Variation in urinary excretion of urea and N-Methyl nicotinamide during the day comparison with fasting levels[1]

MELVIN LEE[2] AND GUILLERMO ARROYAVE[3]
Institute of Nutrition of Central America and Panama (INCAP),
Guatemala, C. A.

SUMMARY

Complete urine collections were taken from 19 healthy young adult volunteers at 8:00 a.m., 10:00 a.m., 12:00 noon, 2:00 p.m., 4:00 p.m., and 6:00 a.m. the following morning, while the subjects were on a regular free diet. Urea nitrogen and creatinine were measured in all samples and N-methyl nicotinamide was measured in the urine samples from 9 of the volunteers.

The rations of urea nitrogen/creatinine and of N-methyl nicotinamide /creatinine were not significantly different in midmorning and late morning samples as compared with the ratios in fasting samples. Early afternoon and mid-afternoon samples had statistically significant higher ratios of urea nitrogen/creatinine, but the corresponding ratios of N-methyl nicotinamide/creatinine did not differ.

The urinary excretion of urea, especially in a fasting sample, has been proposed as an index of the level of protein intake in population groups. Howewer, there is no agreement

[1] This investigation was supported by grant AM-04731 from the National Institutes of Health, Bethesda, Md., U.S.A.

[2] Assistant Professor, Department of Epidemiology and International Health, University of California School of Medecine, San Francisco, California, U.S.A.

[3] Chief, Division of Physiological Chemistry, Institute of Nutrition of Central America and Panama.

INCAP Publication I-387.
Received for publication May 27, 1966.

regarding the most meaningful way to express urea excretion. Platt (1) employed the ratio of urea nitrogen to total nitrogen, but this ratio is not a sensitive way to express changes in urea excretion, because urea is a component of total urinary nitrogen. Arroyave (2) proposed the use of the ratio of urea nitrogen/creatinine, noting that creatinine is a relatively constant component of total urinary nitrogen. He presented data to support the use of this ratio as a valid indicator of previous protein intake. Dugdale and Edkins (3) published values for the urea nitrogen/creatinine ratios for normal and malnourished children and noted that after-supper samples did not differ significantly from fasting samples.

This ratio has also been used in nutrition surveys. However, conditions under which samples are collected differ from one survey to the next, and even within a survey, creating problems in the comparison of data. In some cases (4), only fasting samples were collected. In others (5), urine samples were collected after breakfast; in the late morning; shortly before lunch, or even after lunch.

To study the effect which time of collection of urine samples might have on urea nitrogen/creatinine ratios, these metabolites were measured in urine samples collected at intervals during the day, beginning with fasting samples. In addition, since the excretion of water soluble vitamins is commonly expressed in terms of urinary creatinine (6), the excretion of N-methyl nicotinamide per gram of creatinine was determined in some of the subjects, using the same samples.

MATERIAL AND METHODS

Two groups of healthy young adult volunteers, members of INCAP staff, ages 21 to 38 years, were recruited for these investigations. Group I included 9 subjects, 5 males and 4 females; and group II included 10 subjects, 2 males and 8 females. They were instructed to rise at 6:00 a. m. and to discard the urine voided at that time. Total urine samples were then collected at 8:00 a.m., 10:00 a.m., 12:00 noon, 2:00 p.m., 4:00 p.m., and at 6:00 a.m. the following morning. The volume of each sample was recorded and an aliquot was immediately frozen, without preservative.

The subjects ate breakfast at 8 o'clock, lunch at 12 o'clock, and supper some time after 4 o'clock in the afternoon. No record was kept of the diet composition or of the amount eaten, but previous studies (7) indicate that the average diet of a group such as the one involved in this study is represented by the following pattern of daily nutrient intake: total protein, 76.7 g.; calories, 2660; vitamin A, 6430 I. U.; vitamin C, 138 mg.; thiamine, 1.03 mg.; riboflavin, 1.87 mg. and niacin (preformed), 12.27 mg. Sixty-three percent of the total protein intake is from animal sources. The total consumption of fats is more than 100 g. per person per day, which represents 38% of the total caloric intake.

After thawing, the urine samples were filtered. Creatinine was determined by the method of Clark and Thompson (8), urea nitrogen was determined colorimetrically by the method of Barker (9), and N-methyl nicotinamide was determined fluorimetrically according to the method described by ICNND (6). Analysis of variance was carried out according to the procedure described by Snedecor (10), and tests of significance according to the "t" test, as described by Croxton (11).

RESULTS

Table 1 shows the means and standard deviations for the excretions of urea nitrogen/creatinine (g/g) and N-methyl nicotinamide/creatinine (mg/g) for the six collection periods. N-methyl nicotinamide was determined in only 9 of the subjects (Group I). Values of "t" are shown, in each case, comparing the sample indicated with the fasting sample (period 1). The same ratios are presented graphically in Fig. 1.

TABLE 1

AVERAGES, STANDARD DEVIATIONS, AND "t" VALUES FOR UREA NITROGEN/CREATININE (g/g) AND N-METHYL NICOTINAMIDE/CREATININE (mg/g) EXCRETED BY HEALTHY VOLUNTEER SUBJECTS

EXPERIMENT		PERIOD OF COLLECTION					
		1 [a]	2	3	4	5	6
		6 a.m. to 8 a.m.	8 a.m. to 10 a.m.	10 a.m. to 12 noon	12 noon to 2 p.m.	2 p.m. to 4 p.m.	4 p.m. to 6 a.m.
Group I (9 subjects)							
Urea nitrogen/ creatinine	$\overline{X}$	6.30	6.04	6.93	8.26	7.40	8.72
	S. D.	1.33	2.36	2.33	4.42	2.12	2.69
	"t"		0.58	1.42	2.54*	2.63*	4.84*
N-methyl nicotinamide/ creatinine	$\overline{X}$	5.15	5.94	4.56	4.38	3.67	5.46
	S. D.	4.02	3.98	2.54	1.42	2.04	4.08
	"t"		0.94	0.74	1.08	1.92	0.32
Group II (10 subjects) Urea nitrogen/ creatinine	$\overline{X}$	8.84	8.73	9.72	11.17	12.61	10.74
	S. D.	±2.26	±2.53	±1.90	±3.10	±3.48	±2.04
	"t"		0.20	1.83	3.82**	6.28**	2.69

[a] Fasting urine sample.

* Significant at the 5% level.

** Significant at the 1% level.

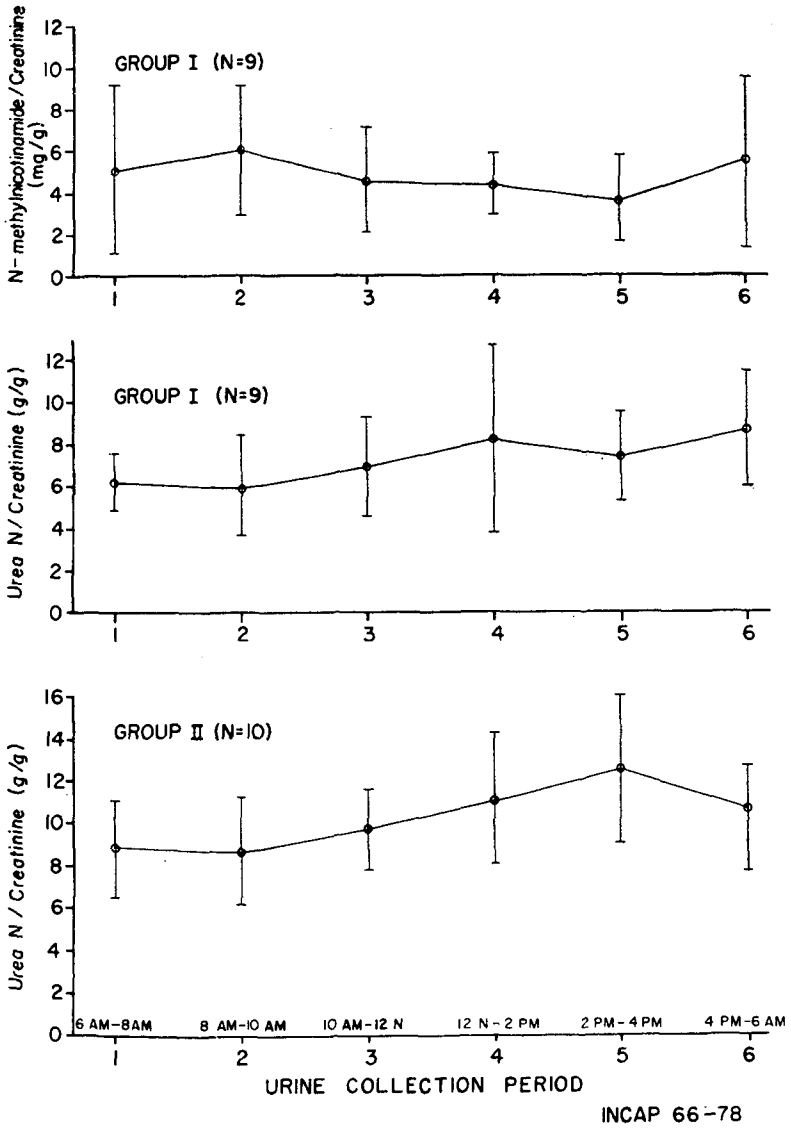

Fig. 1.—Pattern of excretion, by human adults, of urea N and N-methyl nicotinamide during a 24-hour period.

Analysis of variance for N-methyl nicotinamide/creatinine excretion for Group I shows no statistically significant effect of the period of collection, as indicated by an F value of 1.76. Fig. 1 shows that the mean excretion values simply oscillate around an overall mean. The "t" test indicates that there are no significant differences between the means of periods 2-6 and the mean of period 1 (the fasting sample).

Analysis of variance for the effect of collection time gave F values of 3.32 and 7.03 for urea nitrogen/creatinine of groups I and II, respectively. These are significant at a 5% level for Group I and at a 1% level for Group II. Fig. 1 shows that there is a gradual increase in urea nitrogen/creatinine during the day. Applying the "t" test, periods 4, 5 and 6 were significantly higher than period 1 (the fasting sample). However, periods 2 and 3, the early and late morning samples, were not significantly different from the fasting sample in either group.

DISCUSSION

The values of urea nitrogen/creatinine and of N-methyl nicotinamide/creatinine obtained in this study, with healthy young adults, were only slightly higher than those obtained by others (5) for a comparable age group. However, it should be noted that our subjects constituted a more homogeneous group than the population sampled in a general survey, since all belonged to middle income groups and were well nourished.

An adequate breakfast did not appreciably alter the relative excretion of urea nitrogen. This supports previous observations that the rate of urea nitrogen loss is, under ordinary conditions of life, determined mostly by the previous sustained level of protein intake. The nitrogen intake of a regular ample lunch raises the excretion of urea nitrogen.

One might have expected N-methyl nicotinamide excretion to rise markedly after a meal. Nevertheless, in the present study it was even more stable, from period to period, than the excretion of urea. With respect to the parameters measured, there is no apparent advantage to be gained from the inconvenient collection of fasting urine samples, as compared with the more convenient marning samples. Even the statistically significant increase in urea nitrogen/creatinine seen

in the afternoon samples hardly represents a biologically meaningful difference, within the framework of interpretation of this test. Thus, it is recommended that in survey work the measurement under discussion should be made on samples of urine collected in the morning, before lunch, but not necessarily under fasting conditions.

ACKNOWLEDGEMENTS

The authors are indebted to Mr. Rolando Funes for assistance with the laboratory procedures.

RESUMEN

Se recogió la excreción total de orina de 19 voluntarios adultos, jóvenes y sanos, a las 800 a.m., 10:00 a.m., 12:00 m., 2:00 p.m., 4:00 p. m. y a las 6:00 a.m. del día siguiente, estando los individuos bajo dieta libre regular. Se midió el nitrógeno y la creatinina en todas las muestras, y la N-metilnicotinamida solamente en las muestras de 9 de los individuos.

No hubo diferencia significativa al comparar las razones de nitrógeno ureico/creatinina y N-metilnicotinamida/creatinina en las muestras de las 8:00 a.m. y las 10:00 a.m., con las muestras tomadas cuando los individuos estaban en ayunas. Las razones de nitrógeno ureico/creatinina fueron significativamente más altas en las muestras de las 2:00 p.m. y las 4:00 p.m., pero las razones correspondientes de N-metilnicotinamida/creatinina no difirieron.

BIBLIOGRAPHY

1. Platt, B. S.—Nitrogen metabolism in malnourished infants and children. In: **Malnutrition in African Mothers, Infants and Children.** Report of the Second Inter-African Conference on Nutrition. Gambia, November 19-27, 1952. London, Her Majesty's Stationery Office, 1954, p. 153-159.
2. Arroyave, G.—The estimation of relative nutrient intake and nutritional status by biochemical methods: proteins. **Am. J. Clin. Nutrition** 11: 447-461, 1962.
3. Dugdale, A. E., and Edkins, E.—Urinary urea/creatinine ratio in healthy and malnourished children. **Lancet** 1: 1062-1064, 1964.
4. Lee, M.—Unpublished results of a survey of nutritional status of Eskimos relocated to the San Francisco Bay Area.
5. U. S. Interdepartmental Committee on Nutrition for National Development. **Northeast Brazil Nutrition Survey.** Washington, D. C., May 1965.
6. U. S. Interdepartmental Committee on Nutrition for National Defense. **Manual for Nutrition Surveys,** 2nd edition. Bethesda, Md., 1963.
7. Flores, M.—Personal communication.

8. Clark, L. C., Jr., and Thompson, H. L.—Determination of creatine and creatinine in urine. **Anal. Chem. 21: 1218-1221, 1949.**
9. Barker, S. B.—The direct colorimetric determination of urea in blood and urine. **J. Biol. Chem. 152: 453-463, 1944.**
10. Snedecor, G. W.—**Statistical Methods, Applied to Experiments in Agriculture and Biology,** 5th edition. Ames, Iowa, The Iowa State College Press, 1956.
11. Croxton, F. E.—**Elementary Statistics, with Applications in Medicine and the Biological Sciences.** New York, Dover Publications, 1959.