STUDIES OF ACIDOSIS.

THE TITRATION OF ORGANIC ACIDS IN URINE.

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Description of Method.

urine, roughly measured, are thoroughly mixed with nely powdered calcium hydroxide, allowed to stand ninutes with occasional stirring, and then passed ry folded filter. This treatment removes carbonates ates. To 25 cc. of the filtrate in a 125 to 150 cc. clear glass[1] one adds 0.5 cc. of 1 per cent phenol- olution, and 0.2 N hydrochloric acid from a burette ed not be measured) until the pink color just dis- I = approximately 8). 5 cc. of 0.02 per cent tro- olution are then added. As the indicator solution is horoughly mixed with the urine by shaking the tube; ution is omitted some of the tropeolin OO may be . Finally 0.2 N hydrochloric acid is added from the il the red color equals that of a standard solution .6 cc. of 0.2 N HCl, 5 cc. of tropeolin OO solution, o a total volume of 60 cc. When the end-point is sufficient water is added to the titrated solution to lume equal to that of the 60 cc. standard solution nilar tube as a color control.

ring the color of the titrated solution with that in the is convenient during the titration to hold the two

e tubes of Pyrex glass made by the manufacturers for urea s by the Van Slyke and Cullen technique. The tubes are diameter, 200 mm. long, and uniform in size.

tubes side by side between the thumb and fingers, the tube containing the urine being the one held nearer to the tips of the fingers where it can be easily shaken as the 0.2 N acid is run in from the burette.

Sometimes it is desirable to use a similar technique for the phenolphthalein end-point also. In this case a tube of urine filtrate to which no phenolphthalein is added serves as a standard.

We have found that as the final end-point with tropeolin OO is approached comparison of colors is somewhat facilitated by placing the two tubes side by side in a comparator of the form described by Dernby and Avery, although with practice the end-point may be located within 0.1 cc. by merely holding the tubes together as described above.

Calculation.

From the volume of 0.2 N HCl used to titrate from the end-point of phenolphthalein to that of the tropeolin OO, the amount, usually 0.7 cc., is subtracted which is utilized in a similar titration of a control determination in which water is substituted for the urine. The volume of 0.2 N HCl thus corrected represents the approximate organic acid content of the urine sample, plus the creatine and creatinine, and an amount of amino-acids ordinarily negligible.

In order to calculate the results in terms of cc. of 0.1 N organic acid per liter, the figure representing the cc. of 0.2 N HCl used in the titration is multiplied by 80 (by $\frac{1,000}{25} = 40$ in order to transfer figure from 25 cc. to 1,000 cc. of urine, and by 2 to change from 0.2 N to 0.1 N terms).

Correction for Creatinine.—A 0.1 M solution of creatinine (11.32 mg. per cc.) titrates in the above determination as a 0.1 N solution of organic acid. Therefore, in order to correct for the creatinine, the cc. of 0.1 N organic acid per liter calculated from the above titration may be diminished by

$$\frac{\text{mg. creatinine per liter urine}}{11.32} \text{ or by } \frac{\text{mg. creatinine N per liter urine}}{4.2}.$$

The simplest way is to subtract the creatinine correction directly from the cc. of 0.2 N acid used in the titration, and mul-

ifference by 80. In this case the correction is $\frac{1}{80}$ as
e above; *i.e.*, cc. correction =
$\frac{\text{ine per liter urine}}{906}$ or $\frac{\text{mg. creatinine N per liter urine}}{336}$.

	cc.
l used in titration	7.6
on found in blank analysis ..... 0.7 cc.	
ne correction for 500 mg. creatinine N per ine. Correction = $\frac{500}{336}$ cc. = ... 1.2 "	
l correction ..... 1.9 cc.	
l titration figure = 7.6 − 1.9	5.7
anic acid per liter = 80 × 5.7	456.0

OO was preferred by us as indicator for the final In neutral solution it gives nearly the same yellow ne, but so much more intense that a water solution of rochloric acid with the indicator can be used as a color ithout the use of a comparator. Very dark urines greater dilution, but such are not often encountered. vantage of this indicator is that its maximum acid reached even at pH 2.7, so that if too much HCl is ne titration the solution becomes redder than the This particular advantage is possessed in much less ne three indicators mentioned below as alternatives. licators that may be used are methyl orange, tetra-olsulfonephthalein (bromophenol blue, Clark and Lubs), ylaminoazobenzene. To some eyes the color change ese dyes may be more readily detected than that of O. The two azo dyes are not much different in color olin OO, both changing from yellow to red, but the ol blue turns from blue to a clear yellow on acidifying, a very different alternative. To the authors the tro-nd-point appeared the most satisfactory, however.

Theoretical Basis of Method.

The method is based on the following previously know

1. Relatively little strong mineral acid is required to the hydrogen ion concentration of a water solution from 2×10^{-3} if the only electrolytes present are alkali salts o acids, such as sulfates and chlorides.

2. If the salt of a weak acid is present, however, the of nearly a full molecule of hydrochloric acid for each mol such salt is necessary in order to cause the above chang drogen ion concentration. The organic acids known to normal and pathological urines, in amounts sufficient to b titatively significant in the total acid excretion of th belong to the class of weak acids whose salts behave in th manner.

3. The only mineral acids found in significant amounts which belong to the class of weak acids, and therefore fo which show the above behavior, are phosphoric and c acids.

4. Very weak bases form salts which behave like thos weak acids. Creatinine is titrated almost quantitati changing the hydrogen ion concentration from 10^{-3} to 2 and creatine to about 60 per cent. Aside from the t amino-acids, these appear to be the only bases of this kind in considerable amount in human urine.

Effect of the Different Organic Acids of the Urine on the T

The titration figure obtainable by titrating between t drogen ion concentrations a solution containing the sal acid of a known dissociation constant may be calcul follows:

From the law of mass action:

$$(1)\quad H^+ = k\frac{HA}{A'}$$

H^+ = hydrogen ion concentration in terms of norm
A' = anion of acid.
k = dissociation constant of the acid.
HA = free, undissociated acid.
BA = salt of the acid.
λ = degree of dissociation of the salt into Na an

he salt of the acid is present, and dilutions are of the
s used in titrations (0.1 to 0.01 M), the equation be-
ctically

$$(2) \qquad H^+ = \frac{k}{\lambda} \times \frac{HA}{BA}$$

the high dilutions encountered approaches unity, it
proximate calculations be neglected.
n 1 may then be expressed as

$$(3) \qquad H^+ = k \times \frac{HA}{BA} \text{ or } \frac{HA}{BA} = \frac{H^+}{k}$$

tic acid $k = 1.8 \times 10^{-5}$.
pH equals 8, or $H^+ = 1 \times 10^{-8}$, we therefore have in
f acetic acid $\frac{HC_2H_3O_2}{BC_2H_3O_2} = \frac{10^{-8}}{1.8 \times 10^{-5}} = \frac{1}{1{,}800}$. One
801 parts, or 0.05 per cent, of the acid is free.
H = 2.7, $H^+ = 2 \times 10^{-3}$, and we have $\frac{HC_2H_3O_2}{BC_2H_3O_2} =$

$\frac{}{^{-5}} = \frac{200}{1.8}$.

I of 2.7, therefore, $\frac{200}{201.8}$, or 99.2 per cent, of the acid is
anging the hydrogen ion concentration of an acetate
rom the slightly alkaline reaction of 10^{-8} N (or a pH of
acid reaction of 2×10^{-3} ((pH = 2.7) approximately
ion of 0.002 N HCl) therefore requires an amount of
l in molecular equivalents to 99.15 per cent of the total
resent.
e different acids which occur or may occur in human
values in Table I are calculated. The values of the
are for 25° unless otherwise indicated.
rison of the results so calculated with those experimen-
ined in titrating solutions of some of these acids is satis-
s shown in Tables III, IV, and VI of the experimental
his paper. The conclusion seems justified that the
s carried out estimates certainly over 90 per cent of the
ids of the urine, and presumably over 95 per cent, since

a higher titration value by 3 or 4 per cent is obtained for acids excreted as ammonium salts. The data for carboni phosphoric acids indicate the necessity for their removal the organic acids are titrated.

TABLE I.

Calculated Titration Values of Weak Acids of the Urine.

Acids.	Dissociation constant.	Acid free at		Calcu propor ac determ titr from pH
		$H^+ = 10^{-8}$ N pH = 8	$H^+ = 2 \times 10^{-3}$ pH = 2.7	
		per cent	*per cent*	*per*
Organic.				
Uric*	1.5×10^{-6}	0.5	99.9	99
Acetic†	1.8×10^{-5}	0.0	99.2	99
β-hydroxybutyric‡	2.0×10^{-5}	0.0	99.0	99
Lactic†	1.4×10^{-4}	0.0	93.5	93
Acetoacetic‡	1.5×10^{-4}	0.0	93.1	93
Citric§	2.0×10^{-4}	0.0	91.0	91
Formic*	2.1×10^{-4}	0.0	90.6	90
Hippuric†	2.2×10^{-4}	0.0	90.2	90
Mineral.				
$H(NaHPO_4)$‖	2.0×10^{-7}	2.5	100	97
$H(HCO_3)$¶	3.5×10^{-7}	4.2	100	95

* His and Paul.

† Ostwald.

‡ Henderson and Spiro.

§ Shown by Amberg and McClure to occur in amounts equivalen to 70 cc. of 0.1 N acid in a normal 24 hour urine. The titration val citric acid given in Column 5 are those directly determined by Sö The constant is estimated from them.

‖ Sörensen.

¶ Kendall.

Effect of Weak Bases of the Urine on the Titration.

The amount of strong acid required to change the pH solution of a weak base from 8 to 2.7 may be calculated fro dissociation constant K_b.

$$K_b = OH' \times \frac{\text{salt of base}}{\text{free base}} = \frac{10^{-14}}{H^+} \times \frac{\text{salt of base}}{\text{free base}}$$

of base" represents the amount combined with acid. therefore, salt of base, or $\frac{\text{acid combined with base}}{\text{free base}} =$

$\frac{}{} = K_b \times 10^6$. At pH 2.7, or $H^+ = 2 \times 10^{-3}$, the ratio

$\frac{2 \times 10^{-3}}{10^{-14}} = 2\ K_b \times 10^{11}$. The difference between the

ıd by a given base at pH 8 and that bound at pH ents the amount required to titrate between the two Table II contains a list of the weak bases of the urine, constants and the proportion of an equivalent of HCl

TABLE II.

lculated Titration Values of Organic Bases of the Urine.

	Basic dissociation constant. K_b	Base free at		Proportion estimated by titrating with HCl from $H^+ = 10^{-8}$ to $H^+ = 2 \times 10^{-3}$	
		$H^+ = 10^{-8}$	$H^+ = 2 \times 10^{-3}$	Calculated.	Observed.
		per cent	*per cent*	*per cent*	*per cent*
...	0.0015×10^{-11}	100	99.7	0.3	0.2
...	1.81×10^{-11}*	100	24.0	76.0	99
...	3.57×10^{-11}*	100	12.3	87.7	60
...	1.5×10^{-5}†	6.2	0.0	6.2	5.3-6.0

and Wood.
ed at 40°, Wood.
Kato, and Sosman.

o titrate each from pH 8 to pH 2.7, calculated as above The constants are from data obtained at 25°, except ne and creatinine. In Column 5 results are brought om Table V, showing the amounts of HCl bound by the ases in the titration, as determined experimentally. both by observation and calculation, practically with- on the results of the titration, even when the urea con- is at the maximum observed in human urine.

ilable data on the K_b of creatine and creatinine do not ulated results corresponding so closely with those experi- obtained as do the data on the other substances requir-

ing consideration. The divergence is perhaps due to th
that Wood's values for K_b of creatine and creatinine were
mined at 40°, while the titration is performed at 20°.
evident, however, that practically all the creatinine is titra
organic acid. The amount of this substance excreted
between 13 and 27 mg. per kilo of body weight per 24
(Folin, 1905). The mean, 20 mg., would neutralize 1.8 cc.
N acid per kilo or 108 cc. for a 60 kilo individual.

Creatine when present titrates to about 60 per cent
organic acid; but it is excreted by adults only in conditions i
ing rapid autolysis of muscle tissue, and would therefore no
rule, require consideration.

Ammonia is titrated to the extent of 5 to 6 per cent, bu
actual effect of the presence of organic acids as ammonium
than fixed alkali salts is to make the results of the titration
most of the acids approximate more closely the theoretical v
as shown in Table IV. The ammonium salts of the organic
titrate 2.3 to 4.6 per cent more completely than the sodium
not 6.2 per cent more completely, as would be theoretical
pected, and as is approximately realized for the ammonium
of hydrochloric and sulfuric acids. The observed positive am
error is such as to make the results obtained with all but the
est organic acids approximate more closely to 100 per cent th
results obtained in the absence of ammonia. The tender
the ammonia error to correct the opposite error in the o
acid titration is enhanced by the fact that ammonia and o
acid excretion tend to run parallel, particularly when acid
tion is abnormally high, as in diabetic acidosis. For the re
therefore, that the ammonia correction is not great and i
nature actually to diminish, as a rule, the other error i
determination, it has seemed not only simpler but bet
attempt no correction for it in urine analyses.

Effect of Amino-Acids on the Titration.

Amino-acids if present in large amount would be distu
factors, as at an H^+ of 2×10^{-3} they bind with their NH_2 g
considerable amounts of acid. Glycocoll, which does not
much from the other monoamino-acids in this respect,

molecule of HCl at this H^+. The amount is calculated
ys:

id constant for glycocoll is 3.4×10^{-10}, the basic constant $2.9 \times$
calculated by Winkelblech from conductivity measurements.
acid constant we have by calculating as above:

OOH free at $= 10^{-8}$ N	— COOH free at $H^+ = 2 \times 10^{-3}$ N	Proportion of COOH group estimated by titration from $H^+ = 10^{-6}$ to $H^+ = 2 \times 10^{-3}$
er cent	*per cent*	*per cent*
96.7	100	3.3

unction of the NH_2 group is similarly calculated from the
nstant, $K_b = 2.9 \times 10^{-12} = (OH)' \times \frac{\text{glycine chloride}}{\text{free glycine}}$ or

$$\frac{CH_2\text{-}NH_2}{CH_2\text{-}NH_2\ HCl} = \frac{(OH)'}{2.9 \times 10^{-12}} = \frac{10^{-14}}{H^+ \times 2.9 \times 10^{-12}} =$$

9.

these values we calculate:

H_2 free at $= 10^{-6}$ N	— NH_2 free at $H^+ = 2 \times 10^{-3}$ N	Proportion of NH_2 group estimated by titratin with HCl from $H^+ = 10^{-6}$ to $H^+ = 2 \times 10^{-3}$
er cent	*per cent*	*per cent*
100	63.3	36.7

tal consumption of HCl by both COOH and NH_2 groups
tration should be, according to the above calculation,
.367 = 0.40 molecule of HCl per 1 molecule of glycine
The actual amount observed by Sörensen was 0.385

ther monoamino-acids apparently bind similar amounts
The constants for leucine and alanine were determined
elblech as follows: leucine, $K_a = 3.1 \times 10^{-10}$, $K_b =$
$)^{-12}$; alanine, $K_a = 9.0 \times 10^{-10}$, $K_b = 3.8 \times 10^{-12}$.
g to these, leucine would require in the titration 0.38
of HCl; alanine 0.36, nearly the same as glycocoll. The
Table VII for the mixture of all the monoamino-acids
from casein are in the same neighborhood (44 per cent).

The amino-acid nitrogen constitutes 1 to 2 per cent o total urinary nitrogen (Van Slyke, 1913–14; Henriques). daily excretion of 14 gm. of nitrogen, 2 per cent would ind 200 cc. of 0.1 M amino-acids. The neutralizing power of suc amount of amino-acids in the titration would be about 8 of 0.1 N hydrochloric acid.

Our knowledge of the nitrogenous constituents of the indicates the presence of no weak bases, aside from those cussed, in quantities sufficient to affect markedly the or acid titration under discussion, and the nitrogenous excr products have been so thoroughly studied that it is unlikely any quantitatively important substances with definitely properties have been overlooked.

It therefore appears that in titrating the 24 hour urine adult of average size for organic acids, as described in this p about 100 cc. of the 0.1 N organic acid estimated is in realit to creatinine and creatine, 80 cc. or less to amino-acids, an remainder to organic acids.

EXPERIMENTAL.

Titration of Organic Acids in Water Solutions.—A 20 cc. po of each acid, of approximately 0.1 N concentration, was tit in a 100 cc. test-tube with either 0.1 N sodium hydroxide o N ammonium hydroxide to neutrality with 0.5 cc. of 1 per phenolphthalein. 1 cc. of 0.1 per cent tropeolin OO was added, and the solution titrated back with 0.2 N HCl to pH using 0.002 N HCl solution as standard. The results are in Tables III and IV.

Titration of Weak Bases in Water Solutions.—Solutions o bases in 25 cc. portions were brought to pH 8 by addition of NaOH or 0.2 N HCl until a barely visible pink color was rea then tropeolin OO was added and the solution titrated to pH The results are given in Table V.

Effect of Concentration of Phenolphthalein on its End-Poi Presence of Ammonium Salts.—The concentration of ph phthalein to some extent affects the pH at which the pink is just visible. If there is but little indicator present a gr part of it must be in the colored form to give a perceptible

TABLE III.

Titration of Sodium Salts of Organic Acids.

Acid.	(A) 0.1 N NaOH to neutralise acid to phenol-phthalein.	(B) 0.2 N HCl to titrate back to pH 2.7 with tropeolin OO.	(C) Average 0.2 N HCl corrected for blank.	(D) Organic acid determined. $\frac{200\ (C)}{(A)}$	Organic acid theoretically titratable from pH 8 to pH 2.7 (from Table I).
	cc.	*cc.*	*cc.*	*per cent*	*per cent*
Blank	0.1	0.50	0.00		
Acetic	20.00	10.60	9.95	99.5	99.4
		10.50			
Citric	19.86	9.30	8.88	89.4	91.0
		9.35			
Lactic	20.28	9.90	9.40	92.7	93.5
		9.90			
Hydrochloric	20.00	0.70	0.20	1.0	

TABLE IV.

Titration of Ammonium Salts of Organic Acids.

Acid.	(A) 0.1 N acid present.	(B) 0.1 N NH_4OH to neutralise acid to phenol-phthalein at pH 8.	(C) 0.2 N HCl to titrate back to pH 2.7.	(D) Average 0.2 N HCl corrected for 0.5 cc. blank.	(E) Proportion of organic acid determined $\frac{200\ (D)}{(A)}$	Proportion of NH_4 salt theoretically titratable; *i.e.*, that for acid calculated in Table I + 6.2 per cent for NH_4 present.	Differences between average percentage of Na salt and NH_4 salt titrated.
	cc.	*cc.*	*cc.*	*cc.*	*per cent*	*per cent*	*per cent*
Acetic	19.68	20.51	10.70	10.25	104.1	105.6	4.6
	19.68	20.47	10.80				
Citric	21.04	21.55	10.30	9.79	93.0	97.2	3.5
	21.04	21.51	10.27				
Lactic	20.06	20.96	10.03	9.50	94.7	99.7	2.3
	20.06	20.96	10.03				

than when the total amount of indicator is greater. Consequently the amount of extra alkali required to make a solution of an ammonium salt show pink with phenolphthalein is some-

TABLE V.

Observed Behavior of Weak Bases when Titrated from pH 8 to pH

Base.	Amount present in the 25 cc. of solution titrated.		0.2 N HCl required in titrating from pH 8 to pH 7.	Proportion of base titrated.	Proportion of b calculated as titr from dissociati constant (Table
	gm.	*cc. 0.8 N*	*cc.*	*per cent*	*per cent*
Urea.....	1.000	83.3	0.1	0.12	0.3
Creatine.....	0.200	7.6	4.1	60.0	87.7
Creatinine.....	0.100	4.41	4.32	97.8	76.0
	0.200	8.83	8.80	99.7	
Monoamino-acids	0.100	7.37*	3.25	44.2	36.0–40.0 for
from casein	0.200	14.63	6.37	43.5	cine, leucine
	0.200	14.63	6.29	43.0	alanine.
Ammonia (as		12.50	0.67	5.4	6.2
$(NH_4)_2SO_4$).....		12.50	0.75	6.0	
Ammonia (as		9.82	0.53	5.4	6.2
NH_4Cl).....			0.52	5.3	

* Calculated on a nitrogen content of 10.3 per cent. The prepar was made by hydrolyzing casein with sulfuric acid, precipitating the with phosphotungstic acid, and concentrating the filtrate to dryness u reduced pressure after the phosphotungstic and sulfuric acids had removed.

TABLE VI.

Effect of Phenolphthalein Concentration on End-Point in Presence o Ammonium Salts.

0.05 M $(NH_4)_2SO_4$.	1 per cent phenol-phthalein.	0.1 N NaOH to turn pink to phenol-phthalein.	0.2 N HCl to change from phenolphthalein end-point to pH 2.7.		Propo of amm titra from ph phths end-po pH
			Uncorrected.	Minus 0.5 cc. for correction.	
cc.	*cc.*	*cc.*	*cc.*	*cc.*	*per*
25	0.1	0.85	1.42	0.92	7.
25	0.2	0.65	1.36	0.86	6.
25	0.5	0.45	1.20	0.70	5.
25	1.0	0.45	Too cloudy with precipitated phenolphthalein to titrate.		

pendent on the amount of indicator used. This is shown esults in Table VI. It is desirable to use in performing tions 0.5 cc. of 1 per cent phenolphthalein solution, as rather than the indefinitely measured drop or two which n ordinary titrations.

TABLE VII.

Titration of Organic Acids Added to Urine.

0.1 N organic acid added to 100 cc. urine.	0.2 N HCl used in duplicate titrations of 25 cc. urine filtrate.	Average titration figure minus that for urine alone.	0.1 N added organic acid per liter diluted urine.		Proportion of added organic acid determined.
			Found.	Added.	
cc.	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*
0	3.00 3.00				
25	4.55 4.53	1.54	123	125	98.4
50	6.20 6.15	3.17	253	250	101.2
100	9.15 9.10	6.13	490	500	98.0
0	2.87 2.87				
25	4.25 4.20	1.36	109	117*	93.2
50	5.50 5.60	2.68	214	236*	90.7
100	8.30 8.25	5.41	432	472*	91.6

1 N lactic acid used in this experiment had the factor 0.945.

on of Known Amounts of Organic Acids Added to Urine.— ortions of a mixed sample of normal urine were mixed ions of 25, 50, and 100 cc. respectively of acetic or lactic ach mixture was then diluted to 200 cc., and 100 cc. were treated as previously described for determination.

TABLE VIII.

Excretion of Organic Acids with Creatinine Correction.

Data from hospital patients.

Subject.	Weight.	Condition.	Urine excretion.								
			Period.	Volume.	Creatinine N.		0.2 N HCl used in titrating from pH 8 to pH 2.7.		0.1 N organic acid content.		
									Total.		Per kilo.
							Duplicates.	Average minus 0.6 cc. correction for blank.	Uncorrected for creatinine.	Corrected for creatinine.	Uncorrected for creatinine.	Corrected for creatinine.
	kg.		*hrs.*	*cc.*	*gm.*	*cc. 0.1 M*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
Z	60	Myocarditis, decompensation on admission.	12 (day)	658	0.240	57	7.0, 6.9	6.35	334	277		
			12 (night)	946	0.261	62	4.3, 4.1	3.60	272	210		
			24	1,604	0.501	119			606	487	10.5	8.1
O	60	Myocarditis, some decompensation.	12 (day)	707	0.223	53	5.1, 5.25	4.57	258	205		
			12 (night)	744	0.216	51	5.0, 5.0	4.40	262	211		
			24	1,451	0.439	104			520	416	8.2	6.9
C	55	Chronic aortic endocarditis.	12 (day)	242	0.141	34	15.1, 15.3	14.60	282	248		
			12 (night)	332	0.195	46	12.8, 12.9	12.25	325	279		
			24	574	0.336	80			607	527	11.0	9.6
D	62	Chronic myocarditis with decompensation.	12 (day)	647	0.226	54	5.6, 5.7	5.05	261	207		
			12 (night)	750	0.196	47	4.4, 4.3	3.75	225	178		
			24	1,397	0.422	101			486	385	8.8	7.0

are given in Table VII. The results are essentially s those obtained with acetic and lactic acids in pure ions.

id Excretion by Individuals with Normal Metabolism.

given are sufficient only to indicate the usual excre- anic acids; the possible normal variations, particularly ual conditions, may be greater. The figures of Table

TABLE IX.

ır Excretion of Organic Acids by Normal Young Men.

.	Weight.	24 hour urine.			
		Volume.	0.1 N organic acids uncorrected for creatinine.*		Total N.
	kg.	*cc.*	*cc.*	*cc. per kg.*	*gm.*
.....	54.4	1,000	492	9.0	9.3
.....	68.0	1,650	657	9.8	11.5
.....	68.0	975	583	8.5	11.7
.....	62.1	1,500	531	8.5	13.2
.....	68.0	1,150	412	6.1	7.8
.....	56.6	1,500	453	8.0	10.0
.....	68.4	1,000	490	7.2	8.7
.....	57.2	1,400	521	9.1	9.0
.....	82.6	1,100	748	9.1	15.5
.....	87.0	1,300	493	5.7	13.2
.....	56.2	1,100	420	7.5	11.2
.....	61.2	700	499	8.2	10.0
.....	56.6	1,300	547	9.7	12.1
.....				8.2	

atinine correction would reduce the total organic acid figure c. per kilo.

rom afebrile heart patients, with apparently normal . The day periods are from 6 a.m. to 6 p.m., the ds from 6 p.m. to 6 a.m. The data of Table IX are ies of healthy young men. The figures indicate that excretion of organic acids uncorrected for creatinine n about 280 to 750 cc. of 0.1 N acid per 24 hours, 6 to 11 cc. per kilo of body weight. The creatinine reduces the figures to 240 to 600 and 4.7 to 9.6 cc. y.

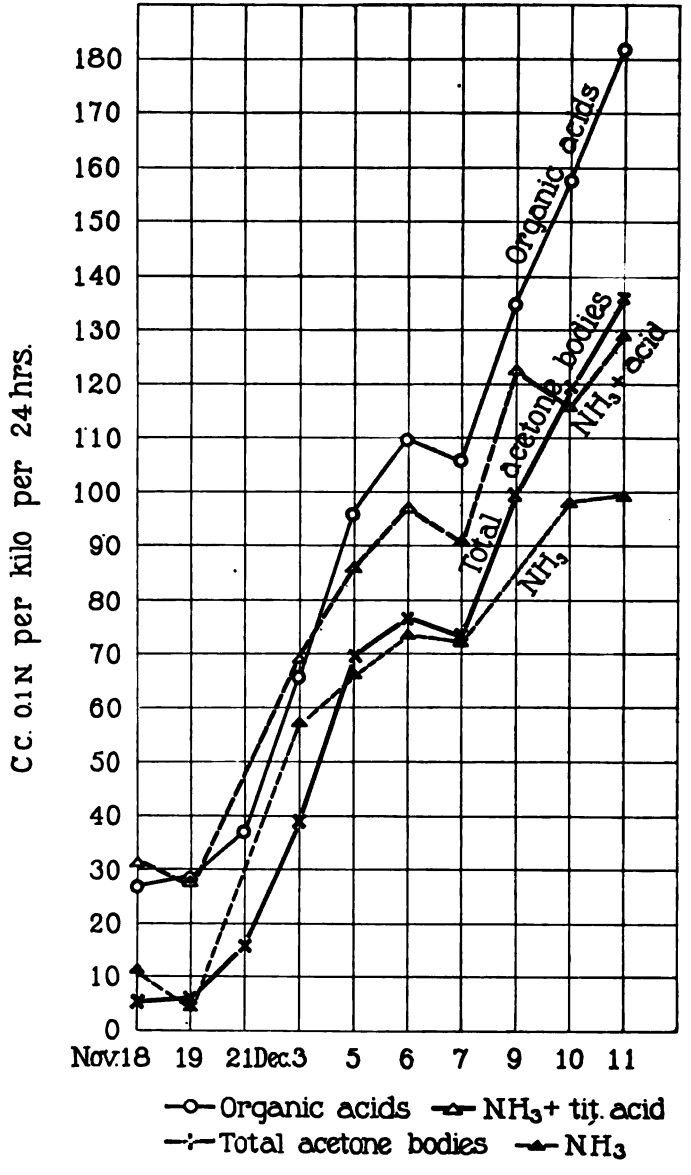

FIG. 1. Excretion in a case of diabetic acidosis.

n of Total Organic Acid Excretion with Acetone Bodies Excretion in Diabetes.

a given in Fig. 1 were obtained with the only case of cidosis which we have studied since the organic acid nethod has been available. Although all the data are case, they nevertheless represent every stage of diabetic rom the time when it was slight, with little ketonuria, e point of coma, with tremendous ketonuria. The as a child of 2 years, weighing 8 kilos. The organic

TABLE X.

d Excretion in a Non-Fatal Case of Methyl Alcohol Poisoning.

	CO_2 bound as bicarbonate by 100 cc. of plasma.	Excretion per liter urine.						
		Creatine.	Creatinine.	Total 0.1 N organic acids.*	0.1 N acetone bodies.	0.1 N lactic acid.	0.1 N formic acid.	Undetermined 0.1 N organic acids.
	cc.	gm.	gm.	cc.	cc.	cc.	cc.	cc.
.	36.4	0.202	0.558	2,042		173	274	1,595
.	36.0	0.283	1.000	2,076	481	83		1,512
.	86.2	0.535	0.800	1,377	143	30	130	1,074
.	76.7	0.300	0.590	262				
.		0.180	0.538	129				
.		0.137	0.557	141				
.				220†				
.		0.105	0.378	86				
.		0.024	0.476	138				
.								

ed for creatine and creatinine.
" " " " estimated not determined.

es recorded in Fig. 1 are not corrected for creatine and , so that they are higher than, though parallel to, the ganic acid excretion. The "total acetone bodies," butyric acid, acetoacetic acid, and acetone were deter- the gravimetric method of Van Slyke (1917), the ammo- cribed by Van Slyke and Cullen, and the titratable acid thod of Folin (1903).

dent from the chart that the organic acids of the urine, d by the technique outlined above, paralleled the ace-

tone body excretion with a high degree of accuracy thro
stages of the acidosis, the parallelism being more accurat
that of the ammonia, or even the ammonia plus titratabl

It appears that the rise above the normal output in
acid excretion may be used as an approximate measure
acetone body excretion in diabetes, the determination of
acids being as simple as that of ammonia and less influen
other factors, such, in particular, as alkali administration.

Organic Acid Excretion in Methyl Alcohol Poisoning.—Th
of Table X illustrate an acidosis caused by organic acid
than the familiar acetone bodies. The data represent
preliminary work on methyl alcohol poisoning and are i
here only for their interest in illustrating a hitherto unf
type of acidosis.

SUMMARY.

The organic acids present both free and as salts in ur
estimated by titrating between the hydrogen ion concent
represented by pH 8 and pH 2.7 respectively, after rem
phosphates and carbonates by means of calcium hydroxi
appears that the titration represents between 95 and 1
cent of the organic acids present. It also includes weal
whose dissociation constants fall within a range in the ne
hood of 10^{-11}, but of this class only creatinine, and at time
tine, appear to be present in significant amounts in human

The average 24 hour excretion of organic acids in t
healthy young men was, per kilo of body weight, 8.2 cc. o
acid uncorrected for creatinine, or approximately 6 cc. co
for creatinine; the extreme range was from 5.7 to 9.8 cc.
rected for creatinine. There appears to be little dif
between day and night periods in rate of organic acid exc

Data from cases of methyl alcohol poisoning and d
respectively are given as examples of acidosis due to
acids of different types. In the case of methyl alcohol po
part of the total organic acid excretion was due to formic,
and hydroxybutyric acids, but the greater part to acids
known nature.

In the case of diabetes, which progressed to coma, the
acetone body excretion was accurately paralleled by the

the titrated organic acids. The parallelism was so close as to indicate the probabilities (1) that organic acids other than the acetone bodies are not excreted in significant amounts in diabetic acidosis, and (2) that the easily performed organic acid titration may be used for approximate estimation of the acetone bodies in diabetic urine.

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STUDIES OF ACIDOSIS.

THE TITRATION OF ORGANIC ACIDS IN URINE.

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Description of Method.

urine, roughly measured, are thoroughly mixed with nely powdered calcium hydroxide, allowed to stand ninutes with occasional stirring, and then passed ry folded filter. This treatment removes carbonates ates. To 25 cc. of the filtrate in a 125 to 150 cc. clear glass[1] one adds 0.5 cc. of 1 per cent phenol- olution, and 0.2 N hydrochloric acid from a burette ed not be measured) until the pink color just dis- I = approximately 8). 5 cc. of 0.02 per cent tro- olution are then added. As the indicator solution is horoughly mixed with the urine by shaking the tube; ution is omitted some of the tropeolin OO may be . Finally 0.2 N hydrochloric acid is added from the il the red color equals that of a standard solution .6 cc. of 0.2 N HCl, 5 cc. of tropeolin OO solution, o a total volume of 60 cc. When the end-point is sufficient water is added to the titrated solution to lume equal to that of the 60 cc. standard solution nilar tube as a color control.

ring the color of the titrated solution with that in the is convenient during the titration to hold the two

[1] e tubes of Pyrex glass made by the manufacturers for urea s by the Van Slyke and Cullen technique. The tubes are diameter, 200 mm. long, and uniform in size.

tubes side by side between the thumb and fingers, the tube containing the urine being the one held nearer to the tips of the fingers where it can be easily shaken as the 0.2 N acid is run in from the burette.

Sometimes it is desirable to use a similar technique for the phenolphthalein end-point also. In this case a tube of urine filtrate to which no phenolphthalein is added serves as a standard.

We have found that as the final end-point with tropeolin OO is approached comparison of colors is somewhat facilitated by placing the two tubes side by side in a comparator of the form described by Dernby and Avery, although with practice the end-point may be located within 0.1 cc. by merely holding the tubes together as described above.

Calculation.

From the volume of 0.2 N HCl used to titrate from the end-point of phenolphthalein to that of the tropeolin OO, the amount, usually 0.7 cc., is subtracted which is utilized in a similar titration of a control determination in which water is substituted for the urine. The volume of 0.2 N HCl thus corrected represents the approximate organic acid content of the urine sample, plus the creatine and creatinine, and an amount of amino-acids ordinarily negligible.

In order to calculate the results in terms of cc. of 0.1 N organic acid per liter, the figure representing the cc. of 0.2 N HCl used in the titration is multiplied by 80 (by $\frac{1,000}{25} = 40$ in order to transfer figure from 25 cc. to 1,000 cc. of urine, and by 2 to change from 0.2 N to 0.1 N terms).

Correction for Creatinine.—A 0.1 M solution of creatinine (11.32 mg. per cc.) titrates in the above determination as a 0.1 N solution of organic acid. Therefore, in order to correct for the creatinine, the cc. of 0.1 N organic acid per liter calculated from the above titration may be diminished by

$$\frac{\text{mg. creatinine per liter urine}}{11.32} \text{ or by } \frac{\text{mg. creatinine N per liter urine}}{4.2}.$$

The simplest way is to subtract the creatinine correction directly from the cc. of 0.2 N acid used in the titration, and mul-

ifference by 80. In this case the correction is $\frac{1}{80}$ as
e above; *i.e.*, cc. correction =
$\frac{\text{ine per liter urine}}{906}$ or $\frac{\text{mg. creatinine N per liter urine}}{336}$.

	cc.
l used in titration	7.6
n found in blank analysis ..... 0.7 cc.	
ne correction for 500 mg. creatinine N per ine. Correction $= \frac{500}{336}$ cc. = ... 1.2 "	
l correction ..... 1.9 cc.	
d titration figure = 7.6 − 1.9	5.7
anic acid per liter = 80 × 5.7	456.0

OO was preferred by us as indicator for the final In neutral solution it gives nearly the same yellow ne, but so much more intense that a water solution of rochloric acid with the indicator can be used as a color ithout the use of a comparator. Very dark urines greater dilution, but such are not often encountered. vantage of this indicator is that its maximum acid reached even at pH 2.7, so that if too much HCl is ne titration the solution becomes redder than the This particular advantage is possessed in much less ne three indicators mentioned below as alternatives. licators that may be used are methyl orange, tetra-olsulfonephthalein (bromophenol blue, Clark and Lubs), ylaminoazobenzene. To some eyes the color change ese dyes may be more readily detected than that of O. The two azo dyes are not much different in color olin OO, both changing from yellow to red, but the ol blue turns from blue to a clear yellow on acidifying, a very different alternative. To the authors the tro-nd-point appeared the most satisfactory, however.

Theoretical Basis of Method.

The method is based on the following previously know

1. Relatively little strong mineral acid is required to the hydrogen ion concentration of a water solution from 2×10^{-3} if the only electrolytes present are alkali salts o acids, such as sulfates and chlorides.

2. If the salt of a weak acid is present, however, the of nearly a full molecule of hydrochloric acid for each mo such salt is necessary in order to cause the above chang drogen ion concentration. The organic acids known to normal and pathological urines, in amounts sufficient to b titatively significant in the total acid excretion of th belong to the class of weak acids whose salts behave in th manner.

3. The only mineral acids found in significant amounts which belong to the class of weak acids, and therefore fo which show the above behavior, are phosphoric and acids.

4. Very weak bases form salts which behave like thos weak acids. Creatinine is titrated almost quantitati changing the hydrogen ion concentration from 10^{-3} to 2 and creatine to about 60 per cent. Aside from the t amino-acids, these appear to be the only bases of this kind in considerable amount in human urine.

Effect of the Different Organic Acids of the Urine on the T

The titration figure obtainable by titrating between drogen ion concentrations a solution containing the sal acid of a known dissociation constant may be calcul follows:

From the law of mass action:

(1) $H^+ = k\frac{HA}{A'}$

H^+ = hydrogen ion concentration in terms of nor
A' = anion of acid.
k = dissociation constant of the acid.
HA = free, undissociated acid.
BA = salt of the acid.
λ = degree of dissociation of the salt into Na an

he salt of the acid is present, and dilutions are of the
s used in titrations (0.1 to 0.01 M), the equation be-
ctically

$$(2) \qquad H^+ = \frac{k}{\lambda} \times \frac{HA}{BA} \quad .$$

the high dilutions encountered approaches unity, it
proximate calculations be neglected.
n 1 may then be expressed as

$$(3) \qquad H^+ = k \times \frac{HA}{BA} \text{ or } \frac{HA}{BA} = \frac{H^+}{k}$$

tic acid $k = 1.8 \times 10^{-5}$.
pH equals 8, or $H^+ = 1 \times 10^{-8}$, we therefore have in
f acetic acid $\frac{HC_2H_3O_2}{BC_2H_3O_2} = \frac{10^{-8}}{1.8 \times 10^{-5}} = \frac{1}{1{,}800}$. One
801 parts, or 0.05 per cent, of the acid is free.
H = 2.7, $H^+ = 2 \times 10^{-3}$, and we have $\frac{HC_2H_3O_2}{BC_2H_3O_2} =$

$\frac{}{10^{-5}} = \frac{200}{1.8}$.

of 2.7, therefore, $\frac{200}{201.8}$, or 99.2 per cent, of the acid is
anging the hydrogen ion concentration of an acetate
rom the slightly alkaline reaction of 10^{-8} N (or a pH of
acid reaction of 2×10^{-3} ((pH = 2.7) approximately
ion of 0.002 N HCl) therefore requires an amount of
l in molecular equivalents to 99.15 per cent of the total
resent.
different acids which occur or may occur in human
values in Table I are calculated. The values of the
are for 25° unless otherwise indicated.
rison of the results so calculated with those experimen-
ined in titrating solutions of some of these acids is satis-
s shown in Tables III, IV, and VI of the experimental
his paper. The conclusion seems justified that the
s carried out estimates certainly over 90 per cent of the
ids of the urine, and presumably over 95 per cent, since

a higher titration value by 3 or 4 per cent is obtained for acids excreted as ammonium salts. The data for carboni phosphoric acids indicate the necessity for their removal the organic acids are titrated.

TABLE I.

Calculated Titration Values of Weak Acids of the Urine.

Acids.	Dissociation constant.	Acid free at		Calc propo a determ titr from pH
		$H^+ = 10^{-8}$ N pH = 8	$H^+ = 2 \times 10^{-3}$ pH = 2.7	
		per cent	*per cent*	*per*
Organic.				
Uric*	1.5×10^{-6}	0.5	99.9	9
Acetic†	1.8×10^{-5}	0.0	99.2	9
β-hydroxybutyric‡	2.0×10^{-5}	0.0	99.0	9
Lactic†	1.4×10^{-4}	0.0	93.5	9
Acetoacetic‡	1.5×10^{-4}	0.0	93.1	9
Citric§	2.0×10^{-4}	0.0	91.0	9
Formic*	2.1×10^{-4}	0.0	90.6	9
Hippuric†	2.2×10^{-4}	0.0	90.2	9
Mineral.				
$H(NaHPO_4)$‖	2.0×10^{-7}	2.5	100	9
$H(HCO_3)$¶	3.5×10^{-7}	4.2	100	9

* His and Paul.

† Ostwald.

‡ Henderson and Spiro.

§ Shown by Amberg and McClure to occur in amounts equivalen to 70 cc. of 0.1 N acid in a normal 24 hour urine. The titration val citric acid given in Column 5 are those directly determined by Sö The constant is estimated from them.

‖ Sörensen.

¶ Kendall.

Effect of Weak Bases of the Urine on the Titration.

The amount of strong acid required to change the pH solution of a weak base from 8 to 2.7 may be calculated fro dissociation constant K_b.

$$K_b = OH' \times \frac{\text{salt of base}}{\text{free base}} = \frac{10^{-14}}{H^+} \times \frac{\text{salt of base}}{\text{free base}}$$

of base" represents the amount combined with acid. therefore, salt of base, or $\frac{\text{acid combined with base}}{\text{free base}} =$

$= K_b \times 10^6$. At pH 2.7, or $H^+ = 2 \times 10^{-3}$, the ratio

$\frac{2 \times 10^{-3}}{10^{-14}} = 2\ K_b \times 10^{11}$. The difference between the

d by a given base at pH 8 and that bound at pH ents the amount required to titrate between the two Table II contains a list of the weak bases of the urine, constants and the proportion of an equivalent of HCl

TABLE II.

lculated Titration Values of Organic Bases of the Urine.

	Basic dissociation constant. K_b	Base free at		Proportion estimated by titrating with HCl from $H^+ = 10^{-8}$ to $H^+ = 2 \times 10^{-3}$	
		$H^+ = 10^{-8}$	$H^+ = 2 \times 10^{-3}$	Calculated	Observed
		per cent	per cent	per cent	per cent
...	0.0015×10^{-11}	100	99.7	0.3	0.2
...	1.81×10^{-11}*	100	24.0	76.0	99
...	3.57×10^{-11}*	100	12.3	87.7	60
...	1.5×10^{-6}†	0.2	0.0	0.2	5.3 6.0

and Wood.
ed at 40°, Wood.
Kato, and Sosman.

o titrate each from pH 8 to pH 2.7, calculated as above The constants are from data obtained at 25°, except ne and creatinine. In Column 5 results are brought om Table V, showing the amounts of HCl bound by the ases in the titration, as determined experimentally. both by observation and calculation, practically with- on the results of the titration, even when the urea con- is at the maximum observed in human urine.

ailable data on the K_b of creatine and creatinine do not lated results corresponding so closely with those experi- obtained as do the data on the other substances requir-

ing consideration. The divergence is perhaps due to th
that Wood's values for K_b of creatine and creatinine were
mined at 40°, while the titration is performed at 20°.
evident, however, that practically all the creatinine is titra
organic acid. The amount of this substance excreted
between 13 and 27 mg. per kilo of body weight per 24
(Folin, 1905). The mean, 20 mg., would neutralize 1.8 cc.
N acid per kilo or 108 cc. for a 60 kilo individual.

Creatine when present titrates to about 60 per cent
organic acid; but it is excreted by adults only in conditions i
ing rapid autolysis of muscle tissue, and would therefore no
rule, require consideration.

Ammonia is titrated to the extent of 5 to 6 per cent, b
actual effect of the presence of organic acids as ammonium
than fixed alkali salts is to make the results of the titratio
most of the acids approximate more closely the theoretical
as shown in Table IV. The ammonium salts of the organi
titrate 2.3 to 4.6 per cent more completely than the sodiu
not 6.2 per cent more completely, as would be theoretica
pected, and as is approximately realized for the ammoniu
of hydrochloric and sulfuric acids. The observed positive am
error is such as to make the results obtained with all but the
est organic acids approximate more closely to 100 per cent th
results obtained in the absence of ammonia. The tende
the ammonia error to correct the opposite error in the o
acid titration is enhanced by the fact that ammonia and o
acid excretion tend to run parallel, particularly when acid
tion is abnormally high, as in diabetic acidosis. For the re
therefore, that the ammonia correction is not great and i
nature actually to diminish, as a rule, the other error
determination, it has seemed not only simpler but bet
attempt no correction for it in urine analyses.

Effect of Amino-Acids on the Titration.

Amino-acids if present in large amount would be dist
factors, as at an H^+ of 2×10^{-3} they bind with their NH_2
considerable amounts of acid. Glycocoll, which does not
much from the other monoamino-acids in this respect,

molecule of HCl at this H^+. The amount is calculated
/s:

id constant for glycocoll is 3.4×10^{-10}, the basic constant $2.9 \times$
calculated by Winkelblech from conductivity measurements.
acid constant we have by calculating as above:

OH free at $= 10^{-8}$ N	— COOH free at $H^+ = 2 \times 10^{-3}$ N	Proportion of COOH group estimated by titration from $H^+ = 10^{-6}$ to $H^+ = 2 \times 10^{-3}$
er cent	*per cent*	*per cent*
96.7	100	3.3

unction of the NH_2 group is similarly calculated from the
nstant, $K_b = 2.9 \times 10^{-12} = (OH)' \times \frac{\text{glycine chloride}}{\text{free glycine}}$ or

$$\frac{CH_2\text{-}NH_2}{CH_2\text{-}NH_2\ HCl} = \frac{(OH)'}{2.9 \times 10^{-12}} = \frac{10^{-14}}{H^+ \times 2.9 \times 10^{-12}} =$$

9.

these values we calculate:

H_2 free at $= 10^{-6}$ N	— NH_2 free at $H^+ = 2 \times 10^{-3}$ N	Proportion of NH_2 group estimated by titratin with HCl from $H^+ = 10^{-6}$ to $H^+ = 2 \times 10^{-3}$
er cent	*per cent*	*per cent*
100	63.3	36.7

tal consumption of HCl by both COOH and NH_2 groups
tration should be, according to the above calculation,
0.367 = 0.40 molecule of HCl per 1 molecule of glycine
The actual amount observed by Sörensen was 0.385

ther monoamino-acids apparently bind similar amounts
The constants for leucine and alanine were determined
elblech as follows: leucine, $K_a = 3.1 \times 10^{-10}$, $K_b =$
$)^{-12}$; alanine, $K_a = 9.0 \times 10^{-10}$, $K_b = 3.8 \times 10^{-12}$.
g to these, leucine would require in the titration 0.38
of HCl; alanine 0.36, nearly the same as glycocoll. The
Table VII for the mixture of all the monoamino-acids
from casein are in the same neighborhood (44 per cent).

The amino-acid nitrogen constitutes 1 to 2 per cent o total urinary nitrogen (Van Slyke, 1913–14; Henriques). daily excretion of 14 gm. of nitrogen, 2 per cent would ind 200 cc. of 0.1 M amino-acids. The neutralizing power of su amount of amino-acids in the titration would be about 8 of 0.1 N hydrochloric acid.

Our knowledge of the nitrogenous constituents of the indicates the presence of no weak bases, aside from thos cussed, in quantities sufficient to affect markedly the or acid titration under discussion, and the nitrogenous excr products have been so thoroughly studied that it is unlikely any quantitatively important substances with definitely properties have been overlooked.

It therefore appears that in titrating the 24 hour urine adult of average size for organic acids, as described in this p about 100 cc. of the 0.1 N organic acid estimated is in realit to creatinine and creatine, 80 cc. or less to amino-acids, an remainder to organic acids.

EXPERIMENTAL.

Titration of Organic Acids in Water Solutions.—A 20 cc. po of each acid, of approximately 0.1 N concentration, was tit in a 100 cc. test-tube with either 0.1 N sodium hydroxide o N. ammonium hydroxide to neutrality with 0.5 cc. of 1 per phenolphthalein. 1 cc. of 0.1 per cent tropeolin OO was added, and the solution titrated back with 0.2 N HCl to pH using 0.002 N HCl solution as standard. The results are in Tables III and IV.

Titration of Weak Bases in Water Solutions.—Solutions o bases in 25 cc. portions were brought to pH 8 by addition of NaOH or 0.2 N HCl until a barely visible pink color was rea then tropeolin OO was added and the solution titrated to pH The results are given in Table V.

Effect of Concentration of Phenolphthalein on its End-Poi Presence of Ammonium Salts.—The concentration of ph phthalein to some extent affects the pH at which the pink is just visible. If there is but little indicator present a gr part of it must be in the colored form to give a perceptible

TABLE III.

Titration of Sodium Salts of Organic Acids.

Acid.	(A) 0.1 N NaOH to neutralise acid to phenolphthalein.	(B) 0.2 N HCl to titrate back to pH 2.7 with tropeolin OO.	(C) Average 0.2 N HCl corrected for blank.	(D) Organic acid determined. $\frac{200\ (C)}{(A)}$	Organic acid theoretically titratable from pH 8 to pH 2.7 (from Table I).
	cc.	*cc.*	*cc.*	*per cent*	*per cent*
Blank	0.1	0.50	0.00		
Acetic	20.00	10.60	9.95	99.5	99.4
		10.50			
Citric	19.86	9.30	8.88	89.4	91.0
		9.35			
Lactic	20.28	9.90	9.40	92.7	93.5
		9.90			
Hydrochloric	20.00	0.70	0.20	1.0	

TABLE IV.

Titration of Ammonium Salts of Organic Acids.

Acid.	(A) 0.1 N acid present.	(B) 0.1 N NH_4OH to neutralise acid to phenolphthalein at pH 8.	(C) 0.2 N HCl to titrate back to pH 2.7.	(D) Average 0.2 N HCl corrected for 0.5 cc. blank.	(E) Proportion of organic acid determined $\frac{200\ (D)}{(A)}$	Proportion of NH_4 salt theoretically titratable; i.e., that for acid calculated in Table I + 6.2 per cent for NH_4 present.	Differences between average percentage of Na salt and NH_4 salt titrated.
	cc.	*cc.*	*cc.*	*cc.*	*per cent*	*per cent*	*per cent*
Acetic	19.68	20.51	10.70	10.25	104.1	105.6	4.6
	19.68	20.47	10.80				
Citric	21.04	21.55	10.30	9.79	93.0	97.2	3.5
	21.04	21.51	10.27				
Lactic	20.06	20.96	10.03	9.50	94.7	99.7	2.3
	20.06	20.96	10.03				

than when the total amount of indicator is greater. Consequently the amount of extra alkali required to make a solution of an ammonium salt show pink with phenolphthalein is some-

TABLE V.

Observed Behavior of Weak Bases when Titrated from pH 8 to pH

Base.	Amount present in the 25 cc. of solution titrated.		0.2 N HCl required in titrating from pH 8 to pH 7.	Proportion of base titrated.	Proportion of b calculated as titr from dissociati constant (Table
	gm.	*cc. 0.2 N*	*cc.*	*per cent*	*per cent*
Urea	1.000	83.3	0.1	0.12	0.3
Creatine	0.200	7.6	4.1	60.0	87.7
Creatinine	0.100	4.41	4.32	97.8	76.0
	0.200	8.83	8.80	99.7	
Monoamino-acids	0.100	7.37*	3.25	44.2	36.0–40.0 for
from casein	0.200	14.63	6.37	43.5	cine, leucine
	0.200	14.63	6.29	43.0	alanine.
Ammonia (as		12.50	0.67	5.4	6.2
$(NH_4)_2SO_4$)		12.50	0.75	6.0	
Ammonia (as		9.82	0.53	5.4	6.2
NH_4Cl)			0.52	5.3	

* Calculated on a nitrogen content of 10.3 per cent. The prepar was made by hydrolyzing casein with sulfuric acid, precipitating the with phosphotungstic acid, and concentrating the filtrate to dryness u reduced pressure after the phosphotungstic and sulfuric acids had removed.

TABLE VI.

Effect of Phenolphthalein Concentration on End-Point in Presence o Ammonium Salts.

0.05 M $(NH_4)_2SO_4$.	1 per cent phenolphthalein.	0.1 N NaOH to turn pink to phenolphthalein.	0.2 N HCl to change from phenolphthalein end-point to pH 2.7.		Propo of amm titra from ph phtha end-po pH
			Uncorrected.	Minus 0.5 cc. for correction.	
cc.	*cc.*	*cc.*	*cc.*	*cc.*	*per*
25	0.1	0.85	1.42	0.92	7.
25	0.2	0.65	1.36	0.86	6.
25	0.5	0.45	1.20	0.70	5.
25	1.0	0.45	Too cloudy with precipitated phenolphthalein to titrate.		

endent on the amount of indicator used. This is shown esults in Table VI. It is desirable to use in performing tions 0.5 cc. of 1 per cent phenolphthalein solution, as rather than the indefinitely measured drop or two which n ordinary titrations.

TABLE VII.

Titration of Organic Acids Added to Urine.

0.1 N organic acid added to 100 cc. urine.	0.2 N HCl used in duplicate titrations of 25 cc. urine filtrate.	Average titration figure minus that for urine alone.	0.1 N added organic acid per liter diluted urine.		Proportion of added organic acid determined.
			Found.	Added.	
cc.	cc.	cc.	cc.	cc.	per cent
0	3.00 3.00				
25	4.55 4.53	1.54	123	125	98.4
50	6.20 6.15	3.17	253	250	101.2
100	9.15 9.10	6.13	490	500	98.0
0	2.87 2.87				
25	4.25 4.20	1.36	109	117*	93.2
50	5.50 5.60	2.68	214	236*	90.7
100	8.30 8.25	5.41	432	472*	91.6

1 N lactic acid used in this experiment had the factor 0.945.

on of Known Amounts of Organic Acids Added to Urine.— ortions of a mixed sample of normal urine were mixed ions of 25, 50, and 100 cc. respectively of acetic or lactic ach mixture was then diluted to 200 cc., and 100 cc. were treated as previously described for determination.

TABLE VIII.

Excretion of Organic Acids with Creatinine Correction.
Data from hospital patients.

Subject.	Weight.	Condition.	Urine excretion.									
			Period.	Volume.	Creatinine N.		0.2 N HCl used in titrating from pH 8 to pH 2.7.		0.1 N organic acid content.			
									Total.		Per kilo.	
							Duplicates.	Average minus 0.6 cc. correction for blank.	Uncorrected for creatinine.	Corrected for creatinine.	Uncorrected for creatinine.	Corrected for creatinine.
	kg.		*hrs.*	*cc.*	*gm.*	*cc. 0.1 N*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
Z	60	Myocarditis, decompensation on admission.	12 (day)	658	0.240	57	7.0, 6.9	6.35	334	277		
			12 (night)	946	0.261	62	4.3, 4.1	3.60	272	210		
			24	1,604	0.501	119			606	487	10.5	8.1
O	60	Myocarditis, some decompensation.	12 (day)	707	0.223	53	5.1, 5.25	4.57	258	205		
			12 (night)	744	0.216	51	5.0, 5.0	4.40	262	211		
			24	1,451	0.439	104			520	416	8.2	6.9
C	55	Chronic aortic endocarditis.	12 (day)	242	0.141	34	15.1, 15.3	14.60	282	248		
			12 (night)	332	0.195	46	12.8, 12.9	12.25	325	279		
			24	574	0.336	80			607	527	11.0	9.6
D	62	Chronic myocarditis with decompensation.	12 (day)	647	0.226	54	5.6, 5.7	5.05	261	207		
			12 (night)	750	0.196	47	4.4, 4.3	3.75	225	178		
			24	1,397	0.422	101			486	385	8.8	7.0

are given in Table VII. The results are essentially s those obtained with acetic and lactic acids in pure ions.

id Excretion by Individuals with Normal Metabolism.

given are sufficient only to indicate the usual excre- anic acids; the possible normal variations, particularly ual conditions, may be greater. The figures of Table

TABLE IX.

r Excretion of Organic Acids by Normal Young Men.

	Weight.	24 hour urine.			
		Volume.	0.1 N organic acids uncorrected for creatinine.*		Total N.
	kg.	*cc.*	*cc.*	*cc. per kg.*	*gm.*
.....	54.4	1,000	492	9.0	9.3
.....	68.0	1,650	657	9.8	11.5
.....	68.0	975	583	8.5	11.7
.....	62.1	1,500	531	8.5	13.2
.....	68.0	1,150	412	6.1	7.8
.....	56.6	1,500	453	8.0	10.0
.....	68.4	1,000	490	7.2	8.7
.....	57.2	1,400	521	9.1	9.0
.....	82.6	1,100	748	9.1	15.5
.....	87.0	1,300	493	5.7	13.2
.....	56.2	1,100	420	7.5	11.2
.....	61.2	700	499	8.2	10.0
.....	56.6	1,300	547	9.7	12.1
.....				8.2	

atinine correction would reduce the total organic acid figure c. per kilo.

rom afebrile heart patients, with apparently normal . The day periods are from 6 a.m. to 6 p.m., the ds from 6 p.m. to 6 a.m. The data of Table IX are es of healthy young men. The figures indicate that excretion of organic acids uncorrected for creatinine n about 280 to 750 cc. of 0.1 N acid per 24 hours, 6 to 11 cc. per kilo of body weight. The creatinine reduces the figures to 240 to 600 and 4.7 to 9.6 cc. y.

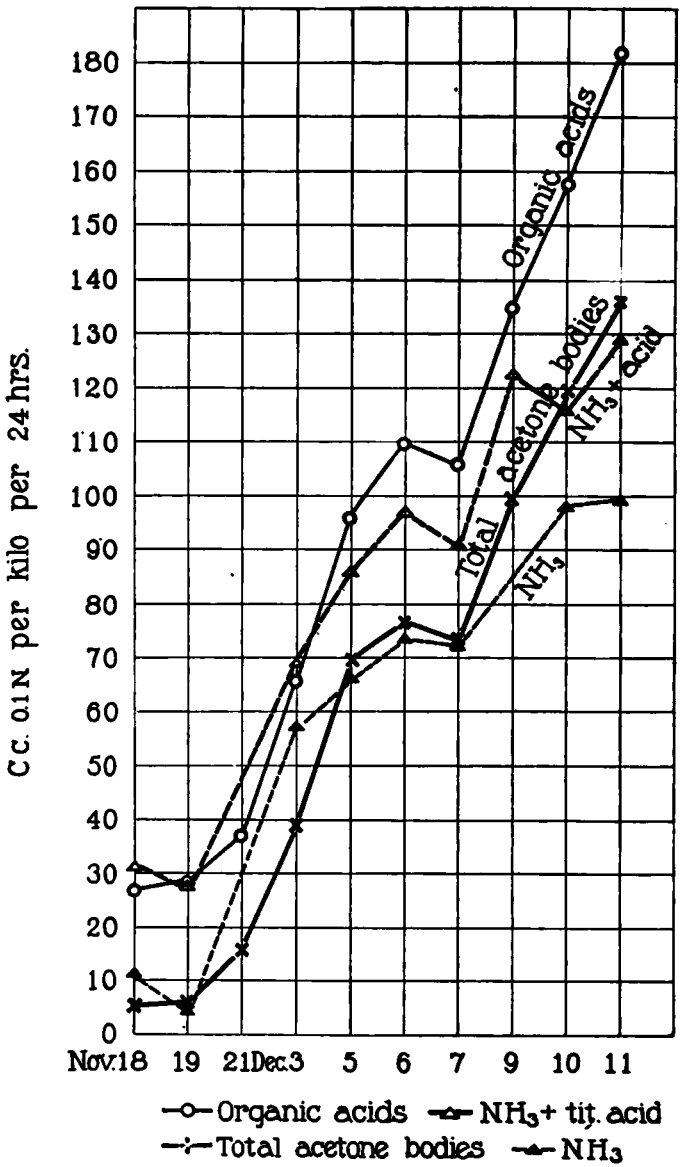

FIG. 1. Excretion in a case of diabetic acidosis.

n of Total Organic Acid Excretion with Acetone Bodies Excretion in Diabetes.

a given in Fig. 1 were obtained with the only case of cidosis which we have studied since the organic acid nethod has been available. Although all the data are ase, they nevertheless represent every stage of diabetic rom the time when it was slight, with little ketonuria, e point of coma, with tremendous ketonuria. The as a child of 2 years, weighing 8 kilos. The organic

TABLE X.

d Excretion in a Non-Fatal Case of Methyl Alcohol Poisoning.

CO_2 bound as bicarbonate by 100 cc. of plasma.	Excretion per liter urine.						
	Creatine.	Creatinine.	Total 0.1 N organic acids.*	0.1 N acetone bodies.	0.1 N lactic acid.	0.1 N formic acid.	Undetermined 0.1 N organic acids.
cc.	gm.	gm.	cc.	cc.	cc.	cc.	cc.
36.4	0.202	0.558	2,042		173	274	1,595
36.0	0.283	1.000	2,076	481	83		1,512
86.2	0.535	0.800	1,377	143	30	130	1,074
76.7	0.300	0.590	262				
	0.180	0.538	129				
	0.137	0.557	141				
			220†				
	0.105	0.378	86				
	0.024	0.476	138				

ed for creatine and creatinine.
" " " " estimated not determined.

es recorded in Fig. 1 are not corrected for creatine and , so that they are higher than, though parallel to, the ganic acid excretion. The "total acetone bodies," butyric acid, acetoacetic acid, and acetone were deter- the gravimetric method of Van Slyke (1917), the ammo- cribed by Van Slyke and Cullen, and the titratable acid thod of Folin (1903).

dent from the chart that the organic acids of the urine, d by the technique outlined above, paralleled the ace-

tone body excretion with a high degree of accuracy thro
stages of the acidosis, the parallelism being more accura
that of the ammonia, or even the ammonia plus titratab

It appears that the rise above the normal output in
acid excretion may be used as an approximate measure
acetone body excretion in diabetes, the determination of
acids being as simple as that of ammonia and less influen
other factors, such, in particular, as alkali administration.

Organic Acid Excretion in Methyl Alcohol Poisoning.—T
of Table X illustrate an acidosis caused by organic acid
than the familiar acetone bodies. The data represen
preliminary work on methyl alcohol poisoning and are i
here only for their interest in illustrating a hitherto unf
type of acidosis.

SUMMARY.

The organic acids present both free and as salts in ur
estimated by titrating between the hydrogen ion concent
represented by pH 8 and pH 2.7 respectively, after rem
phosphates and carbonates by means of calcium hydroxi
appears that the titration represents between 95 and 1
cent of the organic acids present. It also includes weal
whose dissociation constants fall within a range in the ne
hood of 10^{-11}, but of this class only creatinine, and at tim
time, appear to be present in significant amounts in humai

The average 24 hour excretion of organic acids in t
healthy young men was, per kilo of body weight, 8.2 cc. c
acid uncorrected for creatinine, or approximately 6 cc. co
for creatinine; the extreme range was from 5.7 to 9.8 cc.
rected for creatinine. There appears to be little di
between day and night periods in rate of organic acid ex

Data from cases of methyl alcohol poisoning and d
respectively are given as examples of acidosis due to
acids of different types. In the case of methyl alcohol po
part of the total organic acid excretion was due to formic
and hydroxybutyric acids, but the greater part to acids
known nature.

In the case of diabetes, which progressed to coma, the
acetone body excretion was accurately paralleled by the

the titrated organic acids. The parallelism was so close as to indicate the probabilities (1) that organic acids other than the acetone bodies are not excreted in significant amounts in diabetic acidosis, and (2) that the easily performed organic acid titration may be used for approximate estimation of the acetone bodies in diabetic urine.

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