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A Method for the Rapid Determination of Salicylates.

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In the course of some work on the gastric and intestinal absorption of salicylates, existing methods of determining salicylates were found inadequate. Bromine titration methods[1] used on pure solutions of salicylates are not applicable to biological solutions which contain benzene rings. Like Hanzlik,[2] we found extraction with immiscible solvents to be incomplete. The method of Hanzlik and Presho[3] was found unsatisfactory for even after five or six hours of steam distillation from phosphoric acid, salicylic acid was found in the distillate. Furthermore, the depth of color developed with ferric chloride varied with the pH and the time elapsing before the colorimetric reading was made.

The method finally devised depends upon measuring the volume of carbon dioxide liberated by brominating salicylic acid in the Van

* P represents a preliminary, C a complete manuscript.

1 Seidell, *J. Am. Chem. Soc.*, 1909, **31**, 1169.

2 Thoburn and Hanzlik, *J. Biol. Chem.*, 1915, **23**, 163.

3 Hanzlik and Presho, *J. Pharm. Exp. Therap.*, 1923, **21**, 247.

Slyke volumetric gas analysis apparatus.[4] This method was successfully used in this laboratory in determining gastric and intestinal absorption of acetylsalicylic acid, salicylic acid and its salts and mixtures of these compounds with calcium gluconate, sodium bicarbonate, and magnesium oxide.[5]

Solutions. Saturated Na Br solution saturated with Br_2; saturated KI; 1:4 sulfuric acid.

A known volume (10 cc. or less) of the solution containing between 1 and 5 mg. of salicylate as salicylic acid is used. In the case of acetylsalicylic acid or other combined salicylates and urine, in which a large percentage of salicylate is excreted as salicyluric acid, hydrolysis is necessary. Hydrolysis is accomplished by heating in the steam bath with 1 drop of concentrated NaOH for 1 hour. The solution is then acidified with 1:4 sulfuric acid and washed into the chamber of the Van Slyke apparatus. Preformed CO_2 and dissolved air are removed by evacuating the apparatus in the usual way, shaking and then forcing the gas out through the cup. After complete elimination of preformed gas, the levelling bulb is raised so that the upper surface of the solution is at the top of the upper stop cock. The bulb is placed on the lower ring and ¼-½ cc. of the Br_2 solution is run in from the cup by opening the upper stop cock. The two solutions are mixed and allowed to stand 1 minute. Then ¼-½ cc. KI solution is run in so that the excess Br_2 will replace the I^- of the KI with liberation of I_2. This is done so that correction for the vapor pressure of Br_2 will not have to be made. The vapor pressure of I_2 is so small at room temperature that it does not have to be taken into consideration.

Salicylic acid is readily brominated with the formation of tribromphenol and CO_2. One gram mole of salicylic acid liberates one gram mole or 22.4 liters of CO_2. Therefore, 1 cc. of CO_2 at standard conditions is equivalent to 6.15 mg. salicylic acid and the number of mg. of salicylate as salicylic acid in the sample used is obtained by multiplying the reading of the apparatus in cc. reduced to standard conditions by 6.15 or by 8.03 for values in terms of acetylsalicylic acid.

Results. This method was found to give satisfactory results on samples of urine and gastric juice to which known quantities of salicylate were added as well as pure solutions of salicylates in water. Table I shows values obtained upon known solutions of acetylsali-

[4] Van Slyke and Cullen, *J. Biol. Chem.*, 1917, **30**, 289; Van Slyke, *J. Biol. Chem.*, 1917, **30**, 347; Van Slyke and Stadie, *J. Biol. Chem.*, 1921, **49**, 1.

[5] Bradley, Schnedorf and Ivy, *J. Dig. Dis. and Nut.*, 1936, **3**, 415.

TABLE I.
Normal Urine Plus Acetylsalicylic Acid or Na Salicylate.

Cc. sample	Reading of Van Slyke	Barometric Pres.	Temp., C°	Correction factor	Amt. found, mg.	Amt. actually added, mg.	% error		
10	.67	746	26	.862	4.12	4.00	3.0	Sodium	Salicylate
10	.67	746	26	.862	4.12	4.00	3.0	"	"
5	.34	746	26	.862	2.09	2.00	4.2	"	"
5	.34	746	26	.862	2.09	2.00	4.2	"	"
5	.751	744-500	28	.259	1.56	1.50	4.0	Acetylsalicylic	Acid
5	.748	744-500	28	.259	1.55	1.50	3.7	"	"
5	.751	744-500	28	.259	1.56	1.50	4.0	"	"
10	.44	744	28	.850	3.00	3.00	0.0	"	"
10	.44	744	28	.850	3.00	3.00	0.0	"	"
5	.420	743	27	.855	2.56	2.5	2.4	Sodium	Salicylate
5	.420	743	27	.855	2.56	2.5	2.4	"	"
5	.480	743	27	.855	2.93	3.0	2.3	"	"
5	.485	743	27	.855	2.96	3.0	1.3	"	"

cylic acid and sodium salicylate in urine. As can be seen, the amount found by analysis checks very well with the quantity added, the percentage error ranging from 0-4%.

Urine from a subject who was given 15 grains of acetylsalicylic acid was analyzed to determine the amount of salicylate present. Then known quantities of acetylsalicylic acid were added and the

TABLE II.
Urine from Subject After Taking 15 gm. Aspirin.

Cc. sample	Reading Van Slyke	Amount Found	Amount per cc.	Barometric Pressure 745 Temp. 30°
10	.400	2.73	.273	
10	.401	2.74	.274	Aver. .274 mg./cc.
10	.403	2.75	.275	

Same Urine to Which Acetylsalicylic Acid Was Added.

Cc. sample	Reading Van Slyke	mg./cc. Pres. Orig.	mg./cc. Found	mg./cc. Added	mg./cc. Recovered	% error
5	491	.274	.671	.400	.397	.8
5	492	.274	.672	.400	.398	.5
5	491	.274	.671	.400	.397	.8
5	440	.274	.601	.320	.327	.2
5	440	.274	.601	.320	.327	.2
10	.680	.274	.473	.200	.199	.5
10	.681	.278	.474	.200	.200	.0

analyses were repeated. As seen in Table II, the amounts determined check very well with the amount added.

Known quantities of acetylsalicylic acid were added to gastric juice collected from a Pavlov pouch dog and samples analyzed. Here

again analyses checked with actual quantities added to within 0.5-4.0%.

An attempt was made to apply this method to blood without success, for, if hydrolysis is attempted on the blood itself, a mass of denatured protein results, and attempts using blood filtrates were disappointing because of the absorption of the salicylate by the protein precipitate.

TABLE III.
Solutions of Acetylsalicylic Acid in Gastric Juice.

Cc. sample	Reading Van Slyke	Mg. found	Mg. added	% error
5	.410	2.90	3	3
5	.405	2.86	3	4
5	.415	2.93	3	2
5	.280	1.98	2	1
5	.280	1.98	2	1
5	.285	2.01	2	0.5

The sources of error entering into this method are those that enter into any method depending upon the measurement of gas volumes over aqueous solutions. The apparatus must, of course, be tight at all its stop cocks. The liberation of gas must be complete and, in order to accomplish this, the chamber must be shaken vigorously. The high concentration of salts facilitates the liberation of CO_2 and also hinders the absorption of the gas when the mercury is levelled, thus reducing the inaccuracy at this stage of the procedure.

Conclusion. This method enables one to analyze rapidly for salicylates in biological fluids in which there are no suspended solids to occlude the apparatus. The time required is short—one hour for the hydrolysis, the time necessary for the actual analysis depending upon the skill developed by the operator of the Van Slyke apparatus. The accuracy of this method was found to exceed that of other existing methods and does not depend upon one's ability to match colors in a colorimeter.