

PRESERVATIVES IN MILK.

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(Read at the Meeting, March 6, 1907.)

PART I.

THE SOURING OF MILK, AND THE ACTION OF PRESERVATIVES THEREON.

THE amount of acidity in milk is a function of—(1) The number and species of the acid-producing organisms present; (2) the rates at which the organisms multiply and produce lactic (or other) acid; (3) the nature and amount of substances which inhibit the growth of the organisms or their power of producing lactic acid; and (4) the temperature.

Notwithstanding that considerable variation may be expected in all the conditions affecting the production of acidity, we have found from the results of numerous experiments that the acidity of milk can be expressed with fair accuracy as a function of time and temperature.

TABLE I.—ACIDITY DEVELOPED IN MILK AT 20° C.

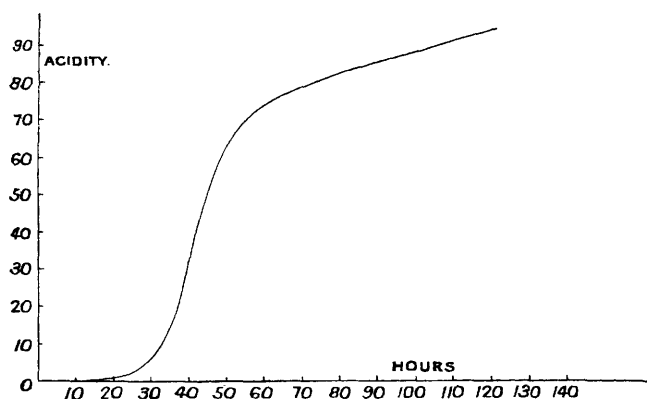
Time in hours	...	10	15	20	25	30	35	40	45	50	55	60
Acidity	...	0	0.1	0.3	2.5	6.0	13.0	29.0	50.0	62.0	70.0	74.0
Time in hours	70	80	90	100	110	120				
Acidity	79	82	85	88	91	93				

In Table I. we give the mean results at 20° C.; our experiments have all been made by titrating 10 c.c. of milk as fresh as possible with approximately $\frac{N}{10}$ strontia solution, using phenolphthalein as indicator, and taking the acidity thus found as the normal acidity of the milk. The milk was kept at known temperatures, and titrated at intervals, and the acidity, less the normal acidity of the milk, taken as the "acidity developed"; all results are expressed as "degrees" or c.c. of N alkali used per litre of milk. The results are also expressed graphically in Curve I.

On examination of the curve, it is seen that there is a break when the "acidity

developed" reaches 45° , and another at 65° ; the first of these points corresponds with the "acidity" at which the micro-organisms cease to increase, and the second to the spontaneous curdling of the milk. The latter point is not absolutely definite, and the curve beyond it shows greater departures from the mean than do the earlier portions. Up to the first break the curve can be expressed with considerable accuracy by a logarithmic equation, and the second portion appears to be expressed by another logarithmic equation; as the third portion is somewhat indefinite, we have not attempted to find an equation.

With different milks the shape of the curve is always practically identical, though the curves do not always quite coincide. We attribute this to the probable



CURVE I.

fact that the number of micro-organisms which enter the milk in its early stages varies widely, as well as to differences in temperature during the interval between milking and the examination of the milk.

The effect of temperature appears to be quite regular between 15°C. and 35°C. ; for each 10° rise of temperature the rate of development of acidity is increased 2.075 times.

We have studied the effect of a number of substances on the souring of milk, which have been used or described as preservatives; these are:

Aromatic acids: Salicylic, benzoic, phthalic.

Phenolic compounds: β -naphthol, abrastol (calcium β -naphthol sulphonate), sodium β -naphthol sulphonate, resorcinol, phloroglucinol.

Basic substances: Cyllin was taken as a type.

Inorganic compounds: Sodium fluoride, potassium acid fluoride, sodium sulphite, potassium metabisulphite, borates.

The method adopted was to make up samples of milk containing 0.2 per cent., 0.1 per cent., 0.05 per cent., and 0.025 per cent. of the substances, and keep them at 20°C. ; at intervals the "acidity developed" was estimated as described above. Control samples of the milk used were also examined. The only exceptions were the experiments with borates, as we found that to obtain strictly comparable figures it

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was necessary to add to 10 c.c. of milk 0.5 c.c. of a 0.5 per cent. solution of phenolphthalein and 30 per cent. of glycerol. To compare the preservative action we plotted out our results, and read from the curves the time taken to reach an "acidity developed" of 13° , which one of us and Harrison have found to be the point at which milk curdles on boiling (ANALYST, 1900, **25**, 116). We can confirm this figure, and in some cases have determined the time at which the milk curdled on boiling by actual experiment; the results were practically identical with those read from the curves. The difference between the time when the control milk reached 13° "acidity developed" and the time when the sample reached this point gives an index of the preservative action.

Some of the substances experimented with caused the milk to turn sour faster than the control, when used in small amounts; these were:

	0.2 Per Cent. Hours.	0.1 Per Cent. Hours.	0.05 Per Cent. Hours.	0.025 Per Cent. Hours.
Sodium fluoride ...	5	0	- 1	- 2
Potassium acid fluoride	$8\frac{1}{2}$	0	- 1	- 2
Sodium sulphite ...	$15\frac{1}{2}$	$5\frac{1}{2}$	$-\frac{1}{2}$	- 2
Resorcinol ...	7	2	0	- 1
Phloroglucinol...	5	$1\frac{1}{2}$	0	$- 1\frac{1}{2}$

Others had no appreciable preservative action; these were:

	0.2 Per Cent. Hours.	0.1 Per Cent. Hours.	0.05 Per Cent. Hours.	0.025 Per Cent. Hours.
Phthalic acid ...	0	0	0	0
Abrastol ...	0	0	0	0
Sodium β -naphthol sulphonate ...	1	$\frac{1}{2}$	$\frac{1}{4}$	0
Cyllin ...	7	2	$\frac{1}{2}$	0

The following substances had an appreciable preservative effect:

	0.2 Per Cent. Hours.	0.1 Per Cent. Hours.	0.05 Per Cent. Hours.	0.025 Per Cent. Hours.
Sodium benzoate, I. ...	16.7	8.9	4.1	1.3
" " II. ...	—	7.5	3.2	1.7
Potassium benzoate ...	15.6	9.4	3.9	1.0
β -naphthol ...	56	9.5	2.5	0.8
Salicylic acid (neutralised with soda) ...	21.5	15.5	7.0	4.2
Potassium metabisulphite ...	134	43	21	5.5
Borate mixture, I. ...	0.0	20.5	0.0	0.0
" " II. ...	0.0	20.5	0.0	0.0
" " III. ...	0.0	17.5	0.0	0.0
" " IV. ...	0.0	21.0	0.0	0.0
" " V. ...	30.5	17.5	8.0	3.5

The benzoates had the composition:

	Sodium Benzoate, I. Per Cent.	Sodium Benzoate, II. Per Cent.	Potassium Benzoate. Per Cent.
Benzoic acid ...	78.9	60.0	75.3
Sodium carbonate ...	—	17.7	—
Moisture ...	5.1	12.0	0.45

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The borate mixtures gave the following results on analysis :

	I.	II.	III.	IV.	V.
	Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.
Na_2O ...	10.7	4.7	5.9	4.2	13.8
B_2O_3 ...	57.7	57.4	49.8	57.2	49.1
Equal to H_3BO_3	102.3	101.7	88.3	101.4	87.0

No. I. contained a little saccharin.

No. III. contained a little saccharin and 1.0 per cent. of salicylic acid.

We give in Table II. the actual figures found, and have plotted out the results in the curves.

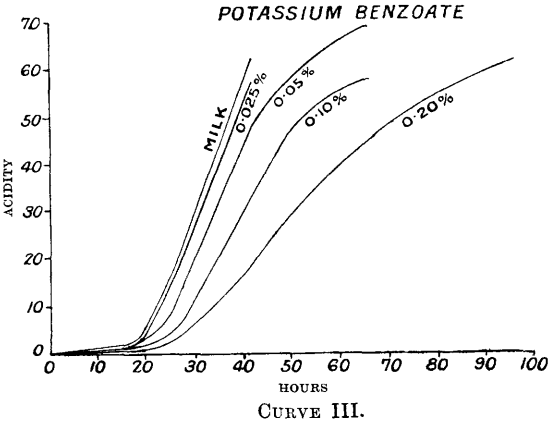
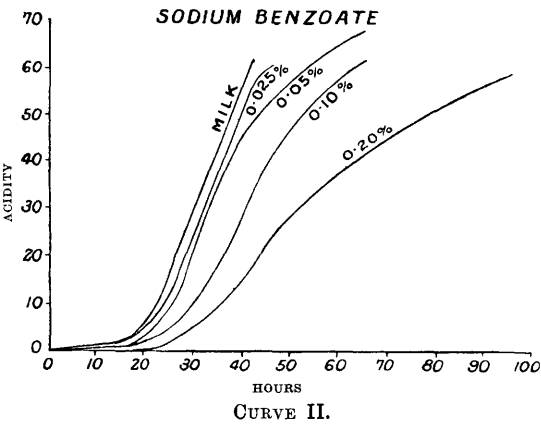
TABLE II.—ACIDITIES DEVELOPED.

Time in hours ...		18.7	23.5	26.5	42.6	47.3	67.4	97.0	—
Control milk	Per Cent.								
	—	4.0	12.5	20.0	63.5	—	—	—	—
Sodium benzoate {	0.2	0.0	2.0	2.5	16.5	26.0	43.0	60.0	—
	0.1	2.0	4.0	6.0	35.0	42.0	62.5	—	—
	0.05	2.0	7.0	11.0	50.0	55.0	69.5	—	—
	0.025	3.0	10.0	15.5	57.0	61.5	—	—	—
Time in hours ...		18.0	23.7	26.7	42.8	47.5	67.6	97.2	—
Potassium benzoate {	Per Cent.								
	0.2	0.5	1.00	2.0	19.5	26.0	47.0	64.0	—
	0.1	1.0	3.5	5.0	34.5	42.5	58.5	—	—
	0.05	2.0	6.5	12.0	49.0	54.5	68.0	—	—
	0.025	2.5	10.0	16.5	58.0	—	—	—	—
Time in hours ...		19.1	23.9	26.9	43.0	47.7	67.8	97.4	—
Control milk {	Per Cent.								
	—	6.0	17.0	25.0	67.0	—	—	—	—
	0.2	0.5	1.0	0.5	2.0	4.0	9.0	28.5	—
	0.1	0.0	2.0	4.5	35.5	43.5	68.5	—	—
	0.05	2.5	8.5	15.0	55.5	59.0	73.0	—	—
β -Naphthol {	0.025	3.2	12.7	19.7	64.2	—	—	—	—
Time in hours ...		17.6	26.6	41.7	50.6	65.7	75.2	89.7	116.7
Control milk	Per Cent.								
	—	1.5	12.5	62.5	—	—	—	—	—
Sodium salicylate {	0.2	1.0	1.0	6.5	10.5	27.0	31.0	39.0	41.5
	0.1	0.0	0.5	10.0	24.0	38.0	47.5	58.0	—
	0.05	0.03	6.0	24.0	43.0	59.0	—	—	—
	0.025	1.0	6.5	47.5	62.0	—	—	—	—

Time in hours ...		19.2	28.3	45.0	52.2	69.2	92.4	115.2	139.2
Control milk	Per Cent.								
	—	1.0	11.5	68.0	—	—	—	—	—
	0.2	0.0	0.5	13.5	23.0	34.0	39.0	45.6	51.5
	0.1	1.5	6.0	26.0	33.0	49.5	55.5	62.8	—
	0.05	1.8	12.3	50.8	56.8	—	—	—	—
Sodium sulphite	0.025	2.1	15.1	62.1	—	—	—	—	—

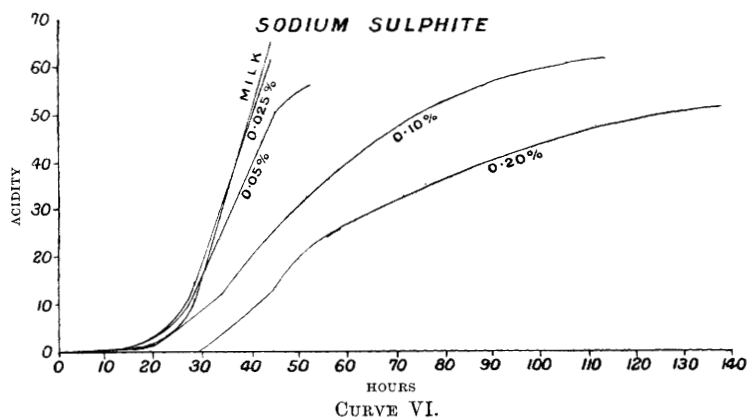
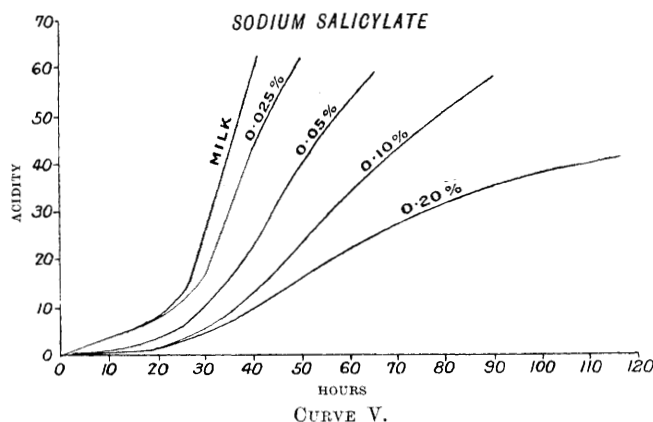
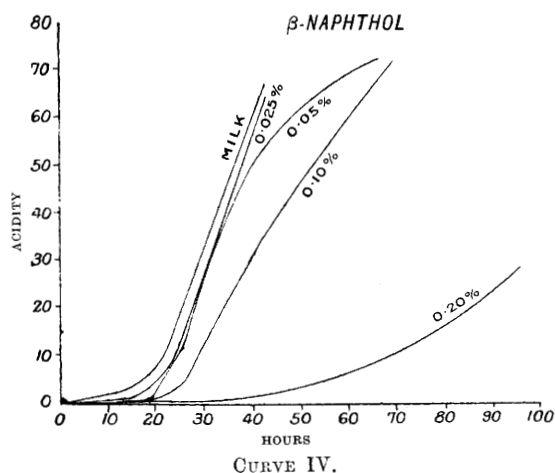
Time in hours ...		19.6	28.7	45.4	52.6	69.5	92.6	115.3	139.2
Control milk	Per Cent.								
	—	1.0	14.0	68.0	—	—	—	—	—
	0.2	0.0	1.0	0.5	0.5	2.0	2.0	8.5	10.0
	0.1	0.0	0.5	3.5	4.0	10.5	16.0	19.5	23.9
	0.05	0.2	2.2	7.7	13.7	26.2	32.7	41.2	51.1
Potassium acid sulphite	0.025	1.4	5.9	29.9	37.9	57.2	—	—	—

Time in hours ...		19.0	27.6	43.1	50.9	67.4	75.2	93.6	—
Control milk	Per Cent.								
	—	0.8	5.2	36.1	—	—	—	—	—
	0.174	—	—	3.0	4.6	13.3	22.0	46.3	—
	0.102	—	1.25	3.95	8.3	29.6	—	—	—
	0.088	—	1.5	4.0	10.3	41.6	—	—	—
Boric acid	0.044	—	0.9	11.7	32.4	—	—	—	—
	0.022	—	1.4	21.4	—	—	—	—	—



The following points are worthy of note :

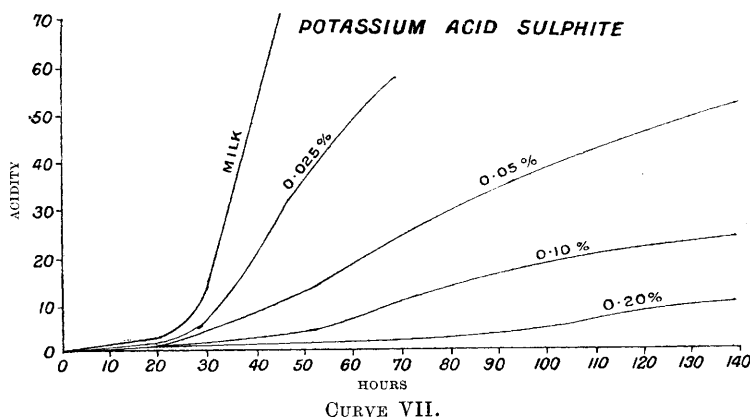
(a) With benzoic, salicylic, and boric acids the preservative effect is approximately proportional to the amount; there is a general similarity between the benzoic and salicylic acid curves, and the first break in these takes place at a much lower acidity than in the case of the milk curve; the breaks in the boric acid curves are much nearer to that in the milk curve. This indicates a difference between the action of the



aromatic acids on the one hand and boric acid on the other. In this connection we may recall that boric acid is a very weak acid, and is strengthened in its

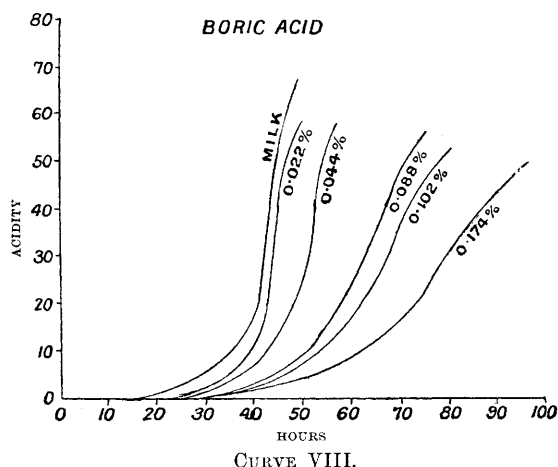
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acidic function in the presence of polyhydric alcohols. We venture to hazard the speculation that micro-organisms secrete a compound which strengthens boric acid, which probably diffuses through their cell walls.



CURVE VII.

(b) Fluorides, sulphites, and phenols have a preservative action which increases with the amount added; in most cases, probably in all, small amounts cause the milk to go sour faster than the control milk. This indicates that an action takes



CURVE VIII.

place between the substance added and a constituent of the milk. Fluorides remove the lime from its combinations with the constituents of milk, and it is probable that calcium fluoride is inert; it also appears probable that the calcium compound of casein which exists in milk in a state of pseudo-solution hinders the activity of micro-organisms.

Sulphites are probably oxidised, and it is worthy of note that in practice it is found that milk tends to go sour faster if air is excluded.

The phenols are probably combined with proteins.

(c) It is remarkable what a small increase preservatives give to the life of milk. At 20° milk keeps for thirty-five hours before becoming sour enough to curdle on boiling, and at 30° seventeen hours. As it is only at the higher temperatures that milk turns sour sufficiently fast to justify in any way an addition of preservatives, it is interesting to compare the additional life conferred by 0.1 per cent. of preservatives at 30°. The figures are:

Boric acid	... 10 hours	β -naphthol	... 4½ hours
Benzoic acid	... 5½ "	Potassium meta-	
Salicylic acid	... 7½ "	bisulphite	... 21 "

When it is considered that the same effect can be attained by cooling the milk down a few degrees, at a cost which certainly does not exceed that of the preservative, it is evident that there is no justification for the use of preservatives in milk.

PART II.

NOTES ON THE DETECTION AND ESTIMATION OF PRESERVATIVES.

Our results show that the only preservatives likely to be used in milk are borates, benzoates, salicylates, and possibly β -naphthol, in addition to formalin and hydrogen peroxide, which we have not investigated. Potassium metabisulphite is probably not used, as its taste is pronounced and objectionable even in small quantities.

Detection of Benzoates.—Sodium benzoate is sold as a milk and cream preservative under various fancy names, and it is stated by the vendors that it cannot be detected. Its detection is a very simple matter. We find that by precipitating the curd by acetic acid, and extracting the whey with either a mixture of ether and petroleum ether or with chloroform, washing the solvent with water, adding a small quantity of water, a little phenolphthalein, and dilute alkali drop by drop till the aqueous portion is pink, we can obtain the benzoic acid present in the milk in a few c.c. of solution, to which can be applied any or all of the usual tests.

(a) Ferric chloride produces a buff-coloured precipitate insoluble in acetic acid.

(b) When reduced by magnesium in acid solution benzaldehyde is formed.

(c) Evaporated to dryness with soda-lime, and ignited in an inert gas, benzene is produced (characteristic smell), which can be converted into nitrobenzene (another characteristic smell), reduced to aniline, diazotised, and condensed with β -naphthol in alkaline solution (red colour).

(d) When evaporated to dryness, 2 c.c. aniline and 0.02 gram rosaniline hydrochloride added, and boiled for twenty minutes, a blue colour is produced (De Brevans, *Journ. Pharm. Chim.*, 1901 [vi.], **14**, 438; ANALYST, 1902, **27**, 56).

(e) When evaporated to dryness, and heated with sulphuric acid and gallic acid, anthragallol is formed. On making alkaline, a red colour turning brown is produced.

We obtained all these reactions with minute amounts of benzoic acid, and have had no trouble in detecting 0.03 per cent. of benzoic acid in milk when using 50 c.c.

Detection of β -naphthol.—A very characteristic reaction of the naphthol is its easy condensation with tetrazonium salts, even in faintly acid solution, to form dark red compounds.

We test as follows: A diazotised benzidine solution is prepared (1 gram benzidine, 1 gram sodium nitrite, 4 c.c. strong HCl, 100 c.c. water) and neutralised, using phenolphthalein as indicator; a little is added to a few c.c. of milk; if β -naphthol is present a red colour will be produced. Alkalinity must be avoided, as milk itself gives a brownish red in alkaline solution. To confirm, a diazotised solution of phenylhydrazine may be used, which produces a red colour when condensed in alkaline solution, but no colour with milk; or the β -naphthol may be extracted with chloroform, and the chloroform solution heated with caustic potash for a few minutes; a deep blue colour is produced with β -naphthol.

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The detection of other preservatives presents no difficulty.

Estimation of Boric Acid.—There are numerous methods for the estimation of boric acid, which depend on the neutrality of boric acid to methyl orange, and its conversion into a strong acid in the presence of polyhydric alcohols. These methods all require the removal of phosphoric acid, and are either rather tedious or involve loss.

One of us and Harrison (ANALYST, 1907, **27**, 179) have described a method in which advantage is taken of the difference of the end-point (using phenolphthalein as indicator) in a hot aqueous solution from that in the presence of glycerol. When working this method we found small but appreciable differences in the "boric acid value" of our alkali solution when standardising on boric acid. We also (*see above*) found it impossible to get concordant results for the acidity of milk containing boric acid; it was found that the amount of phenolphthalein added had an enormous effect on the results.

The following experiments were all made on 10 c.c. of milk, and the results are expressed as degrees of acidity:

1. Phenolphthalein Added.	2. Milk Alone.	3. + 8 c.c. Glycerol.	4. + 0.2 Per Cent. Boric Acid.	5. + H ₃ BO ₃ and Glycerol.	Acidity due to Boric Acid added.
0.025 c.c.	—	—	—	61.4°*	—
0.075 c.c.	21.8°	22.4°	46.2°	59.4°	—
0.15 c.c.	—	—	43.4°	56.4°	—
0.5 c.c.	20.7°	21.2°	38.1°	55.6°	34.4°
1.0 c.c.	19.9°	20.3°	33.4°	55.6°	35.3°
2.0 c.c.	19.1°	19.5°	—	54.7°	35.2°
3.0 c.c.	—	—	—	55.2°	—
5.0 c.c.	18.7°	19.2°	27.6°	54.7°	35.5°

The following results were obtained at the boiling-point:

0.5 c.c.	—	—	24.9°	56.4°	—
5.0 c.c.	—	—	16.2°	51.4°	35.2°

The above experiments (which were confirmed by others) show that (*a*) in the absence of glycerol the "end-point" with boric acid varies greatly with the amount of phenolphthalein, and (*b*) in the presence of much phenolphthalein boric acid is neutral in boiling solution. We therefore use the following method of estimating boric acid: To a measured or weighed amount of milk add half its bulk of phenolphthalein solution (0.5 per cent.), and run in alkali till a pink colour appears; boil and titrate back while still boiling with acid solution until white, and finally with $\frac{N}{10}$ alkali until faintly pink (the colour, though faint, is distinct); add 30 per cent. glycerol, and continue the titration with $\frac{N}{10}$ alkali; subtract, if necessary, the glycerol blank, and the alkali used for final titration multiplied by 0.0062 will give the boric acid.

* This end-point was indistinct and the result doubtful.

Preservatives added as H_3BO_3 .				H_3BO_3 Found.	
0.17	0.168
0.10	0.10
0.086	0.085
0.052	{ 0.06
					{ 0.054
0.026	{ 0.03
					{ 0.026
0.082	0.077
0.049	0.048
0.046	0.048
0.088	0.090

In all cases the substance added was one of the commercial preservatives of known boric acid content (see p. 147).

DISCUSSION.

THE PRESIDENT said if the quantity of boric acid present was very small, say under 20 grains to the gallon, he was inclined to think that the methyl borate process, although not generally adopted, gave good and reliable results. In other processes so many salts were introduced that it was difficult to be quite certain of the presence of minute quantities. With larger quantities, however, the results of the distillation process were not so good, and some loss was liable to occur owing to the number of distillations that were necessary. The author's experiments as to the souring of milk were very interesting. Most of the substances mentioned seemed to have very limited preservative powers. He asked how the results obtained by using preservatives compared with those of sterilisation.

Mr. L. NASH asked whether the accuracy of the boric acid determination would be interfered with if the milk contained other preservatives as well.

Mr. CHAPMAN said, in the case of some other articles with which he had had to deal, his experience had always been that for equal weights salicylic acid was a better preservative than potassium metabisulphite. This seemed to show that different bacteria were more variously affected by preservatives than might be imagined. It had recently been shown that in milk to which a preservative had been added the direction in which decomposition took place depended upon the substance added. In experiments with sodium fluoride and salicylic acid respectively it was found in the one case that there was but slight decomposition of the albuminoid constituents and very extensive decomposition of the milk-sugar, while in the other case the effect was just the opposite.

Mr. A. E. PARKES asked whether the method of estimating boric acid described by the authors was applicable to cases in which borax as well as actual boric acid was added.

Mr. RICHMOND, in reply, said that it was not easy to compare the effects of preservatives with those of sterilisation. In sterilising milk the micro-organisms were destroyed, and the rate of souring afterwards must depend upon the organisms which got into the milk, or those few which escaped destruction. Supposing, however, the sterilised milk to be kept under the same conditions as the preserved milk, he thought it probable that sterilisation, or even heating to such a degree as to kill

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most of the organisms without complete sterilisation, would keep the milk at least as well as any preservative. With regard to Mr. Nash's question, the method depended upon the fact that the acidity of boric acid, while it was nil in hot aqueous solutions containing an excess of phenolphthalein, became very strong in the presence of polyhydric alcohols. He could not say from experiment that there was no other preservative possessing that property, but he did not think there was. The method showed the whole of the boric acid, whether it was present alone or in a mixture of boric acid and borax. Their reason for making alkaline and then acid again was that the excess of acid might combine with the alkali of the borax, if any were present. The comparison referred to by Mr. Chapman was very interesting in connexion with the results they had obtained with cyllin. This was said to be one of the most powerful antiseptics known, but it proved a very weak preservative for milk.

