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Thermal Processing Effects on Folacin Bioavailability in Liquid Model Food Systems, Liver, and Cabbage

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The effects of thermal processing on folacin bioavailability in processed and unprocessed lactose–casein liquid model food systems containing either folic acid or 5-methyltetrahydrofolic acid (5-CH_3-THF) were examined by using a chick bioassay. Microbiological and high-performance liquid chromatographic (HPLC) analyses indicated that folic acid was very stable during thermal processing at 120 °C for 20 min while 5-CH_3-THF was approximately 75% degraded. Both derivatives were found to be biologically available after processing, which indicated that no complexes were formed during processing that inhibited their utilization. For the model systems there was general agreement between chick bioassay, microbiological, and HPLC analyses. Thermal processing effects on naturally occurring folacin in beef liver and cabbage were also examined. Folacin from cooked beef liver appeared to be fully available. Raw cabbage folacin, which had undergone enzymatic deconjugation during diet preparation and storage, was found to be completely biologically available. Approximately 60% of the folacin in cooked cabbage, which corresponded to the polyglutamate fraction, was not biologically available.

The bioavailability of folacin in foods has been examined by many researchers (Scherte et al., 1965; Tamura and Stokstad, 1973; Babu and Srikantia, 1976; Graham et al., 1980). Limited data are available to explain the low availability observed for many food products. Margo et al. (1975) and Colman et al. (1975) reported data that suggested that folacin from fortified rice, maize, and bread was adsorbed more slowly than doses of folic acid alone. Differences in folacin availability from natural and synthetic sources have also been reported (Nelson et al., 1975; Stokstad et al., 1977), suggesting that natural inhibitors of folacin utilization may be present in certain foods. Inhibition of certain γ-glutamyl carboxypeptidases (conjugases) has been reported for various yeast preparations (Rosenberg and Godwin, 1971) and for beans and other pulses (Krumdieck et al., 1973). These data suggest that, in addition to bulk of food alone, various substances contained in natural products may influence folacin utilization.

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It has long been recognized that the folacin activity in foods consists of several fractions (Santini et al., 1964) and that these fractions differ in stability (Ghitis, 1966). Ford (1967) found that heat steriziliation of milk destroyed about 50% of its folacin activity. The loss of folacin was found to be related to the oxidatve destruction of ascorbic acid and could be retarded by exclusion of oxygen from the milk during heat processing and storage.

The stability of folacin derivatives has been examined under various conditions. O'Broin et al. (1975) studied the stability of four major dietary folacin monoglutamate derivatives at room temperature. Their results indicated that folic acid and its formyl derivatives were extremely stable while 5-methylterahydrofolic acid (5-CH_3-THF) and unsubstituted tetrahydrofolic acid (THF) were very labile. Specific buffer ion effects, influencing the degradation of most folacin derivatives, were also observed. Cooper et al. (1978) and Paine-Wilson and Chen (1979) observed similar results regarding the thermal stability of folacin derivatives. Limited kinetic information indicates that the degradation reaction rates vary as a function of oxygen and reducing agent concentration (Chen and Cooper, 1979; Ruddick et al., 1980).

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While the effects of thermal processing on the stability of folacin in foods have been characterized to a limited degree, no information has been reported dealing with processing effects on the bioavailability of food folacin. The present investigation was designed to examine the effect of thermal processing on the bioavailability of folic acid and 5-CH_3-THF. The biological activity of these folacin derivatives in processed and unprocessed lactose–casein model food systems simulating liquid infant formulas was determined by using a chick bioassay. In addition, beef liver and cabbage were also examined to determine the bioavailability of naturally occurring derivatives.

MATERIALS AND METHODS

Model Food System, Liver, and Cabbage Preparation. A model food system formulated to simulate an infant formula was composed of 7.0% lactose and 1.5% potassium caseinate in 0.01 M phosphate buffer (pH 7.0). The system was fortified with either folic acid or 5-CH_3-THF to yield 12 mg of the folacin derivative/450 g of the model system solids. Folic acid containing model systems were also prepared with added ascorbate or iron at levels equivalent to those found in the infant formula (6.38 mg of L-ascorbic acid sodium salt or 6.65 mg of ferrous sulfate per 100 mL of liquid model system).

Model systems were sealed under vacuum in commerically available retort pouch film and were then retorted at 120 °C for 20 min. The pouch material consisted of a lamination of polyester, aluminum foil, and polypropylene copolymer films with the polypropylene exposed to the liquid model system. Temperature history was recorded by using a Digisstrip II programmable recorder (Kaye Instruments, Inc.). The processed model systems and unprocessed controls were then freeze-dried by using a shelf temperature of 40 °C for 48 h. All dried model systems were finely powdered and stored in polyethylene bags at 4 °C.

Frozen, sliced beef liver was baked in a convection oven at 177 °C for 25 min. The liver was then freeze-dried and finely ground. Raw cabbage, which was obtained fresh, was chopped and freeze-dried. Cabbage was also steamed (100 °C) for 75 min to provide a heat treatment thermally equivalent to retorting at 120 °C for 20 min (Lund, 1975) prior to freeze-drying.

HPLC Analyses of Folacin in Model Systems. Folacin concentration in the model systems was quantitatively determined by high-performance liquid chromatography (HPLC) using the reverse-phase procedure of Day and Gregory (1981). HPLC analyses were performed by using an Altex Model 312 liquid chromatograph with 280-nm absorption detection. Model system samples (5 g) were dissolved in phosphate buffer (pH 7.0) containing 1% sodium ascorbate. The pH was adjusted to 4.5 with concentrated HCl, and the solutions were centrifuged at 10000*g* for 5 min at room temperature. The supernatant was filtered through a 0.45-m filter (Gelman Instrument Co.) before HPLC analysis.

A preparative cation-exchange chromatography cleanup procedure described by Gregory et al. (1982) was used for model systems containing 5-CH_3-THF to prevent the coelution of a Maillard browning product. Extracts (pH 4.5) were applied to 0.7 × 12 cm columns of Bio-Rad AG 50W-X8 (100–200 mesh, K^+ form; Bio-Rad Laboratories, Richmond, CA) equilibrated in 0.1 M potassium acetate (pH 4.5, 0.25% ascorbate), followed by a 10-mL wash with the same buffer. 5-CH_3-THF was eluted with 20 mL of 0.1 M potassium phosphate (pH 7.0) containing 9.0% acetonitrile and 0.25% ascorbate.

Table I. Basal Chick Diet

ingredients	%
casein[a]	25.00
L-arginine hydrochloride[b]	1.50
DL-methionine[b]	0.40
glycine[b]	1.00
corn oil[c]	4.00
dextrose[b]	57.54
vitamin mix[d,e]	1.20
mineral mix[d,f]	6.36
cellulose[d]	3.00

[a] Vitamin free; U.S. Biochemical Corp., Cleveland, OH. [b] U.S. Biochemical Corp. [c] Mazola, Best Foods, Englewood Cliffs, NJ. [d] ICN Nutritional Biochemicals, Cleveland, OH. [e] Vitamin mix provided (per kilogram of diet): thiamin, 15 mg; riboflavin, 15 mg; niacin, 50 mg; pyridoxine, 6 mg; biotin, 0.6 mg; vitamin B_{12}, 20 μg; choline chloride, 2.0 g; calcium pantothenate, 20 mg; menadione, 1.5 mg; vitamin E, 50 units, vitamin D_3, 4500 units; vitamin A, 4500 units; antioxidant, 100 mg. [f] Mineral mix provided (per kilogram of diet): $CaHPO_4 \cdot 2H_2O$, 18 g; $CaCO_3$, 19 g; KH_2PO_4, 14 g; $NaHCO_3$, 8.8 g; $MnSO_4 \cdot H_2O$, 0.35 g; $FeSO_4 \cdot 7H_2O$, 0.50 g; $MgSO_4$, 3.0 g; KIO_3, 0.01 g, $CuSO_4 \cdot 5H_2O$, 0.03 g; $ZnCO_3$, 0.15 g; $CoCl_2$, 1.7 mg; $NaMoO_4 \cdot 2H_2O$, 8.3 mg; Na_2SeO_4, 2.0 mg.

Chick Bioassay. The chick bioassay was conducted by using the casein–glucose basal diet described by Scott et al. (1969), as shown in Table I. Graded levels of folic acid (0, 0.5, 1.0, and 1.5 mg/kg of diet) were added to the basal diet to provide the basis for a standard dose–response curve. Each freeze-dried model system was individually blended into the basal diet at a level of 3% (w/w). Freeze-dried liver and cabbage were also individually mixed into basal chick diets to provide the sole source of folacin to the chicks (liver blended at 14.75% w/w, cabbage blended at 7.30% w/w). Ingredients in the basal chick diet were adjusted to account for the contribution of the model system components and the lver and cabbage nutrients with regard to the total energy and protein content of the diets. Diets containing 5-CH_3-THF, liver, and cabbage were stored at 4 °C and removed in small aliquots as required for feeding.

Day-old male, Cobb, color-sexed chicks were randomly divided into groups of 15 each. Each chick was weighed and wing-banded at the beginning of the study. The chicks were housed five chicks per pen (three pen per treatment) in stainless steel, heated batteries and fed ad libitum for 21 days. At the end of the 3-week feeding period each chick was weighed. Feed consumption was determined for each pen. Blood was collected by anterior heart puncture into heparinized tubes. Plasma samples were placed in plastic vials, immediately frozen on dry ice, and stored at −20 °C until assayed for folacin content. Samples of each diet were collected at the beginning and end of the chick bioassay to determine stability of the folacin during the study. Diet samples were stored at −20 °C in polyethylene bags until assayed for folacin content.

Microbiological Assay Procedures. The procedures of Shin et al. (1975) and Lin et al. (1975) were adapted for extraction of chick diets for microbiological assay of folacin. Samples (10 g) of the diet were homogenized in 0.2 M phosphate buffer (pH 6.1) containing 0.5% ascorbate. The homogenates were autoclaved at 121 °C for 10 min and cooled in an ice bath. The samples were centrifuged at 10000*g* for 10 min at 4 °C, and the supernatants were collected and then stored in plastic vials at −20 °C. Prior to microbiological assay the sample extracts were treated with hog kidney conjugase to cleave folacin polyglutamates. Hog kidney conjugase was freshly prepared and used according to the procedure of Eigen and Shockman (1963).

Table II. Chick Bioassay Data for Determination of Available Folacin from Model Systems

diet[a]	wt gain,[b] g	feed consumption,[b] g chick^{-1} day^{-1}	plasma folacin,[b,c] ng/mL	dietary folacin, mg/kg[b,c] initial	dietary folacin, mg/kg[b,c] final
standard					
0.00 mg of FA/kg	180 ± 18	11.3 ± 2.3	3.2 ± 0.6	0.11	0.11
0.50 mg of FA/kg	518 ± 13	32.7 ± 2.3	9.6 ± 1.6	0.50	0.50
1.00 mg of FA/kg	583 ± 13	35.4 ± 1.2	19.9 ± 2.7	0.90	0.90
1.50 mg of FA/kg	538 ± 21	36.7 ± 8.0	31.0 ± 2.7	1.65	1.65
model systems[d,e]					
FA, control	553 ± 17	33.1 ± 4.9	17.7 ± 1.2	0.90	0.74
FA, processed	557 ± 9	35.6 ± 1.2	17.5 ± 2.5	0.84	0.75
FA, processed with ascorbate	573 ± 10	34.4 ± 1.2	14.6 ± 2.4	0.84	0.74
FA, processed with iron	553 ± 13	34.7 ± 1.0	19.2 ± 2.1	0.75	0.62
5-CH_3-THF, control	473 ± 23	29.2 ± 4.0	6.5 ± 1.1	0.44	0.30
5-CH_3-THF, processed	206 ± 23	17.5 ± 7.1	3.4 ± 0.6	0.14	0.12

[a] Abbreviations: FA, folic acid; 5-CH_3-THF, 5-methyltetrahydrofolic acid. [b] Mean and standard error; 15 chicks per group. [c] Microbiological assay values. Mean of duplicate samples. Coefficient of variation, 4%. [d] Processed model systems were retorted at 120 °C for 20 min. [e] Folacin added to model systems to provide 0.8 mg/kg dietary folacin before processing.

A blank containing only buffer was treated with conjugase for reference in the microbiological assay. Recovery values were calculated by using folic acid and 5-CH_3-THF standards added to selected sample homogenates. Recovery of FA was 98 ± 4%; recovery of 5-CH-THF was 50 ± 1% (recovery for 5-CH_3-THF represented biological activity of the *l* isomer only in the racemic *dl*-5-CH_3-THF standard).

The microbiological determination of folacin was based on the method of Herbert (1966) using *Lactobacillus casei* (ATCC 7469). All plasma samples, diet extracts, and folacin standards were assed in duplicate. Diet extracts were assayed with and without conjugase treatment. Folacin concentrations were calculated relative to a folic acid standard curve.

RESULTS AND DISCUSSION

Chick Bioassay: Model Systems. The effect of the thermal processing of foods on the bioavailability of folic acid and 5-CH_3-THF was examined by using a chick bioassay. Plasma folacin values of chicks fed the folic acid standard diets (Table II) were used to quantiatively assess folacin status of chicks consuming experimental diets. Preliminary studies indicated that plasma folacin concentration was closely correlated with liver folacin concentration as an indicator of folacin status for the range of dietary folacin levels used in this study. Reponses based on chick growth did not permit quantiation over the full range of folic acid concentrations employed. Plasma folacin, feed consumption, and weight gain values from chicks that consumed diets containing folic acid standards and folic acid model system are listed in Table II. Microbiological analysis of diets sampled at the beginning and end of the study confirmed the accuracy of diet formulation and adequate retention of standard folic acid and experimental folacin derivatives. Chicks consuming a diet containing an unprocessed folic acid model system (FA control) yielded plasma folacin and weight gain responses that corresponded with a dietary folacin value of 0.8 mg of folacin/kg of diet. Since all folacin was added to the experimental diets by way of the model system to provide 0.8 mg of folacin/kg of diet, the added folcin acid in the unprocessed model system was fully available for inestinal absorption and utilization.

Folic acid in the processed model system also yielded plasma and weight gain values which corresponded with 0.8 mg/kg dietary folacin, indicating no destruction of folic acid with thermal processing and complete availability of the processed folic acid. The high retention of folic acid is in agreement with stability data reported by other researchers (O'Broin et al., 1975; Cooper et al., 1978). The biological availability of folic acid in processed foods has not been throughly examined. These findings indicate that folic acid does not interact with components of the lactose–casein model food system during processing to form complexes that might affect its bioavailability. In addition, these results indicate that fortification with either ascorbate or ferrous iron at nutritional levels did not adversely affect folic acid utilization.

The plasma folacin, feed consumption, and weight gain data for chicks consuming diets containing the 5-CH_3-THF model systems are also presented in Table II. Plasma folacin and weight gain values for chicks consuming the diet containing the unprocessed 5-CH_3-THF model system (5-CH_3-THF control) corresponded to a level of approximately 0.3–0.4 mg/kg dietary folacin when compared with the standard folic acid dose–reponse curve. For chicks consuming the processed 5-CH_3-THF model system, the responses corresponded to approximately 0.1 mg/kg dietary folacin. Microbilogical analysis of the diets containing these model systems confirmed the presence of folacin at the levels interpreted from the bioassay.

The 5-CH_3-THF added to the model systems was a mixture of the *d* and *l* isomers. Previous researchers (Mathews and Huennekens, 1960; Shane et al., 1980) had reported that only the *l* isomer of tetrahydrofolates is naturally occurring and biologically active. If it is assumed that a 50:50 racemic mixture of the isomers was present in the 5-CH_3-THF added to the model systems, the responses obtained for this derivative would indicate full retention and availability if chick and microbiological assays yielded a level of 0.4 mg/kg dietary folacin since the *dl*-5-CH_3-THF control model system was added to provide 0.8 mg/kg dietary folacin.

In view of the inactivity of the *d*-5-CH_3-THF isomer, the results obtained for the 5-CH_3-THF control indicate high stability during diet formulation and storage and full biological availability of this folacin derivative. Results of the microbiological assay for dietary folacin from the processed 5-CH_3-THF model system indicated a loss of approximately 75% of the added folacin, but the utilization of the remaining folacin by the chick was not inhibitied.

The degradation of 5-CH_3-THF observed in this study during model system processing (20 min, 120 °C) was in general agreement with previously reported stability data. Cooper et al. (1978) reported a half-life of 21 min at 100 °C at pH 7 in the presence of unlimited oxygen. Paine-Wilson and Chen (1979) reported a half-life of 9 min for

Table III. Comparison of HPLC and Microbiological Assay Results for Model Systems[a,b]

model system[c]	HPLC, μg/g	microbiological, μg/g
FA, control	25.00	21.00
FA, processed	24.25	17.50
FA, processed with ascorbate	22.75	25.60
FA, processed with iron[d]	ND	ND
5-CH_3-THF, control	26.20	12.45[e]
5-CH_3-THF, processed	6.40	3.13[e]

[a] Values are means of duplicate samples. [b] Folacin originally added to model systems to provide 26.67 μg/g. [c] Abbreviations: FA, folic acid; 5-CH_3-THF, 5-methyltetrahydrofolic acid. [d] Not determined. [e] Values represent a measure of the *l* isomer of 5-CH_3-THF only.

this compound at 100 °C at pH 7.

Methods of measurement of 5-CH_3-THF degradation in previous studies have been either microbiological (Cooper et al., 1978; Chen and Cooper, 1979; Paine-Wilson and Chen, 1979) or radiometric (Ruddick et al., 1980). These methods are based on the assumption that the degradation products are not active for the assay employed. Although degradation products were not identified in this study, the correlation between microbiological, chick, and HPLC assay values (Table II and III) indicates that the 5-CH_3-THF remaining after heat processing was the sole biologically active folacin compound present. Degradation products are apparently inactive for both chicks and *L. casei*. The close agreement of values obtained by these methods for 5-CH_3-THF provides evidence for the usefulness of the chick bioassay in determining folacin bioavailability from foods as 5-CH_3-THF is a form found naturally in many products.

Comparison of Assay Methods for Determination of Folacin. In addition to the microbiological analyses of the diets used for the chick bioassay, the model systems alone were analyzed both by microbiological assay and by HPLC. A comparison of the results obtained from these analyses is presented in Table III. Close agreement between the microbiologocal and HPLC results is shown, providing additional support for the validity of the assay. The difference in values for 5-CH_3-THF between the two assay methods is due to the inactivity of the *d* isomer of this derivative for the microbiological assay. Since the *d* isomer is not naturally occurring, this difference in assay sensitivity would not be important when examining natural food sources for folacin content by these methods. However, this difference must be noted when synthetic derivatives are examined.

The results of the HPLC and microbiological assays for total folacin in the model systems are compared to the chick bioassay results in Table IV. Predictions of available folacin in the diets based on plasma folacin, dietary folacin content as measured by microbiological assay, and model system folacin content as measured by microbiological and HPLC assay (as calculated from Table III) are shown. Microbiological and HPLC assay of the model systems provided predictions of available folacin that were consistently less than predictions from the bioassay or diet analyses.

The extraction of the model systems prior to microbiological and HPLC analysis was performed by using a different procedure than that used for diet analysis. The less rigorous extraction technique employed for direct analysis of the model systems would presumably account for the close correlations observed in Table III yet consistently low values for predicted dietary folacin (Table IV). Further research is in progress concerning the adequacy of such extraction methods for the HPLC analysis of food folacin.

Chick Bioassay: Natural Sources of Folacin. Liver and cabbage were examined by chick bioassay in order to compare the results of the model system studies with similar data for folacin from natural sources. Liver folacin has been shown to be mainly polyglutamate in nature with pentaglutamates predominating (Stokstad et al., 1977). Day and Gregory (1981) showed by HPLC the THF, 5-CH_3-THF, and 5-CHO-THF and/or 10-CHO-THF were the major folacin polyglutamates in beef liver, in agreement with previously reported values (Stokstad et al., 1977). Cabbage folacin is also almost entirely polyglutamate in nature. 5-CH_3-THF hexa- and heptaglutamates account for over 90% of the folacin content of cabbage (Stokstad et al., 1977). It is recognized that THF in liver and cabbage would undergo extensive oxidation to inactive products (Reed and Archer, 1980) during drying and diet preparation in this study. Therefore, the biological availability and activity data reported here would apply mainly to methyl- and formyl-substituted reduced folates, which exhibit greater stability than THF.

Plasma folacin, feed consumption, and weight gain values for chicks consuming diets containing either liver or cabbage as their sole source of folacin are shown in Table V, while values for total and available folacin are shown in Table VI. Microbiological analysis of these diets indicated essentially full retention during the feeding period, as observed with the other diets. These diets were prepared to provide approximately 0.4 mg/kg dietary folacin as estimated from published folacin values for these

Table IV. Comparison of Microbiological, HPLC, and Chick Bioassays for Determination of Folacin in Model Systems

		μg of available folacin/g of diet		
			calcd from model system analysis[c]	
model system[a]	chick bioassay[b]	diet analysis, microbiological[c]	HPLC	microbiological
FA, control	0.94 ± 0.39	0.90	0.63	0.75
FA, processed	0.84 ± 0.53	0.84	0.53	0.73
FA, processed with ascorbate	0.68 ± 0.49	0.84	0.77	0.68
FA, processed with iron	0.97 ± 0.34	0.75	*e*	*e*
5-CH_3-THF, control	0.22 ± 0.23[d]	0.44[d]	0.37[d]	0.79[d]
5-CH_3-THF, processed	0.06 ± 0.09[d]	0.14[d]	0.09[d]	0.19[d]

[a] Abbreviations: FA, folic acid; 5-CH_3-THF, 5-methyltetrahydrofolic acid. Model systems were incorporated into diets at 3% (w/w). [b] Mean and standard deviation; 15 chicks per group. Available folacin calculation from plasma folacin values. [c] Mean of duplicate samples. [d] Values represent the activity of the *l* isomer of 5-CH_3-THF only. [e] Not determined.

Table V. Chick Bioassay Data for Determination of Available Folacin from Natural Sources

diet	wt gain,[a] g	feed consumption,[a] g chick^{-1} day^{-1}	plasma folacin,[a,b] ng/mL	dietary folacin, mg/kg[b] initial	dietary folacin, mg/kg[b] final
liver, cooked[c]	590 ± 13	34.3 ± 1.2	70.9 ± 8.6	1.99 (2.61)[f]	2.06 (1.77)
cabbage, raw[d]	560 ± 14	35.7 ± 2.9	9.8 ± 1.6	0.43 (0.40)	0.26 (0.40)
cabbage, processed[d,e]	539 ± 19	34.2 ± 3.2	5.6 ± 0.5	0.15 (0.40)	0.15 (0.37)

[a] Mean and standard error; 15 chicks per group. [b] Microbiological assay values. Mean of duplicate samples. Coefficient of variation, 4%. [c] Liver cooked at 177 °C for 25 min. Freeze-dried liver blended at 14.75% (w/w) into basal diet. [d] Freeze-dried cabbage blended at 7.30% (w/w) into basal diet. [e] Cabbage steamed at 100 °C for 75 min. [f] Diets analyzed with and without the use of hog kidney conjugase to cleave folacin polyglutamates. Values in parentheses are total folacin values after conjugase treatment.

Table VI. Comparison of Assay Data for Determination of Folacin from Natural Sources

diet	chick bioassay[a]	μg of available folacin/g of diet: calcd from diet analysis,[b] microbiological, with conjugase	calcd from diet analysis,[b] microbiological, without conjugase	direct analysis of folacin source,[b,d] microbiological, with conjugase	direct analysis of folacin source,[b,d] microbiological, without conjugase
liver, cooked	3.51 ± 1.46	2.61	1.99	c	c
cabbage, raw	0.42 ± 0.32	0.40	0.43	0.43	0.37
cabbage, processed	0.18 ± 0.12	0.40	0.15	0.37	0.17

[a] Mean and standard deviation; 15 chicks per group. Available folacin calculated from plasma folacin data. [b] Mean of duplicate samples. [c] Not determined. [d] Direct assay of cabbage after freeze-drying.

foods (Perloff and Butrum, 1977). The results obtained for dietary folacin as measured microbiologically indicate the variability in the tables particularly evidenced by the value obtained for liver folacin.

The plasma folacin value for chicks consuming the liver diet was well beyond the value obtained from chicks consuming the standard diet with the highest level (1.5 mg/kg) of dietary folacin. The validity of the chick bioassay with respect to linearity of the dose–response curves for plasma folacin cannot be determined beyond the range of folacin examined. If linearity were assumed, the plasma folacin value obtained in this assay would correspond to a higher level of dietary folacin than the measured microbiologically (Table VI). Weight gain in chicks consuming the liver diet was not different from that for chicks consuming the standard diet containing an optimal level of 1.5 mg/kg dietary folacin. It should be noted that much of the folacin of this liver sample apparently had undergone enzymatic deconjugation prior to or during diet preparation and storage, as indicated by the microbiological assay data (Table V). The bioavailability of folacin from liver cannot be quantitatively determined from this assay since the level of dietary folacin provided by the liver was greater than the levels used to obtain the standard dose–reponse curve, although the liver folacin appeared to be well absorbed and utilized.

Microbiological analysis of the cabbage containing diets, with and without the use of hog kidney conjugase to cleave folacin polyglutamates, indicated that there was little or no loss of folacin during thermal processing. Tamura and Stokstad (1973) reported a 40% loss of cabbage folacin with a less severe heat treatment and the addition of ascorbate before cooking. However, they discarded the cooking water that probably contained extracted folacin (Leichter et al., 1978), which they regarded as destroyed. The processed cabbage in this study was steamed in trays that were then placed directly into the freeze-dryer (no water was discarded). Because 5-CH_3-THF is the major form of folacin in cabbage, extensive losses might be expected with thermal processing (Cooper et al., 1978; Chen and Cooper, 1979; Ruddick et al., 1980). However, cabbage is also an excellent source of ascorbic acid that would afford natural protection for this labile folacin derivative.

The plasma folacin values for chicks consuming diets with raw and processed cabbage as the source of folacin corresponded to 0.42 and 0.17 mg/kg available dietary folacin, respectively. The plasma folacin values indicate complete utilization of folacin from the raw cabbage and approximately 40% utilization of folacin from the processed cabbage.

Tamura and Stokstad (1973) estimated that the bioavailability of folacin from raw and cooked cabbage was only 50% for humans. They reported wide variability in their assay, however, with a range of 0–100% availability for different subjects. Questions were raised in their study concerning the effect of large amounts of food on the speed of digestion and on the hydrolysis of conjugated folacin, both of which would be expected to influence the absorption of folacin polyglutamates from foods.

One possible explanation of the discrepancies observed in availability of cabbage folacin for these two studies may be provided by different mechanisms of enzymatic folacin deconjugation for chicks and humans (Rosenberg and Neumann, 1974; Halsted, 1980). The importance of this difference cannot be determined from these results. A more likely explanation related to the effect of foods on the hydrolysis of conjugated folacin. The polyglutamates in the raw cabbage appeared to be hydrolyzed during diet preparation and/or storage, as evidenced by the close agreement of the values obtained with and without conjugase treatment (Table V). Several investigators have suggested that conjugase inhibitors present in foods may account for variations in folacin utilization (Rosenberg and Godwin, 1971; Krumdieck et al., 1973; Stokstad et al., 1977). Since the polyglutamates in the raw cabbage were hydrolyzed, probably by endogenous conjugase activity, food effects on this hydrolysis in vivo would be negligible.

The concentration of available folacin observed for the processed cabbage corresponds to the level of folacin monoglutamate measured in the processed cabbage diet by microbiological assay without the addition of conjugase enzyme. This suggests that the folacin polyglutamates in the processed cabbage were not biologically available. Heat treatment would have inactivated the endogenous conjugase in the processed cabbage, leaving the majority of folacin compounds in the polyglutamate form. The fac-

tor(s) responsible for the poor utilization of cabbage folacin polyglutamates cannot be determined from this assay. The possibility of dietary fiber interactions with the folacin polyglutamates in the cabbage may provide an explanation for the low bioavailability observed, but this requires further investigation.

Weight gain values for the chicks consuming cabbage diets corresponded to dietary levels of folacin significantly higher than those expected from the actual folacin content in the diet. Feed consumption for the chicks was also much higher than for the chicks consuming comparable levels of folic acid from standard basal diets. Apparently other factors, such as flavor, affect the acceptance of the diets by the chicks. Chicks consuming diets containing cabbage gained more weight than their standard controls in response to increased caloric intake. This would not be expected to influence the estimate of folacin availability from these diets since the increased intake of folacin due to the consumption of greater quantites of diet would be utilized in maintaining the increased growth.

In summary, thermal processing had little or no effect on the bioavailability of folic acid and 5-CH_3-THF monoglutamates in liquid model systems. The folacin in cooked liver, which was a mixture of monoglutamate and polyglutamate forms, was fully available in the chick bioassay. The bioavailability of cabbage folacin appeared to be a function of sample pretreatment, which affected the extent of folacin polyglutamate deconjugation by cabbage conjugases. The results suggest that folacin polyglutamate utilization may be inhibited by certain compounds of cabbage. Further research concerning folacin bioavailability will deal with food composition variables and specific components that may retard polyglutamate utilization.

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