THE DETERMINATION OF LACTIC ACID.*

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(Received for publication, August 25, 1928.)

Lactic acid is a substance of major significance throughout the orders of life, from microbe to man. Its relation to the carbohydrate metabolism has been very extensively studied; and it is not surprising, therefore, that many methods have been proposed for its quantitative estimation. Most of the methods depend upon the conversion of lactic acid into acetaldehyde (1, 2), the latter being determined as such, or colorimetrically after reacting with some color-producing substance. Of these methods, perhaps the most precise is that of Friedemann, Cotonio, and Shaffer (3, 4). Lactic acid, according to this method, is oxidized by acid KMnO₄ in the presence of MnSO₄. The resulting acetaldehyde is aerated out of the solution, absorbed in bisulfite, and determined by the Clausen (2) titration method. The chief advantages of the method over the older oxidation methods of von Fürth and Charnas and Clausen are (1) its speed, (2) the considerably increased yield of acetaldehyde, and (3) the smaller fluctuations between individual determinations. This method, as in the case of the older methods, determines also the more volatile sulfite-binding substances which may be produced from many other compounds besides lactic acid. Their effect on the determination may be minimized, however, as was shown by Friedemann, Cotonio, and Shaffer, merely by allowing the vapors to pass upward through a cooled reflux condenser, the less volatile substances being condensed with the steam and returned to the solution where apparently they are destroyed by further oxidation.

* A preliminary report of this work was presented as a demonstration at the meeting of the Society of Biological Chemists, April, 1928. (Kendall, A. I., and Friedemann, T. E., J. Biol. Chem., 78, p. lxi (1928).)
In actual practice the materials analyzed for lactic acid contain relatively very large quantities of interfering substances, many of which may also yield bisulfite-binding substances when oxidized. In the case of blood and tissues the chief interfering substances are proteins and carbohydrates. The former may be removed by tungstic acid or trichloroacetic acid and the latter may be removed by treatment with CuSO₄-Ca(OH)₂.¹ The resulting filtrate contains relatively small amounts of nitrogenous substances, and gives fairly concordant results either by the method of Clausen or the method of Friedemann, Cotonio, and Shaffer.

Such simple procedures for removing interfering nitrogenous substances do not apply to many of the other materials which are often encountered in biochemical work. Examples of such are bacterial culture media and urine. The materials mentioned contain very large amounts of non-protein substances. The various mercury reagents, especially acid mercuric nitrate, which are very efficient in removing even the very simple nitrogenous compounds may be used, if care is taken in deproteinizing blood; they are, however, entirely unsuited for the preliminary preparation of culture media or urine. The results for lactic acid are invariably low. Even by the most careful neutralization with sodium bicarbonate, as suggested by Ronzoni and Wallen-Lawrence (4), or with BaCO₃, only about 85 to 95 per cent of the added lactic acid may be recovered. The results are low even with a pure lactic acid standard, with the amount of mercuric reagent necessary for the complete removal of nitrogen from peptone media.

Since the usual precipitants are unsuitable the logical procedure would seem to be extraction by ether. But this is open to some criticism. In addition to the possibility of incomplete extraction there is the possibility also of losing some of the lactic acid which has already been extracted. Known small amounts of lactic acid, for example, often are incompletely recovered if added to ether which has been refluxed for some time and the mixture then evaporated in accordance with the directions of Clausen. Considerable varia-

¹ The CuSO₄-Ca(OH)₂ procedure removes many other substances besides the sugars. Examples of such are tartaric, citric, glyceric, and many of the higher saccharinic acids, and polyatomic alcohols. Some, like tartaric acid, can be removed completely. A large excess of Ca(OH)₂ appears to be essential.
tions are also noted when peptone culture media are extracted by ether and evaporated as before. These effects are possibly due to oxidation by the peroxides which are formed from the ether.

The experience gained in this laboratory indicates that there is at present no satisfactory procedure which will yield reasonably reliable results on such complex materials as culture media or urine. The results are probably too high even in the case of blood (which represents a fairly simple mixture) where it is possible to remove most of the nitrogenous and other interfering material, for, as will be shown, by slightly changing the conditions of the oxidations, the lactic acid values in some instances may be reduced by as much as 10 to 30 per cent.

Although it may be impossible in some instances by present methods to determine the absolute amount, it is often desirable to measure merely the change in lactic acid content, i.e. a gain or a loss in a given solution or medium. By making certain changes in the procedure the interfering effect of the nitrogenous materials may be greatly diminished and, although the lactic acid values obtained by direct oxidation may be slightly higher than those obtained from the ether extract, the results so obtained are believed to be more reliable as a measure of the changes in the lactic acid content than can be determined by any of the present methods. One may thus, for example, follow the changes which occur in a culture medium during the growth of microorganisms.

In studying the oxidation of pure lactic acid solutions it was found that a control of at least four factors is essential for the highest yield; namely, (1) the concentration of the oxidizing agent added, (2) the acidity of the solution, (3) the concentration of MnSO₄, and (4) the amount of lactic acid oxidized. (1), (2), and (3) would appear to follow from the equation

\[
\text{MnO}_2 \text{(solid)} + 4 \text{H}^+ + 2e \rightarrow \text{Mn}^{2+} + 2 \text{H}_2\text{O} \quad (1)
\]

The maximum yield of acetaldehyde is obtained when the lactic acid is oxidized by a very dilute solution of KMnO₄ or MnO₂ in the presence of very large quantities of MnSO₄ and at a relatively low acid concentration. In general, therefore, those conditions which appear to reduce the oxidation potential also reduce the overoxidation and coincidently increase the yield of acetaldehyde.

Phosphoric acid was found to be the most suitable acid, and the
optimum acidity appears to be 0.03 to 0.15 M. Although KMnO₄ may be used for most oxidations, it cannot be used where the amount of interfering oxidizable material is relatively large. For such oxidations the use of colloidal MnO₂ is particularly recommended. If added drop by drop a strong colloidal MnO₂ suspension may be added without causing much overoxidation, as is the case when KMnO₄ is used.

The procedure which will be described is essentially the same as that of Friedemann, Cotonio, and Shaffer. However, a number of changes have been made which increase the precision. Certain details of the older procedure require further emphasis, for it is found that the results are more precise and less subject to slight variations only when these details are followed. For this reason a rather detailed description of the procedure will be made.

**Description of Method.**

**Apparatus.**

The apparatus is the same as that described by Friedemann, Cotonio, and Shaffer. A few precautions are essential for success. First of all, an efficient, well cooled condenser is necessary to insure consistent results. The Hopkins type seems to be the best suited for this purpose. This should have not more than 3 mm. of space between the inside condenser tube and the outside tube, and the height of the condensing surface should be at least 25 cm. With this apparatus all the water vapor is condensed at a point below the tube through which the acetaldehyde passes to the absorption tower, and none is lost through solution in the vapors which are collected beyond.

**Solutions.**

1. **Oxidizing Agent.**—Either KMnO₄ or colloidal MnO₂ may be used. Colloidal MnO₂ is prepared as follows:

   **Method A.**—9 gm. of glucose, dissolved in 1 liter of approximately 0.5 N NaOH, are added to 3 to 4 liters of a solution of KMnO₄ (53 gm.). The mixture is warmed to 70° and kept at this

   2 The oxidation of glucose by alkaline KMnO₄ yields almost 2 molecules of oxalic acid and slightly more than 2 molecules of CO₂: C₆H₁₂O₆ + 10 O + 20 e → 2 CO₂ + 2 C₂H₂O₄ + 4 H₂O. Since MnO₄⁻ + 4 H⁺ + 3 e → MnO₂ (solid) + 2 H₂O, it can be seen that \( \frac{2}{5} \), or 0.7 equivalents of KMnO₄ are required.
temperature for 15 minutes. If the color disappears during this period, saturated aqueous KMnO₄ is added until an excess remains. The solution is then cooled, filtered through a large Buchner funnel, and washed well with water. The precipitate of MnO₂, which is granular at first, becomes more and more dispersed with the removal of alkali and salts. To facilitate the removal of salts, the precipitate is thoroughly broken up in a small amount of water (an egg beater is very effective for this purpose) and again returned to the Buchner funnel and washed. This salt-free precipitate is finally broken up thoroughly in water and diluted to 2 to 4 liters. The coarser particles settle out after a few hours. The upper two-thirds of the suspension, which is practically free from visible particles, is removed by suction after standing 12 to 24 hours. The remainder, containing coarser particles, is again suspended, agitated, and allowed to settle. This may be repeated several times. The supernatant, colloidal suspensions are united and adjusted to about 0.05 N or 0.1 N strength, in the following manner. A known amount of this colloidal suspension is run into a solution of KI acidified with H₂SO₄ and titrated with standard thiosulfate.

\[ 1 \text{ cc. } 0.1 \text{ N thiosulfate} = 1 \text{ cc. } 0.1 \text{ N colloidal MnO}_2. \]

Solutions prepared in this manner are not entirely colloidal, although no particles can be seen in them. Upon standing, a sediment settles out. This sediment is not granular, however, and can be readily resuspended. Electrolytes precipitate it.

The yield of this colloidal MnO₂ is not large; only about 50 to 70 per cent of the theoretical quantity is obtained when the precipitate is suspended and worked over five times. Additional treatment increases this yield somewhat.

Any substance which is readily oxidized by alkaline permanganate may be substituted for glucose in this preparation.

**Method B.**—A fairly stable and satisfactory suspension of MnO₂ may also be prepared by allowing KMnO₄ to react with MnSO₄. A concentrated solution of MnSO₄, containing slightly more than 3 equivalents, is run, with stirring, into a solution of KMnO₄ (2 equivalents). The resulting granular precipitate of MnO₂ is washed free of salts, etc., as in Method A above.

**Method C.**—A fairly satisfactory dilute suspension for use in the analysis of blood and tissue extracts, or in any sample which con-
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tains relatively small amounts of other oxidizable materials besides lactic acid, may be prepared by adding a dilute MnSO₄ solution drop by drop with shaking to a dilute (0.01 N) KMnO₄ solution until the color is almost discharged.

0.01 N to 0.005 N permanganate or colloidal MnO₂ is recommended for the determination of from 1 to 15 mg. of lactic acid. For smaller amounts of lactic acid, more dilute solutions may be used. However, if large amounts of other oxidizable materials are also present a strong colloidal MnO₂ suspension should be used.

2. Phosphoric Acid.—A 2 M solution is prepared by diluting 135 cc. of the syrupy acid (85 per cent, 15 M) to 1 liter.

3. Manganese Sulfate.—A 10 per cent solution of MnSO₄·4 H₂O (the ordinary salt) is used.

4. Talcum.—Finely powdered.

5. Sodium Bisulfite.—1 per cent solution. 5 to 10 cc. (more for large amounts of lactic acid) are used for each determination. Enough water is added to cover the beads in the tower. There should be an excess of bisulfite, equivalent to 5 cc. or more of 0.1 N iodine, over the amount required to unite with the aldehyde.

6. Starch Indicator.—5 gm. of arrowroot starch are suspended in 10 to 20 cc. of cold water and poured into 500 cc. of boiling water. Boiling is continued for 20 minutes. The flask is covered with a beaker and cooled. The supernatant clear solution is used. If care is taken to avoid contamination, this solution will keep for several weeks. If the solution becomes contaminated, the endpoint with very dilute iodine (0.002 N) is pink instead of blue.

7. Alkali to Liberate Bound Bisulfite.—Saturated NaHCO₃ solution.

8. Standard Iodine.—0.1 N iodine. This must be standardized frequently against thiosulfate which in turn must be standardized occasionally against KH(IO₃)₂ or KIO₃. The 0.1 N iodine is diluted each day to 0.01 or 0.002 N strength and should be protected from direct sunlight. Each cc. of the 0.01 N iodine solution is equivalent to 0.45 mg. of lactic acid.

9. d,l-Zinc Lactate Standard, Zn(C₃H₅O₈)₂·3 H₂O.—U. s. p. lactic acid is boiled with an excess of c. p. zinc carbonate and filtered hot. The solution is evaporated to a small volume and allowed to crystallize at room temperature. The crystals are transferred to a Buchner funnel and washed several times with
small portions of ice-cold water. The product is twice recrystal-
lized. The mother liquors, which contain some d, l- and much
$d$- or $l$-lactate, are discarded (5, 6). Its purity may be determined
either by a moisture determination (24 hours at 130°) or by the
determination of zinc as ZnO by ignition to constant weight.

Procedure.

The solution containing lactic acid is placed in a 300 cc. Kjeldahl
flask. If the solution is strongly acid or alkaline (greater than
0.05 N) or contains much buffer, the reaction is adjusted approxi-
mately to neutrality (phenol red) by adding either NaOH or
H$_2$SO$_4$. The indicator may be added directly to the solution.
Alcoholic solutions of indicators must not be used. From 1.5 to
8 cc. of H$_3$PO$_4$ depending upon the total volume of the solution in
the flask, 10 cc. of MnSO$_4$ solution and a pinch of talcum are next
introduced. Sufficient water is added to bring the total volume to
50 or 100 cc. A final acidity of 0.06 to 0.10 m H$_3$PO$_4$, at least 1
per cent MnSO$_4$, and a total volume of 50 cc. are preferable.
The reagents, especially the MnSO$_4$, should be rather carefully
measured.

The flask is now connected to the condenser, and the suction is
started. The solution is brought to boiling, and then the MnO$_2$
(or KMnO$_4$) is allowed to drop in, but only after the vapors are
condensing in the reflux condenser. The oxidizing agent is added
drop by drop at a rate of not more than 1 or 2 per second until an
excess has accumulated in the flask. When colloidal MnO$_2$ is used,
an actual excess should be present for at least 10 minutes. If the
solution becomes decolorized, more MnO$_2$ must be added. The
addition of the oxidizing agent requires up to 10 minutes. A total
of 20 minutes aeration is sufficient, time being counted from the
time the oxidation is started.

When the aeration is finished, the flame is removed, the suction
is stopped, and the tower is washed with water. Usually five
5 to 10 cc. portions of water suffice. The total volume should be

3 If the solution contains acetone, acetoacetic acid, or other volatile
bisulfite-binding substance, it is boiled for 5 minutes while aerating with
an empty flask attached to the tower.
kept as small as possible, because the titration can be carried out more rapidly and the end-point is sharper under these conditions. The excess bisulfite is removed with 0.1 N iodine, starch (1 cc.) being used as an indicator. The iodine is added until it is present in slight excess. This excess in turn is removed by 1 drop of 0.1 N thiosulfate solution. The end-point is finally adjusted with the dilute iodine solution in such a manner that 1 drop of this dilute iodine solution produces a clearly discernible change, but not a deep blue, in the colorless solution. The bound bisulfite is then liberated by adding 5 to 10 cc. of the saturated NaHCO₃ solution (2) and is titrated with the dilute iodine solution (0.01 or 0.002 N). Here again the end-point to be attained is the first change from the colorless. This should persist for 30 seconds.

Frequent blanks should be run on the reagents and the blank titration is subtracted as a correction before calculating the results.

The yield in pure solutions of lactic acid is from 97 to 99 per cent, depending upon the conditions. Correction for the yield is not ordinarily made when blood, muscle, culture media, etc., are analyzed because of the presence of other compounds which also yield bisulfite-binding substances. However, if the amount of such interfering substances is relatively small and if accurate results are desired, the results may be multiplied by a factor which is obtained by analyzing similar solutions containing known amounts of zinc or lithium lactate. The factor thus obtained should not be used in subsequent analyses of lactic acid containing solutions of unknown composition unless the concentration of reagents and conditions are the same.

Factors Which Influence the Oxidation.

Oxidizing Agent.—The oxidation of lactic acid to acetaldehyde, which apparently proceeds according to the following reaction

$$\text{CH}_3 \text{CHOH} \rightarrow \text{CH}_3 \text{CHO} + \text{H}_2\text{O} \quad (2)$$
may be accomplished by many oxidizing agents. The quantitative conversion into acetaldehyde, however, seems to be effected by only a few oxidizing agents. So far KMnO₄ has been found most satisfactory and the results are most reliable when a large amount of MnSO₄ is present (3).

According to Friedemann, Cotonio, and Shaffer, the oxidation of the lactic acid is really due to MnO₂ and not KMnO₄. This is based upon the well known fact that KMnO₄ is decolorized and MnO₂ is precipitated when KMnO₄ is added to an acid solution of MnSO₄. The oxidation-reduction potential also drops until it is approximately equal to that of an MnO₂ suspension.

$$2 \text{Mn}^{2+} + 3 \text{MnO}_4^- \rightarrow 5 \text{Mn}^{3+}$$

"In this reaction by an exchange of electrons, the permanganate (MnO₄⁻) is transformed to a lower intensity level (MnO₂⁺), which is still high enough to oxidize the lactic acid, but not so intense as to oxidize rapidly the acetaldehyde" (3). If MnO₂ is the effective oxidizing agent it follows that it should be possible to oxidize lactic acid to acetaldehyde to the same extent, or better, with MnO₂ as with KMnO₄ + MnSO₄. Boas (7), in 1893, used ordinary black oxide of manganese (MnO₂), obtaining a very good yield of acetaldehyde. The oxidation rate with the latter is slow. Hydrated or colloidal MnO₂, however, prepared as above, is very satisfactory. The reagent can be added more rapidly, the fluctuations in the results are smaller, and the yield is somewhat higher than with KMnO₄.

The oxidation appears to depend upon the physical state of the manganese dioxide. Ordinary very finely powdered MnO₂ oxidizes lactic acid incompletely within a reasonable time, due perhaps to the slow rate of oxidation. The oxidation is very rapid and the yield is greater than 97 per cent (depending upon the conditions) if precipitated (hydrated) MnO₂ is used. The precipitated MnO₂ remains granular if not washed as thoroughly as described in the directions above. Such granular MnO₂ may be added all at once.

4 Acetaldehyde may be obtained by the oxidation with KMnO₄ or MnO₂ of many substances having the general formulas CH₃-C(OH)-CO-R or CH₃-C(OH)-CHOH-R, but apparently not CH₃-C(OH)-CHOH-CH₃-R. β-Hydroxybutyric acid yields practically no acetaldehyde. Rhamnose, propylene glycol, and 1,2-dihydroxybutyric acid, on the other hand, are examples of substances which yield acetaldehyde. 1,2-Dihydroxybutyric acid yields acetaldehyde almost quantitatively (6).
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without very greatly lowering the yield. The colloidal material must be added more slowly.

The results of adding 0.1 N MnO₂ to varying amounts of lactic acid are shown in Chart I. The acidity and MnSO₄ concentration were kept constant. The yield of acetaldehyde decreases as the amount of substance analyzed becomes smaller. It seems to be a function of the logarithm of the lactic acid concentration, the effect being most pronounced when small amounts of lactic acid are analyzed and diminishing as the amount oxidized increases.

The unfavorable effect of strong MnO₂ on the yield (Chart I) from small amounts of lactic acid may be overcome by using more dilute MnO₂. This is shown in Chart II, the acidity and MnSO₄ being kept constant as before. Three analyses were made at each concentration and all results were used to obtain the average. The yield from 0.45 mg. of lactic acid increases from 98.1 per cent to

- The use of the granular hydrated MnO₂ for various reasons is not recommended. The colloidal preparations have proved far more satisfactory.
99.1 per cent in a regular progression as the concentration of added MnO$_2$ is decreased from 0.1 N to 0.005 N. It is therefore advisable to use very dilute oxidizing agent when small amounts of lactic acid are analyzed. This is not so important when larger quantities are present, and in this case the more concentrated MnO$_2$ may be used.

**Manganese Sulfate.**—It has been pointed out that probably one of the chief functions of manganese sulfate, in the oxidation of lactic acid by permanganate, is to reduce the permanganate rapidly. Permanganate has a high oxidation intensity, and by its presence, even if momentary, reduces the yield of acetaldehyde, presumably by further oxidation of some of the acetaldehyde formed from the lactic acid.

From Equation 3 above, it is apparent that the concentration of MnSO$_4$ should greatly influence the reduction to Mn$^{4+}$. The rate of removal of Mn$^{7+}$ should be accelerated as the concentration of Mn$^{4+}$ is increased. Overoxidation, due to the presence of KMnO$_4$, would therefore tend to be diminished as the rate of its removal is increased. Thus, in the presence of a very large amount of MnSO$_4$, good yields may be obtained even with 0.1 N KMnO$_4$.  

*This is clearly shown by the analysis with 0.1 N KMnO$_4$ made by Friedemann, Cotonio, and Shaffer (3) (Table IV, p. 354). From their results it can be seen that the yield of acetaldehyde increases regularly as the MnSO$_4$ concentration is increased.*
Chapt III. Effect of changing the concentration of manganese sulfate on the yield of acetaldehyde. The effect on the oxidation-reduction potential is also shown.
But the MnSO$_4$ has still another function. It affects the action of the MnO$_2$ either added as such or as it is formed from KMnO$_4$. This would follow from Equation 1. The effect of Mn$^{2+}$ can be directly demonstrated by measurement of the electrical potential of known solutions against a platinum electrode. If a small amount of MnSO$_4$ is added to a solution of KMnO$_4$ in $\text{H}_2\text{SO}_4$, it is found that the potential drops from about 1.63 volts (against the normal hydrogen electrode) to about 1.35 volts, which is the potential usually obtained from a suspension of MnO$_2$. Upon further addition of MnSO$_4$ the potential continues to fall at a regular rate, depending upon the concentration of MnSO$_4$ present.

The effect of changing the manganese sulfate concentration on both the oxidation-reduction potential and the yield of acetaldehyde from the oxidation of known amounts of lactic acid is shown in Chart III. The same reagents were used in obtaining both series of results. 4 cc. of 1.67 m H$_3$PO$_4$, a measured quantity of a 10 per cent solution of MnSO$_4$, and enough water to bring the volume to 95 cc. were mixed in a flask. 5 cc. of 0.1 n colloidal MnO$_2$ were added to all of the flasks. Solid MnSO$_4$, instead of the 10 per cent solution, was added to the last three flasks which contained 10, 25, and 50 gm., respectively. With the acidity and MnO$_2$ concentration thus kept constant, and only the MnSO$_4$ concentration varied, the results of potential measurements show, as would be expected, that the oxidation-reduction potential varies as the logarithm of the MnSO$_4$ concentration. By appropriate calculation it can be shown that these results are in accord with the equation

$$E_R = E_0 - \frac{RT}{2F} \ln \frac{[\text{Mn}^{2+}]}{C}$$

in which $C$ is the concentration of Mn$^{4+}$, which is assumed to be constant in all.

Since there are no sharp breaks in the curve it may be assumed that MnO$_4$ (not necessarily the lower oxidation stage, Mn$_2$O$_3$) is the effective oxidizing agent and that MnSO$_4$ decreases the tendency to change from Mn$^{4+}$ to Mn$^{2+}$. In other words, the function of the Mn$^{2+}$ is to reduce the oxidizing intensity of the MnO$_2$.

Such a reduction in oxidizing intensity, as the result of the
presence of Mn²⁺, should be reflected in the yield of acetaldehyde from lactic acid. As can be seen (Chart III), the yield increases regularly as the concentration of manganese sulfate increases. The point A on the chart shows the recovery when 1 gm. (or 10 cc. of 10 per cent MnSO₄) is present in 100 cc., and represents the conditions described in the procedure above (p. 29). The yield may be increased slightly by adding larger amounts of MnSO₄ than are suggested in the procedure. For practical purposes, however, this quantity is sufficient.

Effect of Acidity.—Although the yield of acetaldehyde is affected by the concentration of the MnO₂ dropped in and the MnSO₄ present in solution, it is not so markedly affected, within rather wide limits, by changes of the acidity of the solution. In Table I are shown the results of oxidations carried out at various concentrations of phosphoric acid. As can be seen, the results are lowest at high acidity. They increase as the acidity is decreased, reaching a maximum and remaining quite constant over a range of acidity from 0.167 to 0.042 m. It is almost as high in 0.2 m buffer mixtures of H₃PO₄-KH₂PO₄, pH 2.5 to 3.0. However, a large diminution is noted, and the fluctuations become more pronounced, when the oxidations are carried out in solutions of lower hydrogen ion concentration. In H₃BO₃, for example, the minimum yield

### TABLE I

<table>
<thead>
<tr>
<th>Acid or buffer mixture</th>
<th>Molar concentration</th>
<th>No. of determinations</th>
<th>Average percent yield</th>
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<tr>
<td>H₂SO₄</td>
<td>0.5</td>
<td>6</td>
<td>93.0</td>
</tr>
<tr>
<td>H₃PO₄</td>
<td>0.333</td>
<td>9</td>
<td>95.6</td>
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<tr>
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<td>6</td>
<td>97.8</td>
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<td>9</td>
<td>97.8</td>
</tr>
<tr>
<td>H₃PO₄</td>
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<td>20</td>
<td>97.8</td>
</tr>
<tr>
<td>H₃PO₄</td>
<td>0.042</td>
<td>3</td>
<td>97.7</td>
</tr>
<tr>
<td>H₃PO₄-KH₂PO₄</td>
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<td>3</td>
<td>96.8</td>
</tr>
<tr>
<td>KH₂PO₄</td>
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<td>3</td>
<td>89.5</td>
</tr>
<tr>
<td>H₃BO₃</td>
<td>2 per cent.</td>
<td>6</td>
<td>28.8</td>
</tr>
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</table>
obtained by 30 minutes oxidation and aeration was 17.2 per cent, while the maximum was only 66.5 per cent.

The oxidation, therefore, appears to depend somewhat upon the hydrogen ion concentration of the solution. It is not peculiar only to \( \text{H}_3\text{PO}_4 \). The same results may be obtained with \( \text{H}_2\text{SO}_4 \), provided the acidity is about the same as that of \( \text{H}_3\text{PO}_4 \). However, on account of its high degree of dissociation, the maximum yield with \( \text{H}_2\text{SO}_4 \) is obtained over a much more limited range of total acid concentration. Acetic acid, on account of its volatility and limited dissociation, is not a suitable acid; also, the presence of previously unneutralized alkali in the lactic acid solution may bring the hydrogen ion concentration into a range where the results may be low and variable. Phosphoric acid, therefore, on the whole appears to be best suited for the purpose. It is non-volatile and has a sufficiently low dissociation constant so that considerable variation in the amounts of this acid may be employed without greatly changing the hydrogen ion concentration.

It is interesting in this connection to compare the changes in oxidation-reduction potential with the results shown in Table I. According to Equation 1 the reduction of \( \text{Mn}^{4+} \) to \( \text{Mn}^{2+} \) consists essentially of the reaction of \( \text{Mn}^{4+} \) with 4 \( \text{H}^+ \) and 2 e. The influence of the hydrogen ion concentration is shown by the following measurements.

The solutions were the same as were used in the lactic acid determinations. Water, 10 cc. of 10 per cent manganese sulfate solution, 5 cc. of 0.1 n colloidal \( \text{MnO}_2 \), and the required amount of \( \text{H}_3\text{PO}_4 \) or \( \text{H}_2\text{SO}_4 \) were mixed in a flask and the volume was brought up to 100 cc. The \( E_H \) values for 0.067, 0.167, and 0.333 m \( \text{H}_3\text{PO}_4 \) solutions were 1.144, 1.176, 1.196 volts, respectively. In \( \text{H}_2\text{SO}_4 \) solutions of 0.2, 0.5, and 1.0 normality, the \( E_H \) values were 1.271, 1.306, and 1.321 volts, respectively.

These values lie approximately on a straight line if \( E_H \) is plotted against the logarithm of the hydrogen ion concentration or the \( \mathrm{pH} \), of the solutions. The yield of acetaldehyde, however, does not vary so regularly, although the tendency is in that direction. It is probable that the change in hydrogen ion concentration, in addition to its effect upon the oxidizing agent, also affects the substance reduced (8), in this case, lactic acid.
Effect of Interfering Substances.

The effect of interfering substances, in general, is about the same as by the older procedure. Those compounds which by the older procedure yield none or less than 1 per cent of bisulfite-binding substances yield about the same amount by the newer procedure. The sugars, saccharinic acids, and other sugar derivatives give approximately the same result.

A great difference, however, is noted in the oxidation of certain nitrogenous substances. This is shown by directly oxidizing such

\* The phosphotungstic acid filtrate was made up of 20 cc. of culture medium, 5 cc. of 10 per cent phosphotungstic acid, 20 cc. of 5 N H₂SO₄, and enough water to bring the volume to 100 cc. The acidity due to H₂SO₄ was neutralized by NaOH before adding the H₃PO₄.

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### TABLE II.

**Oxidation of Peptone and Peptone-Meat Extract Culture Media.**

5 cc. of culture medium; 10 cc. of 10 per cent MnSO₄; 4 cc. of 1.67 M H₃PO₄; H₂O to 100 cc. Oxidation by 0.1 N MnO₂. The results are expressed as mg. of lactic acid per 100 cc. of culture medium.

<table>
<thead>
<tr>
<th>Method used ..........</th>
<th>Aeration</th>
<th>Distillation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous treatment of solution</td>
<td>None.</td>
<td>PWo* filtrate.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Acidity of lactic acid solution</th>
<th>n H₂SO₄</th>
<th>0.067 M H₃PO₄</th>
<th>n H₂SO₄</th>
<th>0.067 M H₃PO₄</th>
<th>n H₂SO₄</th>
<th>n H₂SO₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>(2)</td>
<td>(3)</td>
<td>(4)</td>
<td>(5)</td>
<td>(6)</td>
<td></td>
</tr>
<tr>
<td>Peptone-meat extract culture medium (C).</td>
<td>83.8</td>
<td>55.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ether extraction.</td>
<td>46.2</td>
<td>48.2</td>
<td></td>
<td>24.4</td>
<td>16.7</td>
<td>46.7</td>
</tr>
<tr>
<td>I. Ether extract.</td>
<td>34.5</td>
<td>16.0</td>
<td>20.1</td>
<td>2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II. Residue.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ether extraction.</td>
<td>28.2</td>
<td>5.8</td>
<td>1.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0 per cent peptone culture medium (A).</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II. Residue.</td>
<td>29.8</td>
<td>3.6</td>
<td>26.5</td>
<td>2.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

*This is true only when a well cooled condenser is used.*
complex mixtures as bacterial culture media and urine. The results of oxidation of untreated and ether-extracted culture media are shown in Table II. These culture media contain 1000 mg., or more, of peptone and other nitrogenous compounds per 100 cc. Striking differences are noted if separate oxidations are carried out in \( \text{NH}_4\text{SO}_4 \) and 0.067 M \( \text{H}_3\text{PO}_4 \). The yield of bisulfite-binding substances is high when the oxidation is carried out in \( \text{NH}_4\text{SO}_4 \). This can be greatly diminished by precipitation with phosphotungstic acid. The greatest reduction, however, is noted when the oxidation occurs in \( \text{H}_3\text{PO}_4 \). Although phosphotungstic acid has a favorable effect on the oxidation in \( \text{NH}_4\text{SO}_4 \) practically no difference is observed in \( \text{H}_3\text{PO}_4 \). The favorable effect of low acidity is further shown by the fact that only slightly more of bisulfite-binding substance is obtained by distillation, whereas in \( \text{NH}_4\text{SO}_4 \) the result is considerably increased.

**Determination of Lactic Acid in Various Materials.**

A study of the methods heretofore used for the determination of lactic acid shows that the oxidation has been carried out in strongly acid solution. The methods of Boas, von Fürth and Charnas, Clausen, Friedemann, Cotonio, and Shaffer, and others call for an oxidation in a solution of \( \text{H}_2\text{SO}_4 \) of at least 1 per cent strength. The effect of acid concentration, although perhaps recognized, does not appear to have been studied. From the results presented it is evident that the oxidation may be carried out just as well at a considerably lower acid concentration. As a matter of fact, the yield at the lower acid concentration is higher, less subject to variation, and the yield of other bisulfite-binding substances from nitrogenous compounds is greatly diminished. The end-points also are sharper and more permanent.

Still another advantage is gained by carrying out the oxidation at the lower acid concentration. The recovery of added lactic acid from a 1 per cent peptone solution approaches that from a pure solution of lactic acid. However, if the oxidation is carried out in \( \text{NH}_4\text{SO}_4 \), the recovery may be less than 90 per cent. Such effects are not noted with non-nitrogenous materials. It appears to be due to the simultaneous oxidation of the non-protein nitrogenous materials in the presence of the strong acid.

The results of analyses illustrating these points are shown in
Lactic Acid Determination

Table III. Peptone-meat extract, a culture medium commonly used in bacteriological work, was oxidized directly without any preliminary treatment both before and after adding lithium lactate. The details and conditions are shown in Table III. The oxidation in $\text{H}_2\text{SO}_4$ resulted in a recovery of only 87 per cent as compared with about 95 per cent when the oxidation was carried out in $\text{H}_3\text{PO}_4$ solution. The favorable effect of removing some of

| Table III. Recovery of Added Lactic Acid from 1 Per Cent Peptone-0.3 Per Cent Meat Extract Culture Medium. |

| Culture medium (M) | 73.0 | 48.2 | 59.3 | 45.0 |
| Culture medium (M) plus 180 mg. lactic acid | 228.9 | 218.0 | 220.9 | 216.2 |
| Lactic acid recovered | 155.9 | 169.8 | 161.6 | 171.2 |
| Per cent recovery | 86.6 | 94.3 | 89.8 | 95.1 |
| Culture medium (N) | 63.8 | 48.6 |  |  |
| Culture medium (N) plus 90 mg. lactic acid | 147.0 | 135.3 |  |  |
| Lactic acid recovered | 78.2 | 86.7 |  |  |
| Per cent recovery | 86.9 | 96.3 |  |  |
| Lactic acid standard, per cent recovery | 93.0 | 98.1 |  |  |

* Phosphotungstic acid filtrate. See Table II.

the nitrogenous substances is shown by the analysis after precipitation by phosphotungstic acid. The recovery increased by 3 per cent in $\text{H}_2\text{SO}_4$ and a smaller increase of about 1 per cent was also noted in the oxidation carried out in $\text{H}_3\text{PO}_4$. The loss in recovery in the two series of analyses shown, compared with the oxidations of pure lactic acid under identical conditions, is about 6 per cent in $\text{H}_2\text{SO}_4$ and about 3 per cent in $\text{H}_3\text{PO}_4$.

While the results of oxidations in $\text{H}_3\text{PO}_4$ shown in Table III are
not entirely satisfactory, it must be remembered that they represent an extreme. The materials most often studied are blood and muscle, and the filtrates which are finally analyzed contain relatively small amounts of nitrogenous substances. The effect of their presence on the yield is therefore quite small. Nevertheless it is apparent that even in the extreme case (peptone-meat extract)

### TABLE IV.

**Analysis of Urine.**

10 cc. of urine (or an equivalent amount of filtrate or extract); 10 cc. of 10 per cent MnSO₄; 2 cc. of 2 M H₃PO₄; H₂O to 100 cc. Oxidation by 0.1 N MnO₂.

<table>
<thead>
<tr>
<th>Method</th>
<th>Mg. per cent of lactic acid</th>
<th>End-point</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct oxidation of untreated urine</td>
<td>26</td>
<td>Fades very rapidly.</td>
</tr>
<tr>
<td>Phosphotungstic acid filtrate</td>
<td>26</td>
<td>“</td>
</tr>
<tr>
<td>CuSO₄-Ca(OH)₂ filtrate</td>
<td>9.7</td>
<td>End-point sharp and permanent.</td>
</tr>
<tr>
<td>Ether extract.</td>
<td>8.1</td>
<td>“</td>
</tr>
<tr>
<td>CuSO₄-Ca(OH)₂ filtrate</td>
<td>5.5</td>
<td>“</td>
</tr>
</tbody>
</table>

### TABLE V.

**Analysis of Urine.**

Analysis of the CuSO₄-Ca(OH)₂ filtrate. Oxidation by 0.1 N MnO₂. The results are expressed as mg. of lactic acid per 100 cc.

<table>
<thead>
<tr>
<th>Method</th>
<th>N H₂SO₄</th>
<th>0.13 M H₃PO₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aeration</td>
<td>24</td>
<td>13.3</td>
</tr>
<tr>
<td>Distillation</td>
<td>40</td>
<td>12.7</td>
</tr>
</tbody>
</table>

the results are more reliable when the oxidation is carried out at the lower acidity.

The analysis of urine presents a number of interesting points. The substances present in largest amount, urea, creatinine, and uric acid, yield no bisulfite-binding substances on oxidation (3). This is true also of many other substances which are present in smaller concentration (3). Nevertheless the direct oxidation of urine in the presence of N H₂SO₄ results in the formation of a very
large amount of bisulfite-binding substances, but they are of such a nature as to indicate that the products have a very small affinity for the bisulfite. The first end-point fades very rapidly, and becomes fairly permanent only after long continued addition of iodine. A smaller amount of such substances is obtained if the oxidation is carried out in $\text{H}_3\text{PO}_4$. Phosphotungstic acid, although it removes a large part of the nitrogenous materials present, apparently does not remove the materials which give rise to these bisulfite-binding substances. They are, however, largely removed by $\text{CuSO}_4$-$\text{Ca(OH)}_2$, and such a filtrate gives values for lactic acid.

### TABLE VI.

**Analysis of Blood.**

The oxidations were carried out either with $0.0025 \, \text{n} \, \text{KMnO}_4$ or $0.01 \, \text{n} \, \text{MnO}_2$ as indicated in the table. The results are expressed as mg. per cent of lactic acid.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Tungstic acid filtrate</th>
<th>CuSO$_4$-$\text{Ca(OH)}_2$ filtrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$0.1 , \text{M} , \text{H}_3\text{PO}_4 + \text{MnO}_2$</td>
<td>$0.1 , \text{M} , \text{H}_3\text{PO}_4 + \text{MnO}_2$</td>
</tr>
<tr>
<td></td>
<td>Aeration</td>
<td>Distillation</td>
</tr>
<tr>
<td>Sheep</td>
<td>23</td>
<td>26</td>
</tr>
<tr>
<td>Dog*</td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td>Man†</td>
<td>8</td>
<td>7</td>
</tr>
</tbody>
</table>

* Blood taken 5 days after double nephrectomy. The animal was very quiet as in coma. Non-protein nitrogen 273 mg. per cent.
† Sample taken after 45 minutes of sleep.

which agree fairly well with the values obtained on the ether extract. Both titration end-points in the latter instances are sharp and permanent (see Table IV).

The advantage of carrying out the oxidation at the lower acidity is again illustrated in Table V. The CuSO$_4$-$\text{Ca(OH)}_2$ filtrate from urine was analyzed by two procedures, with the apparatus and aeration recommended by Friedemann, Cotonio, and Shaffer and by simple distillation. The details are described in the table. With the aeration method, the results were almost twice as high from N $\text{H}_2\text{SO}_4$ as from $\text{H}_3\text{PO}_4$. They were even higher when the distillation method and $\text{H}_2\text{SO}_4$ were used. The two methods gave identical results in $\text{H}_3\text{PO}_4$. 
The effect of the acid concentration on the oxidation of blood filtrates is not so striking as in the two extreme cases just cited. Nevertheless small differences are noted, especially when the lactic acid concentration is low, as in the three series of analyses shown in Table VI. Analyses were made on both the tungstic acid and CuSO₄-Ca(OH)₂ filtrates. The various procedures and the conditions used are shown in the table. The lowest values were obtained by oxidation in H₃PO₄, by the aeration method of Friedemann, Cotonio, and Shaffer. Triplicate analyses made by this procedure agreed more closely, and the end-points were sharper and more permanent, than by any of the other procedures used.

SUMMARY.

1. A procedure for the determination of lactic acid is described.
2. The factors which influence the precision of the method are discussed.
3. The effect of certain types of interfering substances upon the yield of lactic acid is demonstrated.
4. Data illustrative of the amount of lactic acid in various biological preparations are included.

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2. Clausen, S. W., J. Biol. Chem., 52, 263 (1922).
THE DETERMINATION OF LACTIC ACID
Theodore E. Friedemann and Arthur I. Kendall