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Manometric Method for Quantitative Determination of Lactic Acid.

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Van Slyke and Neill[1] have recently introduced an apparatus for the determination of blood gases by manometric methods. This manometric blood-gas apparatus has been used by Van Slyke and Sendroy[2] in determining blood Ca. The Ca is precipitated as the oxalate, and this is oxidized in the apparatus by the addition of acidified $KMnO_4$. The oxalate is oxidized to CO_2 and H_2O, and the CO_2 so produced determined in the usual way.

A modification of the Clausen method for the determination of small amounts of lactic acid was described recently by Friedemann, Cotonio, and Shaffer.[3] These investigators found that the use of manganous salt increases the rate and yield of aldehyde when lactates are oxidized by acid $KMnO_4$.

The writers have developed a method for the determination of lactates using the manometric apparatus of Van Slyke and Neill, and in part the modified Clausen reagents of Friedemann, Cotonio, and Shaffer.

In selecting a mixture of $MnSO_4$ and $KMnO_4$ that would fully oxidize lactic acid to acetaldehyde and CO_2, an excess of $MnSO_4$ was found to prevent the oxidation or greatly to retard the process and this was also true for equal amounts of $MnSO_4$ and $KMnO_4$. When excess $KMnO_4$ was present good results were obtained over a considerable range of ratios of $KMnO_4$ to $MnSO_4$ as shown in Table I. Shaffer gives the oxidation-reduction potential necessary to oxidize lactic acid as +1.36 at 25° C. but carries out oxidation at the boiling point, *i. e.*, at an Eh of approximately +1.74. The Eh of our oxidizing agent was about +1.44 in the concentration of sulphuric acid employed, namely Normal and at 20°.

TABLE I.

Oxidizing agent used		Eh at pH and temp. of exp.	Millimols of CO_2 found	Millimols of lactic acid used
cc. M/10 $MnSO_4$	cc. M/10 $KMnO_4$			
1.8	1.2	+1.425	4.99	8.70
1.5	1.5	+1.435	6.85	8.70
1.2	1.8	+1.438	8.36	8.70
1.0	2.0	+1.440	8.39	8.70
0.8	2.2	+1.441	8.42	8.70

Procedure: The apparatus was the closed manometer type of Van Slyke gas apparatus. Reagents consisted of 5N H_2SO_4, M/10 $KMnO_4$, M/10 $MnSO_4$, Standard Lactic Acid, 5 N NaOH. The solutions need not be made up with care as great variations in the ratio of the oxidant and reductant affect the Eh very little. Solutions should, however, be carefully protected from dust which would yield CO_2 by oxidation. The Mn solutions were acidified with 1/20 vol. of Normal H_2SO_4 to drive off CO_2. The 5 N NaOH was made air free by evacuation and protected from air as described by Van Slyke and Neill. An approximately Normal Lactic Acid solution was made by dilution and filtered through acid hardened filter paper. This solution was made up to N/100 and titrated against N/100 carbonate free alkali with phenolphthalein as an indicator. During the titration CO_2 was removed by rapidly bubbling CO_2 free hydrogen through the closed titration vessel.

The Van Slyke apparatus was cleaned by evacuating and shaking with 2 cc. N H_2SO_4 and 5 cc. H_2O for a period of 2 minutes and ejecting the washings. One cc. of lactic acid containing 0.783 mg. was introduced near the bottom of the cup, the sides of the cup were washed down with 1 cc. 5 N H_2SO_4, and M/10 $MnSO_4$ and M/10 $KMnO_4$ were introduced. Each of the four solutions was drawn into the evacuation chamber separately. The total volume of solution amounted to 5 cc. The cup was then sealed with mercury, the mercury level in the evacuation chamber lowered to the 50 cc. mark and the instrument shaken for a period of 3 to 6 minutes. The gas was then carefully brought to 2 cc. volume by allowing the Hg to slowly rise and the pressure (p_1) was then read, amounting to about 110 mm. This process was repeated until 3 minutes of evacuation showed no change in pressure. Two cubic centimeters of 5 N NaOH were then introduced into the clean cup. The mercury was lowered to about the 15 cc. point, the lower stop cock of the apparatus closed, and the upper opened allowing the NaOH to enter. The instrument was shaken twice to mix the solutions and the Hg slowly allowed to rise to again reduce the gas to 2 cc. volume and p_2 was read. This usually amounted to about 89 mm. A correction (c) of 1 mm. to be added to p_2 was found by the method of Van Slyke and Neill. The volume of reagents having totaled 7 cc., Van Slyke and Neill's values in column 4 of Table III, page 544 (loc. cit.) may be used to calculate millimols of CO_2 found. The values must be multiplied by 2 as only 1 cc. samples were used in place of 2 cc. as in the table.

Rates of reactions involving gas formation can be very readily studied by this method. In the present instance the development of

gas pressure may be measured at regular intervals and when plotted against time shows a beautiful monomolecular curve. The values for (p_1) in one oxidation, for example being 390, 458, 484, 498, 501, 500 in successive 3 minute intervals.

The agreement of determinations of lactic acid in a total quantity of less than one milligram was very good as shown by Table I. When oxidizing mixtures of the optimum proportions were used the values found agree within 1.0% of each other and 4% of the theoretical value. This compares favorably with any other method of determining lactic acid.

The application of this method to the determination of pure lactates is recommended but it should be pointed out that we obtained high values with silver lactate, probably due to the oxidation of acetaldehyde by silver compounds. A danger also lies in the presence of any other oxidizable compound yielding CO_2 on treatment with the reagents described above. The authors are now studying the use of the method with biological material.

[1] Van Slyke, D. D., and Neill, J. M., *J. Biol Chem.*, 1924, lxi, 523.

[2] Van Slyke and Sendroy, J., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 167.

[3] Friedemann, T. E., Cotonio, M., and Shaffer, P. A., *J. Biol. Chem.*, 1927, lxxiii, 335.

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The Blood of the Normal Guinea Pig.

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It is suggested that inconsistencies in the work of previous investigators on the blood of the normal guinea pig have been due, for the most part, to the use of the usual fixed staining technic. The supravital method of blood study, first used by Pappenheim,[1] and reintroduced by Simpson,[2] overcomes the common difficulties of cell distribution, and identification, as met with in the smear method.

The peripheral blood of the normal guinea pig has been studied by the Neutral Red-Janus Green supravital method. Sixty-four guinea pigs from four different sources, of both sexes, and weighing from 500 to 1000 grams, comprise the series. From 200 to 400 white cells per preparation were counted on each. To compensate