

citrovorum to thymidine in the presence of vitamin B₁₂ was the same quantitatively and qualitatively as that with thymidine alone. Thus the liver activity could not be replaced by thymidine or vitamin B₁₂, 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 B₁₂.

L. leichmannii responded promptly to vitamin B₁₂ 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 B₁₂ content. Thymidine, in the presence or absence of folic acid, supported growth comparable in amount to that produced by vitamin B₁₂ only in the presence of folic acid. Thymine could substitute only partially for the folic acid requirement of this micro-organism. Similar results have been reported by others (1, 4, 5).

Although thymidine and vitamin B₁₂ 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 B₁₂ relationship.

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Partition Chromatography of Anthocyanidins¹

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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

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

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% orthophosphoric 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 metasilicic acid; reported iron content less than 0.006%; reported heavy metal content 0.00%; dried at 100° C), 1.32 ml of 10% orthophosphoric 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.

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