XXV. NITROGEN PARTITION IN THE URINE OF THE RACES IN SINGAPORE.

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INTRODUCTION.

THE observations here recorded form the continuation of a research directed mainly to the question of the nitrogen partition in the urine of individuals belonging to different races, including a Brahmin, a Chinese, a Tamil, a European, a Malay, a Hindoo, a Eurasian, a Bengali and a Sikh. The European has been a lecturer for more than five years and the other subjects have studied medicine for at least two years at the local school.

The first five of these subjects have been under observation since October 1916. Some of the results dealing with their diet, nutrition and excretion have been published [1917] and where comparable they support the evidence of the present work.

PREVIOUS RESEARCH.

A good deal of research has been published connected with diet and nutrition in various tropical climates but scarcely any dealing in detail with the nitrogen partition, the only searching work being that carried out by Young [1915], who studied the protein metabolism of white races living in tropical Queensland. His results exhibit no marked variations from the averages obtained in temperate climates.

METHODS.

In all cases analyses of the urine were made for seven—in most cases consecutive—days. Care was taken to prevent decomposition of the specimens by the addition of 2 cc. of 5 per cent. solution of thymol in chloroform.

Kjeldahl's method was employed for estimation of the total nitrogen, Benedict's method for urea, Malfatti's method for ammonia, Johnson's method for creatinine, Salkowski's method for purine bases, Ludwig-Salkowski's method for uric acid, Folin's method for inorganic and ethereal sulphates, Volhard-Arnold's method for chlorides, the uranium acetate method for phosphates, and Folin's method for the total acidity.

Table I. *Statistics regarding Subjects under Observation.*

Subject	Race	Birthplace	Age in years	Height in inches	Weight in kilos.	Years resident in Malaya	Years resident in Singapore
Brahmin	Tamil	Ceylon	23	63	46.1	4.0	3.5
Chinese	Chinese	Singapore	22	62	41.3	22.0	22.0
Tamil	Tamil	Ceylon	28	62	61.7	9.0	4.0
European	British	Australia	35	67	55.6	5.0	5.0
Hindoo	Tamil	Ceylon	23	62	46.9	6.5	2.5
Malay	Malay	Malaya	20	63	56.5	20.0	6.0
Eurasian	Eurasian	Malaya	20	61	49.5	20.0	2.0
Bengali	Bengali	Bengal	18	68	59.1	17.0	3.0
Sikh	Sikh	Punjab	23	67	48.2	12.0	3.0

The composition of the foods and their heat values were taken from a standard work [Leach 1911].

Statistics regarding the subjects are given in Table I. Table II shows the average figures for the nitrogen partition, and Table III the average figures for the non-nitrogenous excretion.

PRESENT OBSERVATIONS.

Brahmin. This man was a vegetarian, his diet consisting of bread, butter, fruit, vegetables, rice (the staple), milk, jam, pulses, tea, coffee, cocoa, sugar, salt and spices. He was under observation for three years, and although his diet varied considerably in quantity, it did not vary in quality. The daily total nitrogen output for three years varied from 4.3 g. to 8 g. He lost 4 lbs. weight in three years.

He lived in the school hostel at the time the observations here recorded were made, his average daily diet containing protein 54 g., fat 37, carbohydrate 288, the heat value being 1746 kilocalories, and the daily total nitrogen output averaging 5.1 g. (Table II). The ammonia nitrogen and creatinine nitrogen were not so reduced in quantity as the total nitrogen, urea nitrogen and uric acid nitrogen, when compared with the standard for Europe which is shown in Table II. Therefore the percentages of ammonia nitrogen and creatinine nitrogen were higher than the standard percentages, whilst the percentages of urea nitrogen and uric acid nitrogen were not so greatly altered.

Hindoo. This man partook of the same diet as the Brahmin. He belonged to the same race—Tamil. His average daily diet during the week of observation contained protein 63 g., fat 32, carbohydrate 354, the heat value being 2007 kilocalories. His figures for nitrogen partition, on the whole, confirmed the results for the Brahmin. The average daily total nitrogen output was 5.97 g. representing 37.3 g. protein. The ammonia nitrogen and the creatinine nitrogen suffered a smaller reduction in quantity than the other nitrogenous excretions and were increased in percentage when compared with the standard for Europe (Table II).

Chinese. This student was under observation for three years. His diet

was mixed, containing rice (the staple), bread, butter, milk, jam, beef, mutton, pork, chicken, fish, eggs, vegetables, fruit, sugar, salt, tea, coffee and spices. During six months of observation his daily diet averaged protein 60 g., fat 43, carbohydrate 227, the heat value being 1577 kilocalories and the average daily nitrogen excretion 9.25 g. [Campbell 1917]. He gained 3 lbs. weight in three years.

For the week in which the present observations were made the average daily nitrogen output was 8.60 g. (Table II). When compared with the standard for Europe, the nitrogen excretions, with the exception of the ammonia nitrogen and creatinine nitrogen, were much reduced in quantity, so that the percentages of ammonia nitrogen and creatinine nitrogen were higher than the standard for Europe.

European. This lecturer was under observation for five years in Singapore. During the first three years he lost 14 lbs. in weight; during the last two years his weight was fairly constant. With the loss of weight there was a fall in the diet. The nitrogen output fell from 15 g. to 12 g. per diem. He used an ordinary mixed diet, practically identical in type with the diet in Europe.

The average daily diet during the week of observation consisted of protein 80 g., fat 59, carbohydrates 216, the heat value being 1763 kilocalories, and the average nitrogen excretion 11.70 g. (Table II), which accounted for 73.1 g. protein. The ammonia nitrogen, 0.69 g., was higher than the standard. All the other nitrogenous substances were excreted in smaller quantities than the standard for Europe. The creatinine nitrogen 0.47 g. was not greatly reduced in amount.

Malay, Eurasian, Tamil, Bengali. These four students are considered together because they sat at the same table in the school hostel, and used the same mixed diet, resembling in substance that taken by the Chinese student. The Malay and the Tamil had been under observation since 1916. On the hostel diet the Tamil lost 1 lb. and the Malay gained 5 lbs. in weight in three years. The Tamil's daily diet for three months averaged protein 58 g., fat 32, carbohydrate 277, the heat value being 1672 kilocalories and the total nitrogen excretion 8.24 g. per diem. The Malay's diet was similar, averaging protein 57 g., fat 31, carbohydrate 239 per diem [Campbell 1917].

In the present series of analyses the average daily nitrogen output was 8.74 g. for the Malay, 9.34 for the Tamil, 9.89 for the Eurasian and 7.57 for the Bengali (Table II). In all cases the nitrogenous excretions were much smaller in amount than those of the standard for Europe, with the exception of the ammonia and creatinine. In all subjects the ammonia nitrogen was excreted in greater amount and the creatinine nitrogen in somewhat smaller amount than the standard.

Sikh. This student used a mixed diet similar to that taken by the four students mentioned above, but he lived outside the school hostel. His average daily nitrogen output was 8.64 g. (Table II), the nitrogen partition resembling that of the Malay.

Table II. *Nitrogen Partition.* (Average figures.)

Subject	Diet	Total N		Urea N		Ammonia N		Creatinine N		Uric acid N		Purine bases N		Rest.	Total N per kilo of body weight g.
		g.	%	g.	%	g.	%	g.	%	g.	%	g.	%	%	
Brahmin	Vegetable	5.10	76.59	3.90	9.88	0.50	9.88	0.40	7.99	8.8	0.08	1.63	0.013	3.66	0.110
Chinese	Mixed	8.60	81.63	7.02	6.28	0.54	6.28	0.36	4.26	8.7	0.14	1.63	0.008	6.11	0.208
Tamil	"	9.34	82.09	7.67	6.72	0.62	6.72	0.42	4.52	6.8	0.16	1.75	0.015	4.76	0.151
European	"	11.70	83.59	9.78	5.96	0.69	5.96	0.47	4.05	8.5	0.16	1.37	0.016	4.89	0.210
Hindoo	Vegetable	5.97	79.60	4.75	6.60	0.39	6.60	0.38	6.36	8.1	0.09	1.62	0.009	5.67	0.127
Malay	Mixed	8.74	78.91	6.89	7.91	0.69	7.91	0.44	5.08	7.8	0.17	1.91	0.013	6.05	0.154
Eurasian	"	9.89	80.75	7.98	5.86	0.58	5.86	0.50	5.06	10.1	0.14	1.45	0.009	6.79	0.199
Bengali	"	7.57	80.37	6.08	8.33	0.63	8.33	0.45	5.92	7.6	0.13	1.76	0.017	3.40	0.128
Sikh	"	8.64	81.45	7.03	7.76	0.67	7.76	0.43	4.97	8.9	0.11	1.31	0.021	4.27	0.179
Standard for Europe	"	16.00	85.00	13.60	3.30	0.53	3.30	0.57	3.60	7 to 11	0.25	1.56	0.025	6.39	0.200

CONCLUSIONS.

On the various diets, vegetarian and mixed, used by the Singapore subjects, the nitrogen partition differed from the standard for Europe in the high percentages of ammonia nitrogen and creatinine nitrogen. As regards creatinine, the percentage was high because creatinine, being mainly of endogenous origin, is not greatly reduced in absolute quantity when the total nitrogen is reduced. It will be shown that, in most cases, the amount of ammonia nitrogen was excessive.

Race had no influence on the nitrogen partition, the figures for the different races resembling one another fairly closely, after making due allowance for differences in diets.

Nitrogenous Excretions.

Total Nitrogen. In all cases the daily total nitrogen was much lower than that of the standard for Europe; but if we consider the total nitrogen per kilogram of body weight it will be observed that the figures 0.210 g. for the European, 0.208 for the Chinese, 0.199 for the Eurasian, resembled the standard 0.200 (Table II). The figures for the Brahmin 0.110 and for the Hindoo 0.127 were the lowest mainly because the vegetable protein eaten by them was not so easily digested as the flesh protein taken by the other subjects. The vegetarians used quite as much protein in their food as many of the other subjects. Most of the subjects used about half the standard quantity of protein. McCay, Chittenden and others have already pointed out that it is possible to maintain protein equilibrium on less than half the standard for Europe. The Brahmin and the Hindoo, who had the two lowest figures, were the best students of their years. However, the average Singapore student under observation had not the physique and energy of the average student in Europe nor of the average European resident in Singapore. This supports McCay's views [1910], that races who live on diets of high protein content have better physique and energy than those whose diet contains less protein.

Urea Nitrogen. The text-books state that the urea nitrogen, 16 g., amounts to about 85 per cent. of the total nitrogen excreted, both varying directly with the amount of protein absorbed. According to Folin, when the total nitrogen output falls to 7—8 g., the urea nitrogen is reduced to about 79 per cent. My observations are in general agreement with these conclusions.

Ammonia Nitrogen. In all cases the percentages of ammonia nitrogen were higher than the standard, in some cases very much higher. At first sight this might be expected, as Folin found that the ammonia nitrogen forms about 5 per cent. of the total nitrogen, if the total nitrogen is only 7 or 8 g. Moreover, Malfatti's method of ammonia estimation, which I employed, gives higher results than Folin's method because the former includes also the ammonia of any amino-acids in the urine. Making liberal allowance for the

ammonia of the amino-acids, the ammonia nitrogen may form 6 per cent. of the total nitrogen, if the latter is only 7 or 8 g. and if Malfatti's method is used. But in many cases my percentages were much higher than this; and the absolute quantities of ammonia were also higher than the standard, except in the two vegetarians, Brahmin and Hindoo (Table II); even in them the amounts were higher than one would reasonably expect on their diet. In my previous researches on Singapore medical students [1917] and on Singapore labourers [1918] large amounts of ammonia were detected on many occasions.

Normally the absolute amounts of urea and ammonia rise and fall together with the total nitrogen. In most of my subjects there was a fall in the amounts of total nitrogen and urea nitrogen but a rise in the ammonia nitrogen, which condition indicates an acidosis. What was the cause? Taking all the diets into consideration there was nothing in the food to account for the increase of ammonia. Again the analyst's reports showed that the Singapore water was particularly pure. It is held that the sole use of the urinary ammonia is for neutralisation of acids formed during metabolism. Probably in the Singapore subjects there was a disturbance in metabolism. The severe climatic conditions in Singapore may be responsible, seeing that so many different subjects were affected. Singapore climate is noted for the lassitude it produces. Eight months change in a temperate climate is taken every four years, a more frequent change being usual for women. Further experiments are required to determine whether the acidosis explains any of the discomforts and ill-effects of the climate. Young [1915] working in Townsville, Queensland, has not found any increase in the ammonia nitrogen, but he has noted an increase in the neutral sulphur, which he suggests may be due to an increased tissue metabolism. Townsville and Singapore climates are not very much alike. Singapore, which is much nearer the equator, does not enjoy a cool season, the temperature averaging 80° F. for the whole year; nor does the Townsville climate affect the health of Europeans in the same degree as that of Singapore.

It is well known that fever—that is, a rise of body temperature—causes an increase in urinary ammonia. Hot, moist and still air in Singapore produces great discomfort and a rise of body temperature [Campbell 1919]. In the cotton sheds in England a rise of mouth temperature is observed when the wet bulb thermometer exceeds 75° F. [quoted by O'Connell 1913]. In Singapore this wet bulb reading is often exceeded for weeks at a time. Probably this caused the acidosis observed by me.

Creatinine Nitrogen. The creatinine coefficients (that is milligrams of creatinine per kilogram of body weight) of my series resembled the standard coefficient for Europe, which ranges from 7—11. My figures varied from 6·8 for the Tamil to 10·1 for the Eurasian (Table II). The Brahmin and the Hindoo, on a diet containing neither creatine nor creatinine, had practically the same coefficient as the subjects on a diet containing these substances.

Obviously all the creatinine of the former subjects was endogenous. The percentages of creatinine were higher than the standard because creatinine, being mainly of endogenous origin, is not lowered in quantity when the total nitrogen is decreased.

Uric Acid Nitrogen. The quantities obtained were all lower than the standard figures, but the percentages agreed with the standard. This was to be expected since uric acid is decreased by cutting down the protein (nucleo-protein) of the diet. The decrease in quantity was particularly noticeable in the case of the Brahmin and the Hindoo, who used a vegetable diet. Most of their uric acid was evidently endogenous.

Purine Nitrogen. None of the subjects excreted so large a quantity as the standard, because they did not consume so large a quantity of the purine bases contained in tea and coffee as the average man in Europe. The tea taken by the Singapore European was a very weak infusion, and he never drank coffee.

Non-Nitrogenous Excretions.

Phosphates. The figures varied from 1.6 g. for the European to 0.9 g. for the Bengali (Table III). The ratio of phosphoric oxide to nitrogen is given in text-books as 1 to 5 or 6; that is about 2.5 g. P_2O_5 . With the exception of the Brahmin and the Hindoo where the ratio was 1 to 5, the average ratio in my results was 1 to 7.5. The Singapore diets evidently contained smaller quantities of absorbable phosphate than the standard diet for Europe.

Table III. *Non-nitrogenous excretion.* (Average figures.)

Subject	Volume in cc.	Specific gravity	Acidity in cc. N/10 NaOH	P_2O_5	NaCl	Inorganic SO_3 g.	Ethereal SO_3 g.
Brahmin	1207	1.013	194	1.1	8.9	0.541	0.097
Chinese	1113	1.014	285	1.3	7.5	1.250	0.107
Tamil	997	1.017	348	1.1	4.9	1.174	0.117
European	1102	1.017	375	1.6	5.4	1.416	0.108.
Hindoo	1016	1.020	262	1.1	8.5	0.736	0.147
Malay	1006	1.017	260	1.3	7.0	0.957	0.129
Eurasian	1056	1.017	286	1.3	5.7	1.174	0.085
Bengali	1328	1.009	188	0.9	3.2	0.829	0.102
Sikh	593	1.024	332	1.3	3.0	1.056	0.092

Sulphates. Only the inorganic and ethereal sulphates were estimated owing to the failure to obtain the necessary reagents, etc., for the estimations of the neutral and total sulphur.

The inorganic sulphates weigh about 2.5 g. in the standard for Europe and arise chiefly from the oxidation of the sulphur of protein. In my observations the amounts of inorganic sulphate followed closely the variations of the total nitrogen, the average figures ranging from 0.541 g. for the Brahmin to 1.416 for the European, the total nitrogen figures being 5.1 and 11.7 respectively.

The ethereal sulphates, which have their origin mainly in putrefaction taking place in the intestine, varied considerably in my observations. When present in relatively large amounts, constipation was present and a purgative readily reduced the quantity. High proportions were observed in the vegetarians, the Brahmin and the Hindoo.

Chlorides. The standard figure for Europe is 11 g. In my series the average amounts varied from 3 g. for the Sikh to 8.9 for the Brahmin. The latter and the Hindoo excreted the greatest quantities, probably owing to the large amounts of potassium salts in their diet. Bunge has shown that potassium salts deprive the blood of its chloride, whence the craving for more on a vegetable diet [quoted by Howell 1915].

It is well known that individuals have been kept in good condition on 1 or 2 g. per diem with experimental diets, so that the Sikh's figure was not very low.

Acidity. Calculated in cc. *N*/10 NaOH, the acidity varied from 188 in the Bengali to 375 in the European; or, calculated as HCl, it varied from 0.68 to 1.36 g. The standard figures are given in text-books as 1.5 to 2.3 g. HCl. One would expect the acidity to fall in the Singapore subjects because their diet contained less protein than the standard; and abundant perspiration reduces acidity.

SUMMARY.

1. The manner in which the nitrogen is distributed in the urine of a Brahmin, a Chinese, a Tamil, a European, a Hindoo, a Malay, a Eurasian, a Bengali and a Sikh, all attending the Singapore Medical School, is considered.

2. The absolute quantities of total nitrogen, of urea nitrogen, and of uric acid nitrogen, are much lower than the standard figures for Europe, but the percentages of urea nitrogen and uric acid nitrogen do not differ materially from the standard. In the Chinese, the European and the Eurasian the total nitrogen per kilogram of body weight is about the same as the standard; in the other subjects the figures are lower than the standard.

3. The absolute quantity of purine nitrogen is much lower in all subjects except the Sikh, in whom it is slightly lower than the standard. The percentages vary greatly.

4. In all cases the percentage of ammonia nitrogen is increased. In many cases the absolute amount is increased, and in some cases the increase is well marked. This confirms observations on Singapore students and labourers, already published. The diet and the water are not responsible. It is considered that the excess is due to an increase of acid substances in the blood, owing to a disturbance in metabolism, and that the climate of Singapore may be responsible, since so many different subjects, living under such different circumstances and partaking of different diets, are similarly affected.

5. The quantity of creatinine nitrogen is slightly lower than the standard for Europe but the percentage is higher—in some cases much higher—because

the reduction in the creatinine nitrogen is much less than the reduction in the total nitrogen. This supports the theory that creatinine is mainly of endogenous origin and that it is not lowered greatly by reducing the protein intake. The creatinine coefficient for Singapore resembles very closely that given for Europe.

6. Race apart from diet has no influence on nitrogen partition.

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