THE CAUSE OF ACIDITY OF FRESH MILK OF COWS AND A METHOD FOR THE DETERMINATION OF ACIDITY.

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(Received for publication, July 11, 1914.)

The usual method employed in determining the acidity of milk is to add a few drops of a solution of phenolphthalein as indicator to 100 cc. of milk and then titrate with \( \frac{1}{6} \) NaOH. By the use of this method it is found that 100 cc. of milk, when strictly fresh, will require the addition of 15 to 20 cc. of the alkali in order to produce a faint but permanent pink coloration.

The acidity of fresh milk has been commonly attributed to the presence of acid phosphates and casein, and we will now consider the relation of these constituents to milk acidity.

That the acidity of milk is due to the presence of acid phosphates (\( M \text{H}_2\text{PO}_4 \)) is indicated by the fact that milk is strongly alkaline to methyl orange. Further, it is well known that phosphates can not be titrated with any degree of accuracy in the presence of calcium salts, due to the fact that some of the insoluble dicalcium phosphate (CaHPO\(_4\)), which is formed during the titration, hydrolyzes, changing into calcium hydroxide and phosphoric acid, and then the calcium hydroxide unites with more dicalcium phosphate, forming tricalcium phosphate (Ca\(_3\)P\(_2\)O\(_8\)).\(^1\) These facts may be represented by the following equations:

\[
\begin{align*}
(1) & \quad \text{CaHPO}_4 + 2 \text{H}_2\text{O} \leftrightarrow \text{Ca(OH)}_2 + \text{H}_3\text{PO}_4 \\
(2) & \quad 2 \text{CaHPO}_4 + \text{Ca(OH)}_2 \rightarrow \text{Ca}_3\text{P}_2\text{O}_8 + 2 \text{H}_2\text{O}.
\end{align*}
\]

That tricalcium phosphate is formed during the titration of any solution containing phosphoric acid and calcium salts is easily demonstrated by an analysis of the precipitate always appearing; this precipitate is tricalcium phosphate, which is characterized by its appearance, varying from a flocculent to a gelatinous con-

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dition according to the concentration of the calcium and phosphates in the solution.

Dibasic phosphates are neutral to phenolphthalein and monophosphates are acid to this indicator; phosphoric acid, therefore, acts as a diabasic acid to phenolphthalein. In the reaction represented above, we have, in place of the original molecule of neutral dicalcium phosphate, one molecule of free phosphoric acid, whereby the acidity as measured by titration is increased over what it would be if no such reaction occurred. These facts serve to explain some results obtained by us in connection with the study of certain problems relating to milk.

We have found that when we titrate whole milk with alkali, in the usual way and then similarly titrate the serum obtained by filtering the milk through a porous porcelain filter, the titration figure given by the whole milk is about double that obtained with the serum. For example, 100 cc. of whole milk may show an acidity of 17 cc. of $\frac{\pi}{16}$ alkali, and 100 cc. of serum, 8 cc. This difference has ordinarily been interpreted as being due to the acidity of milk-casein, but in a future paper we shall show that casein is present in fresh milk as a calcium caseinate that is neutral to phenolphthalein. The other constituents removed from the milk by filtering through porous porcelain are fat and dicalcium phosphate, both of which also are neutral to phenolphthalein. From the illustration given above, the titration figure of the residue on the filter would appear to be 9 (17–8) for 100 cc. of milk, though in reality the reaction is neutral. We believe that the cause of this discrepancy is to be found in the dicalcium phosphate which is present in the whole milk but which is not present in the serum. Its presence in the milk permits the formation of relatively large amounts of phosphoric acid and tricalcium phosphate, requiring the use of increased amounts of $\frac{\pi}{16}$ alkali (17 cc.) to neutralize the milk, as compared with the amount (8 cc.) needed to neutralize the serum. We have been led by such results to believe that the acidity of milk, as usually determined, is about twice what it should be.

The disturbing influence of calcium salts in the presence of phosphates has been studied by Folin in connection with the

\footnote{Amer. Journ. of Physiol., ix, p. 285, 1903.}
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determination of acidity in urine; he was able largely to overcome
the difficulty by the addition of neutral potassium oxalate, by
which the calcium is removed in the form of the insoluble oxalate.
He showed that by this preliminary treatment, correct titration
figures could be obtained for monocalcium phosphate, which
without such treatment gives figures that are remote from the
calculated acidity.

Making use of Folin’s procedure, and, before titrating with
alkali, adding to milk some saturated solution of neutral potas-
sium oxalate, we are able to obtain figures which conform more
closely to the results indicated as accurate by other considerations.

The method, as modified by us for the determination of acidity
in milk, whether fresh or otherwise, is as follows:
Measure 100 cc. of milk into a 200 cc. Erlenmeyer flask, add 50
cc. of distilled water and 2 cc. of a saturated solution of neutral
potassium oxalate, allow the mixture to stand not less than two
minutes and then titrate with \( \frac{1}{2} \) NaOH. Since most solid potas-
sium oxalate is acid, care must be taken to prepare a solution that
is really neutral, which may be done in the following way: A
saturated solution of ordinary potassium oxalate is prepared and
decanted from the solid residue. To this solution is added 1
cc. of phenolphthalein solution and then, drop by drop, enough
normal NaOH solution to produce a permanent faintly pink
coloration.

In the following table is given the acidity of 21 samples of milk
from individual cows, as determined by the two methods, with
and without addition of neutral potassium oxalate.

SUMMARY.

The acidity of fresh milk is due to the presence of acid
phosphates. Titration of phosphoric acid with alkali, in the
presence of calcium salts, results in hydrolysis of dicalcium phos-
phate formed during the titration, whereby free calcium hydro-
oxide and phosphoric acid are first formed and then calcium
hydroxide unites with more dicalcium phosphate to form insoluble
tricalcium phosphate. As a result of these reactions more alkali
is required to make a solution, containing calcium and phos-
phoric acid, neutral to phenolphthalein than is required in the
Absence of calcium. The calcium must be removed previous to titration by treatment of 100 cc. of milk with 2 cc. of saturated solution of neutral potassium oxalate.

<table>
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<th>NUMBER OF SAMPLES</th>
<th>AMOUNT OF $\frac{N}{10}$ NaOH REQUIRED TO NEUTRALIZE 100 CC. OF MILK</th>
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<td>Before addition of neutral potassium oxalate</td>
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J. Biol. Chem. 1914, 19:73-76.

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