Urinary Excretion of Folic and Folinic Acids in Normal Adults.* (25835)

JOHN S. O'BRIEN† (Introduced by C. M. Wilhelmj)
Depts. of Physiology and Medicine, Creighton University School of Medicine, Omaha, Neb.

Several reports have appeared giving values for urinary excretion of folic (FA) and folinic (CF) acids in human subjects in health and disease(1-7). However, no report has appeared in which a large number of normals has been studied. Consequently, it has not been possible to evaluate statistically resting levels of FA and CF excretion. Furthermore, many values reported for FA excretion are not specific for FA but include also CF(1-3). The purpose of this study is to establish urinary excretion values for both FA and CF in a large number of normal adults.

Materials and methods. A pad-plate technic was used for microbiological assay of FA and CF(8) similar to the riboflavin assay reported in detail by Simpson(9). The assay procedure involves addition of samples to paper-pads which are then placed on an agar media containing the growth factors for the organism (with omission of FA or CF) and seeded with the assay organism. The plates containing the agar are then incubated for 24 hr at 37°C, at which time circular zones of growth are seen around the pads. Diameter of the zone is measured to the nearest 0.1 mm with a vernier caliper, and a standard curve constructed plotting average mm of growth against concentration of FA or CF in millimicrograms per ml. Standard curves were run with each determination. Four determinations were made on each unknown sample, and average value recorded. Average error of the method was found to be 10% for CF assay and 12% for FA assay. The CF assay organism used was *Leuconostoc citrovorum* (A.T.C.C. #8081)‡ while the FA assay organism was *Streptococcus fecalis* (A.T.C.C. #8043). Commercially available folic acid was used as the FA standard, while Calcium Leucovorin‡ was used as the CF standard. The latter was corrected for presence of the inert isomer(10) by multiplying the obtained CF result by 0.5. The CF activity for *L. citrovorum* (corrected) was subtracted from total activity for *S. fecalis* to ascertain FA activity, since *S. fecalis* responds equally well to FA and CF. The subjects studied were 51 healthy adults ranging from 22-63 years, and the great majority were medical students. Twenty-four hour urine samples were collected in brown bottles, pH adjusted to 6.8 and aliquots stored at 4°C prior to assay. No attempt was made to protect labile folates from oxidative-degradation. Determinations were made on some subjects from day to day to assess daily fluctuations. All subjects were on unrestricted dietary regimes.

Results. The 24-hour urinary excretion of FA and CF in these subjects is given in Table I. Excretion of FA ranged from undetectable levels to 5.58 μg per 24 hr with a mean value

TABLE I. Urinary Excretion of FA and CF in 51 Normal Adults.

μg/24 hr		μg/24 hr		μg/24 hr	
CF	FA	CF	FA	CF	FA
.19	2.20	.40	.40	.64	2.04
.22	.21	.40	.66	.66	.16
.24	.81	.41	1.06	.66	.22
.25	.43	.43	.71	.67	3.01
.28	4.37	.45	2.85	.68	1.80
.29	3.78	.47	.15	.72	.08
.30	4.34	.47	1.11	.73	Un
.30	.35	.47	.91	.88	.55
.30	.30	.48	.40	.89	Un
.31	.70	.49	1.00	.92	5.58
.32	.46	.50	.29	.93	Un
.35	.13	.51	Un*	1.00	1.25
.36	.36	.53	.53	1.22	1.00
.36	.36	2.54	.50	1.29	4.72
.37	.12	.57	.72	1.31	.79
.39	.16	.57	Un	1.33	.91
.39	2.23	.62	.02	1.60	5.10

CF: Mean = .59 μg/24 hr ± .47 stand. dev.
Range = .19–1.60 μg/24 hr.

FA: Mean = 1.17 μg/24 hr ± 1.92 stand. dev.
Range = undetectable–5.58 μg/24 hr.

* Un = undetectable.

* Presented in part at Am. Physiol. Soc. Meeting, London, Ontario, 1958.

† Post-Sophomore Research Fellow, U.S.P.H.S. Present address, City of Hope Medical Center, Duarte, Calif.

‡ Generously supplied by Dr. H. P. Broquist, Lederle Labs., Pearl River, N. Y.

TABLE II. Daily Excretion of FA and CF.

Subject	Day	μg CF/24 hr	μg FA/24 hr
JD	1	.30	4.34
	2	.49	1.00
	3	.64	2.04
KI	1	.30	.30
	2	.35	.13
	3	.41	1.06
SM	1	.37	.12
	2	.40	.66
HA	1	.39	.16
	2	.47	1.11
JH	1	.67	3.01
	2	.92	5.58
ME	1	1.22	1.00
	2	.57	.72
MC	1	1.29	4.72
	2	1.33	.91

of 1.17 and a standard deviation of ± 1.92; while CF excretion ranged from 0.19–1.6 μg per 24 hr with a mean of 0.59 and a standard deviation of ± 0.47. These results are comparable to those in other studies(4-7). The results of 24 hour urinary excretion of FA and CF in 7 subjects studied on consecutive days are given in Table II. Variation in FA and CF excretion from day to day may be a result of a varying dietary intake. In general, FA excretion varied from day to day and from subject to subject much more widely than CF excretion.

It has been reported that the bulk of CF activity in urine is in an oxygen-labile form which is degraded rapidly in air(11). No attempt was made in this investigation to protect these forms from oxidation, and the results reported here are representative only of oxygen-stable FA and CF.

Summary. The 24 hour urinary excretion of folic (FA) and folinic acid (CF) was determined in 51 healthy adults on unrestricted diets, using pad-plate microbiological assay. The 24 hour excretion of FA ranged from undetectable levels to 5.58 μg/24 hours with a mean of 1.17 and a stand. dev. of ± 1.92; while 24 hr excretion of CF ranged from 0.19-1.6 μg/24 hr with mean of 0.59 and a stand. dev. of ± 0.47.

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Received May 2, 1960. P.S.E.B.M., 1960, v104.

Isolation of a Virus Closely Related to Powassan Virus from *Dermacentor andersoni* Collected along North Cache la Poudre River, Colo. (25836)

LEO A. THOMAS, RICHARD C. KENNEDY AND CARL M. EKLUND
(Introduced by C. L. Larson)
U. S. Department of H.E.W., Nat. Inst. of Health, Nat. Inst. of Allergy and Infect. Dis., Rocky Mountain Laboratory, Hamilton, Mont.

In Sept. 1958, a 5-year-old boy from Powassan, Ontario, died of an acute encephalitis, and McLean and Donohue(1) isolated a virus from his brain. The agent, which was shown to belong to Casals' group B arthropod-borne viruses and to be related to Russian spring-summer encephalitis (RSSE) virus, was called Powassan virus. Viruses of the RSSE complex, which cause serious human illness characterized by central nervous system and