Carbohydrate.	Mols H_2O.	Standard.	Calc. conc.	% error.
Glucose	..	0.01	0.01	..
Fructose	..	0.01	0.01	..
Galactose	..	0.01	0.01	..
Mannose	..	0.01	0.01	..
Rhamnose	1	0.01	0.0101	1.00
Maltose	1	0.0094	0.009895	1.05
Lactose	1	0.0095	0.01	..
Sucrose	..	....	....	..
Sucrose (hydrolyzed)	..	0.0105	0.009974	0.26
Raffinose	5	....	....	..
Inulin	..	....	....	..
Dextrin	..	0.002	?	?

Lactose in Milk.—Measure out 10 cc. of milk and run it into a 250 cc. volumetric flask with 50–100 cc. of water and 1 cc. of 50% sulfuric acid. Heat on the sand bath until flocks of casein separate or until the mixture boils. Cool and add water to the 250 cc. mark. Pour upon a dry filter and, by means of a graduated pipet, take 6.25 cc. of the filtrate and run it into a 250 cc. volumetric flask. Add 2–3 volumes of water, an excess of sodium carbonate solution, and 10 cc. of the picrate solution. Heat on the sand bath five minutes after the color begins to develope. Cool and dilute to the 250 cc. mark. Estimate the color by the colorimeter or by means of the bottle standards. For the latter, rinse the bottle with some of the solution, then fill and compare with bottle standards. Multiply the percentage marked on the bottle standard by 1000 as the percentage of lactose in the milk.

Advantages of the Method.—A speedy and accurate *colorimetric* method for most carbohydrates is introduced.

Only one oxidizing solution, the picrate solution, is necessary and it is cheap, stable, easily prepared and need not be strictly quantitative.

The oxidizing solution is alkaline with sodium carbonate, avoiding secondary reactions on sugars produced by caustic alkali.

With the use of the bottle standards, the method is not only inexpensive but is easily within the reach of all working chemists.

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[CONTRIBUTIONS FROM THE CHEMICAL LABORATORY OF THE UNIVERISTY OF WASHINGTON.]

A COMPARISON OF VARIOUS PRESERVATIVES OF URINE.

BY WILLIAM M. DEHN AND FRANK A. HARTMAN.

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In certain studies on normal urines, incurring the collection and keeping of hundreds of liters, the necessity of using a non-volatile preservative compelled us to test a number of reagents to determine their preservative power.

The ideal preservative for urine requires continuous conformity to the specifications (1) of being neutral or acidic and non-volatile, (2) of having efficient antiseptic power, (3) of being free from hydrolytic or other chemical effects on the organic components of urine and (4) of avoiding interference with reactions involved in the ordinary analysis of the urine.

Though no single preservative meets all of these requirements, a few approximate the same. However, it is scarcely to be expected that a solution so complex as urine, containing so many organic and inorganic compounds, held in acidic-basic, hydrolytic, precipitative, and oxidative equilibria, can be maintained indefinitely in its original composition. It is rather to be expected that one preservative will be effective with certain components of the mixture, while another preservative must be used for others. Hence a variety of preservatives must be studied and their modifying influences must be noted.

For our purpose sulfuric acid was finally adopted, but our studies led us so far afield that we determined to make a systematic survey of preservatives of urine, of which the following is of the nature of a preliminary report. This undertaking was further justified not only by the the fact that only a little work has been done, at least in a comparative manner, on preservatives of urine, but also for the reason that profound chemical changes resulted when urines were preserved with sulfuric acid and other reagents.

In the first set of experiments a quantity of urine, to which dextrose had been added, was hermetically sealed in flasks with the respective preservatives; the second set was put up in glass-stoppered bottles; all the samples were in 500 cc. volumes. After certain times the changes that resulted were observed (1) directly, (2) microscopically and (3) through chemical analysis.

Direct observation showed that all samples became cloudy or yielded precipitates on standing. Thymol remained clear the longest; the others gave cloudiness or precipitates immediately or after days, inversely in the order of formaldehyde, ether, toluene, hydrochloric acid, chloroform, etc. The following developed mold: hydrochloric acid, hydrogen peroxide, formaldehyde, boric acid and strychnine sulfate.

Microscopical examination showed that bacteria were present in all samples, and in large numbers, except in cases of hydrochloric acid and formaldehyde. Urate crystals were present in the sediments of all samples, but seemed to decrease in volume after many days in samples containing mold, especially in the case of hydrogen peroxide. All the urines, except those treated with strychnine sulfate, formaldehyde, toluene, thymol and sodium benzoate, being acidic in reaction, gave sediments containing calcium oxalate but no triple phosphate; the latter urines being alkaline gave both, except in the case of sodium benzoate which gave neither.

In the following tables are given the chemical data as percentages.

TABLE I.

No.	Preservative.	Grams per 500 cc.	Litmus.[2]	Days.[4]	Total phosphate.	Glucose.	Chlorine 174.[3]	Uric acid.	Ammonia.	Urea 176.[3]	Urea corr. 176.[3]	Creatinine 173.[3]
..	None[1]	..	A	..	0.1499	0.6896	..	0.0370	0.0136	..	...	..
..	None	..	B	83	..	0.2439	0.7302	..	..	2.0890	...	0.1421
1	Salicylic acid	1	A	82	0.1350	0.6890	0.7372	..	0.0146	1.9631	0.938	0.8901
2	Chloroform	3	A	18	0.1414	0.3827	0.8195*	0.0290	0.0158	1.0001	0.972	0.6231
3	Thymol	1	B	53	0.1410	0.9524*	0.7411	..	0.0342	1.9621	0.902	0.9643*
4	Boric acid	1	A	29	0.1370	0.5127	0.7681*	0.0350	0.0166	..	...	0.6750
5	Ether	1.6	A	52	0.1310	0.2777	0.7411	0.0150	0.0259	1.9361	0.891	0.9643*
6	Formaldehyde	2	B	51	0.1362	0.7093*	0.7380	0.0365	0.0300	1.8921	0.842	0.6639
7	H_2O_2 (3%)	2	A	33	0.1458	0.4167	0.7018	0.0252	0.0311	1.9631	0.909	0.8901
8	HCl (conc.)	2.4	A	26	0.1385	0.2890	0.8585*	0.0310	0.0331	1.9441	0.886	0.9000
9	Toluene	1.6	B	46	0.1350	0.9748*	0.7414	0.0370	0.0342	1.9481	0.888	0.8901
10	Sod. benzoate	1	B	44	0.1375	0.3960	0.7459	0.0210	0.0411	1.0341	0.962	0.0040

[1] Analyzed on the day when the samples were put up.

[2] The letter A indicates acidic or neutral reaction; B indicates alkaline reaction.

[3] The numbers in the headings of these tables indicates days.

[4] The days of this column refer to the columns of phosphates, glucose, uric acid and ammonia. The days of preservation of others are given in the respective columns.

TABLE II.

No.	Preservative.	Grams per 500 cc.	Litmus.[2]	Glucose 20.[3]	Picrate 20.[3]	Uric acid 163.[3]	Ammonia 28.[3]	Urea 67.[3]	Urea corr. 67.[3]	Creatinine 29.[3]
.	None[1]	..	A	4.545	...	..	..	1.710	...	0.648
.	None	..	A	4.166	...	0.0080	0.0956	1.635	1.468	0.623
1	Salicylic acid	1	A	4.546	5.5	0.0134	0.0306	1.649	1.596	0.628
2	Chloroform	9	A	4.706*	6.0*	0.0246	0.0308	1.730	1.676	0.487
3	Thymol	1	A	4.420	5.5	0.0190	0.0242	1.700	1.658	0.575
4	Boric acid	1	A	4.255	5.7	0.0125	0.0325	1.643	1.586	0.506
5	Ether	16	A	4.469	5.1	0.0160	0.0319	1.744	1.688	0.566
11	Sod. arsenite	1	A	4.445	5.2	0.2200	0.0356	1.643	1.581	0.623
12	Sod. borate	1	A	4.211	5.4	0.0125	0.0343	1.610	1.530	0.416
13	Strychnine H_2SO_4	1	B	4.520	5.2	0.0040—	0.0220	1.627	1.589	0.529
14	Sulfuric acid	4	A	4.020	6.0*	0.0030—	0.0345	1.560	1.500	0.625*
14	Sulfuric acid	18	A	4.082	5.8	0.0020—	0.0241	1.287	1.245	0.000*
15	Phenol	1	A	4.494	7.0*	0.0130	0.0355	1.600	1.538	0.675*
16	Gallic acid	1	A	4.706*	6.2*	0.0620	0.0310	1.632	1.578	...
17	Sandalwood oil	1	A	4.566	5.5	..	0.0225	...	...	0.506

[1] Analyzed on the day when the samples were put up.

[2] The letter A indicates acidic or neutral reaction; B indicates alkaline reaction.

[3] The numbers in the headings of these tables indicate days.

The total phosphate was determined by the uranium acetate method; the glucose by Purdy's method; the ammonia and creatinine, by Folin's methods; the uric acid, by Ruhemann's method;[1] the urea and chlorine, by Dehn's methods;[2] and the total reduction of alkaline picrate by boiling and estimating colorimetrically.[3]

These analytical methods, except for phosphates and for chlorine, are subject to criticisms. The determination by Purdy's solution includes other oxidation-reactions besides glucose; the aeration of alkaline urines yields other volatile bases besides ammonia; the development of color in cold as well as hot alkaline solutions of picric results from other substances besides creatinine; and with hypobromite, nitrogen is evolved from ammonia as well as from urea. We have corrected for ammonia in the urea reading by multiplying the percentages of the former by 1.75 and subtracting this from the percentages of the latter.

Though some of these methods are known to be somewhat inaccurate, for our purposes, being rapid and applied under the same conditions, they give valuable and comparable data.

Observations drawn from the above tables of data are made upon (1) abnormal indications, (2) effects upon the respective components of urine and (3) estimates of the respective preservatives.

Abnormal Indications.—The most important abnormal indications are such as are produced on the analytical data by the preservatives themselves. These are marked in the tables with the asterisk, at least those that are very apparent and whose causes are evident are so marked. However, it must be remembered that an apparent increase of concentration, owing to quantitative disturbances by the preservative, may be compensated by the loss of material, owing to fermentation and other causes. Therefore, when both factors are operative, the apparent reading of concentration may be above, equal to, or below the original concentration, but is invariably above the true concentration.

Since chloroform yields formic and hydrochloric acids by hydrolysis after standing, its presence vitiates chlorine and glucose determinations. Other preservatives that destroy the accuracy of glucose are gallic acid, phenol, salicylic acid, formaldehyde,[4] hydrogen peroxide, and thymol.[5]

[1] *Berl. klin. Wochsch.*, 1903, Nos. 2 and 3; *Deut. med. Z.*, 1903, No. 8; *Med. Woch.*, 1904, No. 3.

[2] *Z. anal. Chem.*, 44, 604; *Z. physiol. Chem.*, 44, 11.

[3] We have found that hot alkaline solutions of picric acid develops color not only with creatinine but also with glucose, uric acid, other purine bases, acetone and acetoacetic ester. We are investigating the possibility of application of this reaction to the analysis of urine. See preceding article.

[4] For the effect of formaldehyde on Fehling's solution see Rudd and Bolenbaugh, Proc. Virginia Chemists' Club, 220.

[5] For the effect of thymol on acetone see Welker, *J. Biol. Chem.*, 3, 27; *N. Y. Med. J.*, 86, 552.

Since thymol also absorbs iodine and reduces alkaline picrate solutions, its presence will effect not only the glucose, but also the uric acid and creatinine determinations. Owing to hydrolysis, sulfuric acid, in dilute concentrations, converts creatine into creatinine; in greater concentrations sulfuric acid precipitates the creatinine. For these reasons sulfuric acid gives the abnormal results indicated above. All concentrations of sulfuric acid not only effect the creatinine but precipitate the uric acid and most of the coloring matter of urine.

Adequate explanations of the other high results in the tables are impossible at present.

Changes in Urine.—The changes possible in urine through aging and standing with preservatives are almost as numerous as the components of the mixture. Only certain inorganic salts, as for instance sodium chloride, seem proof to chemical decomposition. The easiest observed changes result from mere cooling, when urates and other substances are precipitated. A change in reactivity, as when normally acidic urine become alkaline, is accompanied by numerous chemical transformations, particularly of the urate, the phosphate, and the creatine-creatinine equilibria. For this reason it is evident that an acid preservative most often will be the more desirable.

Important transformations in urine are produced bacterially on glucose, urea and other fermentable substances. The enzymes developed are chemically hydrolytic, dissociative and oxidative, as evidenced by the formation of alcohol, carbon dioxide, ammonia, nitrite and nitrate. Though each of the preservatives in the above studied concentrations, undoubtedly has inhibitory influence, none has perfect bactericidal power, hence none is a perfect preservative.

Reference to the above data shows very little change in the phosphates, except when the reaction of the solution becomes neutral or alkaline; a notable exception is the ether sample. The chlorine analyses are uniformly constant, except when interfered with by such preservatives as hydrochloric acid, hydrogen peroxide, and chloroform; why the boric acid sample is high is not evident. With glucose the compensating influence of the preservatives and loss by fermentation are evident in cases of chloroform, thymol and gallic acid; probably with phenol, sodium arsenite and ether also. Salicylic acid, sandalwood oil and boric acid seem best for the preservation of dextrose. With uric acid, the changes are apparently great with ether, hydrogen peroxide and sodium benzoate; however, it must be remembered that these preservatives, and also thymol, interfere with the analysis of uric acid by the uricometer method, thus giving rise to the discordant data.

The ammonia data, as direct estimates of the hydrolysis of urea, are probably the best indications of the comparative antiseptic value of the

different preservatives, not only on account of the refinement of analysis of ammonia but also on account of the non-interference of preservatives with its determination. However, it must be remarked, that, although sulfuric acid yields but little ammonia, it transforms, possibly by oxidation, exceptional quantities of urea.

Considerable uniformity is met with in the analysis of urea, sulfuric acid alone seeming to have large destructive effect and formaldehyde being next with low results on account of precipitation.[1]

With creatinine there may be either an increase or a decrease of concentration. The increase resulting in acidic solutions involves the hydrolysis of creatine to creatinine. Since the colorimetric method of analysis of creatinine was employed, preservatives like thymol, phenol, ether, etc., gave too large readings of concentration. The decrease, resulting usually from the alkalinity of the solution, involves a transformation of creatinine to creatine; a decrease may also result, through precipitation, as in the treatment with sulfuric acid,[2]

The Respective Preservatives.—(1) Salicylic acid is the best preservative of those studied. This excellence of salicylic acid for urine was described by Jordan.[3] It is of further interest to observe that, finding alkaline salicylate to have no reducing or solvent effect on copper, Kendall[4] uses it instead of alkaline tartrate as the medium for oxidation of sugars. Its use, therefore, as a preservative will not interfere with the estimation of dextrose in urine by copper solutions.

(2) Though chloroform preserves urea, and therefore ammonia, it is ineffective with glucose and creatinine.[5]

(3) Thymol, though used frequently as a preservative for urine, does not seem to merit the value placed upon it; for, although little sediment is formed when it is used, and even if it had considerable bactericidal power, which is doubtful, its presence interferes with too many reactions involved in urine analysis. Gill and Grindley[6] made extensive studies on the preservation of urine by thymol and refrigeration and obtained good results for periods up to thirty-two days. However, it is very prob-

[1] Vide infra.

[2] Edlefsen, *Münch. med. Wochsch.*, **55**, 1615, 2524.

[3] *Biochem. J.*, **5**, 274; *Proc. Roy. Soc. Med. Pharm. Sec.*, **5**, 26. See also Luchrig and Sartori, *Pharm. Centrh.*, **49**, 934; Dafert and Haas, *Arch. Chem. Mikros.*, **1908**, 1; E. von Meyer and H. Kolbe *J. prakt. Chem.*, **12**, 178.

[4] THIS JOURNAL, **34**, 320.

[5] The loss of creatinine when chloroform was used as a preservative was observed by Benedict and Myers, *Am. J. Physiol.*, **18**, 380. See also Gill and Grindley, THIS JOURNAL, **31**, 707. The unsatisfactory preservative power of chloroform for sewage was remarked by Lederet and Hommon, *Eng. Record*, **62**, 319; *J. Am. Pub. Health Assoc.*, **1**, 267.

[6] THIS JOURNAL, **31**, 695; Hawk and Grindley, *Proc. Am. Soc. Biol. Chem.*, **9**, 10 (1907–8).

able that the *cold* and not the thymol was more important in the preservation of the urines studied.

(4) Boric acid has little merit[1] as a preservative of urine, at least in 0.2% concentration.

(5) Ether in large quantities preserves well, but volumetric relations are thereby disturbed.

(6) Formaldehyde cannot be considered a good preservative, because it precipitates urea[2] and is a reducing substance.

(7) Hydrogen peroxide (and its stabilizer) interferes with many reactions in urine analysis and has feeble bactericidal power.[3]

(8) Hydrochloric acid has nothing to recommend it as a urinary preservative.

(9) Toluene preserves uric acid and creatinine well.

(10) Sodium benzoate is a very poor[4] preservative of urine.

(11) Sodium arsenite is a fair preservative of urine.

(12) Sodium borate is a poor preservative of urine.

(13) Strychnine sulfate[5] is a good urinary preservative.

(14) Sulfuric acid has no value as a general preservative of urine; it causes too many reactions of hydrolysis and oxidation and does not prevent bacterial change.

(15) Since many bacteria are phenol-forming,[6] phenol cannot be expected to have much preservative power; moreover, it interferes with the analysis of many components of urine.

(16) Gallic acid has no value as a preservative of urine.

(17) Sandalwood oil, from the limited data given, indicates value as a preservative.

Summary.

(1) The ideal preservative must be soluble, non-volatile, and neutral or slightly acidic.

(2) Different preservatives must be employed for different purposes; no one preservative can prevent the change of all the components.

(3) The following may be considered poor preservatives of urine: for-

[1] For the feeble inhibitory action of boric acid on diastatic ferments see Agullion, *Compt. rend.*, 148, 1340; *Ann. inst. Pasteur*, 24, 495. For other feeble preservative powers see Kuehle, *Pharm. Centr.*, 50, 559; Luchrig and Sartori, *Ibid.*, 49, 934.

[2] Goldschmidt, *Ber.*, 29, 1896; May, *Deut. Arch. klin. Med.*, 1900; de Jager, *Z. physiol. Chem.*, 64, 110. For bacterial resistance to formaldehyde, Tirabosehi, *Il policlin.*, 15, 39, 40.

[3] Croner, *Z. Hyg.*, 63, 319.

[4] For the action of sodium benzoate on bacteria, Herter, *J. biol. Chem.*, 7, 59; see also, Lucas, *Proc. Soc. Exp. Biol. Med.*, 6, 122.

[5] For the effective influence of strychnine on bacteria see Scadikow, *Centr. Bakt. Parasitenk. I Abt.*, 60, 417.

[6] Dobravotski, *Ann. Inst. Pasteur*, 24, 595.

maldehyde, hydrogen peroxide, phenol, boric, gallic, hydrochloric and sulfuric acids, sodium borate and sodium benzoate.

(4) The following are better preservatives: chloroform, toluene, ether and thymol.

(5) The best preservatives are salicylic acid, strychnine sulfate, sodium arsenite and probably sandalwood oil.

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[FROM THE CHEMICAL SECTION OF THE IOWA AGRICULTURAL EXPERIMENT STATION.]

THE EFFECT OF ACIDS AND ALKALIES UPON THE CATALASE OF TAKA-DIASTASE.

BY RAY E. NEIDIG.

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It is well known that extracts from plant and animal tissues contain catalase, an enzyme capable of decomposing hydrogen peroxide. The effect of acids and bases toward this enzyme has been the subject of numerous investigations. In this field, Jacobson[1] was the first to note that acid caused a retarding effect upon solutions of catalase obtained from blood. Later, Senter[2] made a more exhaustive study of blood catalase, in which he determined the effect of three acids, namely, hydrochloric, nitric and acetic, and also of sodium hydroxide upon this enzyme. From this investigation he concluded that the time of contact of the acid with the catalase solution had very little influence upon the reaction, that there was no decomposition of the enzyme during its contact with acid, and, lastly, that neutralization of the acid restored the original activity of the catalase. Sodium hydroxide, he found, exercised an inhibiting effect similar to that of acids.

As these first two investigators dealt wholly with catalase from blood, Euler[3] undertook an investigation to determine whether or not catalase from different sources was identical. His work included catalase from one of the higher fungi, *viz.*, *Boletus scaber*. He found an important difference between the action of acids on the inhibition of the catalase obtained from *Boletus scaber* and that obtained from blood (Senter),[4] tobacco extract (Loew),[5] and yeast (Issaew).[6] He also observed that freshly precipitated magnesium hydroxide greatly increased the catalytic power of the enzyme. In this observation he corroborated the work of Loew,[4] who noted the same increase in enzymic activity when magnesium hydroxide was added to extracts of tobacco.

[1] *Z. physiol. Chem.*, **16**, 340.
[2] *Ibid.*, **44**, 257 (1903).
[3] *Hofm. Beitr.*, **7**, 1 (1905).
[4] *Loc. cit.*
[5] U. S. Dept. of Agr., *Report No.* 68.
[6] *Z. physiol. Chem.*, **42**, 102 (1904).