A METHOD FOR DETERMINING THE TOTAL DAILY GAIN OR LOSS OF FIXED ALKALI, AND FOR ESTIMATING THE DAILY OUTPUT OF ORGANIC ACIDS IN THE URINE, WITH APPLICATIONS IN THE CASE OF DIABETES MELLITUS

By EDWARD S. EDIE, M.A., B.Sc. (Edin.), *Carnegie Research Scholar*;
and EDWARD WHITLEY, M.A. (Oxon)

From the laboratory of Bio-chemistry, University of Liverpool

(Received January 2nd, 1906)

A large number of methods have been published for determining and estimating the reaction of the urine.

The number of such investigations is so large that individual reference to the several papers and an exhaustive review of the literature becomes impossible, but it may be stated that the figures vary enormously according to the method and the indicator used for the purpose.

In addition to the experimental difficulties introduced by the fact that the urine is itself coloured, and hence interferes with the delicacy of the reaction to coloured indicators, there is the more important fact that the reaction of the urine is never due to free acid or to free alkali; but to a varying mixture of salts such as the primary and secondary phosphates of the alkalies. In such a mixture the urine reacts entirely differently to different indicators. Thus the same sample of normal urine is *acid* to a sensitive indicator to weak acids such as phenol-phthalein and *alkaline* to a stable indicator such as methyl-orange or di-methyl-amido-azo-benzol. Also if the titration figures to three such indicators as phenol-phthalein, litmus, and 'di-methyl' be taken, it will be found that there is a high acidity with the phenol-phthalein, a much lower acidity with the litmus, and a high alkalinity with the di-methyl indicator. When similar titrations are undertaken in the case of an artificial mixture of the primary (NaH_2PO_4) and secondary (Na_2HPO_4) phosphates of sodium in water to which such phosphates have been added in known amount and ratio,

the reason underlying the differences in the titration values becomes at once apparent. The neutral point for phenol-phthalein lies at the point where the kations and anions are so distributed as to correspond to Na_2HPO_4 , while for di-methyl (or methyl orange) the neutral point corresponds to NaH_2PO_4 , and for litmus the neutral point lies somewhere intermediate between these two values. It is clear from this statement that it is futile to regard any one indicator as the arbiter of neutrality, and to consider a solution as being neutral because it is neutral to phenol-phthalein when it is alkaline to litmus and 'di-methyl,' or when it is neutral to litmus or laemoid, and acid to phenol-phthalein and alkaline to di-methyl at the same time.

The only true definition of neutrality would be the point at which the concentrations of hydrogen and hydroxyl ions are equal, and no coloured indicator satisfies this condition but indicates neutrality at a point where there is a given ratio other than equality between the acidic and basic ions. The value of this ratio depends on the ease with which the coloured indicator, and its ions, associate or dissociate in solution.

The proper method of determining reaction ought, therefore, to be some method of determination of the concentration of hydrogen or hydroxyl ions which would indicate where these two concentrations were equal.

In the case of a solution of the mixed phosphates, such as the urine, all physical methods for determination of the ionic concentrations fail, however, in accuracy on account of the very slow variation of the concentration in the two ions around the neutral point. In the case of free acid or free alkali, the smallest addition of acid or alkali to the solution in the neighbourhood of the neutral point causes an immense swing in the ratio of the two ions which is at once obvious in the gas battery; whereas, in the case of a solution containing phosphates, the degree of dissociation is low, and an addition of acid or alkali causes not a great swing in the ratio of hydrogen or hydroxyl ions but a decomposition of phosphate in either direction, and the establishment of a new equilibrium in which the ratio of the two ions may not be widely different from the former.

On this account a solution such as the urine, or any of the body fluids in general, behaves as a controlling agent or as a neutralizing agent for either acid or alkali, and prevents large variations in either hydrogen or hydroxyl ion concentration.

The importance of such a regulating mechanism to the organism, the cells of which are so sensitive to such variations, is too obvious to need elaboration.

It is also the slow variation in the ratio of hydrogen and hydroxyl ionic concentration as acid or alkali is added which renders the change in colour, when coloured indicators are used, more gradual than in the case of free acid or alkali and causes a difficulty in determining the exact point of change.

Returning to the indications given by coloured indicators in the case of such physiological fluids as urine, it may be stated that the neutral point to each colored indicator means a different equilibrium point between acid and basic ions. Accordingly, the description of such points as neutral points may lead to serious error and misunderstanding unless it be remembered that neutrality means in such a case an equilibrium with a certain ratio of the two ions as shown by a certain indicator and not an equality in the two ionic concentrations.

With this safeguard, however, valuable information can be often obtained as to the distribution of the two phosphates (or rather of the kations and anions) in the solution, provided indicators be used which react and show their change in colours at definite points, such as phenol-phthalein and methyl-orange or di-methyl, and not those which change at intermediate points as litmus.

While this can be carried out in the case of urine at the phenol-phthalein end showing the amount of alkali necessary to be added to reach the equilibrium point corresponding to Na_2HPO_4 , it cannot be accurately carried out at the other end with a stable indicator so as to give the amount of acid necessary to be added in order to reach the equilibrium point NaH_2PO_4 , on account of the yellow colour of the urine.

There is another and physiologically more important circumstance which renders it impossible to determine by any combined use of

coloured indicators whether acid or alkali is gained or lost to the organism by the channel of the urine.

The organism protects itself from any variation in the ratio between the hydrogen and hydroxyl ionic concentrations to which its cells are habituated by the formation of organic acids, or organic bases (or ammonia), which combine with and neutralize the excess of base or acid and carry it off in neutral form in the urine.

The mere determination of the alkalinity or acidity of the urine gives on this account no real idea of how the balance is being varied between acid and alkali in the body by the action of the kidneys.

It was with the object of discounting in some manner this action of the tissue cells in producing, according to circumstances, organic base and ammonia, or organic acid, and so disguising the real output of fixed alkali or acid, that the methods of determination described in this paper were devised.

The real output of fixed alkali or acid having been once determined, one obtains incidentally, as will be shown later, by comparison with determinations (*a*) of the apparent output as given by the reaction of the urine, and (*b*) by separate determinations of the ammonia, a means of estimating the amount of organic acid or organic base manufactured daily by the body in order to maintain the ratio between hydrogen and hydroxyl ions existing in the body.

This latter determination is of high importance in pathological conditions where factors are at work tending to disturb the balance in the tissue fluids between the two ions, as for example in the large manufacture of organic acids which occurs in diabetes.

Before passing to the description of the details of our methods, we may give one or two illustrations of the fact that the reaction of the urine, determined in the ordinary way, gives no real estimate of the ratio between the amount of acid and alkali being carried off from the body in the urine.

It is proverbial in therapeutics how difficult it is to alter the reaction of the urine. It is only when enormous doses of alkali, in the form of alkaline carbonates, are given that the reaction of the urine becomes alkaline to litmus, and, again, large doses of mineral

acids can be given without appreciably altering the reaction of the urine to litmus. Here the alkali or acid, as the case may be, must leave in some form in the urine, yet the reaction of the urine gives us scarcely any indication of the process.

The explanation is, of course, an easy one, the body in the case where acids are given forms a base (such as ammonia or an organic base), and in company with this, the acid is carried off in the urine, or when alkali is given, less ammonia or organic base is formed in order to leave acid for combination with the alkali, or, if this is insufficient, more organic acid than normal is formed. It is only when the power of these regulatory mechanisms has been surpassed that obvious changes begin to take place in the reaction of the urine, and at about this level acid or alkaline intoxication begins to intervene.

Similar carriage of acid or alkali away in the urine may occur without any indication appearing in the reaction of the urine in cases where the acid or alkali is not given by the mouth, but appears as a result of pathological changes within the organism itself. For example, there is no indication in the reaction of the urine of the amounts of organic acid which are carried off in cases of diabetes. Also when the production of such organic acids exceeds a certain limit, and ammonia can no longer be produced in sufficient amount to neutralize them, a drain commences upon the fixed alkalies of the body, and sodium salts of the organic acids begin to be secreted in the urine. It is at this level that the amount of alkali in the blood becomes reduced and acid intoxication and coma are threatened; yet we possess no signal in the reaction of the urine to give definite warning of the commencement of such a condition of affairs.

An appreciation of the importance of determining the amount of organic acid leaving the body in diabetes is seen in the many attempts that have been made to obtain methods for estimating the amounts of each of the organic acids found in the urine in diabetes.

The general fact appears to have been lost sight of in devising these separate methods for the estimation of the amount of each acid, that the acids concerned are not poisonous or dangerous on account of any special toxic properties attached to any special group in their

molecule, but merely as so much acid which is capable of neutralizing and carrying of alkali, and so causing acid intoxication. They are organic acids which the diabetic has lost the power of oxidizing, they must be neutralized by alkali, and therein lies their danger.

Hence, what is required, is not a method for estimating each acid separately, but a method for estimating the total output of organic acids in the urine, and at the same time for indicating whether the organism is still capable of meeting this output and neutralizing it by ammonia, formed in lieu of so much urea which would have normally been produced, or whether the fixed alkali of the blood and tissues is being utilized and drained away from the body.

Such a method we have devised and carried out with the results shown in the protocols of our determinations.

The first step in the process is the determination of the excess of fixed inorganic acid or alkali leaving the body daily.

This is determined by adding excess of standard alkali to a measured volume of the urine, incinerating, adding excess of standard acid and boiling, in order to re-convert back into orthophosphates any pyro- or meta-phosphates which had been formed in the process of incineration, and then titrating to neutrality. The difference in the total amount of standard alkali and acid used then gives the loss or gain in alkali to the body through the urine. The excess of alkali is added to prevent loss of inorganic acid combined with ammonia or organic base in the process of incineration. In the incineration any fixed alkali combined with organic acids is converted into carbonate and hence appears in the difference between the amounts of standard alkali and standard acid used. Accordingly the figure obtained gives the difference between inorganic acid and inorganic base (minus ammonia) in the urine. Since the ammonia and organic acids and bases of the urine may be regarded as formed in the body, the figure obtained shows whether excess of acid or excess of alkali, other than that formed in the body, is escaping daily by the urine. Thus, if more of the standard alkali than of the standard acid is required to give neutrality in the incinerated urine, then acid is leaving in excess, and *vice versa*.

The figure so obtained gives those variations which ought naturally to follow administration of alkali or acid, a figure which is

never obtained by direct titration of the urine. Thus, in Case II, Table II, it is seen that when sodium bicarbonate is administered less alkali than acid is required in order to obtain neutrality, although the unincinerated urine is still acid.

Also, the decrease in the figure of alkali added *minus* acid added, indicates in pathological cases, such as diabetes, a commencing drain upon fixed alkali, or in other words that the body is commencing to fail to turn out enough ammonia to neutralize the organic acids.

In order to obtain the total amount of organic acids formed, two additional determinations are required, these are (1) the acidity of the urine without previous incineration in the ordinary way, and (2) the amount of ammonia.

In making the determinations of neutrality of the urine before and after incineration the same indicator must be employed in the two cases, in order to leave the balance between the inorganic ions at the same level in the two cases. In making the determinations of ammonia for which we have employed the Schlössing process, any indicator may be used which is sensitive to ammonia and gives the full yield—the indicator used by us was di-methyl-amido-azo-benzol. On account of the yellow colour of the urine we have found phenol-phthalëin the best indicator to use for the two titrations in the urine, and having found that when one proceeded from the acid side towards neutrality there was no appreciable error introduced by using phenol-phthalëin even in the presence of ammonia, we have used this indicator throughout.

Great care was taken to check this before committing ourselves to the use of phenol-phthalëin as an indicator in presence of ammonia; both by titrating dilute ammonia solutions of known strength, by first adding excess of standard acid, and then titrating back in presence of phenol-phthalëin, and by comparing titration results with the same solution, using in one case phenol-phthalëin, and in the other di-methyl. The results were always closely concordant, although the change in colour is much less sharp than in the case of fixed alkalies.

If, however, the titration is carried out from the acid side with standard alkali, and the first faint appearance of pink is taken as the

sign of neutrality, phenol-phthaleïn indicates the amount of ammonia quite accurately. If the phenol-phthaleïn be added in presence of free ammonia and titration with acid be attempted there is, however some effect of the ammonia upon the indicator and wrong results are obtained.

In urine which may contain a great deal of ammonia, however, the reaction is always acid to phenol-phthaleïn, and hence direct titration with alkali can always be undertaken without any interference or error due to the presence of the ammonia.

The manner in which the three determinations mentioned above, yield an estimate of the excess of organic acid above organic base formed in the body may be illustrated by giving a formal statement of what is determined in each process, as follows :—

- I. Direct titration with $\frac{N}{10}$ alkali = Acid salts of inorganic acids + acid salts of organic acids + free inorganic acids + free organic acids.
 - II. Total $\frac{N}{10}$ acid added – total $\frac{N}{10}$ alkali added in incineration process
(i.e. total alkali produced by incineration) = Organic acids combined with fixed bases—free inorganic acids – ammonium salts of inorganic acids—acid salts of inorganic acids.
 - III. Total ammonia = Ammonium salts of organic acids + ammonium salts of inorganic acids.
- On addition, I + II + III = Acid salts of organic acids + free organic acids + organic acids combined with fixed bases + ammonium salts of organic acids.

That is to say, addition of Nos. I, II, III give the total organic acid, combined or free in the urine, and all the other constituents cancel out.

In the scheme above, for completeness in statement, free inorganic and free organic acids are included although these are not usually present, if absent, however, these constituents simply drop out of the scheme, and the addition still gives the total organic acids.

Nos. I and III in the above equations are so obvious as to require no comment ; regarding No. II it may be pointed out :—(1) Any organic salts of fixed bases are converted into carbonates on incineration, and so increase No. II ; (2) any organic salts of ammonium or organic bases disappear on incineration, and so do not affect No. II ; (3) any inorganic ammonium salts are converted into corresponding sodium salts, and so use up alkali and decrease No. II ; (4) any free inorganic

acid or inorganic acid salts require alkali for neutralization, and so decrease No. II.

Before passing to the tables of results, the procedures and precautions in carrying out each of the three determinations enumerated above may be described.

I. *Initial titration of the urine.* Twenty c.c. of urine are taken and diluted to about 100 c.c. in order to prevent the urinary pigments from obscuring the end point of the reaction. On account of the change of colour with phenol-phthalëin being more obvious than that with other indicators in a solution of urine, and of its sensitiveness to organic acids, this indicator has been employed. The titration is made with $\frac{N}{10}$ sodium hydrate solution, and the first appearance of pink is taken as the end point.

II. *Titration after incineration.* Fifty c.c. of urine are taken, and 10 c.c. of N sodium hydrate solution are added, so as to give a considerable excess of free alkali with the object of preventing any loss of acid which may be present in the urine in combination with ammonia or organic bases. The mixture is evaporated to dryness in a platinum vessel, and incinerated at a low red heat. The ash is extracted with boiling water, and any unconsumed carbon is filtered off. The filtrate and washings are roughly titrated with $\frac{N}{10}$ sulphuric acid, with phenol-phthalëin as indicator, and an excess of about twice the amount of acid necessary for neutralization is added. The solution is then boiled for about fifteen minutes in order to remove carbonic acid, and to reconvert back into orthophosphates any pyrophosphate which may have been formed in the process of incineration. The final stage in the operation is the titration back to neutrality with $\frac{N}{10}$ sodium hydrate. The difference between total acid added and total alkali gives the figure for determination No. II. The reason for adding such a large excess of acid after incineration is that it is necessary to have free acid present, because the acid phosphates are so little ionized that the process of conversion into orthophosphates in their presence alone takes a long time, and may be incomplete even at the end of several hours boiling; in the presence of free acid the conversion takes place rapidly, and is complete in a few minutes.

III. *Determinations of total ammonia.* These were made by Schlössing's method, using 25 c.c. of urine, and taking care that the layer of urine and lime was shallow so as to afford a large surface. If this precaution be neglected, the total amount of ammonia is not obtained even in a week's time.

RESULTS WITH ARTIFICIAL MIXTURES

The method was tested in artificial mixtures containing phosphates, ammonium salts, and organic acids, with the results shown below, and was then applied to normal urine, and subsequently to three cases of diabetic urine for long periods.

Mixture No. 1. A solution was prepared containing the following constituents in the amounts stated per litre :—Sodium chloride, 6 grams ; ammonium chloride, 1·15 grams ; primary sodium phosphate (NaH_2PO_4), 2·0 grams ; acetic acid, 340·8 c.c. of $\frac{\text{N}}{10}$. The mixture was analysed as above described with the following results, expressed in amounts per 1,000 c.c. of the mixture :—

	Acidity of mixture expressed in c.c. of $\frac{\text{N}}{10}$ alkali required to neutralise 1000 c.c. of mixture	Alkali produced on incineration, that is acid added – alkali added in c.c. of $\frac{\text{N}}{10}$ per 1000 c.c. of mixture	Ammonia in c.c. of $\frac{\text{N}}{10}$ per 1000 c.c. of mixture	Organic acids expressed in c.c. of $\frac{\text{N}}{10}$ per 1000 c.c. of mixture
Calculated	—	—	215	340·8
Determined	484	– 344	204	344·0*

$$* \text{ c.c. } 484 + 204 - 344 = 344$$

Mixture No. 2. A solution was prepared containing the following constituents in the amounts stated per litre :—Ammonium chloride, 4·61 grams ; primary sodium phosphate (NaH_2PO_4), 2·0 grams ; secondary sodium phosphate (Na_2HPO_4), 2·0 grams ; sodium acetate, 3·2 grams ; urea, 8·0 grams.

The results of the analysis were as follows :—

	Acidity of mixture expressed in c.c. of $\frac{\text{N}}{10}$ alkali required to neutralise 1000 c.c. of mixture	Alkali produced on incineration, that is acid added – alkali added in c.c. of $\frac{\text{N}}{10}$ per 1000 c.c. of mixture	Ammonia in c.c. of $\frac{\text{N}}{10}$ per 1000 c.c. of mixture	Organic acids expressed in c.c. of $\frac{\text{N}}{10}$ per 1000 c.c. of mixture
Calculated	—	—	863	234
Determined	168	– 770	828	226*

$$* 168 - 770 + 828 = 226$$

Mixture No. 3. Solution contained in each litre, ammonium chloride, 3.07 grams; primary sodium phosphate (NaH_2PO_4), 2.0 grams; acetic acid, 676.0 c.c. of $\frac{\text{N}}{10}$.

	Acidity of mixture expressed in c.c. of $\frac{\text{N}}{10}$ alkali required to neutralize 1000 c.c. of mixture	Alkali produced on incineration, that is acid added - alkali added in c.c. of $\frac{\text{N}}{10}$ per 1000 c.c. of mixture	Ammonia in c.c. of $\frac{\text{N}}{10}$ per 1000 c.c. of mixture	Organic acids expressed in c.c. of $\frac{\text{N}}{10}$ per 1000 c.c. of mixture
Calculated	—	—	575	676
Determined	818	- 694	552	676*

$$* 818 - 694 + 552 = 676$$

These three experiments, and others which we have not recorded, show clearly that the method yields accurately the amount of organic acids in a solution such as the urine.

DETERMINATIONS IN NORMAL INDIVIDUALS

No.	Daily output of urine in c.c.	Total daily acidity expressed in c.c. of $\frac{\text{N}}{10}$ alkali required to neutralise	Total daily alkali produced on incineration, that is, acid added - alkali added in c.c. of $\frac{\text{N}}{10}$	Total daily ammonia expressed as c.c. of $\frac{\text{N}}{10}$	Total daily organic acids expressed as c.c. of $\frac{\text{N}}{10}$
1	2280	364.8	- 656.6	428.6	136.8
1	1840	504.2	- 1295.4	368.0	- 423.2
1	1850	303.4	- 329.3	488.4	462.5
1	1930	289.5	- 482.5	409.2	216.2
1	1280	371.2	- 440.3	397.4	328.3
2	1620	366.1	- 602.6	197.6	- 38.9
2	1410	400.4	- 747.3	186.1	- 160.8
2	1020	357.0	- 767.0	444.7	34.7
2	1650	343.2	- 402.6	244.2	184.8
2	1210	350.9	- 363.0	406.6	394.5

The negative figures in the third column show that inorganic acid in excess of base leaves the body daily, and indicate the amount of such excess. Attention may also be drawn to the variation in output of organic acid, even in normal individuals. A negative figure in the final column indicates that the excretion of organic bases other than ammonia is in excess of the excretion of organic acids.

The most probable cause of the variations in health shown in the table is a variation in the amount of acid or base in the diet of the individual, the variation in organic acid excreted expressing the action of the organism in maintaining a normal reaction in its media.

DETERMINATIONS IN DIABETIC PATIENTS¹

The amount of fixed alkali leaving the body daily and the output of organic acids was estimated in three cases of diabetic patients.

In the first case this was done daily throughout a period of six months, an abstract of the results only is given in the tables, containing periods illustrative of special points.

Case I. Male, aged 25. In this case Table I shows effects upon alkali, and organic acid output of administration of alkaline carbonates (sodium bicarbonate, one drachm (about four grammes) three times daily); Table II shows the changes in amount of ammonia and organic acids when not under continued administration of the alkali, and also illustrates the effects of a small dose of alkali occasionally under such conditions, the days on which alkali in the form of a seidlitz powder was administered being shown by an asterisk.

CASE I. TABLE I

Date 19c4	Daily output of urine in c.c.	Total daily acidity expressed in c.c. of $\frac{N}{10}$ alkali required to neutralise	Total daily alkali produced on incineration, expressed as c.c. of $\frac{N}{10}$	Total daily ammonia expressed as c.c. of $\frac{N}{10}$	Total daily organic acids expressed as c.c. of $\frac{N}{10}$
Nov. 1	3400	1029	1224	156	2400
" 3	3580	1647	788	165	2600
" 5	3700	1332	2057	89	3478
" 7	3980	1274	2579	80	3933
" 9	3980	613	3025	64	3702
" 11	3690	517	1727	118	2362
" 12	3410	423	1610	102	2135
" 14	1700	228	442	228	898
" 17	3520	493	1422	77	1992

At this stage the administration of bicarbonate (3 drachms or about 12 grams daily) was stopped

Nov. 19	3350	737	951	121	1809
" 21	2560	589	430	256	1275
" 23	2270	658	354	308	1320
" 25	2380	643	286	409	1338
" 27	2440	791	312	386	1489
" 29	1190	357	55	643	1055
Dec. 1	1360	245	76	530	851

Patient left hospital.

1. The character of the cases is given in the succeeding paper, pp. 28 to 38.

In this table it is to be noted that the alkali produced on incineration gives a positive figure throughout on account of the large amount of alkaline carbonate being administered. Contrast with Table II, Case I, where only an occasional seidlitz powder was being given, and also with Tables I and II, Case II. The presence of this amount of base in the urine is entirely concealed by the formation of organic salts, as is shown by the fact that the reaction of the urine remains acid throughout, although there is a decrease in the amount of acidity. It is also to be noted that during the alkaline carbonate administration the amount of ammonia is very low.

On stopping the administration of alkaline carbonates at the point shown in the Table, there commences a drop in the alkalinity on incineration which falls almost to zero, and, no doubt, would soon have shown a negative value as in Table II, or in Case II, Table II. Also there is a corresponding drop in organic acids which soon reach a steady value, lying much below that found during the administration of the alkaline carbonate. The amount of ammonia shows a great increase when the alkaline carbonate treatment is discontinued.

CASE I. TABLE II.

1905 Date	Daily output of urine in c.c.	Total daily acidity expressed in c.c. of $\frac{N}{10}$ alkali required to neutralise	Total daily alkali produced on incineration, expressed as c.c. of $\frac{N}{10}$	Total daily ammonia expressed as c.c. of $\frac{N}{10}$	Total daily organic acids expressed as c.c. of $\frac{N}{10}$
Jan. 10	2220	1021	- 1732	1763	1052
" 11	1850	932	- 1513	1121	540
" 12*	2500	1150	- 1690	870	330
" 13	1900	1026	- 125	1186	2087
" 14	2270	1008	- 617	1348	1739
" 15	2160	1382	- 1378	920	924
" 17	2190	1117	- 644	907	1380
" 19	2190	1082	- 1310	639	411
" 20*	2100	1079	- 1403	810	486
" 22	1480	1126	- 941	616	821
" 23	1760	1169	- 190	553	1532
" 24	2020	1180	- 170	1709	2719
Feb. 12	1730	1263	- 1547	827	543
" 13*	1870	1384	- 755	838	1467
" 14	2100	1604	- 227	848	2225
" 15	1530	1352	- 1493	927	786

In this table it is to be noted that the ordinary titration figure gives no indication of the administration of the alkali on the days marked by the asterisks. This is shown clearly, however, by the incineration figure in the next column, the drop in the negative value showing increased amount of base in the urine after these days. When the administration of alkali is continuous as in Case I, Table I, and in Case II, Table II, the negative figure after incineration becomes a positive one, showing the increased excretion of base under alkali administration. It is also to be observed that the amount of organic acid excreted becomes increased after the alkali, and then drops, to be again increased by the next dose of alkali. Taken together these facts indicate that the alkali given is neutralized in the body by organic acids, and that the body is protected from alkaline invasion in this way by increased formation of organic acids. This relationship is worth remembering in all attempts to prevent the effects of organic acids in the blood by means of administration of alkaline carbonates.

Case II. Male, aged 7. Table I shows alkali and organic acid output under strict diabetic diet. Table II shows effects upon alkali output of administration of alkaline carbonates, and, in the latter part, the effect of stopping the carbonates.

CASE II. TABLE I.

Date 1905	Daily output of urine in c.c.	Total daily acidity expressed in c.c. of $\frac{N}{10}$ alkali required to neutralise	Total daily alkali produced on incineration, expressed as c.c. of $\frac{N}{10}$	Total daily ammonia expressed as c.c. of $\frac{N}{10}$	Total daily organic acids expressed as c.c. of $\frac{N}{10}$
July 4	1110	546	- 107	706	1145
" 7	1820	510	- 29	903	1384
" 9	1730	467	- 367	837	937
" 12	1700	432	- 394	1129	1167
" 14	1480	524	- 255	1154	1423

CASE II. TABLE II

Effect of administering alkaline carbonates.

Date 1905	Daily output of urine in c.c.	Total daily acidity expressed in c.c. of $\frac{N}{10}$ alkali required to neutralise	Total daily alkali produced on incineration, expressed as c.c. of $\frac{N}{10}$	Total daily ammonia expressed as c.c. of $\frac{N}{10}$	Total daily organic acids expressed as c.c. of $\frac{N}{10}$
July 16	1480	397	512	929	1838
" 19	1990	418	704	788	1910
" 21	2160	432	1041	691	2164
" 23	2160	410	1961	311	2682
" 26	2170	87	2226	286	2599
" 28	1740	14	1688	230	1932
" 30	2000	48	1344	336	1728
Aug. 2	1770	35	1685	287	2007
" 6	1650	50	1432	310	1792
" 10	1980	20	1552	143	1715
" 13	2510	40	1852	251	2143
" 18	2220	-9	1332	195	1518
At this point the administration of alkaline carbonates was discontinued.					
Aug. 20	1310	327	-576	356	107
" 22	1280	338	-867	630	101
" 25	1420	327	-747	517	97
" 29	1030	361	-758	499	102
Sep. 3	1340	322	-825	504	1
" 8	1190	309	-369	367	307
" 12	1080	324	-577	372	119

In Table I it is seen that the alkalinity on incineration gives a negative figure, while the ammonia is high and the organic acids much higher than normal (contrast tables for normal individuals). Under the influence of the alkaline carbonates the alkalinity on incineration acquires a high positive value. The amount of alkali is so great that the reaction of the urine decreases in acidity and finally becomes faintly alkaline, but the change is not nearly so great as that shown by the incineration figures. Corresponding to this there is a marked increase in the amount of organic acids secreted. Finally, on stopping the alkaline carbonates, the alkalinity on incineration becomes a negative figure again, the ammonia increases again, though not back

to its former level, and the amount of organic acid drops not merely to its former level but down to a normal figure. The changes in ammonia and organic acid towards the end was not due solely to stoppage of the alkaline carbonate, but to another factor, *viz.*, treatment by acid extract of duodenal mucous membrane, which also had the effect of causing a disappearance of sugar, as explained in the succeeding paper (pp. 28 to 38).

Case III. Female, aged 9. This case was not put upon strict diabetic diet, and the quantities are from 8 p.m. to 8 a.m. daily. So that for comparison with the other tables they should be approximately doubled.

CASE III. TABLE I

Date 1905	Half-daily output of urine in c.c.	Total half-daily acidity expressed as c.c. of $\frac{N}{10}$ alkali required to neutralise	Total half-daily alkali produced on incineration, expressed as c.c. of $\frac{N}{10}$	Total half-daily ammonia expressed as c.c. of $\frac{N}{10}$	Total half-daily organic acids expressed as c.c. of $\frac{N}{10}$
Nov. 16	850	252	- 29	133	356
„ 21	680	292	301	291	884
„ 24	650	266	241	471	978
Dec. 5	540	268	- 11	272	529

CONCLUSIONS

1. A method is described for determining the output of fixed alkali or acid from the body, and also for determining the total output of organic acid.

2. It is the total output of organic acid in diabetes which is of highest importance in relationship to acidosis, or acid intoxication, and not the nature of the individual acids concerned.

3. Administration of alkali, while it yields base for neutralization of organic acids in the body and so decreases the amount of ammonia, at the same time markedly increases the output of organic acids as salts.

4. This property illustrates the mode in which the body normally protects itself from alkaline invasion.

When alkali is given, unless certain limits are passed, the urine remains acid, the alkali being neutralized by organic acid formed, but incineration shows that fixed alkali is now leaving the body, as organic salts.

This is interesting as forming a parallel to the somewhat similar mode of protection against acids. In the case of acids, urea is converted into ammonia which is used to neutralize the acid, and the ammonium salts can then be excreted without appreciably changing the reaction of the urine. In the case of alkalies, organic acids are formed from carbohydrate, and used to neutralize the alkali, and as before these can be excreted without appreciable change in the reaction of the urine. In this connection an interesting fact is the larger amount of organic salts in herbivorous urine.

5. The reaction of the urine, without previous treatment, accordingly gives no real indication of the amount of fixed acid or alkali leaving the body. The variations in output of organic acids in health are considerable, and probably express the reaction of the organism to variations in the amount of acid or base in the diet, whereby a normal reaction is maintained in the body fluids.