*2*) is now available for experimental use, it seemed desirable to investigate further this presumed relationship of thymidine to vitamin B_{12}, using three strains of lactic acid bacteria, all of which respond to thymidine but which vary in their response to other growth-promoting agents. The microorganisms chosen were *Streptococcus faecalis* R, *L. leichmannii* 313, and *Leuconostoc citrovorum* 8081.

The basal medium for *S. faecalis* R was that of Tepley and Elvehjem (*8*), which contains no folic acid but does contain 100 γ per 10 ml of adenine, guanine, xanthine, and uracil. The basal medium for *L. leichmannii* and *L. citrovorum* was that described by Snell *et al.* (*6*) in which there is 100 mγ folic acid per 10 ml as well as adenine, guanine, and uracil in the same concentrations as above. For *L. citrovorum* the Tween 80 and oleic acid ordinarily present were omitted. In this medium an increase in pH from 6.0 to 6.5 affected adversely the response of *L. leichmannii* to vitamin B_{12}. As *L. citrovorum* grows equally well at pH 6.0 and 6.5, the medium was adjusted to pH 6.0 for both microorganisms. The following supplements were used in the assays: crystalline vitamin B_{12},[1] a commercial liver concentrate containing 15 U.S.P. units of antipernicious anemia factor[2], thymidine,[3] thymine,[4] and crystalline folic acid.[2]

In the case of *S. faecalis* R there was no response in 40 hr to vitamin B_{12}, while in 16 hr thymidine and thymine fully replaced the requirement for folic acid in the ratio of 5000: 1, as described earlier by Stokes (*7*).

Both thymidine and one or more factors in liver supported growth of *L. citrovorum* to the same degree, but as reported previously (*3*) the response to thymidine was characteristically delayed 21–40 hr. Attempts to replace the liver preparations or thymidine with vitamin B_{12} were repeatedly unsuccessful. The response of *L.*

TABLE 1

COMPARISON OF GROWTH-STIMULATING PROPERTIES OF THYMIDINE, VITAMIN B_{12}, LIVER CONCENTRATE, THYMINE, AND FOLIC ACID

10 ml Basal medium plus:			Galvanometer reading*			
thymidine	thymine	other supplements	*Streptococcus faecalis* R	*Leuconostoc citrovorum* 8081		*Lactobacillus leichmannii* 313
γ	γ		30° C 17 hr	37° C 16 hr	37° C 40 hr	37° C 24 hr
0	0	0	98	98	95	83
0	0	10 mγ folic acid	36			
50	0	0	30			
0	50	0	30			
0	0	1–10 mγ B_{12}	97			
0	0	0.01 ml liver concentrate		58	39	
40	0	0†		87	35	
40	0	2 mγ B_{12}		85	39	
0	0	0.1–100 mγ B_{12}		98	95	
0	40–3000	0†		99	95	
0	40, 300	2 mγ B_{12}		96	95	
0	0	2 mγ B_{12}‡				83
0	0	2 mγ B_{12}				44
0	0	0.0012 ml liver concentrate				41
20	0	0†				47
0	20, 200	0				80
0	20, 200	2 mγ B_{12}‡				61

* Turbidity of cultures measured in an Evelyn colorimeter, 660-mμ filter, with an uninoculated tube containing the 10-ml assay volume of basal medium set at 100.

† Growth was the same in presence or absence of folic acid. ‡ Folic acid omitted from medium.

[1] Vitamin B_{12} supplied by Merck & Co.

[2] Supplied by Lederle Laboratories.

[3] Thymidine supplied by Dr. D. W. Woolley of Rockefeller Institute.

[4] Supplied by Schwarz Laboratories.

* Since this report has been submitted for publication, Lyman and Prescott have also indicated that the liver factors for *L. leichmannii* and for *L. citrovorum* are not identical. (*J. biol. chem.*, 1949, **178**, 523).

citrovorum to thymidine in the presence of vitamin B_{12} was the same quantitatively and qualitatively as that with thymidine alone. Thus the liver activity could not be replaced by thymidine or vitamin B_{12}, or by a combination of the two. As indicated in Table 1, thymidine supported growth equally well in the absence of folic acid. Of particular interest was the negative response of *L. citrovorum* to thymine in the presence of vitamin B_{12}.

L. leichmannii responded promptly to vitamin B_{12} in amounts ranging from 0.05 mγ to 2 mγ, at which level there is a maximal response with this strain. The growth-promoting effect of liver appeared to be due to its vitamin B_{12} content. Thymidine, in the presence or absence of folic acid, supported growth comparable in amount to that produced by vitamin B_{12} only in the presence of folic acid. Thymine could substitute only partially for the folic acid requirement of this microorganism. Similar results have been reported by others (*1, 4, 5*).

Although thymidine and vitamin B_{12} can replace each other in supporting growth of *L. leichmannii*, the nature of the data obtained with *L. citrovorum* and *S. faecalis* R does not indicate a specific thymidine-vitamin B_{12} relationship.

References

1. Hoffman, C. E. *et al.* *J. biol. Chem.*, 1948, **176**, 1465.
2. Rickes, E. L. *et al.* *Science*, 1948, **107**, 396.
3. Sauberlich, H. E. and Baumann, C. A. *J. biol. Chem.*, 1948, **176**, 165.
4. Shive, W., Ravel, J. M., and Harding, W. M. *J. biol. Chem.*, 1948, **176**, 991.
5. Skeggs, H. R., Huff, J. W., and Wright, L. D. *J. biol. Chem.*, 1948, **176**, 1459.
6. Snell, E. E., Kitay, E., and McNutt, W. S. *J. biol. Chem.*, 1948, **175**, 473.
7. Stokes, J. L. *J. Bact.*, 1944, **48**, 201.
8. Tepley, L. J. and Elvehjem, C. A. *J. biol. Chem.*, 1945, **157**, 303.
9. Wright, L. D., Skeggs, H. R., and Huff, J. W. *J. biol. Chem.*, 1948, **175**, 475.

Partition Chromatography of Anthocyanidins[1]

Earl C. Spaeth and David H. Rosenblatt

Department of Chemistry, University of Connecticut, Storrs

Because of the recent reports of Bate-Smith (*1, 2*) which have been concerned with partition chromatography of anthocyanins and anthocyanidins on filter paper, it seems advisable to make a preliminary report of our progress in this field.

In the course of some work with the coloring matter of wine and grapes, it became necessary to develop a method for the separation and quantitative analysis of an anthocyanidin mixture. We thought that separation might be accomplished by a modification of the partition chromatography method of Martin and Synge (*5*). The anthocyanidins, malvidin and petunidin, were synthesized according to the methods of Robinson and co-workers (*3, 4*).

When solutions of these two pigments in a mixture of one volume of *n*-butanol and three volumes of ethyl ether were shaken with 10% *ortho*phosphoric acid, almost all of the pigment was transferred to the aqueous layer. An 8.1-cm column was prepared in the usual way (*5*) from 2.38 g of silicic acid (Eimer and Amend precipitated *meta*silicic acid; reported iron content less than 0.006%; reported heavy metal content 0.00%; dried at 100° C), 1.32 ml of 10% *ortho*phosphoric acid, and a mixture of one volume of *n*-butanol and three volumes of ethyl ether. Flow through the column was aided by a positive pressure of about 26 cm of mercury. When 4 μg of malvidin chloride in 0.25 ml of the *n*-butanol-ether mixture was placed on the top of the column and washed through with the *n*-butanol-ether mixture, the pigment moved in a single, slowly widening band with an R value (*5*) of 0.35 ± 0.01. The pigment band was completely eluted by continued flow of solvent.

In a similar experiment with the same column, a solution of 4 μg of petunidin chloride in 0.25 ml of the *n*-butanol-ether mixture gave a single band which moved with an R value of 0.41 ± 0.01 and could be eluted completely. These R values have been duplicated. When a mixture of 2 μg of petunidin chloride and 2 μg of malvidin chloride in 0.25 ml of the *n*-butanol-ether mixture was placed in the same column, the single band which formed began to separate into two bands after moving about 2 cm. The R values of each of the two bands after complete separation were the same as the values determined with the single pigments. The first portions of the colored eluate were decolorized when shaken in air with 10% sodium hydroxide solution. Portions representing about the last fourth of the colored eluate when shaken with 10% sodium hydroxide solution showed a definite blue color which was stable in air for more than 5 min. Similar solutions made up from petunidin chloride showed identical color instability to 10% sodium hydroxide solution, while malvidin chloride solutions gave a blue color which was identical with that shown by the later eluates. Our conclusion is that the lower pigment band on our column is due to petunidin while the upper band is due to malvidin.

Further studies aimed at separation of larger amounts of mixtures of anthocyanidins are in progress.

References

1. Bate-Smith, E. C. *Nature*, Lond., 1948, **161**, 835.
2. ———. *Biochem. J.*, 1948, **43**, xlix.
3. Bradley, W., Robinson, R., and Schwarzenbach, G. *J. chem. Soc.*, 1930, 793.
4. Bradley, W. and Robinson, R. *J. chem. Soc.*, 1928, 1541.
5. Martin, A. J. P. and Synge, R. L. M. *Biochem. J.*, 1941, **35**, 1358.

[1] The work reported here was done under contract between the Wine Advisory Board of the State of California and the University of Connecticut.