LIII. AMMONIA EXCRETION, AMINO-ACID EXCRETION AND THE ALKALINE TIDE IN SINGAPORE.

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INTRODUCTION.

IN a paper on nitrogen partition in the urine of the races in Singapore [Campbell, 1919] it was noted that on many occasions the amount of ammonia excreted by apparently normal individuals was high when compared with the European standards. It was suggested that the climatic conditions—hot, moist and still air—in Singapore might be responsible for this acidosis.

The observations recorded in the present paper were made in the first place to determine the hourly variations in the ammonia excretion and their significance, which might explain the acidosis. The excretion of amino-acids and the reaction of the urine were also considered.

AMMONIA EXCRETION.

The amounts of ammonia excreted hourly by thirty subjects of various races, were estimated by Malfatti's method. In most cases daily amounts, for a week or longer, were also estimated using both Malfatti's and Folin's methods. The former method estimates the ammonia of any amino-acids in the urine in addition. Folin's method was not used in my previous paper [1919], but the results obtained with it corresponded with the results recorded by Malfatti's method, as will be seen from Table I, which shows typical examples of average figures from individuals on their usual diets, mixed or vegetarian, similar to those described in the previous paper. In some cases the average figures for ammonia excreted daily were normal; in others, *e.g.* Chinese II and Tamil IV, the figures were high, due allowance being made for the diets and the total nitrogen excretions. Typical examples of all the figures obtained for ammonia excreted hourly are given in Tables II, III and IV. In Charts I, II and III the results, arranged in the form of curves, cover various periods of time continuous for about four days.

On a total nitrogen excretion of about 8 g. the average daily amount of ammonia nitrogen excreted by a normal person is usually stated to be between 0.5 g. and 0.55 g., if Malfatti's method of estimation be used; this gives

Table I. *Ammonia excretion and reaction of urine (average daily figures).*

Subject	Height in inches	Weight in kilos	Diet	Number of obser- vations	Ammonia N				Amino-acid N g.	Total N per kilo of body weight	Acidity Leather's method %	Volume in cc.	Titr. NH ₃ in tablets of 0.1 N acid		Total acidity A+N	Ratio A N	
					Folin's method		Malfatti's method						A	N			
					Total N g.	g.	%	g.	%								
European	67.0	54.8	mixed	16	10.54	0.55	5.3	0.68	6.5	0.13	1.2	0.192	—	1210	275	396	0.69
Tamil I ...	69.0	54.8	mixed, mainly rice	17	8.64	0.46	5.3	0.58	6.7	0.12	1.4	0.157	—	622	271	328	0.82
Chinese I	62.5	54.8	"	9	8.99	0.44	4.9	0.56	6.3	0.12	1.4	0.164	55	857	347	319	1.08
Chinese II	65.0	50.0	"	9	7.74	0.55	7.1	0.65	8.5	0.11	1.4	0.155	59	839	330	392	0.84
Chinese III	61.5	47.4	"	10	9.83	0.50	5.1	0.63	6.4	0.13	1.3	0.207	51	1164	345	360	0.96
Eurasian...	61.0	51.3	"	10	9.01	0.47	5.2	0.58	6.4	0.11	1.2	0.175	57	1099	343	339	1.01
Sikh I ...	65.0	52.0	"	9	7.17	0.39	5.5	0.50	7.0	0.11	1.5	0.137	52	1016	273	279	0.97
Sikh II ...	61.5	44.0	"	9	5.02	0.31	6.1	0.39	7.7	0.08	1.6	0.111	52	511	193	224	0.86
Tamil II ...	62.0	46.8	vegetarian, mainly rice	7	4.95	0.39	7.9	0.45	9.1	0.06	1.2	0.105	—	807	162	280	0.58
Tamil III	65.0	40.0	"	17	4.57	0.29	6.3	0.35	7.7	0.06	1.4	0.114	—	895	94	207	0.45
Tamil IV	63.5	51.1	"	10	5.02	0.50	10.0	0.60	12.0	0.10	1.9	0.098	39	649	196	357	0.55
Tamil V ...	65.0	52.0	"	10	4.38	0.39	9.0	0.46	10.6	0.07	1.6	0.084	46	699	218	280	0.78
Standards for Europe	—	—	mixed	—	16.00	0.53	3.3	—	—	—	—	0.200	—	1231	278	370	0.75
	—	—	"	—	8.00	0.40	5.0	0.52	6.5	—	—	—	—	—	—	—	—

Cole

Table II.

Chinese Subjects. 21/11/19.

(Mixed diet; small meal 6.15 a.m.; large meal 11 a.m. to 12 noon; total nitrogen, 8 g.)

A.—Urinary Alkalinity per cent. per hour.

	a.m.						p.m.					
Subjects	7	8	9	10	11	12	1	2	3	4	Average	
T.S.W.	36	50	53	34	30	34	44	66	64	34	44	
L.E.K.	40	55	98	66	50	56	60	70	65	36	59	
H.S.	56	71	60	47	37	34	37	42	56	34	47	
T.S.H.	46	86	97	78	23	23	23	71	94	75	61	

B.—Urinary Ammonia mg. per hour.

	a.m.						p.m.				Average	Remarks
	7	8	9	10	11	12	1	2	3	4		
T.S.W.	41	38	33	44	29	33	33	20	34	25	33	Excess
L.E.K.	36	28	14	27	19	23	25	17	22	20	23	Normal
H.S.	25	37	31	34	22	38	40	23	36	28	31	Excess
T.S.H.	22	16	13	16	13	20	20	17	8	13	16	Normal

(total nitrogen 5 g.)

C.—Amount of Urine cc. per hour.

	a.m.						p.m.				Average
	7	8	9	10	11	12	1	2	3	4	
T.S.W.	98	172	54	51	35	36	39	31	48	28	59
L.E.K.	19	29	30	35	23	42	65	42	42	28	35
H.S.	44	92	100	64	26	24	20	15	28	18	43
T.S.H.	46	36	36	34	20	22	22	34	30	25	30

D.—Amount of Fluid taken cc.

		Total	Average
			per hour
T.S.W.	6.20 a.m., 200 cc.; 9, 200 cc.; 11.20, 200 cc.; 3 p.m., 200 cc. ...	800	80
L.E.K.	6.20 a.m., 200 cc.; 8.30, 200 cc.; 11, 400 cc.; 3.30 p.m., 200 cc. ...	1000	100
H.S.	6.15 a.m., 400 cc.; 8.50, 200 cc.; 11, 600 cc. 1 p.m., 200 cc.; 2, 200 cc.; 3, 200 cc. ...	1800	180
T.S.H.	6.30 a.m., 200 cc.; 11.15, 200 cc.; 1.30 p.m., 400 cc.; 3.15, 200 cc. ...	1000	100

the average hourly amount of ammonia nitrogen as 21 or 23 mg. Some of my subjects, whose figures are shown in Tables II, III and IV, excreted quite normal average hourly amounts, whilst others excreted large amounts. The subjects, whose results are given in Charts I, II and III, excreted normal average hourly amounts; and it will be noted that as a rule the ammonia excretion increased as the alkalinity per cent. decreased and *vice versa*; in other words the hourly ammonia varied inversely as the alkalinity or directly as the acidity. This supports the theory that urinary ammonia is there to neutralise acid. The alkalinity per cent. was estimated by Leathes' titration method [1919] in which the reaction of the urine is expressed as so much per cent. alkali or so much per cent. acid.

Attention is now directed to the hourly variations in amount of ammonia excreted when the average was distinctly higher than the normal, that is about 30 mg. As seen in Tables II, III and IV, the hourly variations for large quantities did not differ materially from those for normal amounts, that is the ammonia excreted varied, as a rule, inversely as the alkalinity.

Table III.

Tamil Subjects. 24/11/19.

(Mixed diet; small meal 6.15 a.m.; large meal 11 a.m. to 12 noon; total nitrogen, 8 g.)

A.—*Urinary Alkalinity per cent. per hour.*

Subjects	a.m.						p.m.				Average
	7	8	9	10	11	12	1	2	3	4	
A.B.C.	26	34	62	89	75	53	41	73	66	60	57
A.P.P.	45	30	41	40	44	39	36	55	53	52	43
V.D.S.	40	41	42	48	50	46	40	62	72	71	51
C.S.	44	56	73	66	47	52	37	60	65	65	56

B.—*Urinary Ammonia mg. per hour.*

Subjects	a.m.						p.m.				Average	Remarks
	7	8	9	10	11	12	1	2	3	4		
A.B.C.	68	56	27	33	28	30	42	26	32	31	37	Excess
A.P.P.	21	27	29	33	23	25	25	26	23	28	26	Normal
V.D.S.	33	26	27	40	20	27	23	19	15	17	24	"
C.S.	21	27	19	23	17	26	27	27	12	13	21	"

C.—*Amount of Urine cc. per hour.*

Subjects	a.m.						p.m.				Average
	7	8	9	10	11	12	1	2	3	4	
A.B.C.	74	144	45	84	90	80	120	43	37	40	75
A.P.P.	25	26	43	66	52	50	39	27	29	48	40
V.D.S.	100	52	41	60	35	38	24	26	20	25	42
C.S.	32	24	24	26	18	27	31	164	32	53	43

D.—*Amount of Fluid taken cc.*

										Total	Average per hour
A.B.C.	6.30 a.m., 200 cc.;	11.20, 200 cc.;	2 p.m., 200 cc.	...	...	...	...	...	...	600	60
A.P.P.	6.30 a.m., 200 cc.;	11.30, 300 cc.;	2 p.m., 200 cc.	...	...	...	...	...	...	700	70
V.D.S.	6 a.m., 200 cc.;	8, 200 cc.;	11.30, 200 cc.;	2 p.m., 200 cc.	...	...	...	...	...	800	80
C.S.	6.30 a.m., 200 cc.;	10.30, 200 cc.;	12.15 p.m., 300 cc.	...	...	...	...	...	...	700	70

The main object of the hourly estimations was to endeavour to determine whether the ammonia was excreted in greater quantity during the hours of the day when individuals were most exposed to the effects of the climate. This was not found to be the case. The average hourly ammonia excreted during the period of sleep at night was practically the same as that for the whole day (Charts I, II and III). The total amount for any period of eight consecutive hours of the day was not materially different from that for another period of the same duration. Nevertheless, it must be stated, that

the highest daily amounts of ammonia, in all my cases covering a period of six years, were excreted by those who were, during their work, most exposed to the effects of the climate; they included bakers, rubber estate coolies and rickshaw runners [1918]. In other countries large amounts of ammonia have been detected in the urine of soldiers [Hammarsten and Hedin, 1914]. Koizumi [1919] has found that muscular work at raised temperature causes an increase of urinary ammonia of rabbits.

The subjects described in the present paper attended the medical school, most of them living at the school hostel. Sometimes they showed quite normal amounts of ammonia; at other times there was a distinct increase, the increase lasting sometimes only one day, sometimes several days and sometimes a week, the diet, general routine and total nitrogen excretion remaining almost constant. The average increase for those on a mixed diet

Table IV.

Eurasian Subjects. 26/11/19.

(Mixed diet; small meal 6.15 a.m.; large meal 11 a.m. to 12 noon; total nitrogen, 8 g.)

A.—Urinary Alkalinity per cent. per hour.

Subjects	a.m.						p.m.				Average
	7	8	9	10	11	12	1	2	3	4	
L.	38	44	56	50	50	34	30	41	55	62	46
G.	36	34	35	35	31	38	30	40	41	42	36
N.	35	35	46	38	38	38	38	57	87	78	42
P.	30	40	42	46	51	40	32	54	75	75	48

B.—Urinary Ammonia mg. per hour.

Subjects	a.m.						p.m.				Average	Remarks
	7	8	9	10	11	12	1	2	3	4		
L.	45	39	30	49	20	32	32	40	33	34	35	Excess
G.	62	31	29	45	25	30	34	32	28	32	35	"
N.	25	25	48	36	36	35	35	25	21	21	31	"
P.	33	29	25	37	21	19	19	17	26	19	24	Normal

C.—Amount of Urine cc. per hour.

Subjects	a.m.						p.m.				Average
	7	8	9	10	11	12	1	2	3	4	
L.	40	38	28	49	20	48	44	49	38	45	39
G.	68	45	38	80	30	45	55	27	27	30	44
N.	11	10	54	26	26	40	40	28	44	58	33
P.	24	22	20	30	18	14	12	20	32	26	21

D.—Amount of Fluid taken cc.

		Total	Average per hour
L.	6.15 a.m., 200 cc.; 8.45, 200 cc.; 11.30, 300 cc.; 1 p.m., 200 cc. ...	700	70
G.	6.30 a.m., 200 cc.; 11.30, 300 cc.; 1 p.m., 400 cc. ...	900	90
N.	6.30 a.m., 200 cc.; 8.45, 400 cc.; 12 p.m., 300 cc. ...	900	90
P.	6.30 a.m., 400 cc.; 11.30, 500 cc. ...	900	90

Chart I. *European subject (mixed diet, total nitrogen 10 g.).*

Chart II. *Hindoo subject (vegetarian, total nitrogen 5 g.)*

Chart III. *Tamil subject (mixed diet, total nitrogen 8 g)*

and with a total nitrogen excretion of 8 g. was from 23 mg. ammonia nitrogen per hour to 33 mg. per hour. These variations may be normal, but I do not think that such marked variations in the daily ammonia have been noted before in apparently normal individuals, on a constant and customary diet, mainly vegetarian in nature, as taken by most of my subjects. An increase was observed just as frequently in the strict vegetarians as in those using a mixed diet, the absolute quantities of ammonia being of course lower for the vegetarians. It is noteworthy that my subjects appeared to avoid an acid-producing diet.

It was considered that some tropical disease might be responsible, since the high figures resembled those for ammonia excretion in febrile diseases, as described in text books of clinical medicine. Malaria was the only disease of which a history was obtained in a fair number of my subjects; and these did not excrete large amounts more frequently than those without a history of malaria.

In Table I details of the total acidity as arranged by Palmer and Henderson [quoted by Cole, 1919] are given; the figures show that the total acidity was distinctly high in some cases, indicating an acidosis. As in Europe the acidity, generally speaking, varied directly with the total nitrogen. Also the urine was acid on a mixed diet and alkaline on a vegetable diet (see Table I, acidity, Leathes' method). In Europe the volume of the urine is stated to vary inversely as the acidity; but in Singapore the volume more often varied directly as the acidity (see Tables II, III, IV, and Charts I, II and III). Many of the subjects showed that large quantities of water were lost by perspiration, the amount of urine voided being much less than the amount of fluid swallowed. It is evident that a good deal of acid must have been removed by sweat, since perspiration is known to lower the acidity of the urine. Diminution of sweat in a climate like that of Singapore would evidently cause an acidosis.

THE ALKALINE TIDE.

Recently Leathes [1919] stated "...the traditional ascription of the alkaline tide to the secretion of hydrochloric acid in the stomach appears to be erroneous. An intense alkaline tide is normal in the morning when no food is taken; alkaline tides after other meals than the first in the day are certainly not the rule, and in the experience of the writer do not occur. It is not even *a priori* probable that they should, since the passage of acid from the stomach is the signal for the secretion of alkaline digestive juices into the small intestine and for absorption into the blood from the intestine to begin." Leathes holds that the matutinal alkaline tide is accounted for by depression of the respiratory centre during sleep and increased activity on waking and some time later, so that in this way acid is removed by the lungs and not by the urine to the usual extent.

My results in Singapore were quite different from those of Leathes, and

certainly confirmed the traditional ascription of the alkaline tide to the secretion of hydrochloric acid. I found that an alkaline tide, although not constant, was the rule about three hours after any meal in Singapore. I employed the method for estimation of alkalinity and acidity described by Leathes in his paper, the reaction of the urine being given as so much per cent. alkali or so much per cent. acid. The subjects were selected from those employed in the first part of this research. Some of them took meals as in Europe about 8 a.m., 1 p.m., and 7.30 p.m., with light tea (tea, bread and butter) about 6.30 a.m. and 4.30 p.m.; others, as was their custom, took large meals about 11 a.m. and 7 p.m. with small meals about 6 a.m. and 4.30 p.m.

My figures for subjects under normal conditions may be summarised as follows. In 100 cases a matutinal tide was possible; in 68 cases it was present (68 %); in 32 cases it was absent. In 78 cases a tide was possible after the midday meal and evening meal; in 57 it was present (73 %); whilst in 34 out of these 57 occasions the tide was better marked than the corresponding matutinal tide. These points are illustrated in Charts I, II and III, and in Tables II, III and IV. It will be observed that the tides appeared also in those subjects who excreted large amounts of ammonia. In these Tables and Charts I have stated the quantities of fluid swallowed and the quantities of urine voided.

In another series of experiments no food was taken in the morning before 11 a.m. Although in some cases the matutinal tide still appeared, it was absent in the majority (80 %) of cases. On the other hand Leathes found that a tide is still present if no food is taken in the morning. It is well known that acid is sometimes present in the stomach when there is no food there. It is likely that acid might appear at the customary time, if food be only occasionally omitted, and thus cause an alkaline tide.

It was noted that in a European subject in Singapore the tide was often absent after lunch if work was commenced soon after the meal; but it was present if two hours' interval was taken. This was the custom for the Asiatics, who rested between 11 a.m. and 1 p.m., taking lunch between 11 a.m. and 12 noon.

I repeated Leathes' experiments with hyperpnoea and found that hyperpnoea readily made the urine alkaline as he points out; but hyperpnoea was not responsible for the tides in Singapore, some of my subjects breathing quietly and slowly all day, in fact being half asleep during the experiment.

My results resembled the conclusions of Hasselbalch whom Leathes quotes: "After each meal the urine was without exception more acid than before, but showed the lowest acidity about three hours later." It seems that the secretion of alkaline saliva renders the urine more acid just after a meal and that the secretion of hydrochloric acid renders the urine more alkaline about three hours after a meal, seeing that it is at this time the acid reaches its maximum in the stomach.

I found that, as a rule, the smallest amounts of ammonia were excreted

three hours after a meal, so that the ammonia may be required in the stomach at that time. It seems likely that this is so from the following theory recorded by Macleod [1918]. The stomach mucosa is particularly rich in ammonia which, it is supposed, carries out the hydrochloric acid into the cavity of the stomach, the ammonia passing back again to the cells of the mucosa. Therefore it seems that most ammonia would be required in the stomach three hours after a meal, when the acid secretion is at its maximum. Schittenhelm and A. Loeb and Gammeltoft [quoted by Hammarsten and Hedin, 1914, p. 767] have also observed a fall in the ammonia elimination a few hours after a meal and *vice versa*.

NOTE ON AMINO-ACID EXCRETION.

The amino-acids were estimated by subtracting the result obtained by Folin's method of estimating ammonia, from that obtained by Malfatti's method.

Table I shows that, with the exception of Tamil IV, the amount of amino-acid nitrogen varied directly with the total nitrogen, being about 0.12 g. with a total nitrogen of about 8 g., and about 0.07 g. with a total nitrogen of about 5 g. In Tamil IV the amino-acid nitrogen was 0.10 g., the total nitrogen being 5.02 g. His ammonia nitrogen was also relatively high. When large amounts of ammonia were excreted the amino-acids were sometimes increased; at other times they were not altered.

The average amino-acid nitrogen per cent. of total nitrogen was about 1.2 with a total nitrogen of about 8 g., and about 1.5 with a total nitrogen of about 5 g.

SUMMARY.

1. Further observations were made to endeavour to determine the cause of the frequent excretion of relatively large amounts of ammonia in Singapore, as estimated by Folin's and Malfatti's methods. Some results regarding amino-acid excretion and the alkaline tide were also obtained.

2. The hourly excretion of ammonia in subjects excreting a normal amount of this substance, varied, as a rule, directly as the acidity or inversely as the alkalinity. This supports the theory that urinary ammonia is used to neutralise acid.

3. The hourly variations for those excreting large amounts of ammonia resembled those for individuals excreting normal amounts. Any subject was likely to show an increase, which lasted sometimes for a day, sometimes for several days. The increased excretion was spread out over the whole day and was not confined to the hours of greatest exposure to the effects of the climate. When normal amounts were excreted a similar condition existed. Nevertheless, it is considered that the climate was responsible for the acidosis since the largest daily amounts of ammonia, noted in all subjects, were excreted by the individuals who were most exposed to its effects. It is noteworthy that my subjects appeared to avoid an acid-producing diet.

4. Well-marked alkaline tides, although not constant, were the rule three hours after any meal, being just as frequent after the midday and evening meals as after the morning meal. The traditional ascription of the alkaline tide to the secretion of hydrochloric acid in the stomach was therefore supported.

5. The ammonia excretion in the urine varied inversely as the acidity of the stomach. This supports the theory that ammonia is used to carry hydrochloric acid from the stomach mucosa to the stomach cavity.

6. In Singapore the volume of the urine varied directly as the acidity, in this respect differing from Europe. In some cases, the total acidity was distinctly high, indicating an acidosis.

7. The amino-acids in the urine varied directly as the total nitrogen.

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