AN IMPROVED TEST FOR ACETONE IN URINE.

R. E. LYONS and J. T. BRUNDAGE, Indiana University.

Lieben's test for acetone[1] depends upon the formation of iodoform when potassium iodide, iodine solution, and a few drops of sodium hydroxide solution are added to an acetone containing mixture. The iodoform is recognized by its distinctive odor and, microscopically, by the star, or hexagonal crystals. The test is not specific[1] since both ethyl alcohol and acetic aldehyde also react with these reagents to yield iodoform. This sometimes leads to erroneous results because of alcohol formed through sugar fermentation in diabetic urine.

The difficulty is obviated[2] by substituting ammonium hydroxide for the caustic alkali as proposed by Gunning[3] in a modification of the Lieben Test.

In either test the reaction is much more sensitive if a urine distillate is used. The distillation not only frees the acetone from non-volatile interfering substances, but converts some acetonacetic (di-acetic) acid, if present, into acetone. Protein interferes[4] and, if present, the separation of acetone by distillation or aeration is necessary.

M. Kohlthoff[5] states that 100 mg. each of potassium iodide and chloramine T, 10-20 drops of 4N ammonium hydroxide and 10 cc. of a solution of one part acetone in 10,000 parts of 2 per cent ethyl alcohol when warmed to 60°C. gave an iodoform precipitate in two hours.

The object of our investigation has been to determine (a) whether this reaction could be applied as a specific test for acetone in urine, (b) what urinary constituents or preservatives interfere, (c) if the necessity of distillation, or aeration, of the urine could be dispensed with, and (d) the conditions for attaining the maximum sensitiveness of the reaction.

EXPERIMENTAL.

The acetone used was purified by distilling over anhydrous potassium carbonate. From a constant boiling fraction aqueous and urinary solutions of various concentrations were prepared.

The procedure employed in each experiment was as follows: Two drops (from a medicine dropper) of a saturated solution of potassium iodide, about 0.1 g. of powdered chlorazene[6], and 10-15 drops of 4N ammonium hydroxide[7] were added to 10 cc. of the acetone containing

[1] A. Lieben, Ann. Chem. u. Pharm. Suppl. 7, 226 (1870).

[2] V. Jaksch, Zeit. f. Klin. Med. 8, 115 (1884).

[3] Gunning, by Bardy, J. de pharm. et de chim. (5), 4, 30 (1881).

[4] Rosenbloom, Jour. Amer. Med. Assoc. 59, 445 (1912).

[5] Kohlthoff, Pharm. Weekblad, 62, 652-5 (1925) ; Chem. Abstr. 19, 17, (1925) ; Chem. Zentralbl. 96, 2, 3, (1925).

[6] Product of the Abbott Laboratories: p-toluene sodium sulpho-chloramide.

[7] Ammonium hydroxide of the specific gravity 0.971 is approximately 4N.

"Proc. Ind. Acad. Sci., vol. 36, 1926 (1927)."

solution and the mixture placed, for 3 to 5 minutes, in a bath of water having a temperature of 60°C.

The limit of sensitivity of this test for acetone in aqueous solution was investigated with results, as tabulated:

Acetone g.	Water cc.	Odor of Iodoform	Crystals of Iodoform (Time required for the appearance of)
1	500	present	at once
1	2,000	present	at once
1	10,000	present	at once
1	20,000	present	10 minutes
1	25,000	present	15 minutes
1	33,000	present	20 minutes
1	40,000	present	5 hours
1	50,000	present	no crystals
1	100,000	present	no crystals
1	125,000	present	no crystals
1	150,000	absent	no crystals

The delicacy and dependability of this test when applied directly to urine to which acetone had been added were found greatly impaired.

Concentration		Iodoform	
Acetone g.	Urine (normal) cc.	Odor	Crystals
1	500	present	none
1	1000	absent	none

The minimum concentration, therefore, in which acetone can be detected in urine by the above treatment, without distillation or aeration, lies between 1 in 500 and 1 in 1000. The precipitated phosphates interfere with the recognition of iodoform by either color or crystalline form. There was also an odor from the reaction mixture which made it difficult to differentiate the odor of iodoform. The final solutions were of darker color than the urine. This indicated that but little iodine was actually used and that some normal urinary constituent inhibits the formation of iodoform.

The Effect of Preliminary Removal of Phosphates from the Urine.—A test was first made to determine whether or not the addition of ammonium hydroxide before the potassium iodide and chlorazene would have any effect upon the trend of the reaction in a solution of acetone in water, 1 in 5,000. Equal volumes of the acetone solution receiving the reagents in normal and in reverse order yielded abundant and identical quantities of iodoform.

Urine containing acetone, 1 in 1,000, was made alkaline with ammonium hydroxide and the precipitated phosphates removed by filtration. The test applied to the filtrate gave no iodoform.

Other portions of the urine, containing different amounts of acetone, were treated with the magnesia mixture and the test applied to the filtrates. A slight odor of iodoform was detected in the sample having

an acetone concentration of 1 in 500; none in that having a concentration 1 in 1,000.

Samples of urine, containing varied amounts of acetone, were acidified with hydrochloric acid and sufficient phosphotungstic acid added for complete precipitation. The precipitate was removed by centrifuging and the clarified liquid made alkaline with ammonium hydroxide. This was again certifuged and the iodoform reaction applied to the clear liquid. The limit of sensitiveness following this procedure was found to lie between 1 in 500 and 1 in 1,000. Neither removal of phosphates nor precipitation with phosphotungstic acid increased the sensitiveness of the reaction.

The Effect of Removal of Urinary Pigments.—Half gram portions of various decolorizing agents were added to 10 cc. portions of urine containing acetone, 1 in 1,000. The mixtures were shaken, filtered and the filtrates subjected to the iodoform reaction with results as follows:

	Decolorizing agent	Iodoform Odor	Iodoform Crystals
1.	none	none	none
2.	"Superfiltchar"[8]	strong	abundant
3.	"Darco"	strong	many
4.	Lloyd's Reagent (did not decolorize)	none	none

The greatest increase in sensitiveness of the reaction followed the treatment with superfiltchar. The minimum amount of this bleaching carbon required to completely decolorize 10 cc. portions of normal urine after shaking one to two minutes at room temperature was found to be 0.4 g. The volume of the completely decolorized filtrate was approximately 6 cc. after the use of 0.4 and 0.5 g. of the carbon.

In connection with the tests executed to fix the maximum delicacy of the reaction in which the odor of iodoform, not the formation of a precipitate, was relied upon, it was observed that an odor slightly suggestive of iodoform was produced when the agents were added to decolorized acetone-free urine and even when the reaction was applied to distilled water. The cause of this odor was traced to the presence in the solutions of ammonium iodide and hypoiodite, normal products of the reaction of iodine with dilute ammonium hydroxide.[9]

The addition of sodium hydrogen sulphite not only neutralized the excess of ammonium hydroxide, but decolorized the final solution; and completely removed the odor suggestive of iodoform.

The limit of sensitiveness of this test for acetone in urine was determined, as follows:[10] To 15 cc. of urine, containing a definite amount of acetone, there was added 1 cc. concentrated ammonium hydroxide and 0.6 to 0.7 g. of superfiltchar. The mixture was shaken for one to two minutes, filtered and 10 cc. of filtrate reserved for the iodoform test.

---

[8] Pharmaceutical grade.

[9] Chattaway, Trans., Chem. Soc., 69, 1572 (1896).

[10] This procedure avoided the precipitation of phosphates later and was adopted as standard in the remaider of the experimental work.

Concentration		Iodoform	
Acetone	Urine	Crystals	Odor
g.	cc.		($NaHSO_3$ treatment)
1	1,000	at once	present
1	3,000	at once	present
1	5,000	within 10 min.	present
1	8,000	within 15 min.	present
1	10,000	within 20 min.	present
1	15,000	none (24 hrs.)	present
1	20,000	none	present
1	40,000	none	present
1	50,000	none	none

The iodoform crystals obtained in the reaction are six pointed stars, not the hexagonal plates characteristic of iodoform crystallized from alcoholic solution. To identify the iodoform, in case the crystals were not well formed, the supernatant liquid was decanted, the precipitate dissolved in little warm alcohol and a few drops of cold water added to effect quick separation of the iodoform. A drop of the precipitate was then examined under the microscope for crystals of iodoform.

Effect of Abnormal Constituents of the Urine.—The substance was added to 15 cc. of a solution of acetone in urine, 1 g. in 5,000 cc. 1 cc. concentrated ammonium hydroxide was added and the mixture shaken with 0.7 g. superfiltchar and filtered. The reaction was applied to 10 cc. of the filtrate.

1. Certain urinary preservatives.

Preservative	Preservative quantity of	Iodoform Crystals	Iodoform Odor (after $NaHSO_3$)
Blank		present	present
Thymol	0.1 g.	present	present
Xylene	0.3 cc.	present	none
Sodium fluoride	0.2 g.	present	present
Chloroform	0.3 cc.	none	none
Salicylic acid	0.2 g.	present	present

Xylene, when added in the above concentration, interferes with the detection of iodoform by odor. Chloroform inhibits the formation of iodoform because of its retention of the free iodine in solution. When salicylic acid was used more than the usual amount of ammonium hydroxide was added.

2. Dextrose, Beta-hydroxybutyric acid.

Substance	Quantity in 15 cc.	Iodoform Crystals	Iodoform Odor
Dextrose	3 cc. 2% sol.	present	present
Beta-hydroxybutyric acid	0.1 cc.	present	present

3. Certain synthetic antiseptics and laxatives (or their cleavage products).

Acetone was added, 1 g. in 5,000 cc., to samples of urine passed after the ingestion of, (a) urotropin preceded by sodium acid phosphate, (b) caprakol (hexyl-resorcinol), (c) phenolphthalein. The application of the test to 10 cc. of filtrate, obtained after the usual decolorizing treatment, from each specimen gave a positive result.

4. Protein.

Various amounts of egg albumen were added to aqueous solutions of acetone, 1 g. in 7,000 cc., and 10 cc. portions were subjected to the iodoform test. A Kjeldahl nitrogen determination was made upon the samples which contained just enough protein to completely inhibit the formation of iodoform. From these data the minimum protein concentration preventing iodoform formation from acetone in water solutions, was found to be 0.55 g. per 100 cc.

Different amounts of acetone were added to albuminous (egg albumin) urine. The protein was removed from 15 cc. portions by adding four to five drops of acetic acid and 10 per cent potassium ferro-cyanide solution until precipitation ceased. After 10 minutes the mixture was filtered, the filtrate made alkaline with ammonium hydroxide and shaken with superfiltchar. The iodoform reaction was applied to 10 cc. of the decolorized filtrate. When the acetone concentration was 1 g. in 8,000 cc. urine the precipitate of iodoform appeared within 20 minutes. The minimum concentration of acetone in urine which contains albumin that can be detected by iodoform odor, using this procedure for removal of albumin, was found to lie between 1 g. in 20,000 and 1 g. in 40,000 cc. urine.

The iodoform test was applied to aqueous solutions of di-acetic acid and of beta-hydroxybutyric acid, one drop in 10 cc. water, with negative results. This demonstrates that the "acetone bodies" do not decompose during the operation to yield acetone and that the test is specific for preformed acetone. It was also proven that their presence in a 1 in 5,000 acetone solution did not interfere with iodoform formation from acetone.

Reaction of the Reagents with Acetaldehyde:

The modified iodoform test was applied to 10 cc. of a water solution of acetaldehyde, 20 drops of acetaldehyde in 500 cc of water. A precipitate of iodoform appeared immediately.[11]

Methyl salicylate, sodium salicylate, salol, saliphen, p-cresol, and phenolphthalein are substances given in the literature as interfering with Legal's test for acetone. Lieben's test is given by acetaldehyde and alcohol; it is interfered with by thymol, phenolphthalein and protein. Phenolphthalein and protein interfere with Gunning's iodoform reaction. The iodoform test for acetone described in this paper, when applied to decolorized urine, is inhibited by chloroform and is inter-

[11] Twenty cc. of this aldehyde solution were placed in the acrometer cylinder of Folin's apparatus for the determination of acetone in urine and the procedure carried out as given by Hawk (Physiological Chemistry, 8th. Ed. 567). Iodoform crystals were present in the reagent flask within 15 minutes.

fered with by protein. Acetaldehyde yields iodoform by this method and also by the procedure of Folin.

Procedure.—

(a) Urine free from albumin.

To 15 cc. of urine, contained in a flask, add about one cc. of concentrated ammonium hydroxide and 0.7 g. superfiltchar, shake for one to two minutes and filter through a dry paper. To 10 cc. of the decolorized filtrate add about 0.1 g. chlorazene, two drops of a saturated solution of potassium iodide, mix and place in water at 60°C. for about five minutes. According to the quantity of acetone, the precipitate of iodoform may appear immediately, or after some hours, or quantities of iodoform too small to constitute a visible precipitate may be readily recognized by the characteristic odor. If a precipitate is not observed, add a slight excess of a saturated solution of sodium hydrogen sulphite and examine as to odor.

(b) Urine contains albumin.

To 15 cc. of urine add three to four drops of acetic acid and 10 per cent potassium ferro-cyanide solution until precipitation ceases. After 10 minutes filter, or centrifuge, and proceed with the clear, albumin-free fluid as directed in (a).

SUMMARY.

1. Superfiltchar removes from urine certain substances which interfere with the production of iodoform from acetone.

2. The iodoform reaction as described is adaptable to the detection of acetone in the urine. When applied to a decolorized urine filtrate, iodoform was detected by odor in a concentration of 1 g. acetone in 50,000 cc. of urine. With a concentration of 1 g. acetone in 10,000 cc. of urine a precipitate of iodoform resulted within 20 minutes.

3. The modified test is not sensitive to alcohol, di-acetic acid nor beta-hydroxybutyric acid, but is sensitive to acetaldehyde.

4. It is interfered with by the presence of protein in amounts exceeding 0.5 g. per 100 cc. The protein may be removed with acetic acid and potassium ferro-cyanide and the decolorizing process then carried out without greatly reducing the sensitivity of the reaction.

5. The reaction is not inhibited nor rendered less sensitive through the presence of the commonly used urine preservatives nor by the synthetics urotropin, hexyl-resorcinol, and phenolphthalein.