THE DETERMINATION OF LACTIC ACID*

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In previous papers (1, 2) the conditions for the oxidation of lactic acid to acetaldehyde, quantitatively, and the accurate determination of the latter, were described. It was found that the oxidation to acetaldehyde appears to depend upon factors such as the concentration of oxidizing agent added, the acidity of the solution, the concentration of MnSO₄, and probably also the amount of lactic acid oxidized. The maximum yields were obtained with very dilute solutions of oxidizing agent and a high concentration of MnSO₄. From these studies we were of the opinion that the conditions necessary for the maximum yield of acetaldehyde were also those which tended to reduce the oxidation potential of the oxidizing agent. We described procedures for the determination of lactic acid, based upon these studies, which in our hands we had found to give the best results at that time.

Peculiar difficulties have since then arisen. The proximity to pathological laboratories, in which formaldehyde and acetone were being used, and an unusually cold water supply during the winter months, which made the reflux condensers too efficient, forced us to abandon the aeration procedure first recommended. The oxidation and distillation, which we now use and which is described in this paper, is similar in many respects to the older published methods of Boas (3) and von Fürth and Charnass (4). In order to obtain maximum and reproducible results the oxidation must be carried out under certain prescribed conditions. Since the bisulfite-binding substances produced by oxidation of substances other than lactic acid are not completely removed by a condenser, as in the aeration procedure of Friedemann, Cotonio,

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and Shaffer (1), it becomes necessary not only to remove the interfering and error-producing substances before oxidation but also to regulate the conditions of the oxidation itself more carefully.

The procedure described in this paper has been used in our laboratory for more than 3 years, during which time analyses were made on such materials as meats, tissues, culture media, blood, urine, and milk. The boiling and oxidation require only 15 minutes. The titration of bound sulfite has been somewhat improved. The use of standard iodine-potassium iodide, which must be standardized frequently, has been discontinued, and solutions prepared from stock 0.100 \( \text{N} \) KIO\(_3\) substituted. By this procedure the yields from pure solution, or the recovery of lactic acid added to such materials as culture media, are 99 ± 0.5 per cent.

**Description of Method**

**Apparatus**

Although the ordinary Kjeldahl nitrogen still and flasks may be used, this is not recommended because of the longer time of
boiling necessary and because a larger volume of distillate must be collected to wash down the aldehyde in the condenser tube. For small quantities, such as are present in blood and culture media, we recommend a smaller apparatus, similar to the one illustrated in Fig. 1. It consists of a 300 cc. Kjeldahl flask, a nitrogen bulb, and a small separatory funnel fitted into a 2-hole No. 5 rubber stopper, an 8 inch condenser jacket through which passes an 18 inch length of thin walled block tin tubing, a 2-hole No. 8 rubber stopper, and a 150 cc. extraction flask. The units are assembled as shown and fastened to the support by only one burette clamp. No clamp is needed for the Kjeldahl flask. Heat is applied by means of microburners.

Reagents

Oxidizing Agent—Either KMnO₄ or colloidal MnO₂ may be used. The colloidal MnO₂ of 0.02 to 0.01 N strength is preferred. It may be prepared by methods previously described (2).

Phosphoric Acid-Manganese Sulfate Reagent—100 gm. of MnSO₄·4H₂O (the ordinary salt) are dissolved in about 500 cc. of warm water. To this are added 25 cc. of syrupy phosphoric acid (85 per cent, 15 N). The solution is cooled and diluted to a volume of approximately 1 liter.

Talcum—Finely powdered talc.

Sodium Bisulfite—25 gm. are dissolved in 2 liters of water. The solution should be kept in a stoppered bottle.

Alkali to Liberate Bound Bisulfite—(a) A saturated NaHCO₃ solution; (b) a 10 per cent solution of Na₂CO₃.

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; 500 cc. of hot water are added and boiling is continued for 15 minutes. The flask is covered with a beaker, cooled, and kept in the refrigerator. The supernatant clear solution only is used. The solution will keep several weeks if care is taken to avoid bacterial growth. If contamination occurs (which may take place in a few hours in warm weather) the end-point with dilute iodine (0.002 N) is pink instead of blue.

Strong Iodine Solution—40 gm. of iodine and 75 gm. of KI are dissolved in a small quantity of water and the volume is brought up to about 2 liters. The solution is used to oxidize the excess bisulfite.
Determination of Lactic Acid

Standard Iodine Solution—The weak iodine solutions (0.01 to 0.002 N) may be prepared either by dilution of a standard iodine solution or by liberation of iodine from 0.1 N iodate + KI. The latter method is preferred because of the permanence of iodate solutions. 0.1 N KH(IO₃)₂ solution contains 3.2496 gm. per liter. 0.1 N KIO₃ contains 3.567 gm. per liter.

5 to 10 gm. of c.p. KI, about 200 cc. of cool, distilled water, and 2 to 5 cc. of 5 N H₂SO₄ are placed in a volumetric flask. Standard iodate is added and the volume is brought almost to the mark with cooled water. It is then warmed to 20° and the volume adjusted to the mark. Iodine, it should be remembered, is volatile even from dilute solutions. Dilute solutions should be kept cool for this reason.

Sodium Thiosulfate—An approximately 0.1 N solution.

Lactic Acid Standard—Either zinc lactate, Zn(C₃H₅O₃)₂·3H₂O, or lithium lactate, LiC₃H₅O₃, may be used. The latter is preferred because this salt is anhydrous and not hygroscopic. The preparation of pure dl-zinc lactate has already been described (1, 2). The lithium salt is prepared as follows:

U.S.P. lactic acid (85 per cent) is diluted with an equal volume of water and a few drops of phenol red indicator are added. Saturated (approximately 20 per cent) lithium hydroxide or LiCO₃ (the former is preferred) solution is added to slight excess, as is indicated by the phenol red. The solution is heated to boiling and the alkali is again added to slight alkalinity. It is now cooled. 4 volumes of 95 per cent alcohol are added and after cooling for some time, the mass of crystals is filtered off on a Buchner funnel and washed thoroughly with 95 per cent alcohol. This preparation is recrystallized from water and dried at 100°.

9.60 gm. of lithium lactate are transferred to a 1 liter volumetric flask. Enough H₂SO₄ is added to bring the final concentration to 0.2 N when diluted to the mark. This 0.1 M lactic acid standard will keep for at least 1 year, provided it is kept away from strong light or stored in a refrigerator.

Copper Sulfate Solution—200 gm. of CuSO₄·5H₂O are dissolved and brought to a volume of 1 liter. 10 cc. are used for every 250 cc. final volume; or 4 cc. for every 100 cc.

Calcium Hydroxide Suspension—1 kilo of fresh unslaked lime is slaked with water and immediately afterward sufficient water is
added to bring the volume to approximately 5 liters. The sus-
pension is thoroughly shaken, allowed to stand for a few seconds,
and decanted from the coarser particles. 10 cc. of this sus-
pension are used for every 250 cc. final volume, or 4 cc. for every
100 cc.

Preparation of Sample for Analysis

Blood and Milk—The proteins are removed by any of the com-
moner procedures, such as, for example, the Folin-Wu (tungstic
acid) or the Somogyi (Zn(OH)\(_2\)). The latter is preferred for milk.
The sugars are removed by precipitation with CuSO\(_4\) + Ca(OH)\(_2\)
(Van Slyke) which also removes citric, succinic, malic, maleic, and
tartaric acids, quantitatively, and other substances partially,
which would otherwise give sulfite-binding substances on
oxidation.\(^2\)

10 cc. of the sample are added to exactly 70 cc. of ZnSO\(_4\)-H\(_2\)SO\(_4\)
reagent (14 gm. of ZnSO\(_4\)·7H\(_2\)O per liter and H\(_2\)SO\(_4\) to ±0.1 N
strength). Exactly 20 cc. of a standard NaOH solution (adjusted
to give a distinctly alkaline reaction to phenolphthalein when
added to 70 cc. of the ZnSO\(_4\)-H\(_2\)SO\(_4\) reagent) are now run in. The
contents are mixed thoroughly and then either centrifuged or
filtered. Centrifugation is preferred, as explained below. An
aliquot, 50 cc., is transferred to a 250 cc. volumetric flask. 10
cc. of CuSO\(_4\) solution and 10 cc. of Ca(OH)\(_2\) suspension are added,
and after being mixed by rotation, the volume is brought to the
mark. Centrifugation is again preferred. Aliquots representing
at least 0.5 cc. of blood or milk are taken for analysis. 0.002 N
iodine is used in the titrations.

Urine—A 10 to 25 cc. sample is run into a 250 cc. volumetric
flask, the required amounts of CuSO\(_4\) and Ca(OH)\(_2\) reagents are
added, and the volume is brought to the mark. An aliquot of the
filtrate, representing not more than 5 cc. of urine, is used for
analysis. 0.005 N iodine solution is used for titration.

\(^1\) Data showing the lactic acid content of milk and tissues are not pre-
sented in this paper, but the procedure is described here for the sake of
completeness. Studies in which these procedures are used are now in
progress and will be reported in future papers.

\(^2\) For a list of biochemically important compounds which may affect the
lactic acid values see the following: Friedemann, Cotonio, and Shaffer (1);
Friedemann and Kendall (2), foot-note p. 31; Friedemann (5).
Determination of Lactic Acid

Culture Media—Since bacteria grow so rapidly and produce such great changes in only a short time the analyses should either be carried out immediately or all metabolic activities stopped by strong acidification. Analyses on this acidified sample may then be carried out at any convenient time. Where, as is usually the case, culture media do not contain large amounts of protein, it is not necessary to deproteinize before adding the copper-calcium reagents.

25 cc. of the culture are pipetted into a 250 cc. volumetric flask which contains 25 cc. of \( \text{NH}_3 \). The volume is brought to the mark. This acidified sample will keep indefinitely if stored in the refrigerator. 25 cc. are pipetted into a 250 cc. volumetric flask. To this are added about 150 cc. of water, 10 cc. of CuSO\(_4\) solution, and 10 cc. of Ca(OH)\(_2\) suspension. The contents are mixed by gentle rotation. The volume is brought to the mark. The contents are mixed thoroughly and finally filtered through a dry fluted filter. An aliquot representing not more than 0.5 cc. of culture medium (more may be taken if the peptone content is less than 1 per cent) is taken for analysis. 0.005 \( \text{N} \) iodine is used for titration.

Tissues—Due to the rapid postmortem changes occurring in tissues, a number of procedures, all of which aim to stop enzyme action either by cold (liquid air) or precipitants (HgCl\(_2\) + HCl, trichloroacetic acid, etc.), have been proposed. The following simple procedure has given good results in lung and skin tissues taken from animals immediately after death.

Weighing bottles, each containing a short very thin glass rod, and 10 cc. (pipette) of \( \text{N} \) H\(_2\)SO\(_4\), are numbered and tared. The tissue is immediately either ground in a meat chopper (lung) or rapidly cut into very small pieces with a sharp pair of scissors (skin). An approximately 10 gm. aliquot is then transferred to the tared weighing bottles and immediately thoroughly stirred with the acid. The time elapsing from removal from the animal to acidification is usually not more than 1\(\frac{1}{2} \) minutes. The weighing bottle is now weighed.

In the case of lung tissue the contents of the weighing bottle are emptied into a large (8 inch diameter) mortar which contains 50 gm. of sand. The weighing bottle is washed with three 10 cc. (pipette) portions of distilled water. The tissue is then ground
thoroughly after which 10 cc. of 10 per cent ZnSO₄·7H₂O (6) are added and the grinding is again resumed in order to mix thoroughly. A measured volume of approximately N NaOH (determined by previous titration of 10 cc. of the N H₂SO₄ + 10 cc. of the ZnSO₄ solution + 80 cc. of H₂O and phenolphthalein indicator) is now run in from a burette; the mixture is continually stirred during the addition. Sufficient distilled water is added to make a 10-fold dilution. After being mixed thoroughly again and allowed to stand (covered) about 30 minutes, the contents are filtered through a dry filter.

Skin samples may be treated differently. Grinding apparently does not greatly alter the results. This may be due either to the relatively slow metabolic changes or to the rapid penetration of the acid. 30 cc. of water are added to the contents of the weighing bottle and the tissue is allowed to stand 2 hours in the refrigerator with frequent stirring. Zinc sulfate, alkali, and water are added as described above. This mixture is allowed to stand an additional 2 hours, during which it is frequently stirred, before filtration.

Sugar may be determined directly on these Zn(OH)₂ filtrates. For lactic acid determination it is necessary to treat further with CuSO₄ + Ca(OH)₂ as described for blood.

Procedure

10 cc. of H₃PO₄-MnSO₄ reagent and a pinch of talcum are placed in a 300 cc. Kjeldahl flask. The material to be analyzed is next added. The total volume is brought to about 100 cc. and the flask is attached to the apparatus. 10 cc. of sodium bisulfite solution are placed in the 150 cc. extraction flask. The micro-burners are adjusted to bring the solution to boiling in about 3 minutes (height of flame 3 to 3½ inches).

The addition of oxidizing agent is begun as soon as vapors begin to pass into the condenser. The rate of addition is unimportant, but it is essential to have an excess of oxidizing agent from the be-

³ Normal blood contains no demonstrable quantities of volatile sulfite-binding substances. Normal urine, however, contains from 10 to 25 per cent of such substances. Where it is desirable to correct for their presence distillation of a separate slightly acidified aliquot (by H₂SO₄) is recommended.
Determination of Lactic Acid

ginning up to the end of the distillation. This excess is indicated by a brown or reddish brown (but not gray) color. In order to insure an excess we recommend a continued slow addition of the colloidal MnO₂ throughout the oxidation. From 25 to 40 cc. are usually necessary. Although practically complete in 10 minutes, the results occasionally are low in this period. For this reason we recommend a 15 minute period of oxidation.

The receiving flask is detached from the stopper and lowered to the shelf a few minutes before the end of the oxidation. The glass tip is rinsed with a small amount of water and the flask is removed for titration. The total volume of distillate and washings is 50 to 75 cc. The receiving flask should be cooled in a shallow pan of ice water before the titration is carried out. This precaution appears to be necessary in hot weather. It is not necessary when the temperature of the room is below 25°, although we do it even then.

The removal of the excess bisulfite and titration of acetaldehyde-bisulfite are accomplished according to the Clausen method (7). 1 to 2 cc. of clear starch solution are added. Strong iodine-KI solution is then added to slight excess. This is immediately removed by the cautious addition of thiosulfate. The walls of the flask are now washed down by a thin stream of water, after which the end-point is adjusted to a faint blue by means of the dilute standard iodine solution. The flask is returned to the cooling bath and the contents of the other flasks are adjusted to this same end-point. Since our equipment enables us to make twelve determinations at a time and since the cooling pans can conveniently accommodate these flasks, we adjust all of the determinations to this end-point at the same time. This requires from 5 to 10 minutes and also allows the flasks first adjusted to become further cooled.

The bound bisulfite is now titrated. Approximately 15 cc. of saturated NaHCO₃ are added. The dilute standard iodine solution is run in rapidly, or at such a rate as to keep pace with the decomposition into aldehyde and bisulfite. When this slows up 1 cc. of 10 per cent Na₂CO₃ is added and the addition of standard iodine is continued until the end-point is permanently faintly blue.

See the discussion below (p. 307).
To insure a true end-point 1 cc. more of the Na$_2$CO$_3$ solution is added. The standard iodine is added again if necessary. The end-point should persist for at least $\frac{1}{2}$ minute.

**Blanks**—Oxidations with pure water always yield a small blank. We have found these to be almost proportional to the concentration of standard iodine used. A recent series, for example, gave an average of 0.18 and 0.44 cc. with 0.005 and 0.002 N I, respectively. Filtrates always yield a higher blank. The filter paper apparently either contains lactic acid or a substance which gives an analysis similar to lactic acid (8). It is small and variable in acid filtrates, but is largest and quite constant in neutral or alkaline filtrates. Closely checking results may be obtained with the same size and brand of filter paper. Although the error is negligible when larger quantities are analyzed, the results may be at least 5 per cent too high when less than 0.2 mg. of lactic acid is analyzed. In the latter case (blood, for example) if filtrates are analyzed, we recommend carrying the blanks through the same operations with the same aliquots, filter paper, etc., throughout. On the whole, however, we find centrifugation as suggested by Avery and Hastings (8) more satisfactory for such samples.

**Calculations**—Each cc. of 0.01 N iodine solution used in the titration of bound sulfite is equivalent to 0.45 mg. of lactic acid (1, 7).

**Effect of MnSO$_4$**

The effect of MnSO$_4$ and its probable function have been discussed at considerable length in previous papers (1, 2). Its presence allows a more rapid addition of oxidizing agent, a shorter oxidation period, and higher and more constant results. It appears also to protect the evolved acetaldehyde from further oxidation when other substances, particularly nitrogenous compounds, are being oxidized simultaneously.

The results shown in Table I illustrate its beneficial effect. From a yield of approximately 87 per cent (the same as obtained by the older methods (4, 7)), the results rapidly increase until they approach the maximum at about 0.25 per cent MnSO$_4$ concentration. We recommend a concentration of at least 1 per cent.

Dr. A. B. Hastings (8), who first called our attention to this, finds that filtrates produce sulfite-binding substances as well as CO$_4$ on oxidation.
Determination of Lactic Acid

Culture media and urine are perhaps the most difficult to analyze since they contain a very great variety of different substances, most of which cannot be removed by the usual precipitation methods without also removing some lactic acid. Analysis of these with and without added MnSO₄ are shown in Table II. The values (Columns 3 and 6), with only one exception, are greatly increased by the presence of MnSO₄.

Equally striking increases are also obtained in the yield of added lactic acid (Columns 4 and 7, Table II). From an average recovery of about 60 per cent of the added lactic acid, the yield was increased to about 98 per cent by the oxidation of the culture medium, Sample 2, with MnSO₄ present. An extreme instance of this is illustrated by Urine C. Only about 20 per cent was recovered when the sample was oxidized without MnSO₄; however, added MnSO₄ increased this to 88 per cent.

These results raise a question as to the nature of "lactic acid" in urine determined by this or other methods. According to Friedemann and Kendall (2) direct determination of lactic acid on the CuSO₄ + Ca(OH)₂ filtrate of urine is only slightly higher...
than on the ether extract. In the latter case there is always a loss of lactic acid due to oxidation by organic peroxides formed during extraction and evaporation; volatilization of aldehydes, which are always present, also may occur during evaporation. The results in Column 6, Table II, are, therefore, perhaps as reliable as any obtained on ether extracts.

To test whether this ether-soluble material is lactic acid, the culture medium and Urine C were inoculated with a loop full of feces. Incubation at 37° produced heavy growth in both. Much lactic acid was formed from the sugar in the culture medium. This newly formed lactic acid and the acid initially present in the culture medium were largely removed by the bacteria. However, in spite of heavy growth, none appeared to have been removed from the urine. We may therefore question whether lactic acid is present in measurable amounts in a normal resting urine.

In addition to higher results in an unknown mixture and greater

<table>
<thead>
<tr>
<th>Description of sample</th>
<th>Lactic acid added</th>
<th>Lactic acid recovery, without MnSO₄</th>
<th>Lactic acid recovery, 1 per cent MnSO₄</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg. per cent</td>
<td>mg. per cent</td>
<td>mg. per cent</td>
</tr>
<tr>
<td>Serum-peptone-glucose-broth medium, Sample 2</td>
<td>112.5</td>
<td>1.0</td>
<td>156.2</td>
</tr>
<tr>
<td>Sample 2, 10 days after inoculation with loop full of feces</td>
<td>225.0</td>
<td>0.5</td>
<td>38.3</td>
</tr>
<tr>
<td>Urine B</td>
<td>36</td>
<td>5.0</td>
<td>7.7</td>
</tr>
<tr>
<td>“C, immediately analyzed</td>
<td>36</td>
<td>5.0</td>
<td>7.4</td>
</tr>
<tr>
<td>Urine C, 5 days after inoculation with loop full of feces</td>
<td>36</td>
<td>5.0</td>
<td>5.8</td>
</tr>
</tbody>
</table>
recovery of added lactic acid, still another advantage may be claimed for the use of MnSO₄. In Table I it will be noted that the fluctuations are large when no catalyst is used, but that they become smaller as the MnSO₄ concentration is increased. The greater constancy and the almost quantitative yield make it possible to obtain fairly accurate results with fewer determinations and without the use of a factor in the calculations.

According to Boyland and Gerard and Meyerhof (9) equally satisfactory results may be obtained without the use of MnSO₄. These authors have obtained excellent results by careful addition of KMnO₄ during an oxidation period of 20 to 30 minutes. This is also in accord with our own experience with fresh muscle and blood, which contain relatively less non-protein nitrogen, etc. than such solutions as culture media and urine. As shown above, the presence of MnSO₄ is necessary when more complex materials such as culture media and urine are to be analyzed. We recommend its use in every determination. Certainly no time is lost by its addition, since it is added with the acid. Its use is justified because of (1) the shorter oxidation period, (2) because relatively wide limits in concentration of the oxidizing agent and the rate of its addition are permissible, (3) because of the higher yields, and (4) the smaller fluctuations among determinations.

Size of Sample

The procedure here described applies to samples containing quantities of lactic acid up to about 10 mg. Larger amounts of lactic acid require a greater concentration of MnSO₄ and stronger oxidizing agent. If sufficient material is available, our custom has been to analyze from 2 to 5 mg. The final titration then requires at least 5 cc. of 0.01 or 0.005 N iodine. The color changes are sharper with these stronger iodine solutions. However, this often requires a very large sample; and the interfering substances may then introduce a large error. Examples of this are shown in Table III.

Aliquots were chosen so that approximately 1 to 3 mg. might be analyzed. This required from 1 to 2 cc. of the culture media and at least 10 cc. of urine. Considerable increases were noted in every instance when only one-half as much was analyzed (see Columns 3 and 7).
The effect on added lactic acid is shown in Columns 4 and 8. Here the larger aliquots, with only one exception, caused a lower recovery. Merely reducing the size of aliquot to one-half increased the recovery. The most striking increase is shown by

<table>
<thead>
<tr>
<th>Description of sample</th>
<th>Series A</th>
<th>Series B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactic acid added</td>
<td>Sample analyzed</td>
<td>Without added lactic acid</td>
</tr>
<tr>
<td></td>
<td>mg. per cent</td>
<td>mg. per cent</td>
</tr>
<tr>
<td>1 per cent peptone + 0.3 per cent meat extract</td>
<td>45</td>
<td>2.0 51.2 94.8 96.9</td>
</tr>
<tr>
<td>Serum-peptone-glucose-broth culture medium, Sample 3...</td>
<td>45</td>
<td>1.0 236.0 279.0 95.5 0.5 240.7 285.7 100.0</td>
</tr>
<tr>
<td>Serum-peptone-glucose-broth culture medium, Sample 4...</td>
<td>90</td>
<td>1.0 237.4 326.5 99.0 0.5 241.3 331.6 100.3</td>
</tr>
<tr>
<td>Urine D...</td>
<td>18</td>
<td>10.0 9.5 23.6 78.3</td>
</tr>
<tr>
<td>&quot; E*</td>
<td>18</td>
<td>10.0 6.7 22.8 89.5 5.0 7.1 25.0 99.4</td>
</tr>
<tr>
<td>&quot; F</td>
<td>18</td>
<td>10.0 11.9 23.7 65.5 5.0 15.5 32.4 93.9</td>
</tr>
<tr>
<td>&quot; G†</td>
<td>18</td>
<td>10.0 32.4 47.9 86.1 5.0 35.1 52.7 97.8</td>
</tr>
</tbody>
</table>

* Volatile sulfite-binding substances obtained by distillation of the CuSO₄ + Ca(OH)₂ filtrate acidified by H₃PO₄ were equivalent to 1.0 mg. per cent of lactic acid. The values for "lactic acid" shown above are not corrected for these preformed volatile sulfite-binding substances.
† Volatile sulfite-binding substances were equivalent to 3.1 mg. per cent of lactic acid.

Urine F. The recovery of added lactic acid was only 65.5 per cent when an aliquot of the CuSO₄-Ca(OH)₂ filtrate equivalent to 10 cc. of urine was analyzed. Analysis of 5 cc. increased this to 93.9 per cent.
The importance of testing the procedure for every type of material analyzed is evident from these data. Thus, no matter how small the lactic acid content may be and however desirable it may be to take a proportionately large sample, a knowledge of the maximum size of sample is necessary. These data indicate, for example, that not more than 0.5 cc. of culture medium and not more than 5 cc. of urine should be analyzed.

Effect of Acid Concentration

Although the acidity of the contents of the reaction flask, to which a known quantity of pure lactic acid solution has been added, may vary widely without greatly affecting the yield, its regulation within rather narrow limits is necessary when other organic substances, especially certain nitrogenous compounds, are present (2). This is shown in Table IV.

Oxidations were carried out on the CuSO₄ + Ca(OH)₂ filtrates of culture media and urine. A few determinations were also made directly without previous precipitation, etc. The H₃PO₄-MnSO₄ reagent and the concentration recommended by the authors were used in one of the series. Two other series were set up, sufficient H₂SO₄ being added to bring the total acid concentration to 0.1 and 1 N, respectively. Two types of procedure were used in carrying out the oxidation: the aeration method of Friedemann, Cotonio, and Shaffer and simple distillation, as outlined above.

In reference to Columns 2 and 3, the results show no striking differences between the aeration and distillation methods when carried out on the untreated samples. Data not cited here, however, show large differences when stronger acid is used. The aeration procedure gives lower values at the higher acid concentration. The oxidation at this low (H₃PO₄) acidity is apparently so mild as to yield none of the higher aldehydes which would be removed by the reflux condenser in the aeration procedure.

Removal of the sugars (citric acid, etc.) by CuSO₄ + Ca(OH)₂ appears to have only a slight effect on the results by these two procedures (Columns 4 and 5), illustrating again the mildness of the oxidation in the presence of H₃PO₄.

Increasing the acidity with 0.1 N H₂SO₄ gives slightly higher values than with the H₃PO₄ (Columns 6 and 7). Here again the type of sulfite-binding substances appear to be the same; i.e.,
the results practically agree whether obtained by aeration or distillation.

TABLE IV
Analysis of Culture Media and Urine. Effect of Acid Concentration

The results are expressed as mg. of lactic acid per 100 cc.

<table>
<thead>
<tr>
<th>Material</th>
<th>Quantity analyzed</th>
<th>Direct, no treatment</th>
<th>CuSO₄-Ca(OH)₂ filtrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>H₂PO₄ Aeration (1)</td>
<td>Distillation (2)</td>
</tr>
<tr>
<td>1 per cent peptone only</td>
<td>1.00</td>
<td>5.0</td>
<td>5.5</td>
</tr>
<tr>
<td>1 per cent peptone + 0.3 per cent meat extract</td>
<td>1.00</td>
<td>43.8</td>
<td>47.8</td>
</tr>
<tr>
<td>Same 18 hrs. after inoculation with <em>Bacillus coli</em></td>
<td>1.00</td>
<td>40.8</td>
<td>40.1</td>
</tr>
<tr>
<td>Same 7 days after inoculation with <em>Bacillus coli</em></td>
<td>1.00</td>
<td>7.4</td>
<td>7.4</td>
</tr>
<tr>
<td>Serum-peptone-glucose-broth culture medium, Sample 1*</td>
<td>1.00</td>
<td>246</td>
<td>233</td>
</tr>
<tr>
<td>Urine</td>
<td>10.0</td>
<td>11.0</td>
<td>11.7</td>
</tr>
<tr>
<td>&quot; 2</td>
<td>10.0</td>
<td>8.1</td>
<td>9.2</td>
</tr>
<tr>
<td>&quot; 3</td>
<td>10.0</td>
<td>10.6</td>
<td>7.8</td>
</tr>
<tr>
<td>&quot; 4</td>
<td>10.0</td>
<td>8.5</td>
<td>7.5</td>
</tr>
<tr>
<td>&quot; 5†</td>
<td>10.0</td>
<td>84</td>
<td>85</td>
</tr>
<tr>
<td>Blood from dog. Amytal anesthesia</td>
<td>1.00</td>
<td>12.8‡</td>
<td>7.9</td>
</tr>
</tbody>
</table>

* Meat infusion + 1 per cent peptone + 0.2 per cent phosphate; adjusted to pH 7.8; + 1 per cent glucose + 2 per cent rabbit serum.
† CuSO₄-Ca(OH)₂ filtrate, pale green, suggesting incomplete precipitation.
‡ Folin-Wu filtrate.

Normal H₂SO₄, however, greatly increases the sulfite-binding substances, and it is apparent that the determination is impossible by any procedure at this acidity on such complex materials.
Determination of Lactic Acid

As has been shown previously (2) an increased acidity has many effects. Its effect is most marked when carried out on samples which contain much nitrogenous and other interfering materials. The high acidity first of all decreases the yield from pure lactic acid solution. Secondly, the yield is further decreased by the presence of certain interfering substances, the effect becoming greater the higher the acidity. And thirdly, the interfering substances themselves may yield sulfite-binding substances, thus giving false high values. These effects, especially the first two,

TABLE V

Analysis of Whole Blood and Serum. Comparison of Methods

The results are expressed as mg. of lactic acid per 100 cc.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Description of sample</th>
<th>WoO₄ filtrate</th>
<th>WoO₄ filtrate + CuSO₄, Ca(OH)₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Aeration</td>
<td>Distillation</td>
</tr>
<tr>
<td>1</td>
<td>Human whole blood†</td>
<td>13.5</td>
<td>10.8</td>
</tr>
<tr>
<td>2</td>
<td>“</td>
<td>13.1</td>
<td>10.4</td>
</tr>
<tr>
<td>3</td>
<td>“</td>
<td>13.1</td>
<td>9.8</td>
</tr>
<tr>
<td>4</td>
<td>“</td>
<td>16.0</td>
<td>12.3</td>
</tr>
<tr>
<td>5</td>
<td>“</td>
<td>17.1</td>
<td>13.4</td>
</tr>
<tr>
<td>6</td>
<td>Sheep</td>
<td>16.6</td>
<td>18.8</td>
</tr>
<tr>
<td>7</td>
<td>“</td>
<td>18.8</td>
<td>16.1</td>
</tr>
<tr>
<td>8</td>
<td>“</td>
<td>44.9</td>
<td>44.7</td>
</tr>
<tr>
<td>9</td>
<td>Dog whole blood</td>
<td>25.4</td>
<td>31.0</td>
</tr>
<tr>
<td>10</td>
<td>“ serum; old, sterile</td>
<td>25.8</td>
<td>25.6</td>
</tr>
<tr>
<td>11</td>
<td>“ “ contaminated</td>
<td>31.4</td>
<td>31.3</td>
</tr>
<tr>
<td>12</td>
<td>Rabbit serum; old, sterile</td>
<td>20.3</td>
<td>20.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>87.8</td>
<td>86.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>132.7</td>
<td>133.5</td>
</tr>
</tbody>
</table>

* Folin-Wu tungstic acid filtrate.
† Human whole blood was obtained from laboratory workers about 3 hours after breakfast, without a previous resting period.

may be overcome to some extent by increasing the MnSO₄ concentration.

The H₃PO₄-MnSO₄ reagent, in the quantity (10 cc. per 100 cc. total volume) recommended, contains enough acid to take care of the alkalinity of the CuSO₄ + Ca(OH)₂ filtrates. Should the alkalinity of solutions be higher, we would suggest adjustment to approximate neutrality (phenol red added directly to the sample). The same should be done also if the solution contains much strong acid—say, greater than 0.05 N.
Comparison of Methods

In Table IV a comparison was given of the aeration procedure of Friedemann, Cotonio, and Shaffer and of the distillation as described by us. Analyses of culture media and urine were shown to agree within the limits of experimental error. Additional data obtained on blood filtrates are shown in Table V. These also show that, if precautions are taken to remove carbohydrates and other substances by the CuSO$_4$ + Ca(OH)$_2$ reagents, the results by the two methods agree within the limits of experimental error.

Titration of Bound Bisulfite

The oxidation as outlined above yields acetaldehyde almost quantitatively. In spite of this, however, heavy losses may occur in the final titration. This is due to air oxidation at the time of addition of the carbonate. It seems to be catalyzed by the extremely small amount of manganese (whose action is enhanced presumably by some phosphoric acid, since we have noticed it more often in the oxidations carried out in phosphoric acid) which is carried over in the distillation. It can be prevented by using a large amount of bisulfite, by keeping the volume in the receiving flask as low as possible, and by cooling before the titrations.

Efficient cooling is the most effective method for preventing the losses by air oxidation. This is done just before titration. The low temperature causes condensation of moisture on the flask, but this appears to help rather than hinder the detection of the delicate blue starch-iodine end-point. That cooling increases the accuracy of the titration can be demonstrated by the following simple experiment.

100 cc. of water and 1 cc. of starch solution are placed in a 150 cc. extraction flask (these flasks are preferred to the Erlenmeyer flasks because of the greater depth of solution and the wider neck). The flask is cooled in a pan containing ice and some water. (The large pan, 12 X 24 X 3 inches, used by us conveniently holds twelve flasks.) Titration with 0.002 N iodine requires only a few drops and gives a sharp change from colorless to blue. Another flask, similarly prepared, is now warmed to 37°. Titration of this

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6 A discussion of the theory, technique, and many sources of error is given by Friedemann, Cotonio, and Shaffer.
Determination of Lactic Acid

warmed solution requires more iodine and the color changes gradually, as more is added, from colorless to purple and finally to blue.

SUMMARY

1. The preparation of lithium lactate is described.
2. The use of dilute iodine solutions prepared from KIO₃ or K₂(IO₃)₂ is suggested.
3. A procedure for the determination of lactic acid in various biological materials is described.
4. The factors which influence the accuracy of the method are discussed.

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THE DETERMINATION OF LACTIC ACID
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