STUDIES ON THE ACID-BASE REGULATION IN MENTAL DISORDERS.

(*From the Central Pathological Laboratory of the London County Mental Hospitals.*)

PART I.

The Determination of Urinary Acidity.

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THE following is the first of a series of communications relating to urinary excretion in cases of mental disorder with special reference to acid excretion. In this paper it is proposed only to deal with the make-up of urinary acidity and the methods for the determination of the acid characters. It is well known that respiratory regulation constitutes the first line of defence against change of body reaction, and that the kidneys afford the second protection against alteration by variations in the acid-base ratio of the urine. It is also fully established that urinary acidity is dependent on the nature of diet ingested, but it is not so well recognized that the urinary reaction is also dependent on the adequacy of the respiratory compensatory mechanism, and it would appear that those interested in dietetics and chemical effects on urinary acidity have not appreciated sufficiently the significance of the physiological influences, and those concerned with the respiratory side have been apt to disregard the influence of diet. The organism promptly defends itself against alkalinity by the excretion of bicarbonate, whether the alkalinity be due to ingested alkali or excessive exhalation of acid (CO_2), and this excretion of bicarbonate appears to be independent of any particular renal threshold for bicarbonate (1). The response of the organism to acidity, however, is a much longer process, and in this case the kidney compensation takes the form of retention of base (1) by ammonium base production and (2) the retention of bicarbonate by alteration of the ratio of mono- and dibasic phosphate excreted according to the equation—

$$Na_2HPO_4 + CO_2 + H_2O = NaH_2PO_4 + NaHCO_3.$$

The ammonium base production from the waste nitrogenous substance urea is capable of great expansion and large quantities may

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be created to replace and prevent the loss of Na to the organism, which might result from the effect of invading acids. The phosphate acid compensation is more limited in extent and depends on the amount of available phosphate, and the fact that there is a limit to the effective acidity that can be excreted by the kidney. The phosphate available as shown by the phosphate concentration of the urine may show wide variations in amount; and in the present series of urines examined (a large number representing 24-hour, day and night, and short period urines), the phosphate concentration varied from M/15 to M/150. The ammonium base production deals with the greater part of acid regulation and its excretion rate follows closely the pH of the urine, but the main adjustment of the urinary reaction is effected by the balance of the mono- and di-basic phosphates excreted. It is found that under most conditions the pH of the urine ranges, on the acid side, from 4·5 (the pH of acid phosphate solution) to approximately 8·8, on the alkaline side, the maximum pH for bicarbonate solutions.

Ammonium production has been shown by Benedict and Nash (2), (3), (4) to be a renal function; and its formation bears some relation to the amount of phosphate available. Haldane concludes (5) that the ratio of acid to ammonia in the urine depends on the amount of phosphates available for excretion. In his experimental acidosis the phosphate excretion was increased at the onset, but fell later indicating an exhaustion of phosphate resources. Marriott and Howland (6) fed equivalent amounts of acid in the form of dilute HCl and mixtures of phosphates of different pH to humans, and whereas the acids increased both titration-acidity and ammonia output, acid sodium phosphate increased the output of acid only, and a phosphate solution of pH 7·4 increased the acid but diminished the ammonia output. Hubbard and Munford (7) have shown that the ammonia output varies with urinary volume, which further exemplifies the base-saving object of ammonia production. Ammonia excretion accompanies acidosis, but the output may be even greater during recovery from acidosis (8), in either case it represents the need of the organism for base economy, and Ambard and Schmid (9) regard the renal production of ammonia as being guided by the general composition of the blood and not merely in response to acidosis.

Hasselbalch has suggested that the ratio between ammonia and total nitrogen in the urine varies with the pH, and Rafflin (10) suggests another formula on the same lines, but Polnovski and Boulanger (11) from the analysis of two-hourly by day and complete night urine specimens could obtain no constancy with either formula. Fiske (12) has determined the ratio of ammonia and

sulphate excreted, and suggests that the value of this ratio varies with the pH.

The availability of phosphate and the changes in phosphate excretion are considered to be related to the variations in carbohydrate metabolism dependent upon muscular movement and tone; increased excretion being associated with muscular relaxation when phosphate is not required, or after exercise, it has fulfilled its functions in muscular metabolism (13). As a rule the phosphate excretion and acidity fall during the morning, and rise from noon onwards to an evening maximum.

Lastly, in the make-up of the amount of urinary acidity and less associated with urinary reaction, are organic acids, uric, hippuric and aliphatic acids, which, if the organism tends to alkalinity, will be excreted with their full quota of base, but with increasing acidity and retention of base by the kidney may be excreted in a partially combined state. In very acid urines containing aceto-acetic and β-hydroxybutyric acids, 5% of the former and 20% of the latter may be in the free state (14).

The tidal variations in urinary excretion in normal and psychotic subjects will be recorded in a later communication, but the following figures (Table I) for two-hourly urines, day and night, from a normal and an (acid) psychotic subject show the phosphate and ammonia excretion in relation to the total acidity and urinary pH.

TABLE I.

—	8–10 a.m.	10–12 a.m.	12–2 p.m.	2–4 p.m.	4–6 p.m.	6–8 p.m.	8–10 p.m.	Average for two hours, night.
Normal:								
Volume	105	68	98	102	109	196	56	56·8
pH	7·1	7·4	7·1	7·2	6·2	6·0	5·2	5·3
Total acidity N/10	3·2	2·4	5·4	7·2	21·8	26·5	25·2	27·6
Total phosphates N/10	10	8	17	19	28	24	18	21
Total NH_4 N/10	23	20	21	28	30	42	41	43
Acid psychotic:								
Volume	61	103	87	125	115	65	...	113
pH	5·0	5·0	5·0	5·2	5·0	5·0	...	5·2
Total acidity N/10	25·1	45·9	41·8	38·9	53·5	39·4	...	26·8
Total phosphate N/10	13	21	20	18	26	19	...	17
Total NH_4 N/10	45	75	40	46	70	40	...	34

The factors of urinary acidity usually determined are:

(1) The total acidity and ammonium base, the sum of which represents the excretion of acid without the loss of base to the organism, and indicates the need of the body for base economy.

(2) The phosphate concentration, and the ratio of the mono- and di-basic phosphates representing the main factor in the adjustment of urinary reaction.

(3) The organic acid concentration.

And lastly, the sum total of all these factors, the pH. At a urinary pH more alkaline than 7·4 the organism must tend towards alkalinity, and at a pH at which all available phosphate is excreted in the acid form, pH 5–4·5, there must be a tendency towards acidity, the resulting body reaction depending on the adequacy of respiratory adjustment.

Reference will be made to the methods adopted for the determination of those factors, especially in relation to the total acidity titration of Folin, which appears to be faulty in principle, and to the organic acid titration of Van Slyke and Palmer (15), (16), which may give rise to erroneous conclusions without adequate understanding of the factors involved.

Hydrogen Ion Concentration.

pH determinations have been made by the usual method of addition of indicator to diluted urines and to a series of standard buffer solutions of pH 4 to 9 similarly diluted, and comparison of the colour with the standards. The buffer standards require to be made every few days, and their general preparation is rather beyond the facilities afforded by the ordinary mental hospital laboratory. The B.D.H. capillator method provides a simple and rapid technique of sufficient accuracy for clinical laboratories. It consists in merely mixing equal volumes of urine and the given indicator and comparing the colour produced with the standards provided. Compared with the usual method, this simple process gives very consistent results.

Total Acidity.

The usual method for determination of urinary total acidity consists in the titration of a known quantity of urine with N/10 alkali to the phenolphthalein end-point (pH 7·8). The titration figure is too high and Henderson and Palmer (17) titrate to pH 7·4, the former, however, is the more common practice. This titration with N/10 alkali will include the amount of acid phosphate present, weak organic acids, uric acid, hippuric acid, aliphatic acids, and creatinine. The latter are mainly dietetic in origin, and under ordinary conditions relatively unimportant, but the former depends on the amount of phosphate available and may bear no relation to the pH of the urine, which is mainly dependent on the ratio

of the mono- and di-basic phosphates. It should be noted that ordinary base-forming diet promptly shows an alkaline effect on the urine, whereas an ordinary, acid-forming diet, although raising the total acidity figure (18), does not produce the same change of urinary pH as that noted during sleep which is due to slowing of CO_2 output associated with a less sensitive respiratory mechanism.

In this titration with phenolphthalein Folin states that there is interference with the end-point due to calcium salts, and recommends the following procedure: To 25 c.c. of urine 15–20 grm. finely powdered potassium oxalate and one or two drops of 1% phenolphthalein solution are added. The mixture is shaken vigorously for 1–2 minutes and titrated with N/10 sodium hydroxide until a faint pink colour remains permanent on standing. During the course of hundreds of such titrations it has been noticed that wide discrepancies may occur with this method depending on the concentration and composition of the urine in question. Such discrepancy is considerably more than an interference due to calcium salts and may represent a 30% error. The difference between the titration figure, with and without oxalate addition, bears a relation to the pH of the specimen with consequent ionic concentrations and urate content. The addition of oxalate, as recommended by Folin, gives results less accurate than the simple titration of the urine.

Phosphate Ratios.

In a solution of mixed mono- and di-basic phosphates, titrations with standard alkali to phenolphthalein change will give the amount of acid phosphate, and following titration with standard acid to methyl orange change will give the total amount of phosphate, thus enabling the ratio of the two phosphates to be determined. On the grounds that the phosphates of the urine are the principal constituents concerned with its reaction, and that urinary reactions practically always lie between the pH's represented by the phenolphthalein and methyl orange changes, this method of titration has been applied to urines (19), the results being expresesd as acidity or alkalinity *per cent.* as with the phosphate solutions. Others have termed this double titration the urinary phosphate ratio, inferring that it represents the ratio of urinary mono- and di-basic phosphate. Although these titrations may give an insight into the buffer capacity or potential reaction of the fluid, it is found that they bear not the slightest relation to the actual amount of phosphate present; the titration figure may be as much as five times that of the actual amount, and gives but a very crude index of the actual phosphate ratio. Moreover, there is only rough correspondence between the

pH and the " phosphate ratio " thus determined. The inaccuracy is due to the interference by uric acid, hippuric acid, creatinine, and aliphatic acids (β-hydroxybutyric, lactic, etc.).

Determination of Urinary Ammonium Base.

The estimation of ammonium base excretion is of importance in urinary analysis. The following methods are available.

1. The aeration method of Folin. Alkali is added to the urine and the ammonia set free carried over by aeration into a measured amount of acid, the quantity evolved being determined by titration of the acid excess or colorimetrically by Nesslerization.

2. Distillation *in vacuo* of urine rendered strongly alkaline, and collection of the NH_3 in standard acid and estimation by titration or colorimetrically.

3. A colorimetric method (20) depending on the fact that amines or imines with presence of sodium hypochlorite react with phenol to give first p-nitroso phenol. This in turn reacts with the excess of phenol to give p-benzoquinonoxyphenylimine, which has a blue colour in solution (21).

4. The formol titration method (22), which gives a figure representing both ammonia plus amino-acid nitrogen.

The first two methods are tedious and not practicable for a large number of estimations. The colorimetric method gave promise of being a rapid and accurate one, but an exhaustive investigation has been unsuccessful in arranging the technique to give reliable results, and it has been found that the Malfatti formol titration is the best practical method, and, as will be shown later, accuracy can be approached by a preliminary adsorption of the urine with animal charcoal.

The Effect of Charcoal Adsorption on the Composition of Urine.

Errors in the analysis for acidic factors of urine are mainly due to colour and interference by uric acid, creatinine, etc., and the following analyses were made following successive charcoal clearances to see how far such a procedure could be used as an aid to analytical accuracy. Table II shows the effect of successive 5% charcoal adsorptions on two specimens of urine.

It will be observed that one adsorption with 5% charcoal effects the complete clearance of uric acid. Also successive adsorptions do not appreciably affect the phosphate, amino acid and ammonium contents. Creatinine is not readily adsorbed. After the first adsorption the total acidity remains practically constant.

TABLE II.

Amounts calculated to one litre.—5% purified charcoal added, allowed to stand for ten minutes, and filtered.

Ads.	pH.	Phosphate, mgrm. P.	Total N., grm.	Creatinine, mgrm.	Uric acid, mgrm.	Amino acid, mgrm. N.	Organic acid, c.c. N/10.	Total acidity, c.c. N/10.	P.R.*	Malfatti titration, c.c. N/10.
Specimen 1.										
0	6·3	1,150	11·06	2,000	341	130	620	375	·44	376
1	6·3	1,150	10·50	1,300	*Nil*	122	432	325	·44	375
2	6·3	1,150	10·12	710	,,	126	344	320	·45	371
3	6·3	1,150	9·66	27	,,	122	344	315	·47	368
Specimen 2.										
0	5·6	650	...	1,150	331	105	216	230	·63	343
1	5·6	630	...	527	*Nil*	109	176	195	·65	350
2	5·5	620	...	166	,,	99	84	193	·71	343
3	5·5	590	...	0	,,	95	92	195	·71	336

* N/10 KOH titration to pH 7·8 phenolphthalein
N/10 HCl titration pH 7·8 to pH 4·5 : B.D.H. 4·5 indicator

The effect of the organic acid titration will be referred to later. The slight difference in the pH of the adsorbed and unadsorbed urines in the two examples given is exceptional; in most of the adsorbed samples a distinct reduction in acidity (pH) was noted.

URIC ACID.

The state of purin bodies in the urine causes many reactions referable to the pH. To this can be ascribed the so-called iodine number of urines reported by Weltmann (23), and also probably the curious silver nitrate reactions recorded by Buscaino (24) in mental disorders. In a work to be published on thiosulphate excretion by one of us (S. A. M.) and Dr. Mary Barkas it was found that thiosulphate could be accurately titrated with iodine solution in urine after charcoal adsorption—the interference in unadsorbed specimens depending both on the purin content and the pH of the urine in question.

In addition to the experiments noted, it was found that uric acid was completely removed from a series of urines of varying hydrogen ion concentrations (pH 4·5–pH 7·2) by one 5% charcoal adsorption. Further it was found that hippuric acid was removed up to 80%, and this adsorption could be associated with the more alkaline pH. A saturated solution of hippuric acid adjusted to pH 5 with N/10 KOH, after adsorption had a pH approximating 7·0.

PHOSPHATES, AMMONIUM + AMINO ACIDS, AND AMINO ACIDS.

The inappreciable effects of adsorption on the phosphate content of urines, determined by the colorimetric method of Briggs (25), was further confirmed on a large series of urines of varying pH over the range of ordinary urinary reaction. The constancy of the Malfatti formol titration, and the amino acid by Folin's β-naphthoquinone sulphuric acid method (26) under the same conditions was also confirmed. In each case the absence of colour and interfering substances renders the estimation more satisfactory.

PHOSPHATE RATIOS.

Reference to Table II will show that successive adsorption effects a change in the so-called phosphate ratios as represented by—

$$\frac{\text{titration with N/10 KOH up to pH 7·8}}{\text{titration with N/10 HCl from pH 7·8 to pH 4·5}}$$

There is still no relation between this figure and the actual amount of the phosphates present, and still but rough correspondence with the urinary pH. If the phosphate ratio be taken as the ratio of acid phosphate to the total phosphate as actually determined, and the figure for the titration up to pH 7·8 be taken to express the amount of acid phosphate, in the first case the ratio drops from 1 to 0·85 for a urinary pH 6·3, and in the second case both ratios would indicate that all phosphate was present in the monobasic form at a pH of 5·5. There is still marked interference in the titrations and the investigation of a number of phosphate ratios declared on the basis of the actual phosphate concentration indicates that the so-called phosphate ratio procedure is totally unnecessary and fallacious, and that the pH of the urine is sufficient in itself to denote the ratios of the phosphates present. At a pH less than 5 all phosphate exists in the acid form, beyond which it has exerted its full buffer capacity, the urine has reached its limit of acidity and the organism tends to acidity. The variations in pH and the associated total phosphate concentration are the factors of importance. The so-called "phosphate ratio" is concerned with relatively unimportant dietetic factors, which mask any resemblance to the actual proportions of mono-basic and di-basic phosphates present. In the above titrations methyl orange is generally used for the acid titration, but in this work it has been replaced by the more efficient 4·5 "phosphate" indicator (B.D.H.).

THE "ORGANIC ACID" TITRATION (VAN SLYKE AND PALMER).

This estimation is carried out in the following manner:

To a quantity of urine 2% finely powdered calcium hydroxide is added, and after stirring and allowing it to stand for 15 minutes

the mixture is filtered; in this process carbonates and phosphates are removed. Of the filtrate 25 c.c. are transferred to a 150 c.c. cylindrical glass vessel, and 0·5 c.c. phenolphthalein (1% solution) and N/5 HCl added until the pink colour is just discharged. 5 c.c. of 0·02% Orange IV in alcohol are added and the mixture titrated with N/5 HCl until the red colour is the same as that of a standard made with 5 c.c. Orange IV solution, and 0·6 c.c. N/5 HCl made up to 60 c.c. in a similar cylindrical vessel. The final comparison is made with both solutions at the same volume. The figure for the titration from pH about 8 phenolphthalein to 2·7 Orange IV minus the 0·6 c.c. added to the standard is calculated in terms of N/10 HCl *per cent.* or *per diem.* The titration includes organic acids, creatine, creatinine, and a small amount of amino acids. Normally the excretion for 24 hours corresponds to 280–750 c.c. N/10 HCl or about 8 c.c. per kilo body weight, corrected for creatinine 240–600 c.c. *per diem.*

In diabetic acidosis values from 20 to 180 c.c. per kilo. body weight have been observed.

The method seemed specially attractive for the investigation of mental disorders in relation to disordered metabolism and defective oxidative reactions, *i.e.*, the excretion of β-hydroxybutyric, aceto-acetic, and lactic acids. Further it seemed possible to gauge the nature of the mixture of organic acids by reason of their dissociation constants, and titration to a series of hydrogen ion concentrations between pH 8 and 2·7, as applied to the examination of fruit juices by Goiffon and Nepveux (27). A series of communications have been made by Laignel-Lavastine and Cornélius (28), (29), (30) on the results obtained with anxiety and depressed cases. They record an increased excretion of organic acids as determined by titration, the excretion being more marked with increasing alkalinity of the urine. Further, Goiffon (31) records an increased excretion of organic acids following alkalosis induced by bicarbonate administration. We have not been able to confirm any of these findings. As will be shown later, in our experience the urinary reaction of anxiety and depressed cases is unduly acid, also when adequate correction is made for dietetic and interfering factors, there is no marked increase in the " organic acid " output. Lastly, the organism promptly excretes bicarbonate following alkali administration and the increase of organic acids reported by Goiffon and probably that reported by Laignel-Lavastine and Cornélius is nothing but excreted *bicarbonate which is not cleared* by the treatment with lime, and therefore included in the organic acid titration. We have confirmed the latter fact by experiment and actual determination of the bicarbonate on urines thus treated. Urine of

pH 7·4 may contain the bicarbonate equivalent of 360 c.c. N/10 per litre, whilst at pH 6 the content is negligible, 10–15 c.c. per litre. Palmer in a later paper (16) points out the bicarbonate error of the original organic acid titration and recommends the preliminary acidification of urines tending to alkalinity.

Further, it is clear that considerable correction is necessary for uric acid, hippuric acid, creatinine, glycocoll, factors concerned with diet, if it is desired to gauge the amount and nature of the more important aliphatic acids present. The following table (III) gives the results taken from the analysis figures of a large number of urines in a series showing the proportion of the organic acid titration that is accounted for by relatively unimportant dietetic and other factors.

TABLE III.

Specimen.	Total titration per litre, pH 7·8 to 2·7.	Creatinine.	Uric acid.	Glycocoll (60%).*	Corrected.
1	360 N/10 HCl	96·4	34·0	31·3	218·3
2	560 ,,	114·9	34·0	49·4	361·7
3	700 ,,	151·2	79·5	35·1	434·2
4	760 ,,	159·4	48·6	35·4	516·6
5	720 ,,	139·1	59·5	32·0	489·4
6	640 ,,	146·3	52·3	30·7	410·7
7	620 ,,	176·9	40·6	55·6	346·9
8	420 ,,	96·2	33·3	34·3	256·2
9	344 ,,	93·6	32·4	23·3	214·7
10	400 ,,	88·5	39·4	27·5	244·6

* Over this range glycocoll is only 60% titrated. The above figures need further correction for the hippuric acid content, which was not estimated.

Reference to Table II will show that after adsorption with charcoal the organic acid titration reaches a fairly constant figure, following the removal of uric acid, hippuric acid and a proportion of the creatinine. Salts of aliphatic acids and amino acids are not affected by charcoal adsorption. It may be possible that a more accurate organic aliphatic acid figure may be obtained by preliminary adsorption of the urine with charcoal, determination of the organic acid titration, and correction by actual determination of the remaining creatinine and glycocoll. In its present condition the method can only serve as a rough indication of organic acid excess in marked ketosis in which there is considerable excretion of β-hydroxybutyric acid, etc. The results obtained with the modified technique, and with fractional titration, will be recorded at a later date.

SUMMARY.

In view of the communications on urinary acidity and its relation to respiratory phenomena to follow, it may be that the determination of urinary acidity may have greater significance than heretofore in the investigation of mental disorders.

The factors concerned with urinary acidity have been reviewed, the methods for their determination revised, and certain fallacies regarding the estimation of total acidity and organic acid content noted. It has been shown that the increased excretion of organic acids with alkalosis (reported by the French observers), and in depressive psychotic conditions, is not correct, and arises from faulty principles of technique.

A preliminary charcoal adsorption of urine specimens is suggested to increase the ease and accuracy of certain estimations; also, by this means it may be possible to differentiate the organic acid titration between exogenous organic acids (uric, hippuric acids), and the more important aliphatic acids (aceto-acetic, β-hydroxybutyric and lactic acids).

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