W., *Pub. Health Rep.*, 1947, v62, 1467.
5. Bodian, D., PROC. SOC. EXP. BIOL. AND MED., 1949, v70, 1.
6. Li, C. P., and Habel, K., *ibid.*, 1951, v78, 233.
7. Li, C. P., and Schaeffer, M., *ibid.*, 1953, v82, 477.
8. Koprowski, H., Jervis, G. A., Norton, R. W., and Pfeister, K., *ibid.*, 1954, v86, 238.
9. Li, C. P., and Schaeffer, M., *ibid.*, 1954, v87, 148.
10. Krech, V., *ibid.*, 1954, v86, 488.
11. Wenner, H. A., *Ann. N. Y. Acad. Sci.*, 1955, v61, 840.
12. Sabin, A. B., *ibid.*, 1955, v61, 924.
13. Shelokov, A., Habel, K., and McKinstry, D. W., *ibid.*, 1955, v61, 998.
14. Weller, T. H., Enders, J. F., Robbins, F. C., and Stoddard, M. B., *J. Immunol.*, 1952, v69, 645.
15. Youngner, J. S., PROC. SOC. EXP. BIOL. AND MED., 1954, v85, 202.
16. Habel, K., *ibid.*, 1951, v76, 357.

Received November 7, 1955. P.S.E.B.M., 1955, v90.

Occurrence of Pantothenyl Cysteine in Natural Materials.* (22127)

JOSEPH T. CUMMINS, VERNON H. CHELDELIN, AND TSOO E. KING.
Department of Chemistry and Science Research Institute, Oregon State College, Corvallis.

Pantothenyl cysteine (PA-cysteine)† has been recognized as an early intermediate in the biosynthesis of coenzyme A (CoA) from pantothenic acid (PA). A liver enzyme can convert PA-cysteine to pantetheine (LBF) (1). PA-cysteine has been shown to be a precursor of LBF in *Acetobacter suboxydans* and it is strongly stimulatory to this organism (2,3). It is of interest to determine whether PA-cysteine occurs naturally. Because of the need for specificity and ability to determine materials in small quantities, a bioautographic method was developed to determine various conjugates occurring in natural materials. A strain of *A. suboxydans* 621 was used. This bacterium will grow on most known PA conjugates, and is generally more sensitive to them than to free PA(2,4).

Methods. A synthetic medium previously described(5) with 2% agar was used for the bioautograph. The sterilized medium was inoculated with a heavy culture of *A. suboxydans at* 40° and poured into a sterile container to a depth of 6-8 mm. This container consisted of a 19 x 50 cm glass plate, which rested within a stainless steel frame 15 mm high. After agar had solidified, a developed paper chromatogram of PA conjugates was sterilized by treatment with ether, and then spread on agar surface. After 10 minutes, the paper was peeled off, agar plate covered with a sterile 19x50 cm glass plate and incubated at 30° for 24 hours. The top plate was then removed and agar surface sprayed lightly with a mixture of PA (10 γ/ml) and yeast extract (100 mg/ml). The glass plate was restored and the whole assembly again incubated at 30° for 24 hours. Aseptic conditions were preserved throughout. The bioautograph was then observed against a background of diffuse light. The PA-yeast extract mixture was introduced because *A. suboxydans* otherwise grows very slowly under conditions employed. This mixture stimulated growth throughout the entire agar plate but it exerted its greatest effect in areas where growth had already been initiated, *i.e.*, where prior contact had been made with the PA conjugates on the developed chromatogram. *A beef liver* preparation was used as source of naturally occurring PA-cysteine. It was prepared as follows: 15 g of cold beef liver were ground in a Waring blendor with 50 ml

* Paper No. 17 in pantothenic acid studies. This work was supported by grants from the Nutrition Foundation, and Division of Grants and Fellowships, National Institutes of Health, U. S. Public Health Service. Published with approval of Monographs Publication Committee, Oregon State College, Research Paper No. 281, School of Science, Department of Chemistry.

† The following abbreviations will be used throughout this paper: pantothenic acid = PA; coenzyme A = CoA; pantetheine = *Lactobacillus bulgaricus* factor = LBF; pantetheine-γ-phosphate = LBF-γ-phosphate; pantothenyl cysteine = PA-cysteine.

of ice water. This was poured into 50 ml of boiling water and the liquid decanted from the coagulated protein. Trichloroacetic acid was added to 1% concentration and the solution centrifuged. This yellow liquid was then extracted with ether in a liquid-liquid extractor, and the aqueous layer again centrifuged. The supernatant liquid was passed through a Dowex 1 column (2% cross linked), which had previously been charged with 3 M ammonium formate and washed with water. The column was eluted with formate buffer, prepared by adding 90% formic acid to 0.3 M ammonium formate to pH 3.5. The initial 20 ml of eluate (which was presumably free of coenzyme A(6) as well as other phosphorylated PA derivatives) was selected as the source of natural material used on paper chromatograms. Formate was eliminated by adding excess of HCl and concentrating eluate over KOH in vacuum desiccator. This eluate was reduced, first with Zn-HCl, then with H_2S at pH 7.5, to insure that all PA conjugates present were in the thiol form. About 20 μl of solution were then placed on Whatman No. 1 paper and chromatographed by the ascending technic. 1 γ of PA, LBF, CoA, LBF-γ-phosphate, and PA-cysteine each were used in 20 μl solution, to give zones of reference on chromatogram. To make sure that no reoxidation to the disulfide took place, some chromatograms were run under a nitrogen atmosphere. Results were identical to those obtained with chromatograms run in air.

Results. This method is adequate for detection and identification of low concentrations of PA conjugates. PA, CoA, and PA-cysteine have been detected at 0.2 γ. Since *A. suboxydans* did not grow on PA phosphates (7), it is not expected that this method will detect all possible conjugates of PA.

Table I summarizes the R_f values of standard conjugates and liver preparation in a number of solvent systems. Pyridine-water-isopropyl alcohol (1:1:1), phenol-water (4:1), or acetone-water-urea (59.7:39.8:0.5) proved to be good systems for distinguishing

TABLE I. R_F Values of Some PA Conjugates, Determined by Bioautographic Technic.

Compound	Solvent: Phenol-water (4:1)	Butanol sat. with H_2O	Pyridine-isopropyl alcohol-water (1:1:1)	Acetone 59.7%, water 39.8%, urea 0.5%
PA-cysteine	.48	.17	.75	.76
PA	.75	.35	.80	.66
CoA	.20	0		.16
LBF-γ-phosphate	.94	.81	.96	.46
LBF	.90	.94	.90	.92
Beef liver preparation	.73 .61 .50	0 .29	.80 .73 .60	.70 .63 .54

PA-cysteine from other conjugates tested. Two-dimensional chromatograms using these solvents disclosed three zones of PA activity. One of these corresponded to the free vitamin, another to PA-cysteine. The third zone did not consistently appear throughout the many experiments performed, and it may be present in a much lower concentration. Using butanol-water, it was not possible to resolve the liver preparation sufficiently to demonstrate PA-cysteine.

Summary. A bioautographic technic has been described for the detection of PA conjugates in natural materials. 0.2 γ of PA, PA-cysteine and CoA can be detected. The occurrence of PA-cysteine in beef liver is indicated by this method.

1. Hoagland, M. B., and Novelli, G. D., *J. Biol. Chem.*, 1954, v207, 767.

2. King, T. E., and Cheldelin, V. H., PROC. SOC. EXP. BIOL. AND MED., 1953, v84, 591.

3. Brown, G. M., and Snell, E. E., *J. Am. Chem. Soc.*, 1953, v75, 2782.

4. ———, *J. Bact.*, 1954, v67, 465.

5. King, T. E., and Cheldelin, V. H., *ibid.*, 1950, v59, 229.

6. Novelli, G. D., and Schmetz, F. J., *J. Biol. Chem.*, 1951, v192, 181.

7. King, T. E., and Strong, F. M., *ibid.*, 1951, v191, 515.

Received September 12, 1955. P.S.E.B.M., 1955, v90.