TABLE II. Colicine Sensitivity of S, Induced M, and "Transformed" S Organisms of *Shigella Boydii* Type 5, No. 2132.

	S parent		Induced M variants				Kinetin-induced S variants			
			M1		M2		M1-Tr		M2-Tr	
Colicine	S1	S2	1	2	1	2	1	2	1	2
534	0*	0	9	10	10	6	6	7	5	4
V	0	0	10	10	10	8	3	3	2	1
CF1	0	0	7	0	12	10	5	3	1	5
K	0	0	7	9	9	12	2	2	1	2
67	1	1	11	10	10	10	5	7	5	4

* Zones of inhibition measured in mm.

S organism obtained from the kinetin-passed M variants. The test plates showed a sensitivity gradation in which the S parent organism displayed relative resistance, the kinetin-passed S organisms were distinctly more susceptible and the M variants were markedly antagonized by the same colicines (Table II).

Discussion. The protective function of smooth surface somatic antigen of *Shigella* seems to be the most obvious explanation for the difference in action of colicines against organisms with and without this antigen. Gradual loss of surface somatic antigen by population change, and gradual recovery of this antigen in variant cells whose establishment was promoted by kinetin, results in corresponding colicine sensitivity changes. The validity of any possible characteristic colicine sensitivity spectrum for a certain *Shigella* serotype, therefore, may be regarded as partially dependent upon the protective effect exerted by surface somatic antigen. The amount of this antigen present may account for some of the sensitivity fluctuations observed between cultures of identical serological composition.

Summary. In 58 experiments involving comparisons of 6 smooth parent cultures of various *Sh. boydii* serotypes and their corresponding non-S variants, the variants were significantly more sensitive to colicine action than the smooth parent strains. Reversal of the culture from the non-S to the S form was accomplished by use of kinetin, and the "transformed" cultures reflected, in part, the colicine spectrum of the original S parent culture and in part the spectrum of the non-S variants from which the "transformed" S cultures were immediately derived.

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Effects of Folic Acid Antagonist Therapy on Urinary Excretion of Formic Acid by Humans.* (23968)

HOWARD H. HIATT, JESSE C. RABINOWITZ, RUDOLF TOCH, AND MADELINE GOLDSTEIN

Depts. of Medicine, Beth Israel Hospital and Harvard Medical School, and the Children's Cancer Research Foundation, Boston, Mass., and of Biochemistry, University of California, Berkeley

Although formic acid has been reported to occur in urine of normal human subjects on a mixed diet, the methods used for its determination have involved either extraction of urine with ether and subsequent distillation of volatile acids from acid solution(1,2), or direct distillation of the urine(3-6). Significance of values obtained using these methods is somewhat doubtful since many naturally-occurring substances are known to yield formic acid upon treatment with acid at elevated

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TABLE I. Formic Acid Excretion in Subjects without Leukemia.

Subject	Sex	Age	Clinical data	Urinary formic acid, μmoles/hr
Adults				
60	♂	32	Normal control	16.8, 13.8
61	♂	35	*Idem*	23.4, 13.8
62	♂	29	"	16.3, 19.8
82	♂	65	Arteriosclerotic	32.0
83	♂	65	Metastatic carcinoma	10.4
84	♂	60	? Neurologic disease	20.5
85	♂	59	Angina pectoris	12.3
86	♂	67	Emphysema	14.7
87	♂	19	Idiopathic thrombocytopenia	21.5
88	♂	68	Pneumonia	47.3
91	♀	72	Aortic stenosis, angina pectoris	8.5
92	♀	68	Acute myocardial infarct	15.0
93	♀	64	*Idem*	13.2
94	♀	64	Congestive heart failure	9.9
95	♀	70	Angiocystic hamartoma	9.0
96	♀	60	Acute myocardial infarct	3.6
Children				
2	♂	12	Ulcerative colitis	1.9
5	♂	10	Streptococcal pharyngitis	8.5
8	♀	10	Repair of uretero-pelvic junction	3.8
45	♂	11	Rhabdomyosarcoma	5.9
46	♂	2	Neuroblastoma	1.5
90	♀	10	Fracture of fibula	15.7

temperatures. In animal studies with C^{14}-labeled substrates, sources of urinary formate have been shown to include sarcosine(7), α-carbon of glycine, glycolate, and glyoxylate, and β-carbon of serine, acetone, and methionine(8). A major fraction of formic acid presented to the mammal is further metabolized (9-11), but profound impairment in capacity to utilize formate has been demonstrated in folic acid-deficient rats(11,12). This undoubtedly accounts for the marked increase in urinary excretion of formate by rats on folic acid-deficient diet(13).

The work to be reported indicates that formic acid is present in detectable quantity in urine of all human subjects studied. The levels in adult urine are lower than previously reported values(1,5). In children with acute leukemia undergoing folic acid antagonist therapy, excretion of formic acid is markedly increased. This investigation has been made possible by recent development of a sensitive and specific enzymatic method for determination of formic acid(14).

Methods. Timed urine samples were obtained from 3 healthy adults and from 19 patients on medical and pediatric services of Beth Israel Hospital. In addition, specimens were obtained from 22 children undergoing therapy for acute leukemia at Children's Cancer Research Fn. Sixteen of the latter group were receiving the antifolic acid drug, amethopterin (Methotrexate®; 4-amino-4-deoxy-N^{10}-methylpteroylglutamic acid) in amounts listed in Table III. Pertinent clinical data are summarized in Tables I-III. Urine samples were refrigerated immediately, and either analyzed for formic acid within 18 hours, or frozen within that time and kept at −10° until

TABLE II. Formic Acid Excretion in Children with Acute Leukemia not Receiving Amethopterin.

Subject	Sex	Age	Clinical data	Previous amethopterin and interval since last given	Urinary formic acid, μmoles/hr
21	♂	13	In remission on 50 mg of 6-mercaptopurine daily	Yes; 5 mo	7.8
23	♂	4	In relapse; receiving adrenal steroids, chloramphenicol, and novobiocin	" 6 mo	16.8
24	♂	6	In relapse; receiving intrav. ACTH, chloramphenicol, and novobiocin	" 5 mo	22.1
26	♂	3	No previous therapy, receiving adrenal steroids	No	6.1
40	♀	7	Amethopterin omitted 14 days previously because of severe toxicity (stomatitis); partial remission	Yes; 14 days	11.2
50	♂	1	No previous therapy	No	3.6
53	♀	2	*Idem*	"	2.8

TABLE III. Formic Acid Excretion in Children with Acute Leukemia Receiving Amethopterin.

Patient	Sex	Age	Remission	Daily dosage of amethopterin, mg	Duration of treatment	Urinary formic acid, μmoles/hr
26	♂	3	Yes	1.25	4 wk	10.4
					5 "	8.3
					12 "	119
27	♀	5	"	1.25	3 mo	45.8
28	♂	4	"	2.5	6 wk	26.8
					18 "	85.8
29	♀	4	"	1.25	11 mo	49.5
30	♂	4	No	1.25 (& prednisone)	1 yr	32.0
33	♂	8	Yes	2.5 "	4 wk	8.4
					10 "	6.7
					12 "	6.7
					15 "	36.4
					18 "	66.1
34	♂	3	Partial	1.25	3 mo	123
					3½ "	165
				Amethopterin stopped 3 wk previously		9.7
				" " 4 " "		9.6
35	♂	3	Yes	2.5	6 mo	64
					8 "	117
					9 "	117
37	♀	7	No	2.5 (& prednisone)	2 "	14.0
41	♀	8	Yes	2.5 "	3 "	122
43	♂	9	"	2.5 "	3 wk	12.8
					12 "	72.1
					16 "	125
44	♀	5	"	1.25	6 "	10.5
					15 "	36.6
					22 "	265
47	♂	3	Early relapse	2.5	5 mo	60
					5½ "	204
				Amethopterin stopped 2 wk previously		72
				" " 3 " "		23.8
55	♂	3	In relapse	2.5	8 mo	301
				Amethopterin stopped 3 wk previously		10.5
56	♂	6	Incomplete (improving)	2.5	14 days	34.8
57	♂	10	Yes	2.5 & 5.0 on alternate days	1 mo	193

analyses were performed. Urines were brought to pH 7.0 with 5.0 *N* KOH, and assayed for formic acid according to the method of Rabinowitz and Pricer(14).

Results. Urinary excretion of formic acid by adults ranged from 3.6 to 47.3 μmoles/hour (Table I). Nonleukemic children and leukemic patients not receiving amethopterin excreted 1.5-22.1 μmoles/hour (Tables I and II). The 3 children with acute leukemia who had never received the folic acid antagonist excreted 2.8-6.1 μmoles/hour. Fifteen of 16 children receiving amethopterin excreted more than 32 μmoles/hour, and 9 patients excreted over 117 μmoles/hour (Table III). An increase in formic acid excretion was usually not apparent until after several weeks of amethopterin therapy, and often not until long after remission of the leukemic process. Thus, in subject #33 formic acid excretion was only 6.7 μmoles/hour 12 weeks following start of treatment, but had increased by 10-fold after 18 weeks. A similar phenomenon was observed in patients 26, 43, and 44. This latent period may explain the low excretion of formic acid in patient 37, who had received amethopterin for 2 months. The level of urinary formic acid in treated children bore no apparent

relationship to clinical status of leukemia. Cessation of amethopterin administration to patients 34, 47, and 55 was followed by a sharp fall in formic acid excretion.

No evidence was found in urine of amethopterin-treated patients of substances that interfered with the formic acid assay. This was demonstrated by recovery experiments in which urine samples were reassayed after addition of known increments of formic acid; 100% recovery was obtained in all samples so tested.

Discussion. Our data demonstrate suitability of the enzymatic procedure of Rabinowitz and Pricer(14) for determination of urinary formic acid. Our studies indicate that in normal human subjects and in patients with a variety of disease states, formate may be detected in the urine, although in smaller quantity than previously reported(1,5). The variation in formic acid excretion from one patient to another, as well as in the same patient may be attributable to variations in dietary intake of formate precursors.

Participation of formic acid in metabolic processes is known to require a folic acid derivative(15-18). Formation of this derivative has been shown to be inhibited in animals receiving folic acid antagonists(19). Hence, formic acid would be expected to accumulate in folic acid deficiency and in subjects receiving folic acid antagonist therapy. This explanation undoubtedly accounts for the formaturia reported in folic acid-deficient rats(13) and for that observed in our patients treated with folic acid antagonist, amethopterin. Increased excretion of formic acid was often not seen until after several weeks or months of therapy.

It is of considerable interest that in several patients a complete remission had taken place well in advance of an increase in formic acid excretion, or in formiminoglutamic acid excretion(20). Since animal studies indicate that increase in urinary formic and formiminoglutamic acids is a reflection of folic acid deficiency in the whole organism(13), our observations provide *in vivo* evidence for the thesis that leukemic cells are abnormally sensitive to folic acid deprivation(21).

Summary. An enzymatic procedure has been employed to demonstrate the presence of formic acid in small quantity in urine of normal human subjects and of patients with a variety of disease states. The excretion is markedly elevated in patients with acute leukemia receiving the folic acid antagonist, amethopterin.

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