The determination of lactic acid

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THE DETERMINATION OF LACTIC ACID

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IV. SUMMARY

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The determination of the exact amount of lactic acid in solution has been a subject under investigation since around 1890. The measurement of the amount of this acid in naturally fermented beverages has been and is still being used in Europe as a quality control measure in the fermentation industries. As a result of the importance of this determination to the fermentation industry, many of the early investigations of the determination have been done by this group and appear frequently in the French, German, and other European journals of the food and beverage industries. The normal amount of lactic acid in wines is variously reported as being between 1.2 and 6.4 grams per liter.

More recently, particularly since it has become evident that lactic acid probably plays an important role in the metabolism of human body tissue, the determination of lactic acid has assumed additional importance. In work of this type the amount of lactic acid available for analysis is necessarily small and it has become evident that the methods of the wine industries were inadequate since, in most cases, the probable error would exceed the total amount of lactic acid in the sample. Workers in this newer line of investigation then turned their attention from these older, well-known methods to other
more exact methods which will be described later in the review of literature. These more recently developed methods, together with the old routine methods of the fermentation industries and a few other occasional procedures which have been developed for special use, represent the majority of the work which has been done to date on the determination of lactic acid.

**B. Present Investigation**

Part of the original purpose of the present investigation was to measure the amount of lactic acid which is present in certain fermenting brines as a result of the normal bacterial fermentation of sugars to lactic acid. Since other acids may be present, a titration with standard alkali may not give a true measure of the amount of lactic acid present. There are also other conditions which render the simple titration inaccurate.

The method which appeared best suited for the present needs was chosen from the literature and, in order to establish the proper technique, the method was tried on a known solution of lactic acid. In the course of this preliminary work there arose certain theoretical considerations as well as certain technical problems which seemed worthy of further investigation. It is the plan, therefore, to present the findings of the investigations on the method, and further, to make a comparison of the amount of lactic acid present in typical brines as measured by ordinary titration and by the newer oxidation method which is believed to be more specific for lactic acid.
Many of the early methods for the determination of lactic acid are based on a separation of the barium salt of lactic acid in alcohol. In a solution of 75 per cent ethyl alcohol and 25 per cent water, barium lactate is completely soluble and is separated (as that salt) from such insoluble barium compounds as barium citrate, tartrate, and succinate. Other interfering substances are removed previously. Among these interfering substances are sugars and other ether-insoluble substances which are eliminated by means of an ether extraction, and volatile acids, chiefly acetic and formic, which are removed by steam distillation.

1. Determination of Lactic Acid as Barium Oxide

Method of Moslinger

Moslinger (1) in 1901, working on the determination of the acids of wine, was the originator of a method based on the separation of barium lactate in 75 per cent alcohol. Briefly, his method involved a steam distillation of 50-100 c.c. of wine, followed by neutralisation of the residue with barium hydroxide and the addition of barium chloride. This was followed by evaporation on a steam bath to 25 c.c., and the addition of 75 c.c. of 95 per cent alcohol and filtration.
After evaporation, the filtrate was ashed and the amount of barium oxide present determined by direct titration. The alkalinity of the ash was a measure of the lactic acid present. The method was rather crude but was reasonably simple and served as a method in the routine analysis of wines until improved in 1912 by Roettgen (2) and in 1914 by Baragiola and Schappi (3). Roettgen added calcium ion to the solution just before the addition of alcohol, while Baragiola and Schappi, after a complete examination of the method for errors, made the following recommendations in the procedure: first, removal of the excess of barium chloride before the final evaporation, since it was found that in the ashing some barium chloride was converted by carbon to barium oxide; second, use of a smaller sample to avoid losses of barium lactate due to occlusion, and third, use of a fractionating connection in the steam distillation to avoid losses of lactic acid. These same authors (3) made this statement: "There is an inherent error due to organic bases and tartaric, phosphoric, and malic acids which may form condensation products with lactic acid. This error cannot be avoided at the present time," The error is probably inherent in the method at the present time.

Later, in 1916, Roettgen (4) in a continuation of his previous work found that with slight modifications the results were higher. In 1914, Gasparini (5) found that the method of Noslinger (1) as used in his laboratory was the most suitable for the quantitative determination of lactic acid in wines.
2. Determination of Lactic Acid as Barium Sulfate

Method of Kunz

In the same year that Moslinger (1) published his method and in the same journal another method for the quantitative determination of lactic acid in wine was published by Kunz (6). The technique for the Kunz method is more complicated than that for Moslinger's method and involves the following steps. A 200 c.c. sample of wine is evaporated to 2/3 volume with the addition of an excess of barium hydroxide. The concentrated liquid is acidified and extracted with ether to remove sugars and other ether-insoluble material. Water is added to the ether extract and the mixture evaporated to remove the ether. The aqueous solution is steam distilled to remove the volatile acids, then made slightly alkaline with barium hydroxide, and concentrated to 10 c.c. Sufficient 95 per cent alcohol is added to bring the concentration up to 75 per cent and the solution is filtered. The filtrate is evaporated to dryness and the residue taken up in water. Hydrochloric acid and sodium sulfate are added and the barium sulfate precipitate is weighed. In this procedure the lactic acid is represented by barium sulfate.

In his series of investigations to determine the best method for the determination of lactic acid in wines, Roettgen (7), in 1914, showed that the methods of Kunz (6) and Moslinger (1) had failed to agree in earlier experiments, but later, repeating the work on six different wines, the author found that the two methods agreed.
A.O.A.C. Method. The official method of the Association of Official Agricultural Chemists for the determination of lactic acid in the presence of other organic acids is based on this original method of Kuns (6) and was published in 1926 by Nelson (8). This method differs from the original only in the preliminary treatment of the sample which treatment necessarily varies with the type of material to be analyzed. An improvement is made whereby lactic acid is identified in the final filtrate by crystals of quinine lactate. The method gives from 82.5 to 97.9 per cent on 0.05 to 1.0 grams of lactic acid in the presence of 0.20 to 1.0 grams of each of the following acids: citric, tartaric, malic, acetic, and benzoic.

3. Step Titration Method

Another general method for the determination of lactic acid based on a separation in 75 per cent alcohol solution is the step titration method originated by Tillmans and Weill (9) in 1929. The method was devised for use in routine wine analysis and follows the general steps of the Noslinger (1) method through the separation in 75 per cent alcohol solution. After decolorization, the filtrate is titrated with 0.1N sodium hydroxide until alkaline to phenolphthalein and then titrated back to pH 3.2 with standard hydrochloric acid. The hydrochloric acid used is a measure of the amount of lactic acid present. The authors claim that the results obtained by this method agree with those obtained by other methods.
The claims are supported by an investigation in 1930 by Ruffy (10) as a result of which he recommended the step titration method of Tillmans and Weill (9), for routine wine analysis, although for accuracy he preferred the Moslinger ash method as modified by Baragiola and Schiappi (3) which was discussed on page 3.

Bonifazi (11) in 1936 devised a step titration method for wine analysis similar to that of Tillmans and Weill in which the preliminary steam distillation was omitted and lactic and acetic acids were measured together by the acid used in back titration. Acetic acid was then determined by the usual distillation method and the lactic acid found by difference. The merit of this method was claimed to rest in its rapidity. Results showed it to be as accurate as the official Moslinger (1) method. Fabre and Eremond (12) found the Bonifazi method to be accurate to within 0.32 grams of lactic acid per liter of wine.

A modification of the method of Bonifazi which simulates the original method of Moslinger was described in 1931 by Michel (13). Instead of titrating the alcohol filtrate, Michel (13) precipitated the excess barium with carbon dioxide and ashed the filtrate. The alkalinity of the ash was a measure of the lactic and acetic acids present. As in the Bonifazi method the amount of acetic acid had to be determined by distillation.

Among the reasons for the evaporation of lactic acid samples in the presence of an excess of barium hydroxide is the conversion of an apparently normally occurring lactic acid
anhydride to the free acid. The use of this particular alkali for the conversion was the subject of an investigation by Berg and Schalze (14) who made the interesting observation that the use of barium carbonate instead of barium hydroxide during the preliminary evaporation in the above methods prevented the conversion of sugar into lactic acid but permitted the conversion of lactic acid anhydride into the acid. They also mentioned that a frequent source of error in the Kolsinger method was the use of too high temperatures in ashing. At high temperatures barium chloride may be reduced to barium oxide by organic carbon, thus leading to high results.

Bonvagnin and Capt (15) in 1952 made the most recent summary for lactic acid methods in wine analysis in which they state that, "the method of Bonifazi (11) gives good results when the lactic acid content is between one and four grams per liter and almost theoretical at 2.5 grams per liter. Results are uncertain outside this range and in the presence of large amounts of sugars and proteins. The method of von Pellenberg (17) is satisfactory in any case but the technic is involved." The method of von Pellenberg (17) mentioned in the above summary is part of a simultaneous determination of all the acids of wines. In this comprehensive study, lactic acid is ultimately separated as the silver salt. As the authors mentioned, the procedure is extremely involved and is thus unsuited for routine analysis.
In his paper, von Pollenberg (17) observed that "lacton" was not present in appreciable amounts in wine. It is assumed by the present writer that "lacton" refers to the lactic acid anhydride, which other authors have believed to be present naturally in all wines and the hydrolysis of which is a step requiring specific treatment. As mentioned before, the usual treatment is an evaporation to a small volume in the presence of an excess of barium hydroxide. von Pollenberg is the only author who has been found to deny the presence of appreciable quantities of lactic acid anhydride in wine. Findings on the presence of such an anhydride in dilute lactic acid solution, which have been made by the present writer, will be presented in a subsequent part of this paper.

B. Methods Involving the Oxidation of Lactic Acid

and the Determination of the Various Oxidation Products

1. The Clausen Method

In 1932, Clausen (18), working on the lactic acid content of urine and blood where the amount of lactic acid was extremely small, found that the existing methods for its determination were inadequate. The permanganate method of von Fuertth and Charnass (19), which will be described later, was available for the determination of quantities of lactic acid greater than 10 or 20 mg, but was not suitable for the determination of quantities as little as 0.2 mg, which, in many cases, was the maximum amount available for analysis. The methods involving the separation of barium lactate were also inadequate. The use of the colorimetric methods
was also discouraged. These colorimetric methods, which appear in the more recent literature, will be mentioned later.

In reviewing the literature concerning lactic acid, Clausen found that Meissner (20) had reported a successful quantitative oxidation of lactic acid to acetaldehyde and carbon monoxide by heating in a 50 per cent sulfuric acid solution. From this point the determination of lactic acid depends only on the accurate determination of either the aldehyde or the carbon monoxide produced. The determination of the aldehyde was the more desirable, since, as Clausen pointed out, the quantity of acetaldehyde produced during the oxidation was much more specifically a measure of the amount of lactic acid present in biological material than was the quantity of carbon monoxide which might be produced under like conditions.

To measure, accurately, the amount of acetaldehyde produced, Clausen then made improvements on an old method for the determination of acetaldehyde, which had been published in 1900 by Bipper (21). The Bipper method is based on the following equilibrium reaction

\[
\text{CH}_3\text{CHO} + \text{NaHSO}_3 \rightarrow \text{CH}_3\text{CHOHSO}_3\text{Na}
\]

acetaldehyde  "aldehyde-bisulfite complex"

which proceeds quantitatively towards the right in faintly acid solution, and to the left in alkaline solution.

It is interesting to note at this point that from a consideration of the electronic structure of the three molecules
involved in the aldehyde-bisulfite equilibrium, it could be predicted that the reaction would go, as it does, to the right in acid solution and to the left in alkaline solution. The following schematic drawings show the acetaldehyde molecule and the bisulfite ion with their valence electrons:

![Chemical Diagram]

**Key**

- electrons from C atoms
- electrons from O atoms
- electrons from H atoms
- electrons from S atom

The bisulfite ion with its pair of unshared electrons at 1 and 2 possess the ability to present those electrons to the carbon atom of acetaldehyde at 3 and 4. The transfer, if accomplished, would result in the occurrence of a carbon atom with 10 electrons and would be impossible except for another electron shift which occurs at the same time. Hydrogen ions are known to show strong electron-sharing tendencies, and, therefore, in acid solution, it is possible that one pair of the electrons in the double bond between the C and O atoms of the aldehyde is pulled away from the carbon atom to satisfy the valence pull of hydrogen ions, with the formation of the linkage $\text{C} \overset{\cdot}{\text{O}} \overset{\cdot}{\text{H}}$ in place of the linkage $\text{C} \overset{\cdot}{\text{O}} \overset{\cdot}{\text{H}}$ as before. The electrons at 5 and 6 then, are the pair which originally were part of the carbon-oxygen double bond. By this shift, therefore, it is possible for the sulfite ion in acid solution to impose its two electrons on the carbon atom at
3 and 4 with the formation of a compound with an electronic structure as follows:

\[ \text{H}_2\text{C}:\text{C-H}^{(1)} \]
\[ \text{H}_3\text{C}:\text{C-H}^{(2)} \]

\[ \text{H}_2\text{C}^+:\text{C}^-=\text{H}_2\text{O}^-\]

From the above discussion it follows that the formation of such a compound is possible only in acid solution, and similarly, that the addition of alkali to a solution containing the compound would remove the hydrogen ion from the oxygen of the aldehyde group at (1) and therefore would split off the bisulfite group at (2).

Ripper made use of the reaction towards the right only, absorbing the highly volatile acetaldehyde in an excess of standard bisulfite and titrating the excess bisulfite with standard iodin. The amount of bisulfite bound by aldehyde, and therefore the amount of lactic acid originally present, was determined by the difference between the total amount of bisulfite and the excess as determined by titration. The instability of the bisulfite solution on standing for only a few minutes caused errors which Clausen eliminated by making use of the reverse reaction in equation 1. After removing the excess bisulfite with iodin he added alkali, freeing the bound bisulfite, and immediately titrated the bisulfite, thus liberated, with standard iodin. The amount of iodin used in titrating the bound bisulfite was a measure of the amount of lactic acid originally present.

Clausen has cited data to show that the results thus obtained using the Moissner 50 per cent sulfuric acid oxidation and the modified Ripper aldehyde-bisulfite titration are
accurate to 97 to 100 percent of the theoretical in pure
lactic acid solution containing as little as 0.18 mg. and as
much as 45 mg. of lactic acid. He also pointed out that
there are many sources of error in the determination when
applied to biological material. Among the substances which
might cause an error are beta-hydroxy butyric acid, pyruvic,
malic, tartaric, glycollic, and citric acids, and phenols.
All but the phenols yield bisulfite binding substances which
incur a positive error, while phenols cause a negative error.
Pyruvic acid may be eliminated as a source of error by an
other extraction in the presence of bisulfite. Other inter-
fering substances may be partially removed by prescribed
treatment.

Breine and Brahdy (22) made slight changes in the Clausen
method, the most outstanding of which is the addition of a
second absorption tube to insure complete absorption of all
the acetaldehyde produced in the oxidation. Other changes
were made in an attempt to make the improved method more
specific for lactic acid than was the original method.

2. Permanganate and Manganese Dioxide Oxidations

Boas (23) in 1893 found that lactic acid could be de-
tected, qualitatively, by oxidizing lactic acid to acetalde-
hyde with manganese dioxide in the presence of acid. The
presence of iodoform, formed in the distillation of acetalde-
hyde into an alcoholic iodin solution, was a positive test for
lactic acid.
Jerusalem (24) attempted to make the method quantitative for biological materials by distilling the aldehyde vapors into water, adding an excess of standard iodin, and, after allowing time for the formation of iodoform, titrating the excess iodin with thiosulfate. He reported determinations which were 92 to 98 per cent of the theoretical value in pure solution, but von Fuerth (25) later showed that the method was theoretically incorrect because of an error in the calculations. Von Fuerth also indicated that the results of Jerusalem were approximately correct since only about half of the lactic acid yielded aldehyde.

In 1910, von Fuerth and Charnass (26) evidently continuing the work of Jerusalem, determined lactic acid by using a permanganate oxidation similar to the one published by Jerusalem (24) and measuring the acetaldehyde formed, not as Jerusalem did by conversion to iodoform, but by absorbing the aldehyde in standard bisulfite and titrating the excess bisulfite with iodin according to Rippers method. This method has been previously described (21). A correction factor was necessary, since only 80-2 per cent of a known, added lactic acid solution was found to have been recovered from unknown solutions. The method was, nevertheless, an improvement over that published by Jerusalem.

Later von Fuerth and another co-worker, Mondshein, found an error in the previous method of von Fuerth and Charnass (19) due to the presence of beta-hydroxy butyric acid.
The acetone produced when this acid is subjected to the 
permanganate oxidation was distilled along with acetaldehyde 
from the lactic acid, and formed an addition product similar 
to the aldehyde-bisulfite compound. On titration, therefore, 
acetone-producing substances, such as beta-hydroxy butyric 
acid, were measured as lactic acid and results were too high. 
This difficulty was overcome by these investigators by 
subjecting an aliquot of the aldehyde distillate to treatment 
with sodium peroxide. Under the prescribed treatment all 
the acetaldehyde was destroyed and the acetone remaining was 
again distilled into bisulfite. The difference between the 
amount of iodin used in titrating this fraction and the 
amount required to titrate the fraction containing both the 
acetaldehyde and the acetone was the amount used to measure 
the acetaldehyde, and therefore the amount of lactic acid 
present.

Von Fuertth and Ishihara (28) have applied the original 
method of von Vuerth and Charnass to the determination of 
lactic acid in urine and report good results. The main 
feature of the newer method is the determination of lactic 
acid in an ether extract of the original substance.

The first of a series of articles on the determination of 
lactic acid in biological material, published as a result of 
investigations by Friedemann and co-workers, appeared in 1927 
under the authorship of Friedemann, Cotonio, and Schaffer 
(29). The method fundamentally is that of Boas' original 
potassium permanganate oxidation to acetaldehyde combined with
Clausen's modification of the Ripper titration of the bisulfite-aldehyde compound.

An important feature of this first in the series of paper by these workers is the addition of manganous sulfate to the oxidation medium. The use of this salt is reported to increase, materially, the rate of oxidation, thus allowing a shorter oxidation period. The yield of acetaldehyde is also increased. Another feature is the use of a new simple apparatus designed to affect immediate removal of aldehyde vapors as soon as they are formed. The method is reported to be reasonably accurate for quantities of lactic acid from 0.045 mg. to 180 mg.

In the second of the series of publications, Friedemann (30) showed that in determining the amount of lactic acid in decomposing sugar solution, the analysis using the method previously reported from his laboratory (29) agreed with an analysis made by tediously isolating and weighing the pure zinc salt of lactic acid.

Later, in 1929, Friedemann and Kendall (31) found that in analyzing complex biological materials results were apt to be high because of the oxidation and simultaneous determination of allied substances. In an attempt to eliminate this difficulty these authors made several recommendations, all of them in an effort to cut down the potential of the oxidizing agent, since from their experimental work it seemed probable that those conditions which tended to reduce the oxidation potential of the system also tended to reduce the
amount of oxidation of other substances. At the same time they found that these conditions increased slightly the yield of acetaldehyde from lactic acid. Among the recommendations which were made in an attempt to bring about a reduction of the potential are, first, reduction of the concentration of the hydrogen ion, using phosphoric acid in place of the 1 per cent (or more of) sulfuric acid, second, the use of larger amounts of manganous sulfate than had been used previously, and, third, the use of manganese dioxide in place of potassium permanganate as an oxidant.

The statement is made by these authors that "at the present time there is no satisfactory procedure which will yield reasonably reliable results on such complex materials as culture media or urine." They justify the use of their improved method, which they admit has weaknesses, on the basis of the fact that, although the measurements are not absolute, they are constant, and as a result of this constancy, changes in lactic acid content can be accurately determined. In this respect the method is valuable. It is the belief of the present writer that the statement of the authors, which is quoted above, is an underestimation of the true value of their own method.

The last report on the series of studies by Friedemann appeared in 1932 under the authorship of Friedemann and Graesser (32). They found that the proximity to pathological laboratories, in which formaldehyde and acetone were being used, and an unusually cold supply of water during the winter months,
which made the reflex condensers too efficient, forced us to abandon the aeration procedure first recommended." (29)

To overcome these more recent difficulties they substituted a simple distillation apparatus in the place of the old aeration apparatus. Since this new procedure did not afford the desired separation of interfering aldehyde and acetone vapors that were obtained with the reflex type of condenser as used in the aeration method, a more careful oxidation and more complete and controlled preliminary treatment was necessary in order to obtain good results. After a careful investigation of the proper conditions, the authors reported that there was no apparent difference between the results obtained by the new distillation procedure and the old aeration method.

Modified forms of apparatus for the determination of lactic acid according to the methods of Friedemann et al have been suggested by Fuchs (33), Meck and Zacherl (34) and West (35). West describes a simplified apparatus which was designed for use in the aeration procedure and which is available for purchase at the A. H. Thomas Company in Philadelphia.

For the determination of lactic acid in blood Mueller-Parcham (36) used an oxidation to acetaldehyde similar to that described by Friedemann (29), but measured the bound bisulfite differently. The "aldehyde-bisulfite" complex was dissociated with di-sodium phosphate in the presence of an excess of standard iodin, and the unused iodin was measured with standard sodium arsenite. The improvement is assumed to have been designed to eliminate the error due to air oxidation of sulfite.
Two other methods for the determination of lactic acid as acetaldehyde are of minor interest. Bellet (37) distilled the aldehyde as formed into an ammoniacal solution containing sodium hydroxide. The unreduced silver was determined and subtracted from the amount originally present. The amount of silver reduced by acetaldehyde was a measure of the lactic acid present.

With all the methods existing at the present writing, it seems undesirable to attempt the use of such an unstable substance as ammoniacal silver for the determination of acetaldehyde. The fact that no further use of this method has been reported in the literature since the original is suggestive of the impracticability of such a method.

Leone and Tafuri (39) are the originators of the other method. In their method, the acetaldehyde was distilled into a known amount of hydroxylamine with the resulting formation of an oxime. The excess hydroxylamine was titrated with standard sulfuric acid, using methyl orange as an indicator. This method apparently has no value since its recurrence in the literature has not been observed.

3. Oxidation of Lactic Acid to Oxallic Acid by Permanganate in Alkaline Solution

In 1912, Bacon and Dunbar (39) reported a successful determination of lactic acid in tomato products, based on the quantitative oxidation of lactic acid to oxalic acid by potassium
permanganate in alkaline solution. When the oxidation is complete, the solution is acidified, the excess permanganate and manganese dioxide destroyed with known oxalic acid solution, and the solution titrated back to a faint pink with permanganate. The amount of permanganate used in oxidizing the lactic acid, first to oxalic acid and then to carbon dioxide and water, is found by subtracting the permanganate equivalent of the added oxalic acid from the total amount of permanganate used. The weight of permanganate thus found, multiplied by a factor (0.237) equals the weight of lactic acid. The determination must be preceded by a preliminary treatment with lead acetate followed by an ether extraction. Both of these prescribed treatments are designed to eliminate interfering substances, but, despite these, both malic and tartaric are measured as lactic acid if present in the original material. The method is old and probably is replaced at the present time by newer and simpler methods.

Using an alkaline permanganate oxidation similar to the one just described, Onodera (40) in 1917 determined the lactic acid simultaneously with formic and acetic acids. Of the three acids, lactic acid is the only one to form oxalic acid by this oxidation. The oxalic acid produced is separated as the calcium salt and is determined in the usual manner with permanganate. An ether extraction is necessary to separate the acids from interfering substances. The determination is reported not to be influenced by small amounts of propionic and butyric acids, but the extent of interference of other ether-
soluble substances is not mentioned.

Another specific application of the determination of lactic acid by oxidation in alkaline permanganate solution is the one published by Hartmann and Hillig (46) in 1933 which constitutes a proposed official A.C.A.C. method for the determination of this acid in milk and milk products. These authors found that the method of Friedemann and Grasser (32) (page 17) is too exacting and that the successful determination depends upon too many factors for the method to be recommended as official. As a result of this investigation by Hartmann and Hillig, and others made in the Department of Agriculture in Washington, D. C., a recommendation was made favoring the oxidation in alkaline permanganate solution, and determination of the oxalic acid formed. The inconsistent results obtained by Hartmann and Hillig, and as explained by them, may have been due either to the absorption of lactic acid in the heavy casein precipitate, or to certain difficulties inherent in the oxidation procedure. Since the same Friedemann procedure has been successfully used on casein-free substances by the present writer and others (36, 32, and 35) it seems probable that the poor results must have been caused in this particular case by the inclusion of lactic acid in the casein precipitate.
1. Oxidation by Dichromate to Acetic Acid

Because he was not able to obtain reliable results with either the gravimetric method of Kunz (6) or the oxidation to oxalic acid according to a method similar to that of Onodera (40), Szeberenyi (41), in 1917, originated a method for the determination of lactic acid in wine based on the oxidation of lactic acid by potassium dichromate in moderately strong sulfuric acid solution under a reflux condenser. The acetic acid formed is later distilled and titrated with standard alkali in the usual manner. One mole of lactic acid yields one mole of acetic acid so that the acidity of the acetic acid distillate is the same as the lactic acid content of the original. Tartaric, malic, citric, and oxalic acids do not interfere since they are oxidized directly to carbon dioxide. However, volatile acids, alcohol, acetone, and certain esters must be previously removed by steam distillation. There is a negative error of about 3 per cent due to the complete oxidation of some of the lactic acid to carbon dioxide and water.

The claim is made by Szeberenyi (41) in connection with the dichromate determination that lactic acid may be lost by entrainment in the distilling vapors. In an unpublished paper Anderson (42) shows that appreciable quantities of lactic acid are not lost during a reasonably long period of simple distillation under either reduced or normal pressures. These distillations, however, were not the steam distillations as prescribed in the Szeberenyi method, which fact probably explains the differing
conclusions.

In 1919, Schuppi (43), continuing the work of Szechbery (41) on the dichromate oxidation to acetic acid, found that the method was fairly accurate on solutions containing tartaric, malic, succinic, and citric acids, but reported that the method was unsatisfactory for the determination of lactic acid in wines, since the results were too high when compared with results obtained by Mealingor's (1) method. Sucrose was shown to interfere if present in the solution during oxidation.

In contrast to the failure of Schuppi to make successful determinations of lactic acid by the dichromate oxidation, Senichon and Flanzy (44), in 1932, reported that the same method appears to be satisfactory for use in measuring the amount of lactic acid in wines and fruit juices. They found that no lactic acid was entrained in the volatile acids distillate if the procedure was followed exactly. They also found that pyruvic acid and higher fatty acids were oxidized to acetic acid.

C. Gasometric Method

In the oxidation of lactic acid by permanganate in acid solution, there results one mole of carbon dioxide as well as one mole of acetaldehyde per mole of lactic acid. Using a preliminary treatment and subsequent oxidation similar to that of Friedemann, Cotonic, and Schaffer (29) (page 15), two other workers, Avery and Hastings (46), in 1931, found that a useful but somewhat empirical determination of lactic acid in blood
could be made by measuring the amount of carbon dioxide, rather than the amount of aldehyde produced during the course of the oxidation. Since their results on blood were somewhat higher than those obtained by the method of Friedemann, Cotonic, and Schaffer (29), which method had been shown to be specific for lactic acid by comparison with other methods, it was concluded that some substance present in blood was producing small but significant quantities of carbon dioxide. The amount of this gas seemed to be constant so that when the proper correction factor was applied to the calculation of results, the results were comparable to those obtained by other methods.

It is recalled to mind at this point that Clauson (18), in 1922, discouraged the use of a gasometric method, determining carbon monoxide produced by a 50 per cent sulfuric acid oxidation, because of the failure of such a measurement to be specific for lactic acid.

D. Colorimetric Methods

There appears in the literature a series of qualitative tests for the detection of lactic acid in small quantities, particularly for lactic acid in blood, tissue, and other biological materials, based on the color developed by lactic acid or its decomposition products in the presence of certain specific color reagents. Since, for the most part, the methods are only qualitative, no more than mention of them will be attempted in this paper. By the addition of half a drop of a 2 per cent
solution of guaiacol under prescribed conditions, Hartwig and Saar (47) could detect the presence of lactic acid in concentration of 0.2 per cent and less by the formation of a characteristic red color. Fearon (48) used thiophene for the development of a red color in the presence of acetaldehyde formed from lactic acid. Brauer (49) used resorcinol, and Eickert (50) used pyrocatechol in tests similar to that of Fearon (48). Lactic acid was detected by Germuth (51) in concentrations from 0.5 to 1.5 per cent by the addition of 15 per cent potassium thiocyanate solution. The orange or purplish color developed by this reagent in the presence of lactic acid is not discharged by mercuric chloride.

Mendel and Goldschneider (52) report a quantitative colorimetric method using veratrole. These authors established the fact that the amount of color developed with this reagent is proportional to the amount of lactic acid present, and on this basis, they made accurate comparisons with known standard solutions of lactic acid, and thus proposed a quantitative method for the determination of lactic acid in 1 c.c. of blood.

E. Miscellaneous Methods

Convinced that the existing methods for the determination of lactic acid were not sufficiently specific for the acid, Phelps and Palmer (53), in 1917, attempted a more complete separation of lactic acid from other organic acids. In the course of their work they also introduced a completely different ultimate measure of lactic acid. The separation from formic, acetic, citric, and tartaric acids was made by means of a
fractional distillation of the ethyl esters of these acids and lactic acid. The separation from propionic and butyric was based on insolubility of the quinine salts of propionic and butyric in carbon tetrachloride. Lactic acid was weighed as quinine lactate following the evaporation of the carbon tetrachloride filtrate containing this salt. Quinine lactate was then further identified by its melting point. The results reported varied from 95.7 to 101.6 per cent of the theoretical in solutions containing considerable amounts of the other acids already mentioned.

**Determination by weighing as zinc lactate.** A long and involved method for the determination of lactic acid in tissue or blood has been described by Wolf (54) in 1914. Proteins are removed by one of the usual protein precipitants; the filtrate is evaporated to a convenient volume and extracted with ether; water is then added; the other evaporated, and the solution filtered. The filtrate is treated with lead carbonate at 100°C, and again filtered; the lead is removed from this filtrate, which also contains the lactic acid, and the solution treated with zinc carbonate; zinc lactate is allowed to crystallize out; is then dried at 110°C, and weighed. Since it has been more recently shown by Friedemann (30) and his co-workers that this zinc lactate determination agrees with the acid permanganate oxidation, and since the latter is by far the simpler for routine analysis at least, it is evident that such an involved procedure as Wolf's no longer has any value except, possibly, as an occasional check on some other method.
**Partition Method.** In 1930, Werlman (55), who was working on saturated fatty acids produced by fermentation, developed a method for the determination of lactic and other acids in the presence of not more than one other acid by partitioning between isopropyl ether and water. The water solution of two acids is shaken up with a given amount of isopropyl ether and the mixture allowed to layer. From a previously determined partition coefficient and the titration of an aliquot of the aqueous phase with standard alkali, a very good approximation of the amount of lactic or other acid may be calculated. The advantages of this method are its rapidity and ease of manipulation, as well as the fact that its use may be extended to the determination of any pair of acids which are soluble in water and in isopropyl ether. Its chief disadvantage is that there can be no more than two acids present if the determination is to be successful.

In an attempt to lessen the severity of this limitation, the same worker (56) has extended the determination to include a mixture of three different acids instead of two as before. In the improved method, two different partitions are necessary, using different proportions of water and ether. The percentages of the different acids are obtained as before by titrating an aliquot of the aqueous phase with standard alkali.
EXPERIMENTAL WORK

A. General

1. Choice of Method

It was part of the original idea of the present investigation to apply a known method to the determination of lactic acid in an unknown pickle brine. A glance at the literature convinces one that a large amount of work has been done up to the present time on the determination of lactic acid in a number of widely varied types of materials. No mention has been observed, however, of the measurement of lactic acid in a fermenting pickle brine, although materials which should approach this substance in composition are culture media, naturally fermented wines, fruit juices, and vegetable products, all of which have been successfully analyzed for lactic acid according to various methods which appear in the literature and which have been reviewed in this paper.

To obtain the desired measure of lactic acid in the unknown solution, therefore, it was necessary to choose from among the existing methods and apply the technique of the method chosen to the determination of the lactic acid in the material at hand.

The methods involving the isolation of lactic acid as barium lactate are known to be long and tedious, and at best give results that are only approximately correct. Barium
lactate methods, therefore, did not appear to be the best suited for the use intended. Colorimetric methods have been criticized as not being specific and the use of colorimetric methods applies chiefly to the determination of very small quantities of lactic acid where other methods fail to give an accurate measurement, so that these two possibilities were eliminated. The method of Phelps and Palmer (53) which was designed to be highly specific for lactic acid has its chief advantage in its specificity, but requires the difficult step involving a vapor-phase conversion of all the acids present to the ethyl esters. This and other difficult operations render the method impracticable. Werkman's (56) partition method is very useful for the analysis of certain culture media for which use it was originally intended, but it is limited to the determination of lactic acid in the presence of no more than one or two other acids. Furthermore, these other acids and their partition coefficients must be known. There is also the added possibility of a variable partition coefficient in the presence of the large amounts of salt found in brines which have been analyzed during the present investigation.

By such a process of elimination there remained only the possibility of one of the various oxidation procedures. Of all the known oxidation methods, the permanganate method developed by Friedemann and others (32) appeared to be the most likely to give the best results for the following reasons: First, wide variations in amounts of lactic acid can be determined, the authors reporting successful determinations in solutions containing
from 0.045 mg. to 180 mg. of lactic acid with an accuracy of 97 to 98 per cent of the theoretical; Second, complete information as to the sources of error and the possibility of their elimination; Third, comparative simplicity and adaptability to routine analysis; Fourth, wide variation in the types of material that can be analyzed.

Keeping in mind that the determination of the hydrogen ion is not a specific determination for lactic acid in a ferment where other acids may also be present, one must realize that the determination of lactic acid depends on some chemical reaction that is specific for the lactate radical. For this reason any determination for lactic acid other than titration must also include all the salts of lactic acid as well as the free acid. Such a determination also measures any anhydride or similarly bound lactic acid precursor which may be present. That lactic acid anhydride or an anhydride-like substance is present in dilute lactic acid will be shown in a subsequent part of this paper. The term lactic acid, then, when mentioned in the future in connection with its determination by oxidation, will include salts and "anhydrides" of lactic acid as well as the free acid.

2. Description of Method Chosen

Since the method used in the present investigation was essentially the same as that described in detail by Friedemann and his co-workers (32) it will not be necessary to include more than a brief account of the steps involved in the procedure. The details are omitted. A measured amount of the unknown solution,
containing lactic acid was acidified with sulfuric acid to prevent spoilage by bacterial decomposition, and at the time of analysis an aliquot of the acidified sample was treated with copper sulfate and calcium hydroxide, which treatment removes sugars and some of the di- and tri-basic carboxy acids such as succinic, malic, tartaric, and citric acids, if present. The solution, thus treated, was made up to volume and centrifuged to throw down the precipitate formed by the calcium hydroxide and copper sulfate. An aliquot of the supernatant liquid after centrifuging was ready for oxidation. In general the aliquot was so measured as to contain 5 to 10 mg. of lactic acid. In cases where the standard lactic acid solution was used for analysis no preliminary treatment with copper and lime was necessary. The lactic acid in the prepared sample was oxidized by permanganate at boiling temperature to acetaldehyde in a solution that contained approximately 0.037 molar phosphoric acid and 0.047 molar manganese ion. The removal of aldehyde vapors was effected by means of the aeration procedure as described in Friedemann's earlier papers (29 and 30) rather than according to the simple distillation method as recommended in a later paper (32). The latter distillation method was tried but was found to give inconsistent results. In either case the aldehyde was absorbed, as formed, in a solution of bisulfite, and the resulting solution of bisulfite containing the bound aldehyde was placed in an ice bath until cool. During the winter months it was found convenient to cool the aldehyde bisulfite solution by simply placing the absorbing vessel containing the solution on a ledge outside the window for a few minutes.
After cooling, the excess bisulfite was oxidized, using first strong iodin and later weak iodin and starch, adjusting the solution to the familiar faintly blue end point. This end point is the so-called "first end point." The solution was then returned to the ice bath and later made alkaline with sodium bicarbonate to dissociate the bisulfite-aldehyde complex. The faintly alkaline solution was titrated immediately with standard iodin solution. The number of cubic centimeters of 0.01 N iodin used in this titration multiplied by 0.450 gives the number of milligrams of lactic acid present in the sample. The standard iodin was prepared for use, as needed each day, from a standard solution of 0.1 N potassium bi-iodate as recommended by Friedemann (32), the purpose of this procedure being to overcome the irregularities due to the instability of dilute iodin solutions.

3. Materials Used

A brief description of the materials used follows. Complete procedures for making the reagent solutions are omitted since these are described in detail in Friedemann's account of the method (31).

Iodin - Merck's resublimed U.S.P.
Lactic acid - J. T. Baker's U.S.P.
Manganous sulfate - J. T. Baker's C.P. MnSO₄ · 2H₂O (38 grams of this salt were used in place of 100 grams of MnSO₄ · 4H₂O)
Phosphoric acid - J. T. Baker's C.P. 85 per cent ortho phosphoric acid.

Potassium bi-iodate - C. Frederick Smith So. Reagent Quality.

Potassium iodide - J. T. Baker's C.P.

Propionic acid - Eastman Kodak Company.

Sodium bicarbonate - J. T. Baker's U.S.P.

Sodium bisulfite - J. T. Baker's C.P. meta powder.

Sodium carbonate - J. T. Baker's C.P.

Sodium thiosulfate - J. T. Baker's C.P.

Starch - J. T. Baker's potato starch.

Zinc carbonate - Baker and Adamson's C.P.

Zinc lactate. Pure zinc lactate was obtained by three successive recrystallizations from an aqueous solution of zinc lactate that was obtained by warming together 50 grams of lactic acid and 34 grams of zinc carbonate. After the last recrystallization the salt was filtered on a Buchner filter funnel and washed with small portions of distilled water. The salt was air-dried at room temperature and the product thus obtained was analyzed for moisture, zinc oxide, and "lactate." The amount of "lactate" was determined by the loss in weight on combustion of the anhydrous salt. The result of the analysis, together with the theoretical results for \( \text{Zn(C}_6\text{H}_5\text{O}_3)\text{H}_2\text{O} \), are given in Table 1.
TABLE 1

Analysis of Zinc Lactate (Averages)

<table>
<thead>
<tr>
<th></th>
<th>Found</th>
<th>Theoretical</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{H}_2\text{O}$ (24 hours at 130°C)</td>
<td>18.24%</td>
<td>18.17%</td>
</tr>
<tr>
<td>ZnO (White Ash)</td>
<td>27.43%</td>
<td>27.35%</td>
</tr>
<tr>
<td>&quot;Lactate&quot; (By Combustion)</td>
<td>59.71%</td>
<td>59.86%</td>
</tr>
</tbody>
</table>

The results were slightly higher than theoretical for zinc oxide and were correspondingly low for "lactate." This fact would indicate that there was a small amount of hydrolysis of the zinc lactate at the time of the last recrystallization. Moreover, zinc hydroxide, which would be formed as the result of such a hydrolysis, loses one molecule of water at 125°C, or 5°C. below the drying temperature used in the determination. Therefore, the fact that the analysis for water as well as the determination of zinc oxide was higher than theoretical is evidence that zinc hydroxide was the contaminant responsible for the differing results.

Another possibility is that the salt was contaminated with the salt of some acid with a smaller number of carbon atoms than lactic acid. That the zinc salt of some acid other than lactic acid might have been present is, however, improbable because of the several recrystallizations. To eliminate the error from the presence of zinc hydroxide in the zinc lactate it was necessary merely to weigh out the
sample for the standard solution on the basis of the "lactate" content of the material at hand rather than on the basis of the formula weight of pure zinc lactate.

It is to be noted here that according to table 1, the error to be expected from the use of the impure standard is considerably less than the difference between the amount of lactic acid known to be in solution and that found according to the best of the results given in tables later on. It appears, therefore, that the precaution taken in weighing out the sample on the basis of the "lactate" content rather than on the basis of the formula weight is not necessary except to minimize the effect of an accumulation of a number of errors, most of which are negative.

It may be mentioned at this point in connection with the zinc lactate standard that considerable difficulty was experienced in preventing spoilage of the standard lactate solution made from the zinc salt. Unless special precautions are taken in regard to the proper storage of the standard solution in a cold place, a bacterial or mold growth invariably sets in with a resulting loss in lactic acid content. Such a decomposition had been under way in two different standard solutions before the writer was aware that such a decomposition had been taking place. The use of these decomposed solutions led to several series of results which, although they were lower than they should be, were significant in the comparison of results obtained on the same sample under different sets of conditions. Friedemann (31) has recommended that zinc lactate solutions be kept in a refrigerator to prevent spoilage.
4. Apparatus

The apparatus used for the oxidation of lactic acid and the absorption of aldehyde was essentially the same as the aeration apparatus described by Friedemann, Cotonic and Schaffer (29) in the original paper on the Friedemann method. A few determinations were made with a distillation apparatus which was used to approximate the conditions obtained by Friedemann and others in the later papers (31, 32) in their distillation methods. The distillation apparatus consisted of a Kjeldahl flask and permanganate delivery tube similar to that used in the aeration procedure except that a one-hole stopper was placed in the thistle tube to prevent the aldehyde vapors from being blown out through the permanganate delivery tube. Permanganate was run into the thistle tube through a separatory funnel which was inserted through the hole in the stopper. A simple glass distilling head connected the Kjeldahl flask to the upper end of a small Liebig-type glass-walled condenser. The small end of an ordinary sugar tube was connected to the other end of the condenser and the large end of the sugar tube was placed in a 150 c.c. extraction flask which contained bisulfite solution. The sugar tube acted merely as an improvised delivery tube which presented a large surface of bisulfite solution and therefore enhanced the complete absorption of all the aldehyde vapors which distilled over.

All of the volumetric glassware was calibrated and found in most cases to be sufficiently accurate to make possible its use in the present investigation without any correction. Where the errors were large, the proper corrections were applied.
B. Mechanism of the Oxidation Reaction

It can be shown by a process of elimination of other possibilities that the most serious errors in the determination of lactic acid in pure solution by Friedemann's method occur during the oxidation of lactic acid to acetaldehyde. Friedemann (29) reported that the error in the titration of the acetaldehyde-bisulfite solution is small and, therefore, is not responsible in itself for the failure to recover more than 98 per cent of a known solution of lactic acid. To eliminate the only other possibility, namely that acetaldehyde is not completely absorbed during aeration, the present writer carried out an experiment whereby the stream of air coming from the absorption tower was bubbled through an extraction flask containing 10 c.c. of bisulfite solution. The usual titration of the contents of this flask failed to reveal the presence of any aldehyde in the solution, proving that absorption in the tower was complete. Therefore, in order to explain the low results there remained only the possibility that the oxidation of lactic acid does not follow the exact stoichiometric equation written below:

\[
\text{CH}_3\text{CHOH} + 6\text{H}_2\text{O} + 0 \rightarrow \text{CH}_3\text{CHO} + \text{CO}_2 + \text{H}_2\text{O}
\]

lactic acid \( \text{KMnO}_4 \) acetaldehyde

(equation 2)

Friedemann and his co-workers worked with a number of variable factors in order to obtain a maximum recovery but, as mentioned before, failed to obtain more than 98 per cent
recovery. As a result of their investigation they recommended an optimum acidity, manganous ion concentration, and size of sample, as well as the use of the proper oxidizing agent. It was the hope of the present investigator to further improve the yield of acetaldehyde by a further consideration of some of the points involved in the oxidation mechanism. Actually, no further improvements have been made, although certain observations have been made concerning the reaction which seem worthy of mention at this point.

1. Comparison of the Relative Functions of Manganese Dioxide and Potassium Permanganate

The original method of Friedemann, Cotonio, and Schaffer (29) described the use of an oxidizing medium consisting of a dilute solution of potassium permanganate similar to that used by workers before them. Friedemann, Cotonio, and Schaffer found, however, that the presence of manganous ion in the solution containing the lactic acid had the desirable effect of increasing the rate of oxidation by the permanganate and, for that reason, they recommended its use in the determination. The function of the manganous ion, they pointed out, was to reduce the permanganate to manganese dioxide as soon as it was added to the lactic acid solution. Manganese dioxide, then oxidized lactic acid. The reduction of permanganate to manganese dioxide takes place according to the following reaction:
\[ 2\text{MnO}_4^- + 5\text{Mn}^{4+} + 2\text{H}_2\text{O} \longrightarrow 6\text{MnO}_2 + 4\text{H}^+ \]

(Equation 3)

Since it is the manganese dioxide that is the actual oxidizing agent, it is easy to see why the presence of the manganese ion in the oxidizing medium is effective in increasing the rate of oxidation of lactic acid.

In a later paper, Friedemann and Kendall (31), introduced another change in technique for the purpose of improving their results on complex biological materials. They showed that it was more desirable to use colloidal manganese dioxide in the place of permanganate since extraneous materials present in the lactic acid solution which were unaffected by manganese dioxide might yield bisulfite-binding substances if permanganate were used. The result on lactic acid would be the same whether permanganate or manganese dioxide were used since permanganate, in the presence of high manganese ion concentration, is reduced to manganese dioxide before reacting with lactic acid.

Using the regular Friedemann procedure which has been described on page 30, a series of analyses was made by the present writer in order to compare the results obtained by the use of colloidal manganese dioxide in one case, and dilute potassium permanganate in the other. The preparation of a stable colloidal suspension of manganese dioxide was a source of trouble at the outset of this series of analyses. The preparation described by Friedemann and Kendall (31) involving the reduction of potassium permanganate by sucrose in alkaline solution was first tried, but it was impossible
in two different attempts to prepare a stable suspension of manganese dioxide which was sufficiently concentrated to be of much use as an oxidizing agent. A third attempt, using the reduction of permanganate by manganous sulfate, yielded a suspension which was slightly more stable and was sufficiently concentrated for regular use. This last preparation appeared to be much coarser than either of the other two but, in spite of the apparently larger particle size, it appeared to be quite stable. Although, theoretically, the oxidation is the same whether the oxidizing agent is manganese dioxide or permanganate in the presence of manganous ion, the results in table 2 show that there is a difference of about 13 per cent in the amount of lactic acid oxidized in a given period of time by the two reagents.

Table 2

Amounts of Lactic Acid Oxidized by Coarse Manganese Dioxide and by Potassium Permanganate

<table>
<thead>
<tr>
<th>Mg. L.A. oxidized by .04 N MnO₂ in 15 min.</th>
<th>Mg. of L.A. oxidized by .03 N KMnO₄ in 15 min.</th>
<th>Mg. L.A. oxidized by .04 N KMnO₄ in 30 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.35</td>
<td>8.05</td>
<td>2.93</td>
</tr>
<tr>
<td>7.92</td>
<td>8.07</td>
<td>2.69</td>
</tr>
<tr>
<td>7.51</td>
<td>8.05</td>
<td>2.85</td>
</tr>
<tr>
<td>7.62</td>
<td>8.07</td>
<td>2.90</td>
</tr>
<tr>
<td>8.17</td>
<td>8.94</td>
<td>3.99</td>
</tr>
<tr>
<td>7.83 (± .34)</td>
<td>9.01 (± .06)</td>
<td>3.94 (± .15)</td>
</tr>
</tbody>
</table>
The results shown in table 2, can be explained by a further consideration of the facts involved. The manganese dioxide which was used in this experiment was part of the coarse dispersion obtained in the third attempt as previously described. The rate of reaction of a solid such as manganese dioxide depends, among other things, on the amount of surface area available for reaction. Therefore, as the manganese dioxide is more finely divided the rate of reaction becomes faster. The manganese dioxide which is formed in the case where permanganate is added to the solution in the presence of manganous ion, is in a very finely divided state and is, therefore, readily available for reaction with lactic acid. On the other hand, where the previously prepared manganese dioxide has become coagulated into larger particles, the reaction with lactic acid is slow since a reaction is not possible until there is some sort of physical breakdown of the larger particles into smaller particles. That the difference between the results obtained using manganese dioxide and potassium permanganate is one of reaction rates is brought out by the figures in the third column of table 2 where, after a longer period of oxidation, the results more nearly approach those obtained in the second column. Why Friedemann and Kendall were able to obtain satisfactory results which could not be reproduced by the present writer may possibly be explained by either or both of the following reasons: First, the preparation of colloidal manganese dioxide, which Friedemann and Kendall used, may have been finely divided.
enough to approach the conditions obtained where permanganate is added to a solution containing manganous ion; second, the size of the sample used by Friedemann and Kendall was smaller, in most cases, than were those analyzed by the present writer, which fact may mean that the shorter oxidation period is sufficient for smaller samples but not for the larger samples. This last point, however, has not been confirmed or denied by any experimental work, and therefore is only hypothetical. In either case permanganate appeared to be the better oxidizing agent for use in the present investigation.

The reason that dilute permanganate solutions have been found to give better results than manganese dioxide suspensions is easily explained. It has already been shown that the reason for the low results using a previously prepared manganese dioxide suspension is that the rate of reaction of the relatively large manganese dioxide particles is slow. To speed up the reaction and, therefore, to improve the results it is necessary only to furnish finely divided particles of manganese dioxide which is the case where manganese dioxide is formed in the lactic acid solution by the dropwise addition of dilute permanganate in the presence of a high concentration of manganous ion.

That the state of division of the manganese dioxide suspension is important is shown in table 3. The manganese dioxide used in obtaining these results was some of the small amount of satisfactory suspension which was obtained in one of the two first attempts at making a colloidal suspension. The particle size of this suspension was noticeably smaller than that
of the manganese dioxide particles in the suspension used in obtaining the results in table 2.

Table 3

Amounts of Lactic Acid Oxidized by Finely

<table>
<thead>
<tr>
<th>Divided Manganese Dioxide</th>
<th>Permanganate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ms. of Lactic Acid Obtained by Oxidation with 0.05 N MnO₂ in 17 minutes</td>
<td>Ms. of Lactic Acid Obtained by Oxidation with 0.05 N KMnO₄ in 16 minutes</td>
</tr>
<tr>
<td>9.61</td>
<td>9.83</td>
</tr>
<tr>
<td>9.61</td>
<td>9.82</td>
</tr>
<tr>
<td>9.61</td>
<td>9.85</td>
</tr>
<tr>
<td><strong>Average</strong> 9.66</td>
<td><strong>Average</strong> 9.85</td>
</tr>
</tbody>
</table>

Thus, the results obtained using the more finely divided manganese dioxide more nearly approach those obtained using permanganate than do the results using the coarser manganese dioxide, the difference being only 1.9 per cent in table 3 as compared with a difference of 13 per cent in table 2.

The importance of the particle size of manganese dioxide is still further shown by the following experiment. If all of the permanganate to be used in one of the oxidations similar to those in the second column in table 3 is added at once at the beginning of the oxidation period rather than added dropwise, there results a mass of coagulated manganese dioxide which becomes somewhat dispersed on boiling, but which nevertheless is rather coarse in regard to particle size throughout the oxidation.
In such a determination the results are invariably low, indicating again that this physical state of the manganese dioxide particles is very important in the oxidation of lactic acid.

2. The Occurrence of Manganic Phosphate in the Oxidation Reaction

During the course of the preliminary work on the technique of the method, it was noticed that there was formed during the oxidation of lactic acid a grey or greyish-brown precipitate, the composition of which was unknown at the time it was observed. Later on, analysis of this substance showed it to be manganic phosphate (MnPO$_4$ $\cdot$ H$_2$O).

Friedemann (32) mentioned the occurrence of such a grey precipitate but failed to describe either its appearance or position in the reaction. It was thought that the isolation and identification of this unknown substance might reveal information which would be valuable in working out the mechanism of the reaction. Although the unknown substance actually turned out to be the product of a side reaction and not necessarily a part of the main oxidation reaction between manganese dioxide and lactic acid, a discussion of the compound which was isolated will be included here since, to the knowledge of the writer, such a discussion has not been reported in connection with this determination.

Description and Analysis of Manganic Phosphate - The precipitate occurred as a quite insoluble grey or greyish-brown substance which was very finely divided and which settled out rather slowly
from an agitated suspension. Mollor (57) has mentioned a similar compound of manganese and phosphoric acid which he reported to be manganic orthophosphate (MnPO₄ • H₂O). According to his statements, manganic orthophosphate is a very stable greenish-grey crystalline powder which is insoluble in water and which loses water of crystallization at 300 to 400°C. No accurate solubility measurements have been reported.

A measurement of the approximate crystal size of the precipitate was made possible by a direct microscopic examination, using a previously calibrated eye piece and a high power dry objective. An examination of several different microscopic fields indicated that the average diameter of manganic phosphate crystals is between 0.001 and 0.002 millimeters.

A microscopic examination of the same precipitate in a field of polarized light, by Mr. Stevens of this department, indicated to him that the crystal form of this compound belongs to one of the three following classes of crystalline structures: (1) monoclinic, (2) triclinic, or (3) orthorhombic. The crystals, according to him, could not be isometric, tetragonal, or hexagonal. His conclusions were based on the fact that in no case was there in any microscopic field a crystal which showed complete extinction of the polarized light by rotating the field through an angular rotation of 360°.

According to observations made by the present writer, the compound goes into solution very slowly in the presence of hydrochloric acid which, it is assumed, reduces the manganic ion to manganous ion in the process of solution. A hydrochloric acid solution of the precipitate contained only manganese and
phosphoric acid by a qualitative analysis, and a quantitative analysis of this same solution showed a ratio of grams of manganese to grams of phosphorus of about 1.93. The theoretical ratio for manganic phosphate is 1.77. The difference between 1.77 and 1.93 might be due to experimental error, since only very small quantities of the pure precipitate were available for analysis, but more probably a large part of the difference is due to the inclusion of manganese dioxide in the precipitate. The analysis, in itself, is not sufficient evidence for the conclusion that the compound is manganic phosphate and nothing else; but together with Heilorn's description of a similar compound and other considerations which will be presented later on, the evidence is considered to be sufficient to make it convincing that the precipitate in question is manganic phosphate.

The determination of phosphorus has been done in all cases according to the method of Truog (39) which involves the reduction of phosphomolybdate to molybdenum blue by stannous chloride, and a comparison of the color developed with that developed by a standard phosphorus solution. Manganese was determined colorimetrically by oxidation to permanganate with periodate in strongly acid solution.

**Solubility of Manganic Phosphate.** It was found difficult to obtain accurate information as to the exact solubility of manganic phosphate either from the literature or by experiment. Those measurements which were finally obtained, by experiment, are described immediately following this paragraph. These experimental measurements are only approximately correct, but
will necessarily be sufficient until better measurements are made.

In one attempt to obtain an accurate solubility measurement, a small amount of the pure precipitate was washed free of all soluble substances and the washed residue boiled in 100 c.c. of distilled water for two hours under a reflux condenser at which time the solution was assumed to be saturated with manganic phosphate. The precipitate became darker brown and coarser indicating some undesirable chemical change in the constitution of the manganic phosphate. The chemical change can be shown to be due to hydrolysis of the manganic ion. This hydrolysis was recognized to be a probable source of error; but, regardless of this situation, the amount of phosphate in the saturated solution was measured and from this measurement and the percentage of phosphorus in manganic phosphate by previous analysis, the solubility of manganic phosphate was calculated on the basis of the amount of soluble material present in the saturated solution. The solubility thus calculated has been found to be about 18 milligrams of $\text{MnPO}_4 \cdot H_2O$ per liter. As mentioned before, this solubility figure is recognized to be only approximately correct, and is probably too high, since the hydrolysis of manganic ion to some undetermined manganese oxide or hydroxide will lead to the presence of more phosphate in solution than should normally be present in a saturated solution of manganic phosphate.

Another determination of the solubility of manganic phosphate was made indirectly. Starting with the conditions prescribed for the usual determination of lactic acid, a measured quantity of standard permanganate was added very slowly to the
lactic acid solution until the first traces of the grey-white precipitate of manganic phosphate appeared. At this point the aldehyde-bisulfite receiving flask was removed and the contents titrated as usual. The number of equivalents of permanganate used was calculated and found to be larger than the number of equivalents of iodin necessary to titrate the bound bisulfite. The difference between the number of oxidizing equivalents of permanganate and of iodin was assumed to represent the number of equivalents of manganous ion that had been oxidized to manganic ion. Since the addition of permanganate was presumably halted at the saturation point of manganic phosphate the number of equivalents of manganic ion present in solution at that point should represent the solubility of manganic phosphate under the existing conditions. The solubility figure thus obtained was found to be somewhere between 44 and 48 milligrams of manganic phosphate per liter of solution. This figure is probably high, also, because of certain theoretical assumptions that have been made and which may be incorrect, and also because of the difficulty in determining at just what point manganic phosphate begins to precipitate. It must be remembered that the first solubility figure of 18 milligrams per liter represents the solubility of manganic phosphate in pure distilled water while the second, higher figure represents the solubility under entirely different conditions. At any rate, it is safe to assume that the solubility of manganic phosphate in pure water and in solutions such as the one used in the oxidation of lactic acid lies somewhere between 10 and 50 milligrams per liter at 100°C. The lower figure is probably more nearly the correct one.
Relation of Manganese Phosphate to the Oxidation Mechanism. It was noticed during one particular series of determinations that if the permanganate were added very slowly and each drop allowed to react completely, no manganese phosphate was formed until about 80 per cent of the lactic acid present had been oxidized to the aldehyde. This result was obtained using 10 milligrams of lactic acid as a sample. This observation can be taken to mean that until a certain minimum concentration of lactic acid has been reached, practically all of the oxidizing capacity of the added permanganate or manganese dioxide has been utilized in the oxidation of lactic acid alone. Not until that point has been reached is bivalent manganese oxidized to trivalent manganese by manganese dioxide.

In the process of the reduction of manganese dioxide by reducing agents such as lactic acid there are two possible valence forms which the reduced manganese may assume. It is possible that the reduced form of manganese may have a valence of either two or three, or both. If the trivalent condition is the stable form for the majority of the reduced manganese ions, and if manganic phosphate (Mn₃(PO₄)₂) is as insoluble as has been indicated, then there should be a precipitation of manganic phosphate by the phosphoric acid present soon after the oxidation begins. Such a condition is not the case. This conclusion points, therefore, to one of two possibilities: either that manganese dioxide is reduced directly to the bivalent condition, or, if reduced to the trivalent condition, there is immediately set up the rapid equilibrium reaction:

$$2\text{Mn}^{+++} \rightleftharpoons \text{Mn}^{++} + \text{Mn}^{++} \quad \text{(equation 4)}$$

where some trivalent manganese ions spontaneously regenerate.
tetravalent manganese at the same time that other trivalent ions are reduced to manganous ions. Actually, it is believed there is at all times a condition of equilibrium between the three forms as indicated in equation (4), the equilibrium, however, being affected by the amount of lactic acid present, and that trivalent manganese is not present in sufficiently large quantity to be precipitated by phosphoric acid until the lactic acid concentration is small.

A discussion of the equilibrium reactions involving manganese in the two, three, four, and seven valence conditions was presented by Leumer and Yost (59) in 1934. Fossmoen and Redmon (60) described similar equilibrium conditions in 1935. Their conclusions applied to present conditions indicate, as concluded above, that either bivalent or trivalent manganese, or both, can result from the oxidation of lactic acid by manganese dioxide.

The oxidation of lactic acid, then, takes place as outlined in the preceding paragraphs, until the concentration of lactic acid has become so small that the rate of reaction is slowed up. At this point there is an accumulation of manganese in the tetravalent condition, and a reverse of the reaction in equation (4) takes place, with the result that there is an accumulation of sufficient trivalent manganese to cause its precipitation according to equation (5) below:

\[
\text{\text{Mn}^{4+} + \text{PO}_4^{3-} \rightarrow \text{MnPO}_4 \text{(solid)}} \quad \text{(Equation 5)}
\]
In connection with the work of Leunor and Yost it may be mentioned at this point that a cherry-red color, such as that described by them as being characteristic of manganic ion complexes, has been observed from time to time by the present writer in the oxidations of lactic acid by manganese dioxide where there has been an excess of oxidizing material present. The red compound, in this case, appears to be very unstable, and no attempts have been made as yet by this investigator to isolate the compound or make any further investigation into its nature. It is very probable, however, that the red color is due to the presence of some manganic ion complex, because of the fact that its occurrence has been observed only in the presence of an excess of oxidizing agent. Redmon (61) has isolated the cherry-red manganic ion complex formed by oxalic acid.

Effect of Soluble Phosphate on the Precipitation of Manganic Phosphate. It was thought that the addition of large quantities of soluble phosphate to the usual oxidation medium might, in some way, shift the equilibrium conditions involving the manganic ion. This shift in equilibrium conditions should be indicated by the quantity of acetaldehyde produced as compared with the amount produced by the usual oxidation. By experiment there was a decrease of about 50 per cent in the yield of aldehyde in all cases where 5 or 10 grams of sodium dihydrogen phosphate were added to the usual oxidation medium. This decrease at first appeared to be significant, but later it was concluded that the effect was that due only to a decrease in the acidity of the solution because of the buffering action of
the salt. The hydrogen-ion concentration has already been shown by Friedemann and his co-workers (29) to be an important factor. This particular line of investigation was followed no further.

C. Use of Different Types of Oxidation Apparatus

1. The Aeration Procedure

The aeration apparatus described in the original method of Friedemann, Cotonio, and Schaffer (29) was found later by Friedemann and Graesser (32) to be responsible for erroneous results because of conditions which were peculiar to their laboratory at that time. The difficulties encountered were: First, the presence of aldehyde and acetone vapors in the atmosphere of the laboratory and; second, as quoted in the authors' published paper, "an unusually cold supply of cold water which made the reflux condensers too efficient." These two conditions seemed to be sufficient reason for Friedemann and Graesser (32) to discard the old aeration apparatus and to incorporate the use of a simple distillation apparatus in its place. As a result of investigations carried out by the present writer, this change cannot be justified for the following reasons: First, the simple distillation apparatus cannot possibly afford the desired elimination of vapors other than acetaldehyde which are sometimes produced by the simultaneous oxidation of foreign substances which may be present in the unknown solution of lactic acid. The aeration apparatus makes
possible at least a partial separation. These foreign substances should not be present if it is possible to eliminate them, but if they are present, the added protection of the reflux condenser in the aeration apparatus is a decided advantage: Second, the choice between the two methods should not be dependent on the presence of bisulfite-binding vapors in the atmosphere, since it would be extremely easy in such a case to eliminate this difficulty by washing the in drawn air in a solution of bisulfite previous to its passage through the apparatus: Third, with the lowest possible temperatures obtainable, using water drawn directly from the public water supply, it has not been possible to cool the condensers to the point where there is any indication that the determination is hampered by too low condenser temperatures: Fourth, using a distillation apparatus which was at least approximately the same as that used by Friedemann and Crasser (32), the present writer has been unable to obtain results which are at all consistent or as high as those obtained by the aeration procedure. The reason for this failure to obtain good results with the distillation apparatus is unknown, but it may be a question of some detail in the technique since Friedemann and Kendall (32) reported results of determinations using the two different types of apparatus which they claimed show a general agreement. A description of the experimental work which led to the inclusion of reasons three and four will follow.

Influence of the Temperature of the Condenser Water.
Parallel lactic acid determinations, using some of the same sample in each case, were made according to the usual procedure,
in order to determine the influence of the temperature of the condensor water. In one series the water was allowed to flow through each condenser at a rate of about 600 c.c. per minute, entering the condenser at 7°C, and leaving it at around 10°C to 12°C. The temperatures of the influent and effluent waters was measured at two or three-minute intervals on 100°C thermometers. Those thermometers were held in position in 150 c.c. extraction flasks which were so arranged in the system that water was forced to pass through one of these flasks immediately before it entered the condenser, and through another as it left the condenser.

In the other series all influent water was passed through a two liter Florence flask and heated, as it passed through the flask, with a Bunsen burner which had been carefully regulated so the water was warmed to a reasonably uniform temperature. The temperature thus obtained varied from 17°C to 21°C. Water flowed through the condenser at around 200 c.c. per minute, and reached a temperature of around 30°C, before flowing out of the condenser. The results printed in table 4 are sufficient to indicate that there is no difference between the determination made using a warm condenser, and those made using condensers cooled to a temperature as low as 7°C.
Effect of Condenser Water Temperature on the Analysis of Lactic Acid.

Milligrams of lactic acid as determined by oxidation of 10 milligrams in a known solution using different condenser temperatures.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>17°C - 21°C</th>
<th>9°C - 3°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9.38 mg</td>
<td>9.33 mg</td>
</tr>
<tr>
<td>9°C</td>
<td>9.35</td>
<td>9.32</td>
</tr>
<tr>
<td>9.22</td>
<td>9.34</td>
<td>9.30</td>
</tr>
<tr>
<td>9.24</td>
<td>9.75</td>
<td>9.73</td>
</tr>
<tr>
<td>9.77</td>
<td></td>
<td>9.77</td>
</tr>
<tr>
<td>Avg.</td>
<td>9.31 (± 0.06)</td>
<td>9.30 (± 0.04)</td>
</tr>
</tbody>
</table>

Friedemann and Crasser (32) did not indicate at what minimum temperature water entered their condensers during these determinations which were influenced by the cold supply of water. It is barely possible that their supply was somewhat lower in temperature than that in the writer's laboratory, but it is doubted that any further lowering below 7°C would produce results that are any different than those printed in Table 4. It is to be concluded, therefore, from the evidence at hand, that Friedemann and Crasser (32) were in error in their statement that a cold supply of condenser water can be held responsible for low results in determinations, using the aeration procedure.
Influence of Design of Reflux Condenser Used. Since it was found that the temperature of the water entering the condenser was not the factor which might influence the accuracy of lactic acid determinations by the aeration procedure, it seemed worth while to extend the investigation a little further along this line, and to determine whether or not the dimensions of the condenser parts were a factor which could be varied without harmful effects on the determination. One of the two condensers, (A) and (B), which had been in constant use up to this point was replaced by another Hopkins type condenser (C) with different dimensions and a series of parallel determinations run on the two different condensers (B) and (C). The exact measurements of condensers (B) and (C) are included in table 5.

**Table 5**

<table>
<thead>
<tr>
<th>Diameter</th>
<th>Length</th>
<th>Condenser B</th>
<th>Diameter</th>
<th>Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>31</td>
<td>255</td>
<td></td>
<td>25</td>
<td>245</td>
</tr>
<tr>
<td>35</td>
<td>290</td>
<td>Condenser C</td>
<td>20</td>
<td>255</td>
</tr>
</tbody>
</table>

The results from this series of parallel determinations shown in table 6 indicate that condensers similar in design to (B) are probably the more suited for the best results. In general, the results obtained with condenser (C) were more variable and were apt to be lower than the results of similar determinations on condenser (B). The explanation of the
differences in results is not evident, but it can be assumed for the present that small quantities of acetaldehyde were retained in the drops of water which were always observed to be present as a result of condensation of water vapor over the entire inside wall of condenser (C). In condenser (B), instead of a widespread condensation of vapors on the whole interior of the condenser, all of the condensation took place at the very lower end of the inside cold water jacket, thus allowing very little chance for retention of aldehyde in the condensed vapors. The results of parallel analysis, using the two different condensers (B) and (C) which follow in table 6 show that where possible it is desirable to choose a condenser with the dimensions similar to those of condenser (B) in which the distance between the outer and inner condenser walls is as small as is reasonably possible.

Table 6
Effect of Condenser Design on the Determination of Lactic Acid

- Milligrams of lactic acid recovered from the same solution using different type condensers.

<table>
<thead>
<tr>
<th>Condenser B</th>
<th>Condenser C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.37 mg.</td>
<td>0.30 mg.</td>
</tr>
<tr>
<td>0.40</td>
<td>0.36</td>
</tr>
<tr>
<td>0.36</td>
<td>0.46</td>
</tr>
<tr>
<td>0.40</td>
<td>0.19</td>
</tr>
<tr>
<td><strong>Averages</strong></td>
<td><strong>0.36 (± .02)</strong></td>
</tr>
</tbody>
</table>

The results in table 6 represent analyses made on a partially decomposed sample of zinc lactate.
From time to time, one or two determinations were made on samples of known lactic acid content, using the simple distillation apparatus described on page 32 for the purpose of determining whether the results obtained using the aeration and distillation procedures showed good agreement. Four different sets of analyses printed in table 7 are sufficient to indicate that results using the distillation method are variable and are apt to be lower than those obtained by the aeration method. As mentioned before, the difference may be due to the use of improper technique in the operation of some detail in the distillation procedure. However, this point was not investigated further since for the several reasons already indicated the aeration procedure seemed to be the more desirable for general use.

Table 7

Comparison of Results Obtained by Aeration and Distillation Procedures

Results are expressed as milligrams of lactic acid found to be present in the same samples.

<table>
<thead>
<tr>
<th></th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
<th>Sample 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aeration</td>
<td>9.30 mg.</td>
<td>9.65 mg.</td>
<td>9.71 mg.</td>
<td>8.36 mg.</td>
</tr>
<tr>
<td></td>
<td>9.22</td>
<td>9.55</td>
<td>9.45</td>
<td>8.34</td>
</tr>
<tr>
<td></td>
<td>7.00</td>
<td>9.55</td>
<td>8.35</td>
<td>7.94</td>
</tr>
<tr>
<td>Distillation</td>
<td>9.30 mg.</td>
<td>9.65 mg.</td>
<td>9.71 mg.</td>
<td>8.36 mg.</td>
</tr>
</tbody>
</table>
D. Effect of the Presence of Propionic Acid in the Determination of Lactic Acid

The extent of the error in the determination of lactic acid due to the presence of a number of other compounds was one of the subjects of investigation by Friedemann, Cotonic, and Schaffer (29). They have made no mention, however, of an investigation of the interference of propionic acid, and since it is possible for this acid to be present in some of the pickle brines to be analyzed, it was necessary to establish whether or not propionic acid would be measured as lactic acid. The proof that propionic acid is not normally measured as lactic acid by the usual permanganate oxidation was demonstrated easily and conclusively by adding 5 c.c. of an approximately 0.1 N solution of propionic acid to a solution containing a known amount of lactic acid and carrying out the usual determination. Two such consecutive determinations were made, and in both cases the amount of lactic acid present as measured by titration of the bound bisulfite, was essentially the same as that found to be present without the addition of any propionic acid. It can be concluded, therefore, that propionic acid, even in relatively large quantities, does not interfere with the determination of lactic acid by the usual permanganate oxidation procedure.
E. Determination of Lactic Acid in a Fermenting Pickle Brine

Having established a method of analysis which was believed to be very nearly specific for the lactate radical, the writer was in a position to determine the amount of lactic acid present in an unknown pickle solution for the purpose of comparing the results thus obtained with those obtained by titrable acidity measurements on the same solution. For the purposes of this comparison the writer analyzed nine different brines containing from 0.15 to 1.1 per cent lactic acid.

1. Description of the Samples Analyzed

There follows a description of the nine samples which were analyzed for lactic acid, both by titration with standard alkali and by oxidation with permanganate. Sample #1 consisted of a representative portion of a dill-pickle brine which had been held at a temperature slightly above freezing for several months following fermentation. Yeast microdorma and other suspended material were removed from the sample by filtration through four layers of cheese cloth. This sample was kindly supplied by the Department of Horticulture Manufactures at Massachusetts State College.

Sample #2 was part of the brine drawn from an actively fermenting sauerkraut preparation at the end of 48 hours fermentation. Samples #3 and #9 inclusive represent the brines used in the fermentation of pickle stock prepared on a commercial basis, and were kindly furnished by the D. M. Jewett Pickle Co. at South Deerfield, Massachusetts. These last six samples were
drawn from several large wooden fermentating vats twelve or more feet in diameter at a point about one or two feet below the surface of the liquid in the vat, with the exception of sample #3 which was taken from a point near the bottom of a vat in the process of being emptied at the time. All the vats had been held in storage under normal atmospheric conditions during the months from August 1936 to April 1937, with the exception of the vat represented by sample #7. This vat had been held under similar conditions throughout the same period, and for at least one year previous to that. The vat from which sample #3 was taken contained fermenting cauliflower, and all the rest contained fermenting cucumber stock.

2. Determination of Titrable Acidity

The determination of the titrable or total acidity of the brines was made on 25 c.c. of the sample measured accurately from a pipette. The 25 c.c. sample was diluted with an equal volume of distilled water and titrated with standard 0.1 N sodium hydroxide, using phenolphthalein as an indicator. It was found difficult to observe the exact endpoint because of the development of a yellow color in the solution upon the addition of the alkali. However, by careful dropwise addition of the sodium hydroxide, and the addition from time to time of another drop of the indicator, it was possible to obtain results that agreed in general within 1 per cent.
3. **Determination of Lactic Acid by Oxidation**

The usual oxidation procedure, which has been described previously, was made on an aliquot of each of the original samples which had been previously treated in the prescribed manner. In nearly all cases the condensers used were of the same dimensions as condenser (B) as described on page 56 and in all cases they took water directly from the tap with no preliminary heating. It might be mentioned at this point that in those cases where a condenser of the type (C) was used the results were quite apt to be low and, at best, were not consistent with those obtained by using condensers of the type (B). This observation agrees with that made before, using pure known lactic acid solutions. The oxidizing substance was 0.05N potassium permanganate in all cases.

4. **Comparison of Titrable Acidity Measurements With The Results Obtained by Oxidation.**

There follows in table 8 the results which indicate the amount of lactic acid in the nine unknown pickle brines according to titrable acidity measurements and according to the determination by oxidation.
Table 2

Lactic Acid Content of Nine Different Brines as Measured by Titrable Acidity and by Oxidation

Results are expressed in grams of lactic acid per 100 c.c. of solution.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Titrable Acidity</th>
<th>Oxidation</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1 (Dill Pickle Brine)</td>
<td>.33</td>
<td>.66</td>
</tr>
<tr>
<td>#2 (Cauliflower Juice)</td>
<td>.13</td>
<td>.16</td>
</tr>
<tr>
<td>#3 (Cauliflower brine)</td>
<td>.22</td>
<td>.25</td>
</tr>
<tr>
<td>#4 (Stock Cucumber Brine)</td>
<td>.34</td>
<td>.99</td>
</tr>
<tr>
<td>#5 (Stock Cucumber Brine)</td>
<td>.95</td>
<td>.95</td>
</tr>
<tr>
<td>#6 (Stock Cucumber Brine)</td>
<td>.94</td>
<td>.94</td>
</tr>
<tr>
<td>#7 (Stock Cucumber Brine Two Years Old)</td>
<td>.42</td>
<td>.83</td>
</tr>
<tr>
<td>#8 (Stock Cucumber Brine)</td>
<td>.75</td>
<td>.83</td>
</tr>
<tr>
<td>#9 (Stock Cucumber Brine)</td>
<td>.76</td>
<td>.83</td>
</tr>
</tbody>
</table>

It was the thought at the outset of this part of the investigation that, if there were to be a difference between the results obtained by the two different measurements, the quantity of lactic acid as measured by titrable acidity would probably be higher than the results obtained by the oxidation method because of the presence of other organic acids. The figures in table 2 indicate, however, that such is not the case. It first appeared, therefore, that there was some serious error in one of the determinations until, in reconstructing some of
the previous work, the evidence pointed to the normal occurrence in dilute solution of some lactic acid precursor which could not be measured by titration at room temperature. This observation was the result of an attempt to prepare a standard 0.1 N lactic acid solution by titration with sodium hydroxide, using phenolphthalein as an indicator. In this attempt, sodium hydroxide was added until the typical faint pink endpoint was nearly reached, and at this point the solution was heated to boiling to expel carbon dioxide. Then in bringing the solution to the exact endpoint with sodium hydroxide, it was noticed that the solution had become decidedly more acid than before, instead of more alkaline as it should have by loss of carbon dioxide. More than this, the endpoint in the final titration was uncertain, the solution becoming more acid all the time.

Later, in determining the titratable acidity of some of the pickle brines, the same general tendency was observed, namely, that the pickle brines became more acid on being heated to the boiling point. A second exact endpoint was not evident in the brines, but was easily observed in the pure lactic acid solution. On the addition of an excess of standard alkali at the time of heating, followed by a titration of the excess alkali with standard hydrochloric acid after a period of 4 to 5 minutes heating. By this means it was found that the total amount of lactic acid present was about 10 per cent higher than the amount found by titration at room temperature. It appeared, therefore, that there must have been present in the lactic acid solution some precursor of lactic acid which could be titrated as lactic.
acid only upon warming the solution. It is the contention of the writer that this same lactic acid precursor is measured as lactic acid by the permanganate oxidation leading to the higher results by that method.

The fact that lactic acid in wines may not all be in the free state has been mentioned before. In the literature concerning the determination of lactic acid in wines this precursor of lactic acid has always been referred to as a lactic acid anhydride. That an anhydride can exist in dilute aqueous solution is questionable, however. A more plausible explanation of this observed phenomenon can be made by assuming the formation of a hydrogen bridge similar to those discussed by Huggins (62) in 1936. Unfortunately Huggins did not report any observations on the formation of hydrogen bridges between molecules of lactic acid, but he did indicate that carboxy groups of certain other organic acids show strong tendencies to form condensation compounds. The condensation products are formed as shown by the general formula below:

\[
\begin{array}{c}
R \\
\text{(I)}
\end{array}
\]

\[
\begin{array}{c}
\text{O}^\\\text{0}-\text{H} : \text{O}^\\\text{0}-\text{H} \\
\text{R}
\end{array}
\]

Figure 1.

In this compound the hydrogen bridge is the link between the \(-\text{OH}\) of one carboxy group and the \(-\text{C}=\text{O}\) of another carboxy group. That a hydrogen bridge is possible in the case of lactic acid in aqueous solution is only speculative, but if such a linkage...
between the carboxy groups of two lactic acid molecules could be assumed to exist, the presence of a lactic acid precursor could be explained without assuming the formation of an anhydride in dilute aqueous solution. The loss of acidity due to the hydrogen bridge linkage of two carboxy groups is evident from a glance at figure 1.

Whatever may be the nature of this anhydride-like lactic acid precursor, it has been shown to be present in pure dilute lactic acid solution, and, therefore, it is theoretically possible that this same substance may be present in the pickle brines. The fact that, in attempting to titrate the pickle brines to an endpoint in hot solution, the solution became more acid on being heated is evidence that the substance is present in significant quantities in the brines. An attempt was made to measure the exact amount of the "anhydride" by titrating to an endpoint in the hot solution, but because of the absorption of alkali by the colloidal material present in the brines it was impossible to obtain a definite endpoint. Because of this last uncertainty it is impossible to conclude that all of the difference is due to the presence of an "anhydride", but it is quite certain that, for the most part, the high results obtained by the oxidation procedure can be traced to the presence of this "anhydride" and not to the presence of some other substance entirely unrelated to lactic acid.

It was reasoned that alcohols particularly ethyl alcohol, if present in the brines might be measured as lactic acid by the oxidation. To eliminate the possibility that high results by this method might be due to the presence of alcohols, it was
necessary only to evaporate a measured portion of the original brine to dryness in the presence of an excess of calcium hydroxide and to make an analysis on the dry residue. Such an analysis on two different samples produced results that were almost identical with those made on the samples which had not received this treatment. This evidence indicated, as was expected, that no appreciable quantities of alcohol were present in the brines.

It is worthy of mention that in one case in sample #2, the titrable acidity was higher than the amount of lactic acid determined by oxidation. This sample was taken from a brine that had been fermenting only 40 hours. The fact that the oxidation determination is not higher than the titrable acidity can be taken to mean, therefore, that the "anhydride" begins to form only when the solution stands for a period of time. In agreement with this theory is the fact that the two-year-old brine (sample #7) was the one which contained the largest ratio of anhydride to free acid.
1. Various methods for the determination of lactic acid were reviewed.

2. The permanganate oxidation method for the determination of lactic acid was studied in an attempt to improve its accuracy.

3. A proposed mechanism for the oxidation reaction was described.

4. The occurrence of manganic phosphate in the oxidation reaction was discussed.

5. The use of the aeration type apparatus was found to give better results than the use of the distillation type apparatus.

6. Propionic acid was found not to interfere with the determination of lactic acid by the permanganate method.

7. The amount of lactic acid present in a series of representative pickle brines was found to be greater than that indicated by the ordinary titration with alkali.

8. It was proposed that the high results obtained by the oxidation method are due to a partial association of lactic acid molecules such that all the lactic acid present is not titrable with alkali.
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