of the glycine, and the consequent increase in the amount of methyl alcohol formed. The difference in the strength of the tests, as indicated in the table, was very pronounced. The fact that in jar No. 3, the silage which ripened in the presence of an antiseptic contained no trace of methyl alcohol, even though a similar amount of glycine had been added, seems to show that the formation of this alcohol is brought about by some organism. This is in harmony with the results secured from the water cultures.

Of course, at this stage of the work, we can point, with certainty, only to the presence of an appreciable amount of methyl alcohol in normal silage, and merely indicate the probability of its formation by the hydrolysis of glycine. It is also possible that other substances than glycine may furnish some methyl alcohol in the presence of certain organisms, as for example, the fermentation of glycerine by B. Boöcopricus.[1] Indeed, the presence of methyl alcohol in fermentation mixtures is not new. Wolff[2] found it present in the distillate from fermented fruits, such as apples, cherries, grapes, and plums. Sanglé-Ferriére and Cumiasse[3] found it in absinthe. Takahashi[4] discovered methyl alcohol among the products produced by several varieties of mycoderma yeasts in fermenting rice mixtures. Evidently a number of different species of microörganisms possess the power of producing methyl alcohol in fermenting mixtures.

Summary.

Normal silage is shown to contain small amounts of methyl alcohol. A number of tests were made on the distillates from several samples of silage, the tests having first been standardized by using various known mixtures of alcohols. The number of different tests used precludes the possibility of positive reactions being given by some other substance than methyl alcohol.

The hypothesis is advanced that at least part of the methyl alcohol is formed by the action of microörganisms on glycine. All work done thus far with water cultures and experimental silage shows results which support that hypothesis.

MADISON, WIS.

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF WASHINGTON.]

THE VOLATILE SUBSTANCES OF URINE.

BY WILLIAM M. DEHN AND FRANK A. HARTMAN.

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Freshly voided, normal urines possess low vapor pressures[5] and little odor, thus indicating the presence of only traces of volatile substances.

[1] Emmerling, *Ber.*, **29**, 2726.

[2] *Compt. rend.*, **131**, 1323.

[3] *Ann. chim. analyst.*, **8**, 82 (1903).

[4] *Bull. Coll. Agr. (Tokyo)*, **6**, 387 (1905).

[5] *Vide infra.*

After standing, all urines undergo bacterial changes,[1] develop increased vapor pressures and give off disagreeable odors, thus indicating *decompositions of complex nonvolatile molecules contained in the fresh urines*. These decompositions are indicated not only in the fermentation of urines, but also in the results of evaporation[2] to dryness and ignition. The latter operations develop from the nearly odorless fresh urines intolerably nauseating odors which evidently were not present in the originals. Furthermore, as will be shown below, these characteristic odors may easily be developed by the hydrolytic action of dilute acids and alkalies. Thus we have various conditions and operations intensifying the odors of urine, and the conclusion is inevitable that *these disagreeable volatile substances were not present as such in the original urines but were produced by hydrolysis, reduction, oxidation or splitting of complex conjugated compounds*[3] *contained therein*. Such odor-poducing, conjugated compounds, except in cases of urea, phenol and indol, have not been described in the literature; at least the antecedents of some of the odorous compound of urine seems to have been overlooked by previous investigators.

Studies, first undertaken three years ago to determine these odoriferous substances, have not only confirmed the view that numerous hitherto known volatile substances are present in urine but also have developed the view that no small number of hitherto unknown substances also are present. Some of the latter have now been studied and others are being investigated.

Since no systematic study of volatile substances of urine has been made, it is purposed to make such studies both of freshly voided urine and urines treated with bacteria, acids and alkalies. These studies are undertaken to determine:

1. The volatile substances of urine.
2. The cause of the characteristic odor of urine.
3. The toxic constituents of urine.
4. The molecular forms of conjugated, deodorized, detoxified substances of urine.
5. The influence of the antecedents of such compounds on metabolism.

A large number of volatile substances contained in urines have been described in the literature. These have been classified as:

[1] THIS JOURNAL, **36**, 410 (1914).

[2] Van Nuys, "The Chemical Analysis of Healthy and Diseased Urine," **1888**, p. 10: "The chemical constitution of the body to which urine owes its odor is not known. It volatilizes very slowly, as urine does not lose all of its odor by boiling or evaporation."

[3] Long, "Urine Analysis," **1900**, p. 13: "Normal urine contains traces of complex aromatic bodies, the exact nature of which cannot in all cases be given."

1. Normal physiological constituents.
2. Casual physiological constituents.
3. Pathological constituents.

However, since these run one into the other, since it is sufficiently important to recognize *what* substances can be eliminated by the kidneys, and since our method of study is based on *chemical differences*, the following classification of volatile substances of urine is made.

TABLE I.—VOLATILE SUBSTANCES OF URINE.

Acids.		Phenols.	
Normal.	Abnormal.	Normal.	Abnormal.
Carbonic	Aceto-acetic	Phenol	Catechol
Formic	β-Hydroxybutyric	*p*-Cresol	Naphthol, etc.
Acetic	Valeric	Other phenols	
Propionic	Levulinic		
Butyric	Crotonic		
Benzoic	Phenylacetic		
Other acids			
Bases.		**Neutral substances.**	
Normal.	Abnormal.	Normal.	Abnormal.
Ammonia	Pyridine	Oxygen	Acetone
Methylamine	Trimethylamine	Nitrogen	Ethyl sulfide
Indol[1]	Benzylamine, etc.	Hydrogen peroxide	Methyl mercaptan
Skatol		Urinol hydrosulfide	Alcohol
		Other compounds	Terpenes, etc.

This classification does not assume that the listed substances are invariably found in the urine or that when eliminated by the kidney they are found in the urine in the molecular forms indicated, for it must be remembered that usually it is impossible to observe when uriniferous substances hydrolyze or split into their more simple molecules.

Furthermore, no claim is made that the table includes all of the volatile substances hitherto recognized. It merely suffices to show that *hitherto known substances*[2] *do not produce all the uriniferous odors;* these must be sought for among hitherto undetected substances.

Other substances which either have not been definitely established, or have had their origin from foods and therapeutically administered materials, have been omitted from the list.

Acid Volatile Substances.

When the odor of urine is mentioned in the literature it is vaguely de-

[1] Though indol is a weak base, in the method of separation described below, it appears among the neutral substances.

[2] The words *component* and *constituent* as defined by some authors to designate *mixture* and *compound* cannot be applied to the volatile substances of urine without ambiguity. At first many of the volatile substances are conjugated hence may be termed constituents, but after hydrolysis, they may be termed components. The less-limiting word *substance* is used in this paper.

scribed as being caused by volatile acids. Moreover, the usual acidity of urines is attributed, at least partially, to various organic acids. It is well, therefore, to note what acids are present and here especially to consider what acids may contribute to the odor. However, as will be shown, none of the listed acids possess odors likely to be mistaken for the characteristic odor of urine. Carbonic[1] and benzoic acids are odorless. The lower fatty acids[2] occur in small quantities and, except under rare pathological conditions, are present as salts or in conjugated forms, hence are not appreciably contributory to the odor of urines. It must be added that, in fermenting urines, volatile acids are increased at the expense of carbohydrates,[3] present.

The occurrence of hydrogen sulfide has been observed by various investigators.[4] Salkowski considered that its evolution from urine is to be traced to the action of bacteria on neutral sulfur compounds, as sulfates or thiosulfates. Karplus[5] also observed bacterial formation of hydrogen sulfide in urines. We have observed that *all urines slowly give off hydrogen sulfide* when treated with cold, dilute phosphoric or sulfuric acid. An explanation of this will be made in connection with *urinod*.[6]

Acetoacetic and β-hydroxybutyric acids[7] are both contributory to the pleasant odor of diabetic urines. Levulinic,[8] crotonic[9] and phenylacetic acid have also been reported.

[1] Winter and Schmidt, *Centr. physiol.*, 1, 421; Marchand, *J. prakt. Chem.*, **44**, 250.

[2] For summary of early literature, see Jaksch, *Z. physiol. Chem.*, 10, 536; for other literature see Dakin's "Oxidation and Reductions in the Animal Body" (Monographs on Biochemistry), 1910, pp. 113–16; see also Magnus, *Z. Med.*, **73**, 428; Molnar, *Z. exp. Path.*, **7**, 343; Strisower, *Biochem. Z.*, **54**, 189; Thudichum, *J. Chem. Soc.*, **23**, 400; Pflüger's, *Arch. Physiol.*, **15**, 12; Stadeler, *Ann. chem. Pharm.*, **77**, 17; THIS JOURNAL, **8**, 86.

[3] Salkowski, *Z. physiol. Chem.*, **13**, 264; *J. Chem. Soc.*, **23**, 400.

[4] Salkowski, *Berl. klin. Woch.*, **25**, 722; *Chem. Zentr.*, 1888, 1471; Porcher and Havieux, *Compt. rend. soc. biol.*, **68**, 27.

[5] Karplus, Virchow's *Arch.*, **131**, 210; see also, Sasaki and Otsuka, *Biochem. Z.*, **39**, 208.

[6] See following contribution.

[7] For literature on these acids see Dakin's bibliography quoted above. See also Duchmüller, Szymanski and Tollens, *Ann.*, **228**, 92; Minkowski, *Chem. Zentr.*, 1884, 406; Wolpe, *Ibid.*, 1887, p. 277; Stadelmann, *Z. Biol.*, **32**, 456; Klinger, *Ann. Chem. Pharm.*, **106**, 18; Neubauer, *Ibid.*, **97**, 129; *J. Pharm. chim.*, [3] **29**, 320; Araki, *Z. physiol. Chem.*, **18**, 1; Magnus, *Arch. exp. Path. Pharm.*, **42**, 149; Hilger, *Ann.*, **195**, 314.

[8] Weinstraud, *Chem. Zentr.*, 1895, p. 292.

[9] Stadelmann, *Z. Biol.*, **21**, 140; Salkowski, *Z. physiol. Chem.*, **7**, 450; **9**, 8; Araki, *Ibid.*, **18**, 1.

Phenolic Volatile Substances.

A large number of simple and polyhydric phenols[1] and their derivatives may appear in the urine conjugated either with sulfuric acid or glucuronic acid. For our study here, phenol and *p*-cresol are the most important, since they are odorous, volatile and appear in the largest quantities, as may be seen in the following experiment:

One thousand liters of urine were treated with dilute sulfuric acid and distilled; the distillate gave, by methods described below, phenols of the following fractions:

	Grams.			B. p.
(1) 170°.....about	1.34		phenol.....	183°
(2) 170–200°.....	19.13		*p*-cresol.....	202°
(3) 200–230°.....	8.12		thymol.....	232°
(4) 230–260°.....	0.67	Compare with	catechol.....	240°
(5) 260–320°.....	2.45		resorcinol.....	276°
			α-naphthol.....	282°
Total,	31.71		β-naphthol.....	288°

It need only be observed here that the odors of phenols do not give the characteristic odor of urine.

Basic Volatile Substances.

Ammonia[2] is the most important base positively known to occur in urine. During the hydrolysis of urea in fermenting urines, ammonium carbonate is formed in large quantities—this decomposes into free ammonia which almost universally has been mistaken for the characteristic odor-producing substance of urine. Although ammonia contributes to the disagreeable odor of alkaline urines, such contribution is only secondary, as may be easily proven by the experiment of heating urines with dilute sulfuric acid—this retains the ammonia but liberates the uriniferous odor.

The occurrence in urine of methylamine,[3] trimethylamine[4] and other

[1] For bibliography of phenols see Dakin's *Loc. cit.*, 1910, p. 130; Baumann, *Ber.*, 9, 54, 1389, 1715, 1747; 10, 685; 11, 1907; 12, 2166; Pflüger's *Arch.*, 12, 63, 69; 69, 285; *Z. physiol. Chem.*, 1, 60; 2, 335; 10, 123; Monfet, *Compt. rend.*, 137, 386; Salkowski, *Ber.*, 9, 1595; 10, 842; Labby and Vitry, *Compt. rend. soc. biol.*, 62, 699. For quantities of phenols in urines see Rumpf, *Z. physiol. Chem.*, 16, 220; Neuberg, *Ibid.*, 28, 123; Mooser, *Ibid.*, 63, 155. For naphthol in urine, see Edlefsen, *Chem. Zentr.*, 1905, p. 1341; Desesquelle, *Compt. rend. soc. biol.*, [9] 2, 101. For catechol, see Baumann, Pflüger's *Arch. Physiol.*, 12, 63; Moscatelli, Virchow's *Arch.*, 128, 181.

[2] Neubauer, *J. prakt. Chem.*, 64, 177, 279; Salkowski and Munk, Virchow's *Arch.*, 71, 500; Rumpf, *Ibid.*, 143, 1; Salkowski, *Z. physiol. Chem.*, 1, 26; Gumlich, *Ibid.*, 16, 19; Rumpf and Kleine, *Z. Biol.*, 39, Jubelband, 63; Camerer, *Ibid.*, 43, 13; Tidy, Meymott and Woodman, *Proc. Roy. Soc.*, 20, 362; Folin, *Am. J. Physiol.*, 13, 45, 66; Grafe and Schläffer, *Z. physiol. Chem.*, 77, 1; Wills and Hawk, *J. Biol. Chem.*, 9, 30; Boussingault, *Ann. chim. phys.*, [3] 29, 472; Heintz, *J. prakt. Chem.*, 64, 399; Hallerwarden, Virchow's *Arch.*, 143, 705.

[3] Schiffer, *Z. physiol. Chem.*, 4, 237; Erdmann, *J. Biol. Chem.*, 9, 85; Folin, *Ibid.*, 3, 83; Takeda, Pflüger's *Arch.*, 129, 82; Schmiedeberg, *Arch. exp. Path. Pharm.*, 8, 1.

[4] de Fillippi, *Z. physiol. Chem.*, 49, 433; Erdmann, *J. Biol. Chem.*, 8, 57; 9, 85; Dessaignes, *Compt. rend.*, 43, 670; Takeda, Pflüger's *Archiv.*, 129, 82.

aliphatic amines has not definitely been established. Indol and skatol[1] are excreted in chemical combination with sulfuric or glucuronic acids. Pyridine[2] and benzylamine[3] have been reported in rare cases.

Neutral Volatile Substances.

This division, including *urinod*, and at least two other new and closely related substances, will be shown to contribute the most important volatile substances of urine.

Oxygen, nitrogen and hydrogen peroxide, possessing no odors, are unimportant in this discussion.

Acetone,[4] one of the most important and most investigated substances of pathological urines, contributes to the aromatic odor of diabetic urines.

Ethyl alcohol[5] sometimes appears in the urine of diabetics. It is also of interest to know that a small portion of the alcohol of imbibed beverages[6] is eliminated in urine either in the free or the combined form.

Other neutral volatile substances which have been reported as present in urine under special conditions are: chloroform,[7] iodoform, ethyl sulfide,[8] methyl mercaptan[9] and the terpenes.

From the foregoing discussion it may be concluded that the volatile substances hitherto known to be present in urine are not responsible for its characteristic odor. Though some of these are contributory to it, the following experiments will show that the newly discovered substance *urinod*[10] is the *id ipsum* of the odor of urine:

[1] For voluminous bibliographies on indol and skatol see Hammarstein's physiological chemistries. A regular occurrence of indol in the distillate of normal urine is maintained by Jaffe, *Arch. exp. Path. Pharm.*, Suppl., **1908**, p. 299.

[2] Kutscher and Lohmann, *Z. Nahr. Genussm.*, **13**, 177.

[3] Schmiedeberg, *Arch. exp. Path. Pharm.*, **8**, 1; **14**, 288, 306; Mosso, *Ibid.*, **26**, 267.

[4] For voluminous bibliographies on acetone see (*a*) Huppert-Neubauer, *Harn-Analyse*, 10 Aufl.; (*b*) v. Noorden's *Lehrb. d. Pathol. des Stoffwechsels*, Berlin, 1893; (*c*) Dakin's "Oxidations and Reductions in the Animal Body" (Monographs of Biochemistry), **1912**, pp. 113–116; also see Jaksch, *Z. physiol. Chem.*, **6**, 541; Müller, *Berl. klin. Woch.*, **1887**; *Chem. Zentr.*, **1907**, p. 366; Jägerroos-Björneborg, *Arch. Gyn.*, **94**, No. 2; Piper, *Lancet*, **185**, 535, 602; Wallace and Gillespie, *Practitioner*, **84**, 2; Abram, *J. Path. Bact.*, **3**, 420.

[5] Markownikoff, *Ann.*, **182**, 362; Pohl, *Arch. exp. Path. Pharm.*, **31**, 281; Völtz, Baudrexel and Dietrich, *Arch. ges. physiol.*, **145**, 210; Dupré, *Proc. Roy. Soc.*, **20**, 268; Béchamp, *Compt. rend.*, **75**, 1830; Maignon, *Ibid.*, **140**, 1063, 1124.

[6] Dupré, *Proc. Roy. Soc.*, **20**, 268; Völtz and Baudrexel, *Arch. ges. Physiol.*, **142**, 47; **145**, 210.

[7] Vitali, *L'Orosi*, **16**, 299; Nicloux, *J. pharm. chim.*, [6] **24**, 64.

[8] Neuberg and Grosser, *Chem. Zentr.*, **2**, 835; Abel, *Z. physiol. Chem.*, **20**, 253.

[9] Karplus, Virchow's *Arch.*, **131**, 210.

[10] Moor described a substance obtained from the residue of urine by extracting with alcohol. He called it *ureine*. It was shown by Gies and Haskins to be a mixture containing large quantities of urochrome. Moor, *Z. Biol.*, **44**, 123; **45**, 420; *Med. Record*, **58**, 336, 471; *Le physiologiste russe*, **2**, 128, 131; Gies, *Med. Record*, **59**, 329; THIS JOURNAL, **25**, 1295 (1903); Haskins, *Am. J. Physiol.*, **12**, 162. It probably contained traces of *urinod hydrosulfide*, as will be shown below.

Experimental Part.

Preliminary studies were made on the vapor pressures and the volatile acidities of normal urine. Our important experimental results, however, were obtained by hydrolysis and distillation of urines with dilute sulfuric acid.

Vapor Pressures of Normal Urines.

Vapor pressure determinations[1] were made under the following indicated conditions:

Sample.	Age. Hours.	Temperature.	Vapor pressure of Urine.	Vapor pressure of Water.	Difference.	Remarks.
A	1	24.0	12.2	22.2	10.0	Acid reaction
A	67	21.8	16.4	19.5	3.1	Acid reaction
A	120	23.0	19.3	20.9	1.6	Acid reaction
A	504	23.0	16.4	20.9	4.5	Containing mold
B	13	20.2	13.4	17.6	4.2	Acid reaction
B	60	21.6	15.9	19.0	3.9	Alkaline reaction
C	0.1	20.5	16.6	18.0	1.4	Acid reaction
C	3.2	21.1	11.0	18.7	7.7	Acid reaction
C	24.0	20.3	10.8	17.7	6.9	Alkaline reaction
C	30.0	20.8	15.5	18.4	2.9	Alkaline reaction

It is observed that the vapor pressures: (1) are not constant throughout the times observed; (2) their variations are not large; and (3) they are lower than aqueous pressures at the same temperature. Since many chemical reactions are going on in urine, involving fermentation, hydrolysis, oxidation, reduction, etc., especially developing ammonium carbonate from urea and multiplying the oxygen-consuming bacteria—it is to be expected that disturbances of the vapor pressure equilibria will result shortly after the urine is voided, and will continue until all fermentations cease. Since the reaction products may have opposite effects upon the original vapor pressure, the equilibrium may not be disturbed or may be disturbed only slightly, therefore, such determinations may have little or no value. For our purpose they show that no large quantity of easily volatile substances is present in normal urine.

Volatile Acidity of Urine.

Since the odor of urine is often judged to be caused by "volatile aromatic acids" it was considered of importance to measure such volatile acidity. Air was, therefore, drawn through a series of bottles supplied with Folin's aspiration tubes. The first bottles contained appropriate solutions for fixing atmospheric acids and bases; next followed the bottle containing the urine; and finally the bottle containing 0.1 N alkali. Thus the acids, volatile at ordinary temperature, were carried over into the alkali and the loss of alkalinity was measured.

Fresh urines and fermented urines were tested in this manner; both were

[1] For method employed see THIS JOURNAL, 29, 1052 (1907).

aspirated directly or after treating with dilute sulfuric acid to liberate free acids from their various combinations.

Sample.	Volume urine.	Kind of urine.	Treated with H_2SO_4.	Time of aeration. Hours.	Volume 0.1 *N* NaOH. Cc.	Calc. as benzoic acid per 1,000,000.
A.....	100	Fresh	+	36	0.14	17.08
A.....	200	Fresh	+	17	0.25	15.25
B.....	100	Fresh	(*a*)	9	0.14	17.08
B.....	200	Fresh	(*b*)	6	0.25	15.25
C.....	2000	Fresh	+	16	0.40	2.44
D.....	1500	Fresh	+	16	0.20	1.63
E.....	2000	Fermented	+	45	0.00	0.00

(*a*) Sample (B) was collected in dilute sulfuric acid and was permitted to stand for 2 mos., then 10 cc. of 20% sulfuric acid was added to the 100 cc.

(*b*) Here 20 cc. of 20% sulfuric acid was added.

Since benzoic acid was found in normal urine in the largest quantities of any acid, it is here taken as the basis of calculations. Of course, when urines are treated with sulfuric acid and are then aspirated or distilled, hydrochloric acid is volatilized, hence the above concentrations, as indicating organic volatile acidity, are too large. These experiments, however, show that: (1) the normal acidity of urines is not largely due to volatile acids; (2) the odor of urines at ordinary temperature cannot be caused by numerous aromatic acids, at least, it cannot be caused by such acids present in any considerable quantity.

In fact, the extremely low concentration of volatile acidity may be caused almost entirely by hydrogen sulfide; this will be shown in the following experiments:

The Occurrence of Hydrogen Sulfide in Urine.

It was observed that all urines treated with dilute sulfuric acid slowly evolved hydrogen sulfide. It became necessary to determine: (1) whether such hydrogen sulfide was formed from the added sulfuric acid; (2) whether the presence of acids accelerated the evolution of hydrogen sulfide; and (3) whether other odors were developed at the same time. Samples of urine were treated in the following indicated manner:

Fraction of same sample.	Volume used. Cc.	Treated with	Effects on lead acetate paper after			
			3 hrs.	4 hrs.	48 hrs.	11 days.
A...	100	4 g. H_3PO_4	edges blackened	darker	very dark	black
B...	100	4 g. H_2SO_4	edges blackened	darker	very dark	black
C...	100	.....	.....	...	.....	edges blackened

Here it is concluded: (1) the hydrogen sulfide was not formed by reduction of the sulfuric acid; (2) its evolution was accelerated by acids, and (3) the untreated sample gave off little or no hydrogen sulfide. The further observation was made that both (A) and (B) but not (C) gave off urinod-like odors.

From these and other observations the conclusion is drawn that treat-

ment of urines with sulfuric acids yields hydrogen sulfide, urinod, a dark brown precipitate and other reaction products.

Methods Used for the Isolation of Volatile Substances.

Two methods have been employed by us to obtain volatile substances in urine:

1. Direct extraction of urines with ether.
2. Extraction of distillates of urine with ether.

Since large quantities of urine and ether were handled in both cases, considerable thought and labor were given to the best methods of experimentation. Three methods were pursued:

Method I.—Three carboys each holding about 40 liters were arranged at different levels (see Fig. 1), so that the contents of the topmost one (A) siphoned into the middle one (B) and the latter into the lowest one (C).

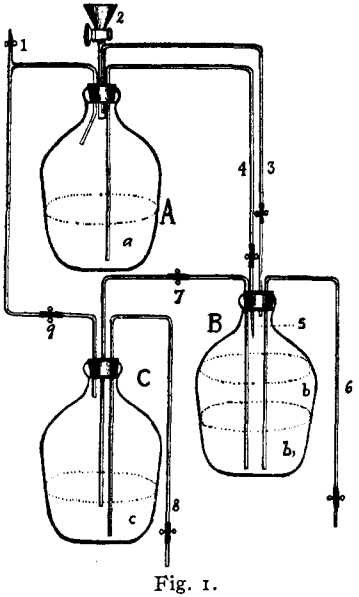

Fig. 1.

The purposes of this apparatus were these: (1) to extract large quantities of urine or its distillates *automatically* by making use of gravity; (2) to avoid loss of ether attending the handling in open vessels; and (3) to avoid danger of fire in working with large quantities of ether.

A sufficient quantity of ether (*b*) is drawn into (B) through (6), by closing (3), (4) and (8) and drawing through detached (9). With screw-clamp (1) opened, bottles of urine or distillate are inverted in funnel (2) and are permitted to empty into carboy (A). When the latter is nearly full, screw clamps (3), (6) and (8) are closed and urine or distillate is siphoned to (4) by drawing at detached (9). A steady stream of urine or distillate spurts through the finely drawn nozzle (5) and, with proper adjustment, break to a fine spray when striking the surface of the ether. With (9) reattached, (3) opened and (1), (6) and (8) and stopcock (2) closed, carboy (A) will empty into (B) and if siphon (7) is filled by drawing at (7) carboy (B) may simultaneously deliver into (C). Hence, without any attention or danger of loss, the apparatus may be set in operation during the day and be permitted to run all night. Since sediment from (A) may clog the nozzle (5), the siphon of (A) should not dip too deeply, nor should this siphon be opened before the sediments have had time to settle. Of course, when such clogging results, tube (5) may be detached and cleaned.

When the ether layer (*b*) becomes much reduced, it may be drawn off

and fresh ether may be added, or better, more and more ether may be added when necessary, without removing any of the layer present.

The extracted liquor from (B) and (C) may be drawn off at (6) and (8), most advantageously when (A) is refilling at (2). This lower aqueous layer contains large quantities of dissolved ether, hence should be distilled to recover the same. This may conveniently be effected by running the liquor directly into the vessel used as a still or the liquor may be preserved for distillation in bottles. The distillation was most conveniently carried on with a battery of flasks such as used for Kjeldahl digestions. The tubes from the different condensers were connected so that one tube only delivered into the receiver and this was surrounded by flowing tap-water and carried a return condenser. The ether distillates containing water were assembled and returned to carboy (B).

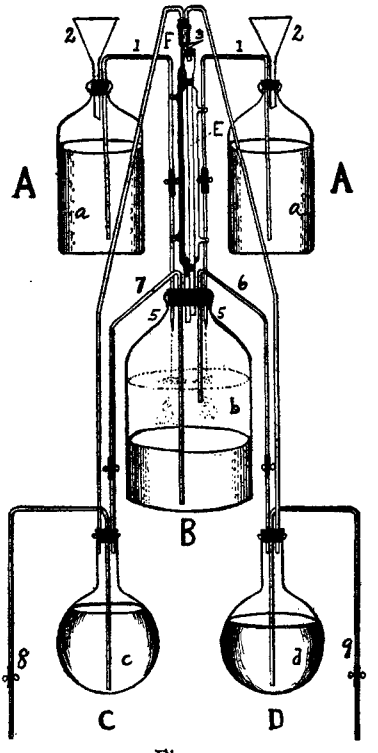

Fig. 2.

Method II.—This, like Method I, is safe and ether-saving. It is semiautomatic, prevents disagreeable odors being liberated in the laboratory but is not so rapid as Method III.

The apparatus (see Fig. 2) consists of bottles (A) delivering urine by siphons (1) through jets (5) into the large bottle (B), which carries two return condensers, one of which (E) opens to the air by a capillary tube (3) at the top to accommodate changes of pressure in (B). The urine having passed through the ether layer (*b*) is siphoned into flask (C); here the dissolved ether is distilled back into the extraction bottle (B). The waste material is then withdrawn through siphon (8). After a number of extractions, the ether (*b*) is siphoned from (B) to (D) where it is concentrated, the ether returning to (B) through the condenser (F).

All siphons are filled by drawing with the suction pump and are controlled by screw clamps. The effectiveness of the extraction depends upon the size and force of the stream striking the ether layer.

Method III.—This method, though most wasteful of ether, was found to be rapid and convenient. The distillates from the urine were placed in ordinary 2.5 liter acid bottles, whose stoppers and necks had carefully been freed from paraffin. The bottles ordinarily were filled about four-

fifths with distillates and nearly to the neck with ether. The bottles, tightly cork stoppered, were shaken vigorously from time to time and finally were set aside, or were permitted to stand until the mixture settled in sharply defined layers. These layers were separated by one of three methods: (1) one or two bottles were emptied into a large separating funnel and the two layers were drawn off in the usual manner; (2) a siphon was used to draw off the aqueous layer from each bottle; (3) the contents of many bottles were poured into a carboy and the two settled layers were separated by the siphon.

The aqueous part was distilled to recover dissolved ether and this ether was used for the other extractions. In all cases the hot residual aqueous solutions gave off uriniferous odors, showing that the extraction of volatile substances was not complete with a single extraction.

Collection and Chemical Treatment of the Urine.

The collection of urine was managed so that it was treated either directly with sulfuric acid, or within a few hours, thus avoiding fermentation.[1] Concentrated sulfuric acid was diluted with one volume of water, and 100 cc. of this solution were placed in each 2.5 liter acid bottle. Then funnels were placed in the necks of the bottles for direct collection or the bottles were filled from large flasks or bottles containing fresh urine. Thus the urines were treated almost immediately with 3–4% of sulfuric acid, enough to unite with all of the reactive constituents of urine, and to yield a definite though weak hydrolyzing medium.

The bottles thus filled were set aside, preferably in a warm, dark, place for a number of days. During this time the color of the solution becomes chocolate-brown or brown-black, a brown precipitate is formed, and characteristic uriniferous odors are developed. If moist lead acetate paper is held in the vapors of these bottles, blackening results, showing invariably the presence of hydrogen sulfide.[2]

Since urines extracted directly gave only small quantities of volatile substances, it was found that some hydrolytic treatment, as with sulfuric acid, was not only productive of but necessary for the evolution of the desired volatile substances.

Three lines of study were followed—the urines were:

(1) Extracted directly with ether.

(2) Digested with dilute sulfuric acid and then were subjected to distillation—practically to dryness.

(3) Permitted to ferment and then were subjected to distillation.

The first method was nearly unproductive of volatile substances—showing that these are largely formed by fermentation, hydrolysis, etc., from

[1] Fermented urines give little urinod and much indol in the "neutral" portion.

[2] See following contribution for further evidence that hydrogen sulfide is found in all urines.

conjugated compounds. The treatment with dilute sulfuric acid, distillation, and extraction with ether was found to be a very satisfactory method for the isolation of volatile substances of urine. The third method was unsatisfactory for the production of volatile substances of urine.

The Distillation of Urines.

The urine, treated with sulfuric acid and permitted to stand many days, was decanted into round-bottomed flasks connected by Hopkins' bulbs to ordinary condensers. These flasks rested on tripods covered with sand baths, wire gauzes, or wire gauzes supplied with circular-hole asbestos shields.

With fermented urines, foaming-over, necessitating redistillation, was a source of much trouble, until the volume was reduced to about one-third. Such difficulty was not met with in case of acid-treated urines. Since the distillation was continued practically to dryness, trouble from bumping was experienced after some two-thirds of the volume was obtained as distillate. When the bumping stage was reached, the concentrated urines were cooled, assembled and permitted to stand so as to precipitate the sediments which were usually dark brown in color. The urine was then siphoned off and the distillation was continued. This process was repeated until the urines were so concentrated that they solidified almost completely on standing. The liquid part of this, however, was filtered off and distilled further.

The first distillate contained traces of floating oil, which tended to cling to the wall of the receivers. It possessed the characteristic urine odor and exercised nauseating and depressing effects upon the worker. The middle fractions seemed to yield less oil and possessed a modified odor suggestive somewhat of creosote. The final fractions contained some light yellow solid[1] and possessed a putrid odor mixed with the odor of sulfur dioxide. Throughout the distillation the odors, at different times, resembled urine, old peanuts and most varieties of intestinal gas.

Treatment of the Ether Extracts.

After concentrating by distilling, the ether solutions were repeatedly and successively treated with aqueous solutions of (A) sodium carbonate, (B) sodium hydroxide, and (C) hydrochloric acid. The residual ether solution was distilled first directly to remove the excess of ether and finally with steam to separate (D), the very volatile, from (E), the less volatile neutral substances. In this manner were obtained fractions containing: (A) volatile acids, (B) volatile phenols, (C) volatile bases, (D) urinod, etc., (E) urinod hydrosulfide, etc.

To illustrate the complexity and variety of the volatile substances of urine, the boiling points of these fractions are given:

[1] See page 2134.

(A) up to 260° mostly at 220–260°.
(B) up to 320° mostly at 170–200°
(C) up to 100°.
(D) up to 170° at 29 mm. mostly at 108° 29 mm.
(E) above 170° at 29 mm.

(A) *The Volatile Acids of Urine.*

From 125 liters of urine, the aqueous solution in sodium carbonate (A) gave with an excess of dilute hydrochloric acid:

(*a*) A copious precipitate of white crystals (mostly benzoic acid).
(*b*) An aqueous acid-containing solution.

(*a*) *The Solid Insoluble Volatile Acids.*—After filtering and drying 9.8 g. of crystals were obtained or one part in 12,700 parts of urine. This solid contained at least three substances, *viz.*, benzoic acid, a *putrid acid*, and dark odorous substances insoluble in ammonia. The melting point of the mixture was 117° (benzoic acid, 121°).

(*b*) *Soluble Volatile Acids.*—These were separated from the aqueous solution by extraction with ether. After evaporating the ether, an oily residue was obtained; it was dissolved in an excess of dilute alkali and then filtered through paper moistened with water. The alkali solution was treated with hydrochloric acid and extracted with ether. The ether solution was dried with fused calcium chloride and distilled. The following fractions were taken:

(*a*) From 125 liters.

Fraction.	B. p.	Weight.
(1).....	50°	0.02
(2).....	50–80°	0.50
(3).....	80–120°	0.50
(4).....	120–220°	?
(5).....	220–255°	?
(6).....	255–270°	(m. p. 47°)
(7).....	270°	

(*b*) From the last third[1] of 1000 liters.

Fraction.	B. p.	Weight.
(1).....	90°	0.50
(2).....	90–120°	0.62
(3).....	120–220°	3.62
(4).....	220–245°	7.84
(5).....	245°	3.24
Total.....	.....	15.82

Fractions 1 and 2 gave, with ammoniacal silver nitrate, a black precipitate of silver indicating formic acid.[2] All the fractions with neutral ferric chloride gave reddening and precipitates of basic ferric salts, thus indicating the presence of fatty acids or merely weak volatile acids. Fractions below 120° gave, with alcohol and hydrogen chloride, a heavy oil but no perceptible odor of ethyl acetate. Fractions above 220° solidified to white, soapy crystals of a peculiar fetid odor. These were identified as largely benzoic acid, as containing members of the fatty series of acids and possibly as containing hexahydrobenzoic or some closely related acid.

Some of the fractions boiling above 220° were dissolved in ammonia,

[1] Unfortunately, the acids from the first two-thirds of the distillate from the 1000 liters were lost.

[2] Strisower, *Biochem. Z.*, **54**, 189; *C. A.*, **8**, 174.

filtered and boiled with an excess of silver nitrate and the precipitated silver salts were extracted with successive portions of hot water; thus there were obtained, on cooling, the following fractions:

	I.	II.	III.	IV.
Weights taken.....	0.1144	0.1355	0.1048	0.4069
Found, Ag.....	45.63	46.86	47.04	46.94

Calculated for C_6H_5COOAg, 47.12; $C_6H_{11}COOAg$, 45.91; $C_6H_{13}COOAg$, 45.52.

These data indicate that: (1) the original mixture of solid acids consists of benzoic acid largely; (2) the other acid or acids have greater molecular complexity than benzoic acid.

From some of the 7.84 g. of solid acids obtained from the 1000 liters of urine, silver salts were prepared in different fractions and thoroughly washed with water. After decomposing the respective fractions with an excess of hydrochloric acid, the acids were extracted with ether and then were isolated in the usual manner.

Fractions.	Crystals.	M. p.	Remarks.
(1)	Transparent flakes.....	124°	Melted sharply.
(2)	Transparent flakes.....	106°	Remelted at 119°, optically inactive.
(3)	Compact crystalline mass.....	105°	Sublimed to plates and needles.
(4)	Compact crystalline mass.....	122°	
(5)	Compact crystalline mass.....	121°	Sublimate melted at 122°.

Since none of these fractions gave precipitates with bromine water, they contained no salicylic or other hydroxy acids. These experiments confirmed the conclusion that the solid volatile acids of urine consist largely of benzoic acid. The putrid-smelling, low-melting acid was suspected to be hexahydrobenzoic acid.[1]

Some of the fraction boiling between 220–250° and freed from benzoic acid as much as possible by crystallizing from hot water, was extracted with ether, brought into water and treated with an excess of silver oxide. The hot aqueous solution was filtered and the first crop of crystals was discarded, for silver benzoate is less soluble than silver hexahydrobenzoate.[2] The second crop separated as white flakes.

0.1732 and 0.1103 g. gave 0.0794 and 0.0508 g. Ag; Calc. for $C_6H_{11}COOAg$: Ag, 45.91%; Calc. for C_6H_5COOAg: Ag, 47.12%; found: 45.84 and 46.05%.

[1] This acid was first prepared by Aschan and is described as forming colorless leaflets melting at 30° and boiling at 232–233°. It is sparingly soluble in cold water and readily soluble in hot water. It is more easily volatile with steam than benzoic acid and possesses an odor resembling valeric acid and ethyl crotonic acid. Aschan, *Ber.*, **24**, 1864, 2617; **25**, 886; *Ann.*, **271**, 261; Markownikoff, *Ber.*, **25**, 370, 3357; Haworth and Perkin, *J. Chem. Soc.*, **65**, 103; Bucherer, *Ber.*, **27**, 1231; Einhorn and Meyenberg, *Ber.*, **27**, 2829; Einhorn and Lumsden, *Ann.*, **286**, 264; Sabatier and Murat, *Compt. rend.*, **154**, 922; Godchot, *Bull. soc. chim.*, **9**, 261.

[2] Bucherer, *Ber.*, **27**, 1231.

Some of the silver salt was heated in a sealed tube with an excess of ethyl bromide; the ethyl ester[1] boiled up to 195°.

Though apparently the presence of hexahydrobenzoic is indicated the evidence is not conclusive. For instance, when the free acid was separated from the silver salt, it did not solidify at ordinary temperatures. It contained no sulfur and no nitrogen. It responded to the ferric chloride test. It did not react with a chloroform solution of bromine. It did not react with a sodium carbonate solution of potassium permanganate. Small, white needles of its lead salt, melting at 40°, contained 42.34% Pb; lead benzoate melts at 114° and contains 46.10% Pb; lead hexahydrobenzoate contains 44.89% Pb; lead heptylate contains 44.49% Pb; lead caprylate melts at 84° and contains 41.98% Pb.

The fraction boiling at 120–220° (last third of 1000 liters) was refractionated and converted into silver salts.

		Silver salts contained % Ag.	
Fraction.	Boiling.	First crop.	Last crop.
(1)	120–160°	51.94	56.45
(2)	160–190°	51.70	51.67
(3)	160–220°	47.00	46.46

Calc. for $C_6H_{13}COOAg$: Ag, 45.52%; C_5H_9COOAg: Ag, 48.38%; C_4H_9COOAg: Ag, 51.63%; C_3H_7COOAg: Ag, 55.34%.

These boiling points and analyses indicate the presence of butyric acid in (1), the presence of valeric acid in (2), and the presence of caproic and heptylic acids, and possibly hexahydrobenzoic acid in (3).

The above experiments show that the volatile acids of urine consist mostly of benzoic acid and fatty acids up to and including caproic acid, probably heptylic and possibly hexahydrobenzoic or some closely related acid.

From 200 liters of fermented urine, 0.53 g. of volatile acids were separated. Since most of this solidified to needles of benzoic acid, it is seen that fermentation is nonproductive of volatile acids of urine.

Since benzoic acid is the most voluminous volatile acid of hydrolyzed urine but is not detected in normally acidic urines subjected to direct extraction with ether, it is concluded that all the benzoic acid of urine is conjugated as hippuric acid.

(*B*) *Volatile Phenols of Urine.*

The aqueous alkaline solution (see above) was acidified with hydrochloric acid and extracted with ether, After drying with anhydrous sodium sulfate, it was fractionated as follows:

[1] Aschan gives 195° as the boiling point of ethyl hexahydrobenzoate, *Ann.*, **271**, 264.

		I. 125 liters (H_2SO_4).	II. 1000 liters (H_2SO_4). (*a*) first 2/3;	(*b*) last 1/3;	(*c*) total.	III. 200 liters (fermented).
(1)....	170°	0.00	1.00	0.34	1.34	0.02
(2)....	170–200°	0.95	17.32	1.81	19.13	4.23
(3)....	200–230°	0.39	6.38	1.74	8.12	0.58
(4)....	230–260°	0.20	0.20	0.47	0.67	0.33
(5)....	260–320°	0.80	1.29	1.16	2.45	1.00
		2.34	26.19	5.52	31.71	6.16

It will be observed that *phenols occur about 31 parts in a million of urine.* All the lower distillates gave turbidity with bromine water, thus indicating the presence of phenols. Some tarry residue remained in the distilling flasks, this indicating decomposed higher phenols. The third fraction, when treated with an excess of bromine and boiled, gave an oil which solidified on cooling. When treated with alcohol, golden flakes, softening at 264° and melting at 280°, were obtained. It has not been identified. It and the other phenolic compounds will be studied further.

(*C*) *Volatile Bases of Urine.*

Only a little basic volatile substance is given off when urines are distilled with sulfuric acid. Of course, this is formed by the hydrolysis of sulfates. Since the distillates of such urines are acid, the bases remain in the ether-extracted, aqueous portion as salts of hydrochloric acid, etc. No effort was made to recover such bases from distillates after extracting with ether. However, in the preparation of neutral fractions of the volatile substances of urine, the ether solution was always washed with dilute hydrochloric acid to remove bases possibly present.

(*a*) An effort was made to obtain volatile bases from urine. Forty liters of urine, collected over sufficient potassium hydroxide to maintain a strong alkaline reaction, was extracted twice with ether after the phosphates had been removed. The ether from both the extracted material and the aqueous portion was distilled over dilute hydrochloric acid to collect low-boiling bases. Evaporation of this aqueous solution gave 2.38 g. of hydrochloride. After extracting the residue with absolute alcohol, an insoluble portion was obtained. It formed a platinum salt containing 43.15% Pt; calculated for $(NH_4)_2PtCl_6$, 43.96% Pt. The portion soluble in absolute alcohol (0.091 g.) yielded a platinum salt containing 41.17% Pt; calculated for $(CH_3NH_3)_2PtCl_6$, 41.32% Pt. This experiment shows that *methylamine is present in alkali-treated unfermented urine only about one part in a million of urine.*

(*b*) Fourteen liters of urine were allowed to ferment one month. After freeing from earthy phosphates, it was extracted twice with ether. The ether was distilled into receivers containing hydrochloric acid; some ammonium carbonate and rectangular crystals of a musty odor formed in the condenser. From 3.259 g. of the hydrochlorides, 0.108 g. of alcohol-

soluble crystals were obtained. Its platinum salt contained 41.26% Pt; calculated for $(CH_3NH_3)_2PtCl_2$, 41.32% Pt. This experiment shows that *methylamine is present in alkali-treated fermented urine one part in 100,000 or ten times as much as in case of the unfermented urine.*

(*c*) From 200 liters of fermented urine, 0.15 g. of bases was obtained; here no effort was made to obtain low-boiling bases. The residue solidified largely on cooling and possessed the odor of indol.

(*d*) From 1000 liters of acid-treated urine 0.5 g. of bases was obtained.

(*D*) *Volatile Neutral Substances of Urine.*

The ether solution from the original distillates of urine, after concentrating and treating with acids and alkalies, was further concentrated and distilled with steam. In this manner easily volatile substances were removed from difficultly volatile substances contained in the original distillate and from paraffin and other impurities introduced during the course of the experiments.

The steam distillation gave at least five different substances, *viz.*, sulfur and urinod hydrosulfide in the residual liquor, and a low-boiling thio-compound, urinod, and a high boiling compound in the steam distillate. The latter distillate also contained some sulfur. The presence of sulfur was observed at various stages of the distillation of the urine. It collected as a light yellow solid in the distillate and the condenser, especially during the final distillations. It melted at 114.5–115° and by the Carius method gave 100% S. Since sulfur itself is not volatile with steam, its formation must result from decomposition of hydrogen sulfide or from some other sulfur-containing compound. Since acids were removed by previous treatment with alkalies, hydrogen sulfide[1] cannot be present as such in the neutral fraction; hence it must be formed by decomposition of some neutral complex substance.

The residue from the steam-distillation was light brown in color, was easily soluble in alcohol but difficultly soluble in water. When warmed with acids it slowly gave off the odor of urinod and hydrogen sulfide. It was obtained in larger quantity from 40 liters by direct extraction than from 1000 liters by distillation. If it is *urinod hydrogen sulfide*, this latter result is to be expected. Since it has not been freed from impurities its further investigation will be postponed. Should it prove to be urinod hydrogen sulfide it will account not only for the sulfur and hydrogen sulfide obtained but also for the conjugated form of urinod.

After the neutral fractions were distilled with steam, they were extracted with ether. This ether solution was dried with calcium chloride and shaken in a separating funnel with metallic mercury to remove sulfur. The ether solution was concentrated and finally distilled *in vacuo*.

The first distillate up to about 100° at 30 mm. always contained oil,

[1] See "The Occurrence of Hydrogen Sulfide," p. 2125.

with an allyl mustard oil or ethyl xanthate odor. Since about 0.5 g. of it has been obtained it will be further investigated. The middle fraction contained the urinod, which will be described in the following paper. The final fraction contained an oil boiling at 170° with 29 mm. pressure. The final fraction did not react with semicarbazide.

The yield of neutral substances from urine directly extracted with ether was practically nil. The yield from fermented urines was 2.44 g. from 200 liters; about equal quantities of urinod and indol were obtained. The yield from sulfuric acid-treated urines was 9 g. from 1000 liters.

(E) Residues from Urine.

When urines were distilled with dilute sulfuric acid in the manner described above, two residues were obtained: (1) a chocolate-brown precipitate was formed when the urines were concentrated and (2) a salt-like residue was formed when the urines were evaporated to dryness.

The first,[1] containing chromophoric substances, is a mixture of acids, such as uric, benzoic, etc., for it is completely soluble in alkalies and is reprecipitated by acids. After long standing at ordinary temperatures, an enveloping sublimate of pure crystals of benzoic acid was observed.

The brown portion of the mixture has not been investigated. Apparently its formation is contemporaneous with the formation of urinod. If the latter proves not to be combined with hydrogen sulfide in fresh urine, it may possibly be combined with the chromophoric substance.

The second residue, obtained by evaporating urines and consisting largely of sodium sulfate, was redissolved in water, treated with dilute sulfuric acid and extracted four times with ether. After concentrating the ether solution, needle-like crystals, melting at 183° and identified as hippuric acid, were obtained. Since no other substance could be obtained from the ether, it is concluded that ether extracts from urine only volatile substances and hippuric acid.

Summary.

(1) A review of hitherto known volatile substances of urine shows that they are not responsible for its characteristic odor. Some of these, as ammonia, indol and possibly the phenols, contribute to the composite nature of the odor.

(2) Fresh and fermented urines possess lower vapor pressures than water.

(3) At ordinary temperatures the volatile acidity of urine is very minute.

(4) Direct extraction of urine with ether yields only a little volatile substance.

(5) Distillation of urines with dilute sulfuric acid yields the largest

[1] Edlefsen, *Münch. med. Wochsch.*, **45**, 1615, 2524, mentions a precipitate of creatinine when urines are treated with sulfuric acid. See also THIS JOURNAL, **36**, 415 (1914).

quantities of volatile substances. These distillates were extracted with ether, concentrated and distilled.

(6) The volatile substances were separated into four major fractions: (*a*) acids, (*b*) phenols, (*c*) bases, and (*d*) neutral substances.

(7) The four fractions distil over a wide range of temperatures, hence, they are, respectively, mixtures of many substances.

(8) The principal volatile acid was found to be benzoic acid (formed by hydrolysis of hippuric acid); hydrogen sulfide, the fatty acids up to heptylic acid, and possibly hexahydrabenzoic acid.

(9) The principal phenols are phenol and *p*-cresol; other higher phenols occur in notable quantities.

(10) Methylamine and indol occur as a trace in fresh urines and in larger quantities in fermented urines.

(11) The neutral substances of urine are the most important contributors to the odor of urine. Urinod and at least three other new substances were indicated.

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URINOD, THE CAUSE OF THE CHARACTERISTIC ODOR OF URINE.

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Though many references are made in the literature and text-books[1] to the characteristic odor of normal urine, the direct association of this odor with chemical compounds is loosely made only to ammonia and to several volatile acids.[2] The odor of fresh urine is described as not unpleasant, while the offensive odor of fermented urine is described as ammoniacal. Apparently, the substance that imparts to all urines the characteristic odor has been overlooked by previous investigators. This substance we have isolated and analyzed; its formula is C_6H_8O and we have given to it the name *urinod*.[3]

[1] Black, "The Urine in Health and Disease," **1896**, p. 25: "In the normal condition fresh urine has a peculiar aromatic odor. The smell of urine is said to be due to the presence of phenylic, taurylic and demoluric acids....when it has undergone decomposition in the bladder it exhales an ammoniacal odor and sometimes gives off sulfuretted hydrogen;" Menninger, "Diagnosis of the Urine," **1900**, p. 17: "The odor is sharp and slightly aromatic, and its cause is at present unknown;" Long, "Urine Analysis," **1900**, p. 13: "The odor of urine is not easily described, as in health it is *sui generis* and characteristic;" Holland, "The Urine," **1904**, p. 18: "Putrid urine has the odor of ammonia modified;" Tyson, "Practical Examination of Urine," **1903**, p. 33: "The characteristic odor of urine....is putrescent and ammoniacal, the former (resulting) from decomposition of mucus."

[2] Salkowski, *Z. physiol. Chem.*, **13**, 264 (1889).

[3] Latin, *urina*, urine; *odor*, smell.