The occurrence of folic acid (pteroyl-L-monoglutamic acid) in human blood serum after small oral doses¹

R. J. LEEMING, HEATHER PORTMAN-GRAHAM, AND J. A. BLAIR

From the General Hospital, Birmingham, and the Department of Chemistry, University of Aston in Birmingham

SYNOPSIS Human serum folates analysed by bioautography after oral doses of 0.5 mg folic acid (pteroyl-L-monoglutamic acid) show folic acid to be present.

Folic acid is probably only a minor dietary component but it has important therapeutic effects. Its mode of absorption and subsequent metabolism have caused much discussion. According to Baker, Frank, Feingold, Ziffer, Gellene, Leevey, and Sobotka (1965) folic acid is converted to tetrahydrofolates on transport across the human intestine but, as large amounts of 5-methyltetrahydrofolic acid were found only after two hours, the possible effects of passage through the intestine and liver could not be accurately defined. Similar results have been reported by Perry and Chanarin (1970).

If hamster everted sacs are incubated with folic acid 5-methyltetrahydrofolic acid and citrovorum factor can be demonstrated by microbiological assay (Cohen, 1965). However, these could have been derived from bacteria or from the folates in the intestinal mucosa, for although the hamster intestinal content of folates is unknown, rat intestinal mucosa contains 2.1 µg/g folates of which 0.46 µg is 5-methyltetrahydrofolic acid and 0.13 µg is citrovorum factor (Sotobayashi, Rosen, and Nichol, 1966).

In two human subjects Whitehead and Cooper (1967) observed that folic acid as determined by microbiological assay appeared in serum obtained from the portal vein after oral folic acid. Butterworth, Baugh, and Krumdieck (1969), comparing the specific microbiological and radiochemical activities of ¹⁴C folic acid, concluded that folic acid was not metabolized on transport. With rat everted sacs, Matty and Blair (1968), Smith, Matty, and Blair (1970a and 1970b) found only folic acid in the serosal solution when folic acid was incubated in the mucosal solution.

Bioautographic techniques (Leeming, Portman-Graham, Swan, and Blair, 1970) enable more specific chemical confirmation of the presence of folic acid

and 5-methyltetrahydrofolic acid to be made and avoid the assumptions and ambiguities of identification by microbiological assay.

Materials and Methods

There were 42 normal subjects, 18 males and 24 females, with ages ranging from 17 to 52 years. Seven of the females were taking oral contraceptives.

COLLECTION OF SPECIMENS

A venous sample of blood was taken from each volunteer and 0.5 mg folic acid given orally. Samples of blood were taken at 15 minutes, 30 minutes, 45 minutes, 60 minutes, and 120 minutes, and the serum was separated.

SERUM EXTRACTION

Of freshly separated serum, 10 ml was added to an equal volume of 1% aqueous ascorbic acid. The mixture was heated in a boiling water bath to 80°C, cooled in a refrigerator, and passed through 8/32 in. Visking tubing under negative pressure in the dark. The clear filtrate was frozen and freeze-dried. For use the dry material was dissolved in 0.2 ml of 1% aqueous ascorbic acid.

BIOAUTOGRAPHS

Bioautography was carried out as previously described (Leeming *et al*, 1970). The chromatograms were developed in 3% aqueous ammonium chloride but the extracts of the six sera from one subject were also developed in 0.5% aqueous sodium carbonate and phosphate buffer 0.1 M, pH 7.0 (Leeming *et al*, 1970). The R_f values of the two moieties in the sera corresponded to those of the folic acid and 5-methyltetrahydrofolic acid standards in the three solvent systems.

RECOVERY AFTER EXTRACTION

A sample of pooled serum from subjects not given

¹Requests for reprints should be addressed to R. J. Leeming, The General Hospital, Birmingham, B4 6NH.

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folic acid was divided into four and an equal volume of one of the three folate analogues—citraovorum factor, 5-methyltetrahydrofolic acid, and folic acid—(10 ng/ml in 1% aqueous ascorbic acid) was added to each of three and to the fourth an equal volume of 1% aqueous ascorbic acid alone. These bioautographs were compared with others in which folates were added to serum after concentration. The fourth sample only showed a small spot corresponding to 5-methyltetrahydrofolic acid. The added folates survived the concentration process as shown by their having the same R_F values as those added after concentration. In the samples where 5-methyltetrahydrofolic acid had been added only the added 5-methyltetrahydrofolic acid could be detected and folic acid was absent. Therefore the folic acid determined in later bioautographs could not have arisen by decomposition reactions.

DIFFERENTIAL MICROBIOLOGICAL ASSAYS

Serum folate assays were carried out using *Streptococcus faecalis* (NCIB 6459) and *Lactobacillus casei* (NCIB 8010).

Results

The results of the bioautographs are shown in Fig. 1

and the results of the microbiological assays given in Tables I and III.

The microbiological assays showed a rise in both *Streptococcus faecalis* and *Lactobacillus casei* active folates 15 minutes after ingestion. The *Streptococcus faecalis* active folate increased approximately linearly with time to a maximum level at 60 minutes and thereafter declined. As this rise and fall of *Streptococcus faecalis* assay paralleled the appearance of folic acid on the bioautographs, this activity was ascribed solely to the folic acid present. *Streptococcus faecalis* is inactive and *Lactobacillus casei* active for 5-methyltetrahydrofolic acid and their difference measures this compound (Table III). The serum 5-methyltetrahydrofolic acid level showed little increase in the first 15 minutes compared with folic acid levels but increased rapidly thereafter.

Discussion

The folic acid dose was chosen as the smallest giving clearly defined results. These experiments showed that when small oral doses of folic acid were given folic acid appeared in the serum and that this was slowly converted to 5-methyltetrahydrofolic acid (Blair, 1970). When the serum levels of folic acid and 5-methyltetrahydrofolic acid were plotted against

Fig. 1 Bioautograph results showing the number of subjects demonstrating the two moieties at each time interval.

Subject	Time in Minutes					
	0	15	30	45	60	120
42 Total	5.6 ± 0.32	6.3 ± 0.39	8.2 ± 0.49	10.1 ± 0.66	11.5 ± 0.76	13.2 ± 0.89
18 Males	5.6 ± 0.53	6.4 ± 0.65	8.3 ± 0.95	10.4 ± 1.32	10.4 ± 1.09	12.1 ± 1.20
24 Females	5.5 ± 0.41	6.2 ± 0.49	8.1 ± 0.51	9.8 ± 0.64	12.3 ± 1.03	14.1 ± 1.27
7 Females on oral contraceptives	6.5 ± 0.99	7.4 ± 0.93	9.3 ± 1.27	10.5 ± 1.61	10.4 ± 1.91	11.1 ± 1.36
17 Females not on oral contraceptives	5.1 ± 0.39	5.8 ± 0.55	7.7 ± 0.48	9.5 ± 0.65	13.1 ± 1.21	15.3 ± 1.63

Table I Mean levels of folate acid in *L. casei* assay¹

¹Results are given in ng/ml with the standard errors of means.

Subject	Time in Minutes					
	0	15	30	45	60	120
27 Total	1.5 ± 0.13	2.0 ± 0.21	2.4 ± 0.22	3.0 ± 0.24	3.3 ± 0.31	2.7 ± 0.16
10 Males	1.5 ± 0.25	2.0 ± 0.39	2.6 ± 0.54	3.2 ± 0.47	3.5 ± 0.58	2.6 ± 0.21
17 Females	1.5 ± 0.15	2.0 ± 0.24	2.1 ± 0.15	2.8 ± 0.27	3.2 ± 0.37	2.7 ± 0.22
5 Females on oral contraceptives	1.4 ± 0.19	1.6 ± 0.19	1.8 ± 0.19	2.2 ± 0.25	2.3 ± 0.54	2.3 ± 0.27
12 Females not on oral contraceptives	1.6 ± 0.21	2.1 ± 0.33	2.4 ± 0.18	3.1 ± 0.35	3.6 ± 0.44	2.9 ± 0.29

Table II Mean levels of folic acid in *Str. faecalis* assay

Time in Minutes						
0	15	30	45	60	120	
4.1	4.3	5.8	7.1	8.3	10.5	

Table III *L. casei* assay minus *Str. faecalis* assay (5-methyltetrahydrofolic acid)

Fig. 2 Mean serum folic acid and 5-methyltetrahydrofolic acid levels plotted against time.
● *S. faecalis*-folic acid; X = *L. casei* less *S. faecalis*-5-methyltetrahydrofolic acid.

time the level of folic acid in the serum rose in the first 10 minutes while the level of 5-methyltetra-

hydrofolic acid did not (Tables II and III, Fig. 2). Therefore in these experiments folic acid was transported across the intestine without being metabolized to 5-methyltetrahydrofolic acid and the conversion of folic acid to 5-methyltetrahydrofolic acid took place elsewhere than in the intestine.

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