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Determination of Threonine.

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Criegee, Kraft and Rank[1] showed that glycols and related compounds are split by lead tetraäcetate to yield ketones or aldehydes. Threonine ($CH_3CHOHCHNH_2COOH$) should, therefore, yield acetaldehyde when oxidized by lead tetraäcetate. Eegriwe[2] described a highly sensitive color reaction for the qualitative estimation of lactic acid based upon the oxidation of the acid with hot concentrated sulfuric acid to acetaldehyde; from the acetaldehyde an intense violet color is produced by condensation with p-hydroxydiphenyl. Miller and Muntz[3] modified Eegriwe's method and they have shown that the color is not given by a number of other acids such as glycolic, oxalic, formic, 2,3-dihydroxybutyric, etc. The apparent specificity of the p-hydroxydiphenyl test for acetaldehyde suggested that it would be of value in the quantitative estimation of threonine.

The following method can be used for the determination of threonine alone or in the presence of other amino-acids: 25 cc of glacial acetic acid containing from 0.5 to 1.5 mg of threonine and 1 g of lead tetraäcetate are kept at 30° for one hour. The acetaldehyde is removed from the reaction mixture during the course of the oxidation by moderately rapid aeration and taken up in 10 cc of concentrated sulfuric acid containing 5 drops of water and 100 mg of p-hydroxydiphenyl in suspension. At the end of the hour, the excess p-hydroxydiphenyl is dissolved by warming to 100°. The solution may be diluted to volume with concentrated H_2SO_4. The amount of color is in direct proportion to the quantity of threonine used. Control tests with alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, hydroxyproline, isoleucine, leucine, lysine, methionine, norleucine, phenylalanine, proline, serine, tryptophane, tyrosine, valine and mixtures of the same were negative. Hydroxyglutamic acid was not available for test. Preliminary experiments suggest the applicability of these reactions to the determination of threonine in proteins and other biological material.

1 Criegee, R., Kraft, L., and Rank, B., *Lieb. Ann.*, 1933, **507**, 159.

2 Eegriwe, E., *Z. anal. Chem.*, 1933, **95**, 323.

3 Miller, B. F., and Muntz, J. A., *J. Biol. Chem.*, 1938, **126**, 413.