

Urinary Excretion Studies Following the Administration of Pterioic Acid.

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A number of reports have dealt with the apparent urinary excretion of pteroylglutamic acid as measured with *Lactobacillus casei* or *Streptococcus faecalis* R. In normal subjects on "average diets," the excretion is quite low, usually in the neighborhood of 4 to 5 μ g daily. The urinary excretion rises following the administration of a few milligrams of the pure substance. In one investigation, assays with *S. faecalis* R indicated that from 44 to 57% of an oral dose of 5 mg was excreted in six hours.¹

The name pterioic acid² has been given to the portion of the pteroylglutamic acid molecule exclusive of glutamic acid, more specifically 4(((2-amino-4-hydroxy-6-pteridyl)methyl)amino)benzoic acid. It may be synthesized by condensing p-aminobenzoic acid with α - β -dibromopropionaldehyde and 2,4,5-triamino-6-hydroxypyrimidine.² Pterioic acid can replace pteroylglutamic acid in promoting growth of certain microorganisms, notably *S. faecalis* R, but it is without appreciable growth-promoting action on *L. casei*.² The presence of pterioic acid in the urine would therefore produce a response in the "folic acid" assay when carried out with *S. faecalis* R but would be without effect if the assay were carried out with *L. casei*. No studies have been reported describing the urinary excretion of pterioic acid following its administration and such an investigation was the subject of the present study.

Experimental. Pterioic acid was synthesized as described above* and was obtained in a

state of approximately 90% purity as estimated by chemical assay³ and extinction coefficient measurements. The compound was administered as a solution of the monosodium salt to normal adult male subjects. The urine was collected under toluene and the samples were assayed for "folic acid" with *S. faecalis* R⁴ and for pteroylglutamic acid with *L. casei*.⁵ The results are summarized in Table I.

Discussion. Pterioic acid has been found to stimulate the growth of certain lactic acid bacteria under conditions in which a response also occurs to pteroylglutamic acid. In one class of organisms, typified by *L. casei*, a response is produced by pteroylglutamic acid but not by pterioic acid or p-aminobenzoic acid. A second class, typified by *S. faecalis* R, responds to pteroylglutamic acid and pterioic acid, but not to p-aminobenzoic acid. A clue to the function of pterioic acid in this class of organisms may be afforded by the observation⁶ that certain enterococci in "resting cell suspensions" convert the "S.L.R. factor," which is pterioic acid with an added formyl group,⁷ to pteroylglutamic acid. A third type of organism, exemplified by *L. arabinosus* 17-5⁸ responds to either pteroylglutamic acid, pterioic acid, or p-aminobenzoic acid. Studies

* Kindly supplied by Dr. C. W. Waller.

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⁶ Stokes, J. L., and Larsen, A., *J. Bact.*, 1945, **50**, 219.

⁷ Wolf, D. E., Anderson, R. G., Kaczka, E. A., Horris, S. A., Arth, G. E., Southwick, P. L., Mozingo, R., and Folkers, K., *J. Am. Chem. Soc.*, 1947, **69**, 2753.

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¹ Jukes, T. H., Franklin, A. L., Stokstad, E. L. R., and Boehne, J. W., III, *J. Lab. Clin. Med.*, 1947, **32**, 1350.

² Angier, R. B., Boothe, J. H., Hutchings, B. L., Mowat, J. H., Semb, J., Stokstad, E. L. R., SubbaRow, Y., Waller, C. W., Cosulich, D. B., Fahrenbach, M. J., Hultquist, M. E., Kuh, E., Northey, E. H., Seeger, D. R., Sickels, J. P., and Smith, J. M., Jr., *Science*, 1946, **103**, 667.

TABLE I.
Assays of Urine Following the Administration of Pterioic Acid.

Amt administered* mg	Route	Assay result†						24-hr urine vol., ml	Apparent excretion of pterioic acid‡		Apparent conversion of pterioic acid to pteroylglutamic acid§	
		<i>S. faecalis</i> R		<i>L. casei</i>					% admin- istered dose	Avg %	% admin- dose	Avg %
		24 hr		Basal mγ/ml	24 hr sample mγ/ml	Basal mγ/ml	24 hr sample mγ/ml					
		Basal mγ/ml	mγ/ml									
2	Oral	2	19	11	19			970	2.7	0	1.4	Insignificant
2	"	2	2	8	6			2330	0			
5	"	2	18	5	8			1680	1.7	2.1	"	"
5	"	2	46	8	12			890	2.4			
5	"	3	32	9	5			1260	2.3			
10	"	2	4	8	8			1770	0.2	0.3	"	"
10	"	4	14	10	13			620	0.4			
2	Intravenous	2	150	6	20			2000	46	33	1.1	1.4
2	"	2	208	5	38			1200	39			
2	"	2	85	11	36			1170	15			

* The compound was prepared as described in the text. It was found to be 90% pure by the chemical reduction test³ and by measurement of the extinction coefficient at 365 mμ. When the preparation was assayed in comparison with pteroylglutamic acid values of 32% and .004% of the activity of pteroylglutamic acid were obtained, respectively, with *S. faecalis* R and *L. casei*.

† Calculated in equivalence of pteroylglutamic acid.

‡ Calculated from the assay with *S. faecalis* R, using a factor of 32% (see above) to convert the pteroylglutamic acid equivalence into terms of pterioic acid.

§ Calculated from the assay with *L. casei*, since this organism responds to pteroylglutamic acid and not to pterioic acid. The calculation was adjusted for the purity of the pterioic acid and the molar weight ratios of pterioic acid and pteroylglutamic acid.

of the relative potencies of these 3 compounds for *L. arabinosus*⁸ showed that the first 2 are less active on a molar basis than is p-aminobenzoic acid.

In experiments with chicks, pterioic acid² and p-aminobenzoic acid^{9,10} have been found ineffective as a replacement for pteroylglutamic acid in the diet. Pterioic acid was administered,¹¹ 5 mg daily for 10 days without effect, to a patient with pernicious anemia in relapse. The patient subsequently responded to treatment with 5 mg of pteroylglutamic acid daily for 6 days.

A study of the urinary excretion of pteroylglutamic acid indicated that about half of the dose, as estimated by assay with *S. faecalis* R, appeared in the urine during 6 hours following the oral administration of 5 mg of the substance. Similar results were obtained when intravenous administration was used.¹ In contrast, pterioic acid when fed by mouth did not appear to be excreted in significant amounts in the urine, but when it was given intravenously a considerable proportion of the administered dose appeared in the urine. This may indicate that the rate of uptake from the gastrointestinal tract is low, or, less probably, that the compound is destroyed in the tract before being absorbed. The results are interesting in view of the low solubility of the monosodium salt of pterioic acid which is in contrast to the moderate solubility of sodium pteroylglutamate. At the pH of the small intestine, the ion formed by the dissociation of only the carboxyl group would be the predominant form of pterioic acid.

A possibility to be considered in the metab-

olism of pterioic acid is its conversion to pteroylglutamic acid. The respective activities of the 2 compounds for *S. faecalis* R and *L. casei* enable a differential assay to be conducted, especially if pterioic acid is present in amounts considerably in excess of pteroylglutamic acid. In this investigation, the *L. casei* assay value of the urine was very low following the intravenous administration of pterioic acid in contrast to the *S. faecalis* R assay value. This indicated that the urine contained predominantly pterioic acid rather than pteroylglutamic acid. However, a distinct rise in the *L. casei* value was observed, which indicated probable conversion of a small proportion of the pterioic acid to pteroylglutamic acid (Table I).

Summary. 1. Pterioic acid was administered to normal adult human males and the urine was assayed for "folic acid" with *S. faecalis* R and for pteroylglutamic acid with *L. casei*. On the basis of the relative response of these organisms to pterioic acid and pteroylglutamic acid, the apparent concentration of these compounds in the urine was determined.

2. The oral administration of from 2 to 10 mg of pterioic acid resulted in only a very small amount of urinary pterioic acid.

3. Following the intravenous administration of 2 mg of pterioic acid the microbiological assays of the urine indicated that from 15 to 46% of the compound was excreted as pterioic acid and that only about 1% appeared to be converted to pteroylglutamic acid.

4. The excretion studies indicated that only a small proportion of an administered dose of pterioic acid was converted to pteroylglutamic acid and that pterioic acid is poorly absorbed from the gastrointestinal tract.

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⁹ Lillie, R. J., and Briggs, G. M., *Poultry Sci.*, 1947, **26**, 289.

¹⁰ Unpublished investigations in this laboratory.

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