

A CONTRIBUTION TO OUR KNOWLEDGE OF THE ACETONE BODIES.

WITH A CLINICAL METHOD FOR THE QUANTITATIVE ESTIMATION OF
DIACETIC ACID AND ACETONE.*

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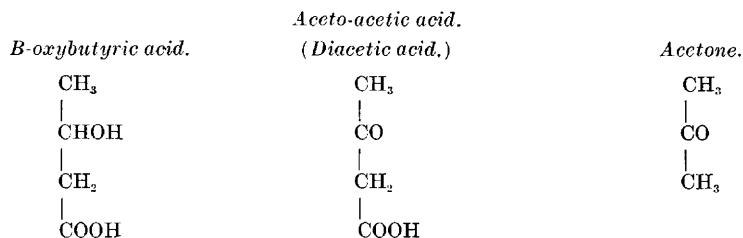
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The scientific and clinical importance of acetone bodies, viz.: acetone, diacetic and oxybutyric acids, is demonstrated by the many investigations which have been made regarding them in the last decade. As evidences of disordered metabolism they are of significant value. The Germans have been the leaders in this promising field of research, but many valuable studies have come from our own countrymen, among which may be recalled the work of Herter, Joslin, Brewer, Baldwin, Hubbard, Brackett, Stone, Low and others.

While much of the earlier work led to conclusions more or less contradictory, the more recent observations permit us to coordinate our knowledge and are full of promise for a rational system of prophylaxis and therapy. It seems worth while, therefore, to review some of the facts as we know them to-day.

THE RELATION OF THE ACETONE BODIES TO ONE ANOTHER.

The relationship of the acetone bodies is a result of their chemical constitution and is made evident by examining their graphic formulæ.



By oxidizing oxybutyric acid we obtain diacetic acid, and when

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diacetic acid is heated to 100 degrees C. it is readily decomposed into acetone and carbonic acid. The latter reaction occurs so readily that hitherto no satisfactory method has been devised of separating the acetone and diacetic acid of the urine and, therefore, all quantitative estimations of acetone represent the combined output of diacetic acid and acetone.¹ By a laborious process we are able, however, to estimate separately the amount of oxybutyric acid.

TABLE 1.—Showing Appearance of Diacetic Acid When Acetone Is Excreted in Considerable Quantities.

Case.	Date.	Diagnosis.	Acetone Tests.		Diacetic Acid Tests.				Amount of Acetone in 24 hours in grams.	Rotation.	
			Lieber's.	Legal's.	Arnold's.	Gerhardt's.	Diazo.	Riegler's.		Before Fermentation.	After Fermentation, Indicating Presence of Oxybutyric Acid.
C. C. K. . .	5, 25, '05	Diabetes. . .	+	0	0	0	0.018		
M. E. M. . .	5, 9, '05	Diabetes. . .	+	0	0	0	. . .	0	0.017		
L. H.	6, 7, '05	Diabetes. . .	+	?	0	0	0.021*		
B. B.	6, 25, '05	Diabetes. . .	+	+	0	0	0.037*		
C. C. K. . . .	6, 27, '05	Diabetes. . .	+	+	0	0.060		
L. B.	5, 10, '05	Diabetes. . .	+	+	+	0	0	0	0.068		
C. C. K. . . .	6, 13, '05	Diabetes. . .	+	+	+	0	0	0	0.399		
F. J. M. . . .	6, 8, '05	Diabetes. . .	+	+	+	0	0	. . .	0.160*		
G. L.	5, 1, '05	Diabetes. . .	+	+	+	+	+	. . .	0.800		
S. C.	5, 5, '05	Diabetes. . .	+	+	+	+	+	. . .	0.616		
G. L.	5, 15, '05	Diabetes. . .	+	+	+	+	+	?	0.657		
G. L.	6, 5, '05	Diabetes. . .	+	+	+	+	+	?	1.113		
S. B. C. . . .	5, 3, '05	Diabetes. . .	+	+	+	+	+	. . .	1.111*		
G. L.	5, 8, '05	Diabetes. . .	+	+	+	+	+	+	1.333*	+0.5	—1.2
J. F.	2, 12, '04	Diabetes. . .	+	+	+	+	+	. . .	0.292	+6.0	—1.0
J. F.	2, 22, '04	Diabetes. . .	+	+	+	+	0.962	+6.8	—0.4

NOTE. +, positive reaction; ?, doubtful reaction; 0, negative reaction; *, amount in one litre.

It seems to be established that oxybutyric acid is the antecedent body from which diacetic acid and acetone are formed. Schwartz² has given diacetic acid by mouth to diabetic animals and has seen a partial conversion into acetone in the urine. Geelmuyden³ gave sodium salts of diacetic acid to normal men who were on a mixed diet and a diet without carbo-

TABLE 2.—Conditions Under Which the Acetone Bodies Are Excreted.

Condition or Disease.	Acetone.	Diacetic Acid.	Maximum Amount of Acetone in 24 hours in grams. Estimations Include Diacetic Acid.		Oxybutyric, Amount of Oxybutyric Acid in 24 Hours in Grams.	Observer.
			Urine.	Breath.		
Normal.	+	..	0.010	v. Jasch.
Normal.	+	..	0.015	Boeri.
Normal.	+	..	0.018	v. Engel.
Normal.	+	0.023	..	Müller
Starvation.	+	..	0.703	Hirschfeld
Starvation.	+	..	0.784	Müller.
Starvation.	+	+	+	2.98 Waldvogel.
Starvation.	+	+	0.699	..	+	1.60 Waldvogel.
Starvation.	+	..	0.431	0.469	..	Joslin.
Starvation and oleic acid	+	..	0.926	0.847	..	Joslin.
Digestive disturbances.	+	+	+	Lorenz.
Digestive disturbances..	+	+	+	Magnus-Levy.
Diarrhea.	+	+	4.000	Beauvy.
Auto-intoxication. . . .	+	+	0.500	Beauvy.
Appendicitis.	+	+	0.400	Beauvy.
Typhoid fever.	+	+	1.000	Beauvy.
Typhoid fever.	+	v. Noorden.
Erysipelas.	+	+	0.800	Beauvy.
Variola.	+	+	0.500	Beauvy.
aricella.	+	+	0.600	Beauvy.
Measles.	+	+	0.800	Beauvy.
Measles.	+	Kulz.
Scarlatina.	+	+	0.700	Beauvy.
itis.	+	+	0.500	..	+	Beauvy.
Diphtheria.	+	+	0.700	Beauvy.
Pneumonia.	+	+	1.000	Beauvy.
Tubercular meningitis. .	+	+	0.600	Beauvy.
nesthesia.	+	+	+	Waldvogel.
Diabetes mellitus. . . .	+	+	10.+	Naunyn.
Diabetes mellitus. . . .	+	+	19.000	Magnus-Levy.
Diabetes mellitus. . . .	+	+	4.200	3.600	..	Schwartz.
Diabetes mellitus (mixed diet)	+	+	+	12.1 Wolpe.
Diabetic coma.	+	+	+	22.8 Wolpe.
Diabetic coma.	+	+	+	16.0 Minkowski.
Diabetic coma.	+	+	+	188.0 Naunyn.
Diabetic coma.	+	+	+	100 to 150 Magnus-Levy.

hydrates, and found an increase in the acetone output in each instance. Minkowski⁴ gave a dog with pancreatic diabetes 10 grams of sodium oxybutyric acid, obtaining as a result a large amount of diacetic acid and acetone in the urine.

The relationship of the acetone bodies is also evident when we consider the order in which they make their appearance in the urine. For example, in a progressive case of diabetes mellitus, the first of these bodies which we find is acetone. As the process advances we next detect diacetic acid, and when diacetic acid has increased to a considerable amount oxybutyric acid appears. When a bad case of diabetes begins to improve, the acetone bodies disappear in the inverse order in which they occurred; oxybutyric acid is the first to go, followed by diacetic acid and finally by acetone. These sequences, ascending and descending, indicate respectively the progressive failure and improvement of oxidation. I think we may look on the excretion of oxybutyric acid and diacetic acid as invariably due to pathologic conditions, and the appearance of any considerable amount of acetone must be regarded in the same light.

CONDITIONS IN WHICH ACETONE BODIES OCCUR.

Recent observation has demonstrated that the excretion of the acetone bodies occurs in a wide number of diseases and under many conditions. Acetone may be excreted in the urine in very small amounts in health under normal conditions. v. Engel⁵ puts the normal limits at from 0.006 to 0.018 gram of acetone in 24 hours. In pathologic conditions, acetone is thrown off in the expired air as well as in the urine, but diacetic and oxybutyric acids can not escape by way of the lungs and are excreted by the kidneys alone. There seems to be no constant ratio between the amount of acetone excreted by the kidneys and by the lungs. Schwartz,⁶ in some of his determinations, found 70 per cent. of the acetone in the expired breath. The acetone bodies are excreted in considerable quantity in starvation; in certain digestive disturbances; in many febrile diseases, such as the exanthemata, pneumonia and typhoid fever; after surgical operations; during pregnancy and labor; after the administration of certain drugs, among which are benzol, antipyrin and morphin; following poisoning with phosphorus, phloridzin, atropin, coal gas, curare and lead. In diabetes their amount may be very large. Naunyn⁷ reports a case in which there was an output of 188 grams of sodium oxybutyric acid in a single day.

Table 2 will indicate the various conditions under which acetone bodies are excreted, with the maximum amounts as noted by numerous observers.

IMPORTANCE AND PRACTICAL VALUE OF ESTIMATING THE ACETONE BODIES.

If acetone bodies are excreted in any considerable quantity, it is positive evidence of improper metabolism. Their study is not only of value because it furnishes us with knowledge important in making a correct diagnosis and prognosis, but also because it affords us a means of following our patient's condition from day to day and of estimating the value of the therapeutic measures which we are using.

In following a case of diabetes mellitus, observations on the acetone body excretion are as important as a knowledge of the sugar output. As soon as diacetic acid appears in the urine, however small may be the amount of glucose excreted, we should recognize that an improper metabolism of fat is in progress. The appearance of oxybutyric acid indicates that the body cells are burning their fuel even less well, that a condition of acid intoxication is imminent, and that coma, however remote, may threaten at any moment.

Recent observations on acid intoxication during confinement and following surgical operations makes their estimation of importance in these conditions. Such studies should be useful in estimating the value of the dietary of large institutions. Here the alienist may find evidence of starvation in cases in which it is suspected but unproved. Knowledge of the progress of acetone body excretion is of value in all chronic diseases with tissue destruction and in acute disease with rapid wasting.

I think that from what has been pointed out it will be conceded that the estimation of the acetone bodies is important and of wider application than is usually supposed.

A CLINICAL METHOD OF ESTIMATING THE ACETONE BODIES.

For the purpose of following the acetone body excretion at short intervals in general practice the usual quantitative methods are too laborious. Furthermore, as it has already been pointed out in many cases, a very large part of the acetone is excreted by the lungs, the determination of the acetone of the breath is an undertaking impossible in general practice, but, unless this is estimated, the exact quantitative determination of the amount of acetone in the urine is in itself of little value. If, however, we could estimate the acetone bodies excreted by the kidneys by means of a short, simple method, which requires little time and is approximately accurate, it would afford us knowledge of great value in following quantitatively the changes from day to day. The need of such a method has induced the present study and has led me to devise a simple clinical method based on the delicacy of the well-known test-tube reaction in urine containing the acetone bodies.

TABLE 3.

Name.	Date.	Acetone.		Diacetic Acid.		Oxybutyric Acid.	Acetone. arms per liter.	
		Lieben's.	Legal's.	Arnold's.	Gerhardt's.			
C. C. K. . .	5, 25, '05	+	0	0	0	. . .	0.014	Lieben's positive, Legal's negative. Acetone = less than 0.030 gms. to 1 liter.
M. E. M. . .	5, 9, '05	+	0	0	0	. . .	0.015	
E. R. W. . .	3, 26, '06	+	0	0	0	. . .	0.019	
N. R. . . .	9, 24, '05	+	0	0	0	. . .	0.029	
L. H. . . .	6, 7, '05	+	?	0	0	. . .	0.021	
E. R. W. . .	3, 12, '06	+	+	0	0	. . .	0.031	Lieben's and Legal's positive, Arnold's negative. Acetone = 0.030 to 0.100 gms. to 1 litre.
St. M. . . .	1, 5, '06	+	+	0	0	. . .	0.031	
E. R. W. . .	1, 29, '06	+	+	0	0	. . .	0.040	
C. C. K. . .	4, 27, '05	+	+	0	0	. . .	0.039	
B. B. . . .	4, 25, '05	+	+	0	0	. . .	0.037	
E. S. . . .	11, 29, '05	+	+	0	0	. . .	0.080	Arnold's positive, Gerhardt's negative. Acetone = 0.100 to 0.200 gms. to 1 liter.
E. R. W. . .	3, 2, '06	+	+	+	0	. . .	0.100	
C. C. K. . .	6, 13, '05	+	+	+	0	. . .	0.155	
F. J. M. . .	6, 8, '05	+	+	+	0	. . .	0.160	
C. C. K. . .	12, 18, '05	+	+	+	+	. . .	0.214	
C. C. K. . .	1, 13, '06	+	+	+	+	+	0.331	Lieben's, Legal's, Arnold's and Gerhardt's all positive. Acetone = more than 0.200 gms. to 1 liter.
G. H. M. . .	6, 16, '06	+	+	+	+	. . .	0.340	
G. L. . . .	4, 1, '05	+	+	+	+	. . .	0.500	
A. B. . . .	1, 29, '06	+	+	+	+	. . .	0.640	
G. L. . . .	5, 1, '06	+	+	+	+	. . .	0.700	
G. L. . . .	3, 19, '06	+	+	+	+	. . .	0.800	
N. R. . . .	12, 7, '05	+	+	+	+	. . .	0.888	
S. B. C. . .	5, 3, '05	+	+	+	+	. . .	1.111	
G. L. . . .	5, 8, '05	+	+	+	+	+	1.333	
G. L. . . .	1, 8, '06	+	+	+	+	+	3.200	
J. F. M. . .	1, 3, '06	+	+	+	+	+	4.000	

The following conclusions are based on the examination of over 600 specimens of urine for the acetone bodies: In 350 of these cases, comparative tests were made with Lieben's, Legal's, Arnold's, Gerhardt's and Ehrlich's diazo tests, in a few Frommer's, Riegler's, Lipliawsky's and Lindemann's reaction were also employed. Quantitative acetone determinations were made in over 100 specimens by the Huppert-Messinger method. Oxybutyric acid was tested for by determining the levo-rotation after fermentation. Of the 350 specimens in which comparative tests were made, 250 were from diabetics; the remaining 100 were from

TABLE 4.—Case of Diabetes Mellitus.

Name.	Ten c. c. Urine + 1 c. c. Standard Ferric Chlorid Solution Diluted with Distilled Water to—	Diacetic Acid and Acetone in Terms of Ace- tone in Grams per Liter.
E. P. A.	Undiluted	0.200
R. B. . .	Undiluted	0.250
E. A. S. .	Undiluted	0.500
J. W. T. .	Undiluted	0.532
S. B. . .	20 c. c.	0.400
J. W. T. .	20 c. c.	0.424
J. W. T. .	20 c. c.	0.532
A. E. S. .	30 c. c.	2.500
M. B. . .	30 c. c.	2.664
F. R. . .	40 c. c.	2.575
L. D. . .	50 c. c.	2.665
M. B. . .	50 c. c.	3.200
G. L. . .	60 c. c.	5.000
M. F. . .	90 c. c.	13.330
G. L. . .	120 c. c.	12.800

TABLE 8.—Case of Cyclic Vomiting.

Date, 1905	Ace- tone.		Diacetic Acid.		
	Lieben's.	Legal's.	Arnold's.	Gerhardt's.	
April 12	+	+	+	+	Height of Attack
April 13	+	+	+	0	Less severe.
April 14	+	0	0	0	Still better.
April 15	0	0	0	0	End of attack.

miscellaneous cases, including starvation, abdominal neoplasms, cyclic vomiting, pregnancy, operative cases, obesity, febrile (grippe, tonsillitis, typhoid, pneumonia, cerebrospinal meningitis) and normal cases.

The reactions finally selected on account of their relative delicacy and ease of application were as follows:

FOR ACETONE.

1. *Lieben's Test*.—Five c. c. of commercial hydrochloric acid were added to 50 c. c. of urine and distilled. To the first 5 c. c. of the dis-

tillate there are added 5 drops of a 30 per cent. solution of sodium hydrate and 10 drops of iodopotassic iodid solution. If acetone is present, a yellowish precipitate of iodoform will be thrown down which may be recognized macroscopically, or under the microscope if present only in traces. This will detect acetone when present only in traces found in normal urine, viz., under 0.020 gram to the liter. Unless special precautions are taken,⁸ alcohol and aldehyd will precipitate iodoform in the same manner; therefore, this should be borne in mind as a possible error.

2. *Legal's Test*.—To 5 c.c. of the native urine there are added 5 drops of a freshly prepared 20 per cent. watery solution of sodium nitroprusside and 5 drops of a 30 per cent. solution of sodium hydrate. This is poured into a white porcelain dish and glacial acetic acid added, drop by drop. The development of a purplish color indicates the presence of acetone. This will detect acetone when present in amounts over 0.030 grams to the liter, i. e., whenever acetone is present in pathologic quantities. A positive Legal's reaction is also given by urines containing creatinin in considerable amounts, aldehyd and alcohol.

FOR DIACETIC ACID.

3. *Arnold's Test*.—Two solutions are necessary. The first solution is made by dissolving one gram of para-amido-aceto-phenon in 100 c.c. of distilled water and adding hydrochloric acid, drop by drop, until the solution, which at first is yellow, becomes colorless; an excess of the acid should be avoided. The second solution is a 1 per cent. watery solution of sodium nitrite. Mix 2 c.c. of the para-amido-aceto-phenon solution with 1 c.c. of the sodium nitrite solution; to this add 3 c.c. of the urine to be tested, then 3 drops of strong ammonia. This is poured into a white porcelain dish and an excess of concentrated hydrochloric acid is added. In the presence of diacetic acid a purplish violet color appears which may readily be seen against the white porcelain.⁹

In my hands this has proved the most delicate reaction for the detection of diacetic acid which we possess. Lipiawsky's modification takes a little more time and seems to be no more delicate. Arnold's test does not react to acetone or β -oxybutyric acid, nor is this color developed in the urine of patients taking salicylic acid compounds.

4. *Gerhardt's Test*.—In this test a stock solution of 50 grams of ferric-chlorid dissolved in 50 c.c. of distilled water is used. One cubic centimeter of this solution is added to 10 c.c. of urine. In the presence of diacetic acid, in considerable amounts, a purplish red color is developed.

This is not as delicate a test as Arnold's. Gerhardt's reaction also

occurs in the urine of patients taking the salicylic compounds, phenacetin, antipyrin, etc.

In my routine work I have come to make use of the above-mentioned four simple reactions. Frommer's test¹⁰ has not, in my hands, proved satisfactory. It seems to answer fairly well to detect acetone when it is present in large amounts, but the color reaction is uncertain and exceedingly slow when only small quantities of acetone are present. Ehrlich's diazo reaction, as first pointed out by Munson and Oertel,¹¹ is a fairly good test for diacetic acid, but is neither as reliable nor as delicate as Gerhardt's test.¹² Riegler's test¹³ for diacetic acid is less delicate than the ferric chlorid test. The same may be said of Lindemann's test.¹⁴

In the 500 specimens of urine examined, whenever diacetic acid was found, acetone was invariably present. A much smaller number were examined for oxybutyric acid; whenever this was present the specimen invariably contained both diacetic acid and acetone. I believe we may feel positive that whenever oxybutyric occurs in the urine it is always associated with acetone and diacetic acid. With an excretion of diacetic acid there is always an acetonuria. Reversely, however, we may have acetone without diacetic acid or oxybutyric acid. Or acetone and diacetic acid without the excretion of β -oxybutyric acid.

We have at present no satisfactory means of estimating the amount of diacetic acid in the urine. All quantitative estimations of acetone represent acetone plus diacetic acid (if this is present) in terms of acetone. Oxybutyric acid may be accurately estimated by a laborious process or may be roughly determined by the degree of polariscopic levorotation after fermentation.¹⁵

In the above series a great many quantitative acetone determinations were made with the purpose of obtaining, if possible, a simple method of following the increase and diminution clinically of the acetone bodies. A few of the typical results, which could be greatly extended if space would allow, are shown in Table 3.

From this table it is evident that there is a fairly definite relationship between the delicacy of the above reactions and the amount of diacetic acid and acetone present, estimated as acetone. This relationship holds good only for fairly fresh urines. After standing for two or three days diacetic acid is broken down into acetone and the amount of acetone estimated is greater than is indicated by the application of the above tests.

When we come to consider a simple method for estimating the acetone bodies when they are present in larger amounts, we meet with the difficulty that the relative amounts of acetone and diacetic acid in these

TABLE 5.—Case of Obesity (Reduction Treatment).

Date. 1906	Amount in 24 hours, c.c.	Specific Gravity.	Reaction.	Albumin.	Ace- tone.		Dia- cetic Acid.		Acetone, grams per liter.	Acetone, grams in 24 hours.	Urea, grams in 24 hours.	Weight, pounds.	
					Lieber's.	Legal's.	Arnold's.	Gerhardt's.					
Jan. 29 .	1825	1026	ac	0	+	0	0	0	0.040	0.073	25.5	241	Mixed diet unre- stricted.
Feb. 4. .	1550	1021	ac	0	+	+	+	0	15.5	237	No carbohydrates for 1 week.
Feb. 9. .	1700	1019	ac	0	+	+	+	+	0.800	1.360	27.2	235	Fat-proteid + 100 gms. wheat bread.
Feb. 12 .	1350	1025	ac	0	+	+	+	0	24.2	233½	Fat-proteid + 200 gms. wheat bread.
Feb. 20 .	1215	1022	ac	0	+	+	0	0	21.3	230	Fat-proteid + 3 slices rye bread and ½ grape fruit.
Mar. 2. .	1320	1024	ac	0	+	+	0	0	0.100	0.132	26.4	226½	Fat-proteid + 3 slices rye bread and ½ grape fruit.
Mar. 12 .	1560	1023	ac	0	+	?	0	0	0.031	0.048	34.3	224½	Fat-proteid + 3 slices rye bread and 1 apple.
Mar. 26 .	1440	1026	ac	0	+	0	0	0	0.019	0.027	34.6	228½	Fat-proteid + 3 slices rye bread and 1 apple.
Apr. 23 .	1410	1021	ac	0	+	0	0	0	216	Fat-proteid + 200 gms. wheat bread.

Loss of weight in 3 months 25 pounds.

TABLE 6.—Fever Case.

Date. 1906	Amount, 24 hours, c.c.	Specific Gravity.	Reaction.	Albumin.	Sugar.	Indican.	Diazo.	Ace- tone.		Dia- cetic Acid.		Acetone, per Liter.	Acetone 24 hours.	Temperature, F.	Diet.
								Lieber's.	Legal's.	Arnold's.	Gerhardt's.				
April 16..	—	1026	ac	0	0	0	0	+	+	+	+	1.132	. . .	101.5	Fluid diet.
April 18..	385	1023	ac	0	0	0	0	+	+	+	+	0.280	0.098	100.5	Fluid diet.
April 19..	440	1023	ac	0	0	0	tr	+	+	+	+	0.300	0.132	100	Fluid diet.
April 20..	770	1011	ac	0	0	0	0	+	+	+	0	0.125	0.096	99.8	Chop, eggs, etc.
April 23.	—	1016	ac	0	0	0	0	+	+	0	0	98.6	Full diet.

TABLE 7.—Very Severe Case of Diabetes Mellitus.

Date, 1904	Amount of Urine in 24 hours, c.c.	Specific Gravity.	Reaction.	Albumin.	Sugar.			Polarization.			Acetone.		Weight, Pounds.	Ammonia, Grams in 24 hours.	Urea, Grams in 24 hours.	Oxybutyric Acid.	Diacetic Acid.		Grams per Liter.	Acetone, Grams in 24 hours.
					Fehling's.	Fermentation.	Grams in 24 hours.	Before Fer- mentation.	After Fermen- tation.	Difference.	Lieber's.	Legal's.					Arnold's.	Gerhardt's.		
Oct. 10, 1905	1380	1031	ac	0	+	+	69.0	+	+	130.	1.17	23.5	..	0	0
Mar. 13.	1100	1028	ac	0	+	+	7.7	+	+	139.	..	30.8	..	+	+
April 10.	1000	1029	ac	0	+	+	6.9	+	+	137.	..	30.0	..	+	+
May 1., .	1600	1028	ac	0	+	+	18.4	+0.3	-0.8	1.1	+	+	136.5	+	20†	20†	0.500	0.800
May 8., .	..	1029	ac	0	+	+	..	+0.5	-1.2	1.7	+	+	+	30†	30†	1.333	..
May 15.,	1200	1022	ac	0	+	+	+	+	20†	20†	0.598	0.657
June 5.,	1300	1028	ac	0	+	+	20.9	+	+	136.	20†	20†	0.856	1.113
Dec. 18, 1906	1500	1025	ac	0	+	+	30.0	+	+	128.5	..	19.5	..	20†	20†	0.700	1.050
Jan. 1., .	..	1030	ac	0	+	+	+	+	20†	20†	0.664	..
Jan. 8., .	1550	1030	ac	0	+	+	9.4	+	+	127.	..	21.8	..	50†	50†	3.200	4.992
Mar. 19.	..	1027	ac	tr.	+	+	+	+	126.	20†	20†	0.800	..
May 31.,	..	1023	ac	0	+	+	+	+	111.	..	20.7	..	120†	120†	12.800	..
June 4.,	2300	1023	ac	tr.	+	+	143.8	+	+	60†	60†	6.400	14.720
June 11.,	3210	1024	ac	tr.†	+	+	106.9	+3.2	-0.9	4.1	+	+	108.2	6.55	32.1	57.8	60†	60†	5.000	16.050
July 7.,	2340	1029	ac	tr.	+	+	124.0	+3.7	-0.5	4.2	+	+	106.0	3.68	18.8	21.4	40†	40†
July 13.,	1900	1026	ac	tr.	+	+	45.6	+1.4	-1.0	2.4	+	+	105.0	4.39	20.0	38.0	50†	50†
July 29.	3340	1028	ac	tr.	+	+	100.0	+	+	100†	100†
Aug. 14.	3000	1025	ac	tr.	+	+	81.0	+	+	70†	70†
Sept. 1.	3700	1025	ac	tr.	+	+	130.0	+	+	80†	80†

Died in coma, Sept. 15, 1906. † Number of cubic centimeters to which Gerhardt's test was diluted as described.

cases is very variable. A rough idea, however, can be obtained in the following manner:

To 10 c.c. of the urine to be tested is added 1 c.c. of Gerhard's test solution of ferric chlorid (made as described above). This is thoroughly mixed and allowed to stand for about two minutes (to permit the color reaction to fully develop). Then filter into a test-tube, one-half inch in diameter, and compare the color with a similar test-tube filled with the standard solution of ferric chlorid described above. If the color of the urine mixture is darker than that of the ferric chlorid solution, distilled water is added until the color is approximately of the same depth as that of the ferric chlorid solution.

From Table 4, made from the results obtained from a number of specimens examined by this method, a rough idea may be had of the amount of acetone and diacetic acid in terms of acetone which such dilutions indicate.

The practical application of this method is conducted in the following manner: The urine under examination is tested by Gerhard's reaction. If this is positive, we know that diacetic acid and acetone are present in an amount in terms of acetone in excess of 0.200 gram per liter. If there is a strong Gerhard's reaction, we dilute the test solution in the manner described above until the color approximates that of the standard ferric-chlorid solution.

If the final dilution indicates an acetone content of half a gram per liter or over, we may be reasonably sure that oxybutyric acid is present and may proceed to its determination by means of the polariscope. If Gerhard's test is negative, we apply successively Arnold's, Legal's and Lieben's tests, in the order named. A positive Arnold's reaction indicates something over 0.100 gram of acetone per liter. A positive Legal's reaction indicates 0.030 gram of acetone per liter. Lieben's test, if positive while Legal's is negative, indicates the presence of acetone in an amount within the normal limits.

Lieben's.	Legal's.	Arnold's.	Gerhardt's.	Amount of Acetone in Gram per Liter.
Positive.	0	0	0	Less than 0.030
—	Positive.	0	0	0.030 to 0.100
—	—	Positive.	0	0.100 to 0.200
—	—	—	Positive.	More than 0.200

The above tests, with the exception of the polariscopic determination of oxybutyric acid and the distillation required by Lieben's test, may easily be completed inside of ten minutes.

Tables 5, 6, 7 and 8 indicate the method of recording cases and the comparative results obtained by actual quantitative determinations of acetone by the Huppert-Messinger method.

I wish to express my obligation to Dr. E. G. Janeway, in whose laboratory this work was conducted and to whom I am indebted for a large part of the material on which these observations were made.

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