this solution is taken as the standard. From this the count rate expressed as counts per minute per milligram of elemental arsenic can be calculated.

The amount of arsenic in the samples can be found by comparing the activity of the processed samples with the count rate.

Detection of Activity. Of the two detection methods, Geiger counter (M-6 tube accepting liquid samples) and scintillation counter, the former was more efficient and was used in all experiments. The number of counts recorded by a sample in both systems was the same, but when the scintillation counter was used, the background was ten times as large as with the Geiger counter.

Test of Complete Method. Filter papers were impregnated with known weights of arsenic in the form of sodium arsenite. The arsenic content was calculated and the test pieces sent for irradiation. The analysis was carried through completely, including all the usual steps. Because the digestion and the separation were satisfactory, it was felt that good results from this test would confirm the accuracy of the method (Table I).

The complete method is a relatively rapid simple technique for determining arsenic in biological material with an accuracy above 98%.

As a further check, the activation method was compared with two chemical methods in an investigation of the arsenic content of detergents (*7*). The procedures included a Gutzeit separation and titrimetric estimations using the method of Thomas and Collier (*11*) and a Gutzeit separation and British Drug Houses stain comparison method (*1*). Results are shown in Table II.

Table II. Comparison of Methods

Thomas and Collier's Method		B.D.H. Method		Activation Analysis	
As, p.p.m.	Sample, grams	As, p.p.m.	Sample, gram	As, p.p.m.	Sample, gram
31.0	1.0	30	0.10	35.9	0.00467
25.0	1.0	25	0.10	24.8	0.00839
13.5	1.0	14	0.25	13.8	0.00454
18.5	1.0	17.5	0.25	18.2	0.00889
1.5	1.0	1.2	0.25	1.87	0.00768
0.85	1.0	1	0.25	0.67	0.00540
1.6	1.0	...	...	1.64	0.00762
0.5	6.0	...	...	0.57	0.00381

Table II shows the close agreement between the results. It also demonstrates the use of very small weights of sample in the activation method. This is important when samples of tissue from a living subject are to be analyzed, and in medico-legal cases where the amount of available material is often limited. The method has also been used in investigations of medical and general interest (*6*).

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Determination of Gluconolactone, Galactonolactone, and Their Free Acids by the Hydroxamate Method

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▶ **The sensitivity of gluconolactone and galactonolactone to hydrolysis under alkaline conditions indicated that the hydroxamates of these compounds could be formed at a near-neutral pH reaction rather than under the alkaline conditions which have been used. The data indicate that there is loss of sensitivity for quantitative determination of these compounds under alkaline conditions and that this results from competition between hydrolysis and hydroxamate formation. The effect of variation of temperature and acidity upon the formation of the lactones from the corresponding free acids is described.**

HYDROXYLAMINE reacts with esters and various related compounds to form hydroxamic acids which react with iron(III) in acidic solution to give colored complexes. Lipmann and Tuttle (*8*) used these reactions for the quantitative determination of acetyl phosphate. Hestrin (*5*) investigated the method in more detail and developed a procedure for the determination of acetylcholine which was also suitable for the determination of the esters, lactones, and anhydrides of short-chain fatty acids. The two procedures differed primarily in that Hestrin used an alkaline hydroxylamine reagent (0.75*N* with respect to sodium hydroxide) while Lipmann and Tuttle used a hydroxylamine reagent adjusted to pH 6.4.

Goddu, LeBlanc, and Wright (*4*) developed an hydroxamate method in which the reactions take place in an

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anhydrous rather than an aqueous system and ferric perchlorate rather than ferric chloride is used as a source of ferric ion. Hilf and Castano (*6*) have applied this method to the determination of sugars and sugar acids.

While the use of an anhydrous system extends the applicability of the hydroxamate determination to the derivatives of fatty acids of higher molecular weight, it also requires that the sample be dried. This constitutes an unnecessary operation when the samples are water-soluble, because such samples will react with hydroxylamine in an aqueous menstruum.

Cori and Lipmann (*2*) and Brodie and Lipmann (*1*), stating that the conversion of the lactone to the hydroxamate was quantitative in the alkaline hydroxylamine reagent, used Hestrin's modification of Lipmann and Tuttle's method for the determination of gluconolactone in water solution. However, in this laboratory, the use of Hestrin's modification of the hydroxamate method to follow the appearance and disappearance of gluconate in bacterial cultures was unsatisfactory. Studies indicated that the alkaline hydrolysis caused the variation in results and that this method could be suitably modified for the determination of both gluconolactone and galactonolactone.

REAGENTS

Hydroxylamine hydrochloride, 4*M*, ACS or photographic grade.

Sodium hydroxide, 4*M*.

Hydroxylamine reagent. Equal volumes of the above solutions mixed and adjusted to pH 8.0 with the appropriate component. This solution is stable for at least 4 hours.

Ferric chloride, 100 grams per liter in 0.1*N* hydrochloric acid.

Tris(hydroxymethyl)aminomethane (purified), 0.1*M*, adjusted to the desired pH with sodium hydroxide or hydrochloric acid.

Phosphate buffer, monopotassium phosphate, 0.1*M* adjusted to the desired pH with sodium hydroxide.

Potassium gluconate, pure, Fisher Chemical Co., 0.1*M*, adjusted to pH 1.5 with hydrochloric acid.

δ-Gluconolactone (purified), Nutritional Biochemical Corp., 0.01*M* in 0.05*M* hydrochloric acid.

Calcium galactonate, C.P., Pfanstiehl Chemical Co., 0.06*M*, adjusted to pH 1.5 with hydrochloric acid.

γ-Galactonolactone, C.P., Pfanstiehl Chemical Co., 0.005*M* in 0.05*M* hydrochloric acid.

APPARATUS

pH was measured with a Beckman pH meter (Model H-2). Photometric measurements were made with a Klett-Summerson colorimeter equipped with a green (540-mμ) filter and 12 mm. × 75 mm. cuvettes.

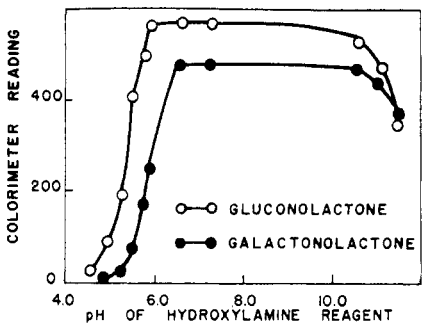

Figure 1. Effect of pH of hydroxylamine reagent on reaction of hydroxylamine with gluconolactone and galactonolactone

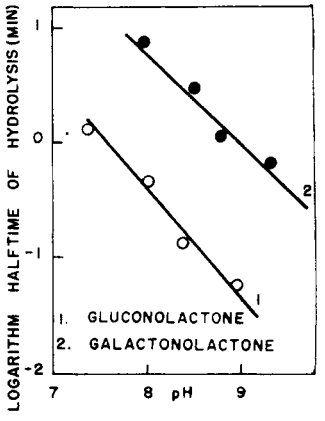

Figure 2. Effect of pH on rate of hydrolysis of gluconolactone and galactonolactone

EXPERIMENTAL

Method. The method presented is essentially that of Hestrin (*5*), as used by Cori and Lipmann (*2*) and by Brodie and Lipmann (*1*), except that the free acids were converted to their lactones at pH 1.5 to 2.0 and 120° C. (autoclave, 15 p.s.i.), and a 2*M* hydroxylamine reagent adjusted to pH 8.0 was used. The modified method for determining the combined free acid and lactone is described below. To determine lactone only, the initial acidification and heating steps were omitted.

The sample was acidified with hydrochloric acid to pH 1.5 to 2.0 and 1-ml. aliquots were autoclaved at 15 p.s.i. to form the lactone. Upon removal from the autoclave, the samples were placed in a cold water bath and 2 ml. of hydroxylamine reagent were added. This was followed by the addition of 1 ml. of 4*M* hydrochloric acid, and 1 ml. of the ferric chloride solution. The pH of the reaction mixture at this stage should be 1.2 ± 0.2 (*5*). The photometric determination was made within 10 minutes after addition of the ferric chloride solution.

Alternatively, the lactone may be formed by heating the samples in a boiling water bath for 15 minutes rather than in an autoclave, or this conversion can be accomplished in a more acid solution. The sample can be added to the hydroxylamine reagent without affecting the determination.

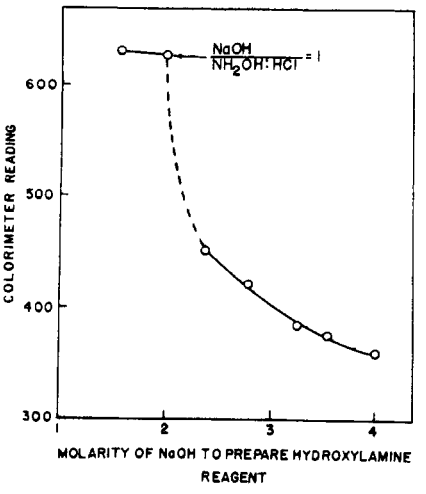

Figure 3. Effect of sodium hydroxide concentration of formation of hydroxamate of gluconolactone with alkaline hydroxylamine reagent

Interrupted line represents an extrapolation of lower portion of curve through range of rapid change.

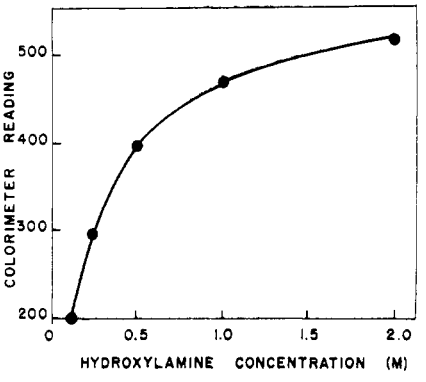

Figure 4. Effect of hydroxylamine concentration in 0.1 M sodium hydroxide on formation of hydroxamate of gluconolactone

For the requisite final pH, 1.2 ± 0.2, the concentration of hydrochloric acid solution used to acidify the sample after formation of the hydroxamate can be varied from 4*M* to accommodate variations in the acidity of the sample. In this work, variations in the concentrations of the sodium hydroxide and hydroxylamine necessitated a compensating adjustment in the acidification step.

Effect of Variation of pH of Hydroxylamine Reagent. Solutions of hydroxylamine reagent varying in pH from 4.5 to 11.5 were prepared by varying the concentration of the sodium hydroxide used to prepare the hydroxylamine reagent. An acidified, 0.01*M* gluconolactone or 0.005*M* galactonolactone solution was used as a sample. A 2-minute interval was allowed for formation of the hydroxamate before the reaction mixture was acidified.

Because hydroxylamine is a weak base ($-\log k = 7.97$) (7), the reagent solution had sufficient buffer capacity in the range pH 4.3 to 7.7 that the addition of the acidified sample had little effect on the pH at which hydroxamate formation occurred. At pH 10 and above, addition of the sample decreased the pH of the reaction medium, ranging from 2 units at pH 10 to 0.8 unit at pH 11.

The results (Figure 1) show that in the 2-minute interval the maximum formation of hydroxamate from gluconolactone occurred when the pH of the hydroxylamine reagent was between 5.8 and 10. With galactonolactone the range of maximum hydroxamate formation was pH 6.5 to 10.5 (Figure 1).

Hydrolysis of Gluconolactone and Galactonolactone under Alkaline Conditions. A 0.1*M* solution of potassium gluconate or a 0.06*M* solution of calcium galactonate acidified to pH 1.5 was converted to the lactone. A 10-fold dilution of this was made in the desired buffer [phosphate below pH 8.0 and tris(hydroxymethyl)-aminomethane, pH 8.0 and above]. The change in lactone concentration of the solutions at 22–3° C. was determined at appropriate intervals, using the method given above for determining only lactone. A rapid delivery pipet equipped with a 1-ml. rubber bulb was used for withdrawing samples which were blown into the hydroxylamine reagent solution. The average of the pH initially and after three fourths of the lactone had hydrolyzed was taken as being the pH at which the reaction occurred. Figure 2 indicates that the half time of hydrolysis (the time in which the lactone concentration decreased one half) is a function of pH. At pH 8.6 this was 0.1 minute for gluconolactone and 1.1 minutes for galactonolactone.

Effect of Concentration of Sodium Hydroxide on Formation of Hydroxamate of Gluconolactone. Because of the problems of accurate measurement of pH in very alkaline solutions, these data are treated separately rather than being incorporated in the section on "Effect of Variation of pH of Hydroxylamine Reagent" and in Figure 1. The two sections are also dissimilar, in that the earlier section deals with the relationship between pH and hydroxamate formation, while this paragraph describes the competition between the reactions of hydrolysis and hydroxamate formation.

Hydroxylamine reagent solutions 1.0*M* with respect to hydroxylamine [to duplicate Hestrin's (5) conditions] and varying from 0.00 to 1.0*M* with respect to sodium hydroxide were used in place of the neutral hydroxylamine reagent. An acidified 0.01*M* gluconolactone solution was used as a sample. The color formation decreased as the concentration of sodium hydroxide in the hydroxylamine reagent was increased (Figure 3). At the composition used for Hestrin's (5) reagent (hydroxylamine 1.0*M*, sodium hydroxide 0.75*M*) the color formation was about 60% of maximum.

Effect of Varying Hydroxylamine Concentration in Alkaline Hydroxylamine Reagent. Hydroxylamine reagent solutions 0.1*M* with respect to sodium hydroxide and ranging from 0.125 to 2.0*M* with respect to hydroxylamine were used in place of the recommended neutral hydroxylamine solution. An acidified 0.01*M* gluconolactone solution was used for the sample. The color formation increased as the hydroxylamine concentration of the reagent increased (Figure 4).

Effects of Time, Temperature, and pH Formation of Lactones from Free Acids. One-milliliter aliquots of solutions of 0.01*M* gluconolactone and of 0.006*M* calcium galactonate, were adjusted with hydrochloric acid to pH 1.5, 2.0, and 2.5, and were heated at 120° C. (autoclave) or at 100° C. (water bath) for up to 15 minutes. At the intervals indicated in Table I, the lactone concentrations were determined by the method given above. Time of heating in the autoclave was considered to have started when the indicated temperature was 115° C. and in the water bath at the time of immersion. The rate of formation of the lactones increased as the acidity increased and was also more rapid at higher temperatures (Table I).

Table I. Effect of Time, Temperature, and pH on Formation of Gluconolactone and Galactonolactone from the Free Acids

Temp., ° C.	Time of Heating, Min.	Gluconolactone[a] Formed, μmoles/Ml.			Galactonolactone[b] Formed, μmoles/Ml.		
		pH 1.5	pH 2.0	pH 2.5	pH 1.5	pH 2.0	pH 2.5
120[c]	0	5.4	5.1	4.5	0.7	0.7	0.7
	2	9.8	9.8	8.8	5.9	5.5	4.2
	5	9.9	10.1	9.9	6.0	5.9	5.2
	10	10.1	10.0	10.1	6.0	6.0	6.0
	15	10.0	10.0	...	6.0	6.0	5.8
100[d]	0	5.4	5.1	4.5	1.0		
	2	8.4	7.5	6.6	...		
	5	9.6	8.9	7.5	5.7		
	10	9.8	9.5	8.3	5.9		
	15	9.9	9.8	9.1	6.0		

[a] Sample, 10.0 μmoles/ml. gluconolactone adjusted to indicated pH with HCl.
[b] Sample, 6.0 μmoles/ml. calcium galactonate adjusted to indicated pH with HCl.
[c] Autoclave, 15 p.s.i.
[d] Water bath.

DISCUSSION

Hydroxamate formation from gluconolactone and galactonolactone decreases when the pH of the reaction medium exceeds about 9.0 and under conditions in which the hydroxylamine concentration is decreased in the presence of a fixed concentration of sodium hydroxide. Extrapolation of the curves of half time of hydrolysis (Figure 2) indicates that hydrolysis of these lactones becomes extremely rapid as the OH^- concentration increases.

It appears that the decrease in hydroxamate formation under alkaline conditions results from such an increase in the rate of hydrolysis of the lactones that the reaction of hydrolysis becomes competitive with that of hydroxamate formation.

While this indicates that the formation of hydroxamate from gluconolactone under alkaline conditions is not quantitative, it does not negate the possibility of determining gluconolactone by the hydroxamate method involving an alkaline reaction medium. However, formation of the hydroxamate under neutral or very slightly alkaline conditions eliminates the possibility of introducing error as a result of hydrolysis and takes advantage of the fact that the sensitivity of the method is increased significantly. Furthermore, solutions of these lactones cannot be neutralized with a strong base, because this, upon addition to the sample, will cause local areas of high alkalinity resulting in some hydrolysis.

The decrease in the hydroxamate formation from galactonolactone above pH 10.5 (Figure 1) and the data relating to the half time of hydrolysis of galactonolactone under alkaline conditions (Figure 2) indicate that although galactonolactone is less sensitive to alkaline conditions than gluconolactone, it would be advisable to form the hydroxamate under the conditions recommended for gluconolactone.

The amount of hydroxamate formed from gluconolactone decreases rapidly below pH 5.5 and similarly below pH 6.0 for galactonolactone (Figure 1). However, under mildly acidic conditions, hydroxamate formation from these lactones was time-dependent. This has been demonstrated with gluconolactone by Cori and Lipmann (2). That this decrease in the rate of formation of hydroxamate occurs in the presence of an amount of free hydroxylamine

which is more than adequate for complete reaction, indicates that the limiting condition is related to the lactone rather than to the hydroxylamine. The variation in the minimum pH at which hydroxamate formation from various compounds will take place at a relatively rapid rate—e.g., acetyl phosphate, pH 4.4 (*8*) and acetylcholine, pH 9.6 (*5*)—also supports this assumption.

In view of this variation of pH at which various compounds will react with hydroxylamine, to adapt this method of determination to a given compound it would be advisable to establish the limits of pH within which the hydroxamate formation for that compound is maximal. With this information, necessary changes in the composition of the hydroxylamine reagent and the hydrochloric acid solution added subsequently can easily be made.

The conversion of the free acids to their lactones is no problem. This can be effected rapidly and under relatively mild acidic conditions.

This method has been satisfactorily applied to the determination of gluconolactone and galactonolactone in biological systems (*3*).

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Determination of Silver in Photographic Papers and Films by Wet Ashing

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▼ A rapid method for the determination of silver in silver-bearing materials (photographic films, papers, emulsions, etc.) makes use of wet oxidation of organic matter and halides by boiling in a sulfuric-nitric acid mixture and titrating the resulting silver sulfate solution. Both micro- and macroprocedures are outlined. The method avoids all separation and solution transfer steps. It is carried out from beginning to end in the same vessel. The precision of the method is on a par with established silver determination methods.

THE rapid determination of silver in undeveloped photographic papers and films has long been a problem. The usual approach has been to dissolve silver halides and other silver compounds in solvents such as aqueous ammonia, cyanide, concentrated solutions of alkali bromides, iodides, and thiosulfates to complex the silver halides or to form addition products. The resulting solution is separated from the paper or film support by filtration or centrifuging and the silver is titrated by potentiometric or visual end points, polarography, or other means. Generally, little attention is devoted to the preparation of the sensitized paper, film, or emulsion sample prior to silver determination. The latter is usually straightforward, once silver has been separated from the supporting medium, but initial preparation of the sample is difficult because frequently silver is lost by manipulating errors or adsorption of silver compounds on paper fibers or on the baryta (barium sulfate) coating of photographic paper base. Frequently, it is difficult to make this adsorbed silver available for subsequent determination.

These losses have led to attempts to destroy the silver-bearing photographic bases. Ashing the materials under controlled conditions proved not only objectionable from a handling standpoint, but also tedious because of the time required. Losses due to spattering and volatilizing of silver halides were unavoidable. Frequently, the crucible materials were attacked and were a contributory source of error. The large amounts of gelatin present in photographic materials made the ashing procedure difficult to manage because overfoaming was barely preventable.

Ashing was short of being carried to completion because of inclusions of silver compounds and metallic silver in the sintered residue which proved difficultly soluble in nitric acid.

Both solvent extraction and dry-ashing procedures were time-consuming and conditions were difficult to control. They did not afford a really rapid and efficient silver determination by chemical methods.

The literature is replete with suggestions, but so far as is known, no procedure has been published along the lines of wet ashing whereby all silver halides are transposed into ionic silver sulfate.

The method of digestion was first worked out on a micro scale, using film and paper areas 1 to 5 sq. cm. in size and was subsequently expanded to a macroprocedure by using areas of 25 to 100 sq. cm. During the digestion, not only the organic constituents are oxidatively destroyed but also the halides are oxidized and the resulting halogens are completely expelled from the system in gaseous form. A photographic emulsion thus treated will no longer give any positive tests for halide ions. Silver losses are not incurred if spattering is avoided by gradual and careful addition of the oxidizing acids. The method is extremely simple and requires only minimum attention. The procedure lends itself well for running a larger number of determinations simultaneously and is easily mastered by relatively inexperienced laboratory technicians. No special equipment or apparatus is required. During the digestion period—30 to 90 minutes depending on the size of the sample—no special attention or manipulation is