mydriatic value of the alkaloids of belladonna, (2) the effect of different hydrogen-ion concentrations on the deterioration and stabilization of the belladonna preparations and (3) the assay of myotic alkaloids.

CONCLUSIONS.

1. A study of the bioassay method for testing drugs that have a mydriatic action is reported.

2. The mydriatics, atropine sulphate, homatropine sulphate, scopolamine hydrobromide, hyoscyamine sulphate, ephedrine alkaloid, pseudoephedrine alkaloid, ephedrine sulphate and pseudoephedrine sulphate have been studied, and our results correlate with those of Munch.

3. Tinctures of belladonna have been assayed by this method and seem to show no deterioration, but do not correlate-with the chemical method. Further work is necessary to determine this discrepancy.

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A STUDY OF THE COMPOSITION OF SODIUM BISMUTH TARTRATE.*

BY JOHN H. YOE AND JOHN H. MOTE.

INTRODUCTION.

Although many compounds of bismuth with tartaric acid have been prepared very little has been accomplished towards establishing their composition and structure. The date at which bismuth tartrate compounds were first made seems to be about 1847. Since that time numerous other compounds of bismuth and tartaric acid have appeared whose compositions seem to vary widely, dependent upon the mode of preparation. A number of the earlier preparations are reviewed by Warren[1] in his paper entitled "The Composition of Some Complex Bismuth Tartrates Used in the Treatment of Syphilis." In this paper Warren gives the results of his examination of several commercial specimens of bismuth tartrate. The results of his analyses for bismuth, tartaric acid and sodium or potassium content of the various samples are then compared with the theoretical composition of the compound as claimed by the manufacturers. According to Warren only one of the specimens examined proved to have the composition claimed by the manufacturers. This sample was believed to have the composition approximately represented by the formula:

$$CH(OBiO)—COOK$$
$$| \qquad\qquad\qquad + 4H_2O$$
$$CH(OBiO)-\ COOBiO$$
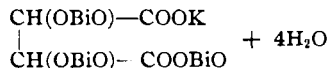
Warren's examination confirmed this view.

It may be noted that in the analysis of this sample, Warren did not add ammonia water to bring the sample into solution, as he did with the other samples. The aqueous solution itself reacted alkaline to litmus.
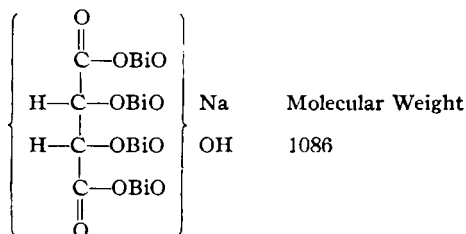
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[1] JOUR. A. PH. A., 14 (1925), 478-487.

Among the earlier preparations discussed the most noteworthy seems to be that prepared by Rosenheim and Vogelsang[1] which they claimed to be potassium tri-bismuth tartrate and to which they assigned the formula:

$$\begin{array}{l} CHO(BiO)-COOK \\ | \\ CHO(BiO)-COO(BiO) \end{array} + 4H_2O$$
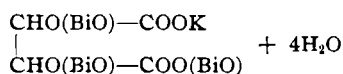
This compound contains 67.15 per cent bismuth and 4.21 per cent potassium.

P. A. Kober[2] has prepared a compound of bismuth and tartaric acid which he claims to be sodium tetra-bismuth tartrate. He proposes the following formula which is analogous to that of Rosenheim and Vogelsang for the tri-bismuth tartrate.

$$\left\{ \begin{array}{c} O \\ \| \\ C-OBiO \\ | \\ H-C-OBiO \\ | \\ H-C-OBiO \\ | \\ C-OBiO \\ \| \\ O \end{array} \right\} \quad \begin{array}{l} Na \\ \\ OH \end{array} \quad \begin{array}{l} Molecular\ Weight \\ \\ 1086 \end{array}$$

Kober considering the method used by Rosenheim and Vogelsang which "consisted in digesting bismuth subnitrate with tartaric acid and an excess of alkali, while heating" came to the conclusion that from thermodynamic considerations the reaction should be carried out without heating. Kober prepared his compound using bismuth hydroxide instead of the subnitrate. The bismuth hydroxide with a slight excess of sodium hydroxide, was added to the tartaric acid and the whole agitated by means of a mechanical shaker, the temperature not being appreciably raised during the process. The sodium-bismuth tartrate was precipitated by adding 95 per cent alcohol.

The present paper is based upon a study of the composition of the sodium-bismuth tartrate prepared by Kober.

EXPERIMENTAL.

Three samples of sodium-bismuth tartrate were used in this study. They were furnished by P. A. Kober and were prepared by his method outlined above.

Dissolving the Compound.—All three samples were found to be very soluble in cold water. When placed in a beaker with a few drops of water the compound first formed a gel which dissolved on the addition of more water (usually 2–5 cc. were sufficient to dissolve a one-Gm. sample). After the sample was in solution it was found that it could be diluted to any desired extent without hydrolysis, but if an attempt be made to dissolve it in a large quantity of water at once, a white precipitate would form. These solutions appeared to be stable up to temperatures at least as high as 70° C. The solutions reacted alkaline to phenolphthalein.

Determination of Bismuth.—The bismuth content was determined by two methods. (Two other methods failed to give consistent results.)

[1] *Z. anorg. Chem.*, 48 (1906), 205.

[2] *J. Lab. Clin. Med.*, 12 (1927), 962.

(a) *Hydrosulphite Method.*—The hydrosulphite method, which makes use of the reducing action of sodium hydrosulphite to get metallic bismuth, is the method used by Kober[1] in the analysis of his bismuth-tartrate compound.

Procedure.—About 0.5 Gm. of the compound was accurately weighed, dissolved in water and diluted to 25 cc. The solution was warmed to 50–70° and 1.5 Gm. of sodium hydrosulphite added. (The sodium hydrosulphite was dissolved in 10 cc. of 2.5 per cent ammonia water. The hydrosulphite was allowed to stand in a stoppered flask and the clear solution pipetted off as needed.) The precipitate was collected in a Gooch crucible, washed, dried in an oven at 100° and weighed as metallic bismuth. The results follow in Table I.

TABLE I.

	Lot 3.	Lot 4.
Per cent Bi	72.60	73.12
	72.73	72.80
	72.82	73.46
	72.85	73.98
	72.65	73.74
Average	72.72	73.70

(b) *Precipitation as Bi₂S₃ from Acid Solution.*—This method is a modification of that used by Warren[2] in the determination of bismuth. Warren precipitated bismuth sulphide from alkaline solution, ammonia being used to bring the substances into solution. We attempted to precipitate bismuth sulphide from alkaline solution but the values were two or three per cent higher than those obtained by the hydrosulphite method and varied widely among themselves. In order to test the method we precipitated the bismuth in alkaline solution, and then filtered, washed and weighed the bismuth sulphide obtained. A sample from Lot 5 was used. We got 75.00 per cent when the results were calculated to bismuth. This precipitate was then dissolved in acid, the solution made alkaline with ammonium hydroxide and then slightly acid with hydrochloric acid and the bismuth precipitated with hydrogen sulphide in acid solution. We obtained 73.26 per cent bismuth, which is in excellent agreement with the average value of 73.24 per cent obtained by the acid-sulphite method (see Table II).

We then made a number of determinations by precipitation as bismuth sulphide from acid solution.

Procedure.—About 0.5 Gm. of the compound was accurately weighed, dissolved in water and diluted to 25 cc. Hydrochloric acid was added until the precipitate which first formed on the addition of the acid had dissolved. About 3 cc. of concentrated hydrochloric acid were then added and hydrogen sulphide passed into the solution under pressure until a clear supernatant solution showed that all the bismuth had been precipitated. The precipitate was collected in a weighed Gooch crucible, the filtrate being tested for any unprecipitated bismuth. The precipitate was washed with water, alcohol, ether, carbon bisulphide, alcohol, ether in the order named. The precipitate was dried in an oven at 100° and weighed as Bi₂S₃. The results were calculated to per cent bismuth and are given in Table II.

[1] *Loc. cit.*
[2] *Loc. cit.*

TABLE II.

	Lot 3.	Lot 4.	Lot 5.
Per cent Bi	72.16	72.63	73.26
	72.87	72.63	73.30
	. . .	. . .	73.27
	. . .	. . .	73.13
Average	72.51	72.63	73.24

The values in Table II are seen to be in fair agreement with those obtained by the hydrosulphite method and given in Table I.

In addition to the above methods we attempted to determine the bismuth content by the electrolytic method but never succeeded in getting all the bismuth out of the solution.

Determination of Tartaric Acid.—The method used for the determination of tartaric acid was a modification of that used by Warren.[1]

Procedure.—The filtrate from the bismuth sulphide was made neutral and the solution evaporated to half its volume, then placed in a graduated flask and made up to volume. Half of this solution was evaporated to 25 cc. (The other half was reserved for the sodium determination), an excess of potassium chloride was added followed by 2–3 cc. of glacial acetic acid. About 100 cc. of 95 per cent alcohol was then added and the solution allowed to stand for two or three days. The potassium bitartrate was then collected in a Gooch crucible and washed with 95 per cent alcohol until free from acetic acid. The contents of the crucible were placed in a beaker containing hot water and the acidity determined by titrating with standard sodium hydroxide, using phenolphthalein as indicator. The results obtained were calculated to tartaric acid. The results follow:

TABLE III.

	Lot 3.	Lot 4.	Lot 5.
Per cent $H_2C_4H_4O_6$	14.40	14.38	13.42
	14.40	. . .	13.47
Average	14.40	14.38	13.45

In his determination for tartaric acid Warren used 50 per cent alcohol instead of 95 per cent alcohol. When we attempted the analysis using 50 per cent alcohol our results were very discordant, which is not surprising when one compares the solubility of potassium bitartrate in 50 per cent alcohol with its solubility in 95 per cent alcohol. The solubility at 25° C. is given in Seidell's "Solubilities" as 0.064 Gm. per 100 Gm. of solvent for 50 per cent alcohol and 0.012 Gm. per 100 Gm. of solvent for 95 per cent alcohol.

In order to allow for the solubility of potassium bitartrate in the alcohol used we ran a series of four determinations for tartaric acid on solutions of known strength. The amounts of tartaric acid used were the same as found in the analysis of sodium-bismuth tartrate and the experimental conditions were also the same as those present when sodium-bismuth tartrate was analysed for tartaric acid. We found that the value of the tartaric acid should be raised by 6.06 per cent in order to give

[1] *Loc. cit.*

nearer the true per cent of tartaric acid in the compound. This amount is something less than would be the case if we calculated the amount dissolved in the alcohol from Seidell's solubility data, which is to be expected as it is hardly likely that the alcohol we used ever became completely saturated.

TABLE IV.

$H_2C_4H_4O_6$ (taken)	0.04287	0.04287	0.04287	0.04287 Gm.
$H_2C_4H_4O_6$ (found)	0.04046	0.04037	0.04066	0.04018 Gm.

The average for all tartaric acid determinations made was 14.07 per cent which when corrected for solubility becomes 14.92 per cent.

Determination of Sodium.—In the determination of sodium we employed the method used by Warren[1] in the analysis of complex bismuth tartrates.

Procedure.—One-half of the diluted filtrate from the bismuth determination was evaporated to dryness in the presence of an excess of sulphuric acid, the residue heated to dull redness, a fragment of ammonium carbonate added and the residue again heated. The sodium sulphate was weighed, its sulphate content determined as barium sulphate in the usual way and the relative proportions of potassium and sodium calculated by the method described in Fales' "Inorganic Chemical Analysis" (Century Co.), pages 202–203.

Two determinations on Lot 5 by this method gave results as follows:

TABLE V.

Per cent Na	2.84	2.84
Per cent K	0.63	0.63

Inasmuch as no potassium was used in the preparation of the sodium-bismuth tartrate and due to the fact that the samples were small, we assumed that the potassium found was within the error of calculation by the method of mixtures described. The other determinations were calculated as sodium from the weights of sodium sulphate found. The results follow in Table VI.

TABLE VI.

	Lot 3.	Lot 4.	Lot 5.
Per cent Na	2.94	2.46	3.03
	3.08	2.58	3.34
	3.08	2.70	2.81
	2.96	2.70	2.86
	2.97	. . .	2.87
Average	3.01	2.61	2.98

Carbon Dioxide.—In the preparation of the sodium-bismuth tartrate under investigation, Kober used an excess of sodium hydroxide. This being the case one would expect to find a certain amount of normal carbonate or bicarbonate in the compound which would correspond to the excess of sodium hydroxide. We therefore made direct determinations for carbon dioxide contained in carbonates by the usual method of adding acid, absorbing the carbon dioxide evolved in "Ascarite" and weighing. The results obtained are given in Table VII.

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[1] *Loc. cit.*

TABLE VII.

	Lot 3.	Lot 4.	Lot 5.
Per cent CO_2	0.41	0.30	0.64
	0.52	0.28	0.40
	. . .	0.30	0.49
	. . .	. . .	0.55
Average	0.47	0.29	0.52

In order to check some of our other results we then calculated the amounts of normal sodium carbonate and sodium bicarbonate that would correspond to this per cent of carbon dioxide. We then calculated the amount of sodium that would be equivalent to the normal carbonate assuming that the normal carbonate was the form which would most likely be present. These calculations are recorded in Table VIII.

TABLE VIII.

	Lot 3.	Lot 4.	Lot 5.
Per cent carbon dioxide	0.47	0.29	0.52
Corresponds to Na_2CO_3	0.83	0.51	0.91
Corresponds to $NaHCO_3$	0.66	0.41	0.73
Corresponds to Na	0.36	0.22	0.40

The values calculated above for sodium will be applied as corrections in the case of the sodium determinations given in the previous section.

Carbon and Hydrogen.—The method used to determine carbon and hydrogen was the usual combustion analysis employed in organic chemistry, the per cent carbon being calculated from the weight of carbon dioxide evolved and the per cent of hydrogen being calculated from the weight of the water formed. The results are given in Table IX.

TABLE IX.

	Lot 3.	Lot 4.
Per cent carbon	5.56	5.29
	4.49	5.21
Average	5.02	5.25
Per cent hydrogen	0.29	0.83
	0.44	0.81
Average	0.36	0.82

Titrations for normal carbonate and bicarbonate were attempted but were unsuccessful as the substance hydrolyzed on adding acid.

Loss of Weight on Heating.—In order to determine the water of hydration the following procedure was adopted:

Procedure.—One-Gm. samples of the sodium-bismuth tartrate were placed in weighed dishes and heated in an oven at 100° C. for a period of two hours. After being heated the dishes containing the samples were placed in a desiccator over calcium chloride and allowed to remain for thirty minutes. The dishes were then weighed again and the process repeated until successive weighings showed constant weight. The loss in weight was calculated as percentage of the original weight. The results are summarized in Table X.

TABLE X.

	Lot 3.	Lot 4.	Lot 5.
Per cent loss in weight at 100° C.	3.97	4.31	3.47
	4.61	...	3.30
	3.69	...	3.42
	3.76	...	...
	3.98	...	...
	4.16	...	...
Average	4.04	4.31	3.40

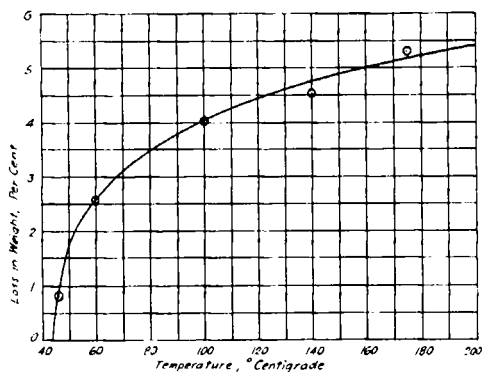While heating the samples in order to determine the water of hydration it was noticed that the color changed to a reddish brown or buff. This, together with the fact that the values did not agree among themselves, led us to suspect that there must be decomposition of the compound. In order to verify this we decided to determine the loss in weight at different temperatures and see if on plotting loss in weight against temperature the curve would show any sharp break, indicative of a definite hydrate. As can be seen from the curve, there is apparently no definite hydrate, and we therefore conclude that the loss in weight was due to decomposition and loss of adsorbed water. The values obtained for loss in weight of Lot 3 at various temperatures are given in Table XI.

Fig. 1.

TABLE XI.

Temperature, ° C.	Loss in weight (per cent).
46	0.81
60	2.61
100	4.04
140	4.56
175	5.36

On heating the compound at 100° or above the color changes from white to a reddish brown or buff. This color is faintly evident at 60° but no change in color was noticed at 46°. This would seem to indicate that the compound does not begin to decompose to any appreciable extent until the temperature is above 60°. Inasmuch as the loss in weight at 46° is 0.81 per cent and the loss in weight at 60° is 2.61 per cent, it seems to us that the loss in weight below 50° may well be ascribed to adsorbed water which would amount to at least one per cent.

It might also be noted that there is a marked decrease in solubility when the compound is heated to 100° or above, further indicating chemical decomposition.

GENERAL DISCUSSION.

For convenience in discussing the results and making comparisons with the theoretical formulas that have been suggested, we have prepared the table below

which shows the theoretical compositions of the substance based on the formula of Rosenheim and Vogelsang for sodium-tri-bismuth tartrate and the formula suggested by Kober for sodium-tetra-bismuth tartrate. This summary is presented in Table XII.

TABLE XII.

	Lot 3.	Experimental. Lot 4.	Lot 5.	Average of Lots 3, 4 & 5.	Theory for anhydrous tetra-Bi.	Theory for anhydrous tri-Bi.
Bismuth	72.61	72.64	73.24	72.84	76.97	74.29
Sodium, corrected for Na_2CO_3	2.65	2.39	2.58	2.54	2.12	2.73
Tartaric acid, corrected for solubility	15.27	15.25	14.26	14.93	13.81	17.80
Carbon	5.02	5.25	. . .	5.14	4.42	5.69
Na_2CO_3 calc. on basis of CO_2	0.83	0.51	0.92	. . .	. . .	. . .
$NaHCO_3$ calc., on basis of CO_2	0.66	0.41	0.73	. . .	. . .	. . .
Extra sodium in Na_2CO_3	0.36	0.22	0.40	. . .	. . .	. . .

The bismuth content of the sodium-bismuth tartrate was found to be 72.84 per cent (average of Lots 3, 4 and 5). The theoretical per cent of bismuth for the anhydrous tetra-bismuth compound is 76.97 and that for the anhydrous tri-bismuth compound is 74.29. It may be noted that the per cent of bismuth increases with the sample numbers. The samples used in this investigation are numbered in the order of preparation. There is apparently an increase in purity of the product as skill and experience are obtained in the preparation. This should easily account for the higher bismuth content found in Lot 5.

The results obtained from the carbon dioxide determinations indicate that about one per cent of the weight must be due to the presence of sodium carbonate or bicarbonate. In addition, the curve relating loss in weight with increase in temperature would seem to indicate that at least one or two per cent of the weight is due to adsorbed water. This being the case, we see that the per cent of bismuth would be in excess of that required for pure sodium-tri-bismuth tartrate.

TABLE XIII.

RATIO OF BISMUTH TO TARTARIC ACID.

	Found.	Theory for tetra-Bi.	Theory for tri-Bi.
Lot 3	4.77	5.57	4.18
Lot 4	4.77	. . .	. . .
Lot 5	5.15	. . .	. . .

The analysis for tartaric acid gave a value of 14.93 per cent (corrected for solubility) as an average for the three lots, as compared with the theoretical value of 17.80 per cent for the anhydrous tri-bismuth tartrate. The value for the bismuth content of Sample 5 was *higher* than that for Samples 3 and 4. The per cent tartaric acid for Sample 5 was *less* than that for Samples 3 and 4. This is in keeping with the assumption that Lot 5 is purer than the others and should approach more nearly the theoretical values for the tetra-bismuth compound, if it is the tetra-bismuth compound. On the other hand, if it were a tri-bismuth compound the values should approach the theoretical values for the tri-bismuth compound as a limit. It will

be seen that the values for tartaric acid are much below that required by tri-bismuth tartrate and they approach that required for the tetra-bismuth tartrate as the purity increases.   For the purpose of comparison we have listed (Table XIII) values for the ratio of the per cent bismuth to the per cent tartaric acid.

It is evident that in every case the ratio of bismuth to tartaric acid is too great to be explained on the assumption that the compound is anything less than a tetra-bismuth tartrate.   If one assumes that the tri-bismuth compound is anhydrous and absolutely pure, the ratio is still smaller than the observed ratio.   Furthermore, it has been shown that the samples with which we worked must not have been over ninety-eight per cent pure.   The value for Lot 5, which is presumably the purest of the three, agrees even more closely with the theoretical ratio for tetra-bismuth tartrate.   We take this as evidence in favor of a sodium tetra-bismuth tartrate.

The value for sodium was found to be 2.54 per cent as an average for the three samples, after a correction had been applied to take into account the excess of sodium present in the form of carbonate.   This value is seen to be below the theoretical value of 2.73 for tri-bismuth tartrate and above the value of 2.12 for the tetra-bismuth tartrate.   We are inclined to believe that the values for sodium are too high rather than too low and regard the sodium determinations as indicative of a sodium tetra-bismuth tartrate.

The determinations for carbon also bear out the contention of Kober that the compound is a sodium tetra-bismuth tartrate.   The average value obtained for Lots 3 and 4 experimentally gives us a value of 5.14 per cent.   The theoretical value for tri-bismuth tartrate is 5.69 per cent, while the theoretical value for tetra-bismuth tartrate is 4.42 per cent.   This value for carbon also checks the value for tartaric acid very well.

The calculations of the Na_2CO_3 and the extra sodium have already been explained elsewhere.

On making determinations for water of hydration by heating to constant weight we were never able to get concordant results.   Also the color of the compound changed, thus indicating chemical decomposition.   On making determinations at different temperatures and drawing a curve of temperature against per cent loss in weight we got a smooth curve with no break and hence no indication of a definite hydrate.   We came to the conclusion that one might reasonably assume that the loss in weight was due to adsorbed moisture amounting to from 1.0 to 1.5 per cent and to loss by chemical decomposition of the compound.   At any rate, the compound does not seem to be a hydrate.

CONCLUSIONS.

The samples of sodium-bismuth tartrate examined proved to be very soluble in cold water and apparently hydrolyzed at temperatures above seventy degrees. Therefore the compound could not have been the same as prepared by Rosenheim and Vogelsang which was prepared at 100°.

The per cent bismuth approaches the theoretical value for sodium-tetra-bismuth tartrate when calculated to the dry basis and sodium carbonate free.

The per cent tartaric acid agrees more closely with the theoretical value for sodium-tetra-bismuth tartrate than with the theoretical value for sodium-tri-bismuth tartrate.

The ratio between the per cent bismuth and the per cent tartaric acid indicates that the compound is sodium-tetra-bismuth tartrate.

The per cent sodium agrees more closely with the value for sodium-tetra-bismuth tartrate than with the value for sodium-tri-bismuth tartrate.

The loss in weight at various temperatures indicates that the compound may contain as much as 1.0 to 1.5 per cent of adsorbed water, but loss in weight at the higher temperatures seems mainly due to chemical decomposition.

The determinations for carbon dioxide indicate that the compound contains about one per cent of sodium carbonate or bicarbonate.

SUMMARY.

A study has been made of the composition of a sodium-bismuth tartrate prepared by the method of Kober. Indications are that the compound is a sodium-tetra-bismuth tartrate.

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LACTIC ACID TESTS.

BY H. V. ARNY AND MARGUERITE C. DIMLER.[*,1]

While lactic acid has been the subject of much work by food analysts and physiological chemists, the literature of pharmacy has furnished very few papers on tests for this acid. This is undoubtedly largely due to the fact that the average market prices of lactic acid have run somewhat higher than citric and tartaric acids and that there has been no temptation to use it as a substitute for the popular fruit acids or preparations containing them. On the other hand, there was at least one period during the past decade when the potential sources of lactic acid were more available to American users than were those of the fruit acids; in fact, it seems probable that larger supplies of and lower prices for lactic acid would prevail should a more pressing demand be created.

In view of this possibility, tests for lactic acid in the presence of other organic acids are desirable and requests for the study of this problem were made a few years since by the U. S. P. Committee on Revision (a). One answer to this request came from Germuth and it is his procedure upon which we began our studies. Finding the Germuth test insufficient for all circumstances, we proceeded to an examination of the literature and to a checking up of the testing methods suggested. This paper reports our findings.

The lactic tests found in the literature may be grouped as follows:

A. *Thiocyanate Tests.*—These tests, reported on by Mendelsohn (b) and by Germuth (c) are based upon the red coloration produced with a strongly acidulated (HCl) solution of potassium thiocyanate when lactic acid is present. Of course, a similar red color is produced when even traces of iron are present, and this Germuth not only recognizes but also provides for in his method by directing that the red