

When isotonic sodium chloride followed hypertonic magnesium sulphate the usual contractions present during sodium chloride were absent in some experiments and present in others. The fluid content of the intestine was markedly increased with each of the hypertonic solutions but slightly more so after the magnesium. The magnesium also appeared to lower the tone of the intestine.

Specific effects of both magnesium and sulphate ions have thus been demonstrated as well as the usual osmotic effects.

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Determination of Lactic Acid in Presence of Certain Interfering Substances.

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Probably the best technique now available for the determination of lactic acid is that of Friedemann, Cotonio, and Shaffer¹ as modified by Friedemann and Kendall² and, more recently, by Friedemann and Graeser.³ There are certain substances which interfere with the lactic acid determination; these have been listed in the original and some of the subsequent articles. Some of these substances, notably the sugars, may be removed from solution by the copper sulfate-calcium hydroxide procedure.² Appreciable amounts of others, however, remain in solution even after this treatment. Important examples of these substances are malic and citric acids, both of which give large yields of bisulfite-binding substances under the conditions used for the oxidation of lactic acid to acetaldehyde. In fact, by some slight modifications of the conditions it is possible to obtain very nearly a quantitative yield of acetaldehyde from malic acid (90 to 93%).⁴ The product formed by the oxidation of citric acid is apparently acetone⁵ and by the lactic acid method a yield of about 60% of the theoretical may be obtained.⁴ However, both malic and citric acids may be quite completely removed from solu-

¹ Friedemann, T. E., Cotonio, M., and Shaffer, P. A., *J. Biol. Chem.*, 1927, **78**, 335.

² Friedemann, T. E., and Kendall, A. I., *J. Biol. Chem.*, 1929, **82**, 23.

³ Friedemann, T. E., and Graeser, J. B., *J. Biol. Chem.*, 1933, **100**, 291.

⁴ McChesney, E. W., unpublished observations.

⁵ Kuyper, A. C., *J. Am. Chem. Soc.*, 1933, **55**, 1722.

tion by means of basic lead acetate as follows: An amount of solution thought to contain 20-50 mg. of lactic acid and any reasonable amount of the interfering substance is placed in a 100 cc. volumetric flask, and diluted to a volume of about 50 cc. A drop of aqueous phenol red is added, then 10% NaOH is added drop by drop until the solution is neutral followed by one drop excess. A saturated solution of basic lead acetate (Haden) is added in 5 cc. portions until there is an excess; usually 5 cc. is sufficient and a large excess is to be avoided. Zinc ions should be absent from the solution as some precipitation of lactic acid seems to result if they are present. The solution is now diluted to the mark, mixed, and filtered. Lactic acid is determined in the usual way on aliquot portions of the filtrate. With the addition of the $MnSO_4 \cdot H_3PO_4$ reagent, a precipitate forms and this causes some bumping during the subsequent boiling but does not seem to interfere otherwise. Table I shows the completeness of the separation of lactic and malic acids by this method.

TABLE I.

Mg. lactic acid in 100 cc. vol.	Mg. malic acid added	Mg. lactic acid found
40.8	0	40.8
0	100	0.8
20.4	100	20.3
40.8	100	40.1
81.6	100	78.6
122.4	100	119.4
40.8	20	40.2
40.8	50	40.0
40.8	75	40.2

The average recovery of lactic acid from the mixtures was 98%.

Other substances. Of the other substances reported in the original article¹ to interfere with the lactic acid determination, the following have been found to give precipitates with basic lead acetate when 10 mg. of the substances are present in the final volume of 100 cc.: tartaric acid, citric acid, tyrosine, cystine, and maleic acid. The following have been found to yield precipitates with basic lead acetate when 5 mg. of the substance are present in the final volume of 100 cc.: tartaric acid, citric acid, tyrosine, and cystine (trace). The sugars glucose, fructose, xylose, galactose, and arabinose are of course not precipitated. This method is not likely to be of value in eliminating the various interfering alpha-hydroxy acids since in addition to lactic acid, alpha-hydroxy isovalerianic acid is not precipitated, nor is the acid derived from the deamination of leucine (alpha-hydroxy isocaproic acid).