The metabolism of some folates in the rat

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1. A number of folates labelled with ^{14}C were administered orally to rats, at various doses, and urinary, faecal and hepatic folates examined.

2. 10-Formylpteroylmonoglutamic acid (10CHO—PGA) entered the folate pool very slowly, and is thought to be relatively ineffective in nutrition.

3. 10-Formyl[2-^{14}C]tetrahydrofolic acid (10CHO—[2-^{14}C]THF) entered the folate pool very rapidly. 5-Methyl[2-^{14}C]tetrahydrofolate ($5CH_3$—[2-^{14}C]THF) was the major urinary folate.

4. 5-Formyl[2-^{14}C]tetrahydrofolic acid (5CHO—[2-^{14}C]THF) entered the folate pool only to a small extent. 5CHO—[2-^{14}C]THF, given intravenously, produced no urinary $5CH_3$—[2-^{14}C]THF in the first 6 h.

5. 5,10-Methylidyne[2-^{14}C]tetrahydrofolic acid was metabolized to an extent which was dependant on the dose. At doses of 3 and 30 μg/kg body-weight, $5CH_3$—[2-^{14}C]THF represented 5·4 and 20% respectively of urinary folates and for 10CHO—[2-^{14}]PGA, the values were 16% of total urinary folates after the higher dose, and 78·5% after the lower dose.

6. Results obtained for the metabolism of $5CH_3$—THF varied depending on the position of the labelling: $5^{14}CH_3$–THF gave no labelled urinary folate, the methyl group being lost rapidly. When $5CH_3$—[2-^{14}C]THF was given, it appeared as the major urinary folate.

7. Folates found in the liver after oral administration of labelled folates were identified by thin-layer chromatography; only folate monoglutamates were identified.

Although the metabolism of folates has been studied since their discovery in 1946, much controversy remains, because of the non-specificity of the microbiological assay which has been the chief analytical method, and the lability and ready interconversion of many of the reduced folates. Synthesis of labelled tetrahydrofolates (Beavon & Blair, 1971; Nixon & Bertino, 1971) has enabled more precise studies to be made of the fate in the rat of orally and parenterally administered folates. Since normal diets usually contain a number of different folates, it is of nutritional importance to determine their metabolic fate, storage and excretion.

5-Methyl tetrahydrofolate ($5CH_3$—THF) is generally regarded as the principal initial metabolite produced after folate or tetrahydrofolate administration, and in this paper is used as an indicator of the extent of metabolism, and thus entry into the folate pool, of the compounds administered. The 'folate pool' is regarded as the body folate which is readily available for use by the body and which may be presumed to contain different folates in dynamic equilibrium.

EXPERIMENTAL

Materials and methods

Chemicals. [2-^{14}C]Pteroylmonoglutamic acid ([2-^{14}C]PGA; 55·3 mCi/mmol), $5^{14}CH_3$—THF (63 mCi/mmol) and [1-^{14}C]hexadecane (1·10 μCi/g) were purchased

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from the Radiochemical Centre, Amersham, Berks.; 2-mercaptoethanol and pteroylmonoglutamic acid were purchased from Koch–Light Laboratories, Colnbrook, Bucks.; 5-formyltetrahydrofolic acid (5CHO—THF) was a gift from Lederle Laboratories Inc., Pearl River, New York, USA; biopterin and tetrahydrobiopterin were gifts from Roche Products, Welwyn Garden City, Herts.

Labelled folates were prepared from [2-^{14}C]PGA by the method of Beavon & Blair (1971), except 10-formyl[2-^{14}C]tetrahydrofolic acid (10CHO—[2-^{14}C]THF) which was prepared as follows. Portions of a solution of 5,10-methylidyne[2-^{14}C]tetrahydrofolic acid (5,10CH=[2-^{14}C]THF) equivalent to 1 μCi were freeze-dried in small vials. To each vial was added 0·1 M-Tris buffer (pH 8) containing 0·1 M-2-mercaptoethanol, and the mixture was kept in the dark at room temperature for 3 h (Rowe, 1971). It was then used immediately. The product could not be chromatographed without decomposition, but ultraviolet spectroscopy at pH 7 gave a single peak with a maximum extinction at 260 nm (literature value 258 nm; Blakley, 1969). Acidification of the sample gave a peak at 355 nm due to 5,10CH=THF; calculations from maximum extinction values showed that the original 10CHO—[2-^{14}C]THF was about 85% pure.

10-Formylpteroylmonoglutamic acid (10CHO—PGA) was prepared by the method of Blakley (1969), 5,10CH=THF by the method of Roth, Hultquist, Fahrenbach, Cosulich, Broquist, Brockman, Smith, Parker, Stokstad & Jukes (1952), 7,8-dihydrofolic acid (7,8DHF) by the method of Futterman (1963), and 5CH$_3$—THF by the method of Blair & Saunders (1970).

Animals. The animals used were adult male Wistar rats weighing 200–250 g (Scientific Products Farms, Ash, Canterbury) which had previously been given diet 41B (Heygates Ltd, Northampton) *ad lib.*

Oral administration. The radioactive materials were given in solution (0·5 ml) by stomach intubation. 5CHO—[2-^{14}C]THF, 10CHO—[2-^{14}C]PGA and [2-^{14}C]PGA were given as the sodium or potassium salts in water, 5^{14}CH$_3$—THF was given as the barium salt in water, 5,10CH=[2-^{14}C]THF was given as the hydrochloride in 0·1 mM-hydrochloric acid (pH 4), and 10CHO—[2-^{14}C]THF was given in 0·1 M-Tris buffer (pH 8) containing 0·01 M-2-mercaptoethanol.

Intravenous administration. The dose was administered by rapid injection of an aqueous solution (0·25 ml) into the femoral vein of rats anaesthetized with a nitrous oxide–oxygen–fluothane mixture, and the wound was closed with a suture clip. The animals were immediately transferred to metabolism cages (see below), where they regained consciousness within 3 min and behaved normally after 10 min.

Housing. After treatment animals were housed individually in metabolism cages ('Metabowl'; Jencons Ltd, Hemel Hempstead, Herts.). In experiments with 5^{14}CH$_3$—THF, exhaled CO_2 was collected for radioassay by circulating CO_2-free air through the cages and washing the exit gas with 1 M-sodium hydroxide solution. Food and tap-water were given *ad lib.* The food was Heygates' diet 41B, made into a stiff dough with whole egg, formed into rods about 15 mm in diameter and baked until hard. The use of food pellets was avoided as most of them were dropped through the cage floor, and the faeces became contaminated or the urine–faeces separator was blocked.

In each experiment in which different doses of the compound were compared eight rats were used, four/dose; in other experiments, eight rats were used for each compound. The urine and faeces were collected and extracted every 24 h; the rats were killed 72 or 144 h after administration of the folate, and the livers and kidneys were removed and dried immediately. They were extracted the following day.

Measurement of radioactivity. Counting was done using a scintillation spectrometer (Model NE8305; Nuclear Enterprises, Edinburgh) operating at 0°. Standardization was internal, using [1-^{14}C]hexadecane.

Urine samples. The scintillation fluid used was NE220 (Nuclear Enterprises, Edinburgh). Urine from the collection flask (15–20 ml/24 h) was made up to a standard volume with distilled water, and a portion (0·2 ml) was added, with vigorous agitation, to 10 ml scintillation fluid.

Faecal samples. After collection, faeces were freeze-dried and then powdered thoroughly. The combustion apparatus was a modification of that of Kalberer & Rutschmann (1961). The side-arm contained 25 ml absorbing solution consisting of monoethanolamine (140 ml) made up to 1 l with methanol (analytical grade). The monoethanolamine was redistilled before use (b.p. 170°). Samples were combusted in duplicate using 2 l oxygen.

The amount of radioactivity was determined by adding 5·0 ml of the absorbing solution to 5·0 ml scintillation fluid consisting of 2,4-diphenyloxazole (4 g) and 1,4-bis-(5-phenyloxazol-2-yl)-benzene (100 mg) dissolved in 1 l toluene (analytical grade; Kalberer & Rutschmann, 1961). If the scintillation fluid–absorber mixture was not homogeneous, methanol was added dropwise until it was. Because quenching by methanol was heavy, counting efficiencies were low (35–40%).

Liver and kidney. Livers and kidneys were freeze-dried; the kidneys were first freed of adherent fat and capsule. The dry tissues were treated in the same way as the faeces; samples were analysed in duplicate.

Exhaled air. Air was circulated through the cages at 400 ml/min, and the exit gas washed in an absorbing bottle containing 1 M-sodium hydroxide (100 ml), which was renewed after 6 h and again after 24 h. The amount of radioactivity in a 1 ml sample in 10 ml scintillation fluid (NE240; Nuclear Enterprises) was measured.

Other examples. The amount of radioactivity in eluates from thin-layer chromatography (TLC) plates and chromatography columns was measured, using NE220 scintillation fluid.

Isolation and purification of folates

Urine samples. Urine collection flasks contained ascorbic acid (0·5 g), concentrated hydrochloric acid (1 ml) and toluene (5 ml), and were wrapped in aluminium foil to exclude light. The flasks were changed every 24 h. After making up to a known volume, the toluene was removed and the samples pooled and freeze-dried. The toluene layer from the original samples was occasionally checked for radioactivity, but radioactivity was not detected.

Desalting of urine samples by Florisil chromatography

Florisil (60–100 mesh; Koch–Light Laboratories Ltd, Colnbrook, Bucks.) was washed by decantation twice with 0·1 M-hydrochloric acid, five times with water, once with 3 M-ammonium hydroxide, then with water to neutrality, and twice more with 0·1 M-hydrochloric acid. The wash-liquid was about ten times the volume of the Florisil; all particles which did not settle from suspension (400 ml) within 15 s were removed by decantation. The washed Florisil was packed into a column of 15 mm diameter as a slurry in 0·1 M-hydrochloric acid.

The dark-brown viscous residue from freeze-dried acidic urines was taken up in 0·1 M-hydrochloric acid (20 ml) and centrifuged. The clear red supernatant fraction was applied to a prepared Florisil column (8 g for up to four rats, 15 g for five–eight rats); the first fraction, which corresponded to the application volume and which should be colourless, was discarded. The column was eluted successively with 0·1 M-hydrochloric acid (containing 10 g ascorbic acid/l; 30 ml/8 g column), water (containing 10 ml 2-mercaptoethanol/l; 30 ml/8 g column), and 3 M-ammonium hydroxide (containing 10 ml 2-mercaptoethanol/l), until the eluate was colourless. Each eluate was made up to a known volume with water and the amount of radioactivity in a portion of eluate was measured. About 18% of the total ^{14}C appeared in the acid fraction, 2% in the neutral fraction, and 80% in the ammoniacal fraction. As the result of the pH changes, the proportions of formyltetrahydrofolates in the three fractions differed, but the changing pH did not affect the over-all proportion of folates that appeared as $5CH_3$—[2-^{14}C]THF in the three fractions (Blair & Dransfield, 1971). The ammoniacal eluate was freeze-dried.

Extraction of faeces. Powdered, dried faeces were extracted in a liquidizer (Moulinex Ltd, Sydenham, London SE 26) with 3 vol. sodium ascorbate solution (10 g/l, pH 6·0). The suspension was centrifuged, and the supernatant fraction acidified to pH 1 with concentrated hydrochloric acid, and applied to a prepared Florisil column (20 g). The elution procedure was the same as that previously described and the ammoniacal eluate was freeze-dried.

Extraction of liver. Powdered, dried liver was homogenized with 3 vol. sodium ascorbate solution (10 g/l, pH 6·0) in an all-glass homogenizer. The homogenate was quickly transferred to a stoppered tube, and heated at 95° for 10 min. The mixture was cooled in ice-cold water, stirred, and centrifuged. The clear supernatant fraction was acidified to pH 1 with concentrated hydrochloric acid, centrifuged if necessary, and applied to a prepared Florisil column (15 g). It was eluted by the procedure described previously and the ammoniacal eluate was freeze-dried.

TLC and radioautography. TLC was done using cellulose (MN300 (Macherey-Nagel; supplied by Camlab, Cambridge) for fluorescent compounds, and MN300UV for ultraviolet-absorbing compounds). Solvent systems used were: (1) *n*-butanol–acetic acid–water (4:1:5, by vol.); (2) *n*-propanol–ammonium hydroxide solution (sp.gr. 0·880)–water (200:1:99, by vol); (3) 0·1 M-phosphate buffer, pH 7·0, (4) 0·46 M-ammonium chloride solution. All solvent systems contained 10 ml 2-mercaptoethanol/l. After radioautography, the appropriate area of cellulose was transferred

Table 1. *Amounts of metabolites in urine, liver and kidneys and tissue radioactivity at intervals after the oral administration of 3 μg 2-^{14}C-labelled folates/kg body-weight to rats*

(Mean values and standard deviations; no. of animals in parentheses)

	Compound administered					
	10CHO—[2-^{14}C]PGA		5CHO—[2-^{14}C]THF		5,10CH=[2-^{14}C]THF	
Urine (% dose)	Mean	SD	Mean	SD	Mean	SD
Day 1	15·08	5·63 (4)	32·52	9·64 (8)	9·12	0·78 (8)
Day 2	5·99	1·14 (4)	5·89	3·15 (8)	8·58	2·97 (8)
Day 3	0·12	0·19 (4)	4·18	1·41 (8)	4·82	1·72 (8)
Liver						
3 d (nCi/g)		—	4·96	1·36 (8)	1·84	0·60 (8)
(% dose)		—	13·92	5·42 (8)	8·30	4·09 (8)
6 d (nCi/g)	24·82	4·38 (3)		—		—
(% dose)	61·27	4·71 (3)		—		—
Kidney						
3 d (nCi/g)		—	6·83	2·67 (8)	1·42	0·86 (8)
(% dose)		—	4·80	2·34 (8)	1·06	0·61 (8)
6 d (nCi/g)	13·58	3·43 (4)		—		—
(% dose)	7·76	0·78 (4)		—		—

Faecal radioactivity was not determined.

10CHO—PGA, 10-formylpteroylmonoglutamic acid; 5CHO—THF, 5-formyltetrahydrofolic acid; 5,10CH=THF, 5,10-methylidynetetrahydrofolic acid.

to a small sintered-glass funnel, eluted into a small tube or flask, and a portion used to measure the amount of radioactivity, if required; the remaining portion was freeze-dried. The residues thus obtained were taken up in two or three drops of water (containing 10 ml 2-mercaptoethanol/l), and centrifuged if necessary, in preparation for characterization using marker compounds.

RESULTS AND DISCUSSION

The levels of radioactivity obtained after doses of 3 and 30 μg ^{14}C-labelled folates/kg body-weight are shown in Tables 1 and 2. The urinary metabolites were in all instances identified by co-chromatography with authentic compounds using at least three TLC solvent systems.

10CHO—[2-^{14}C]PGA. At doses of 30 and 3 μg/kg body-weight, 5CH$_3$—[2-^{14}C]THF was absent from the urine and none of the four compounds found in purified day-1 urine (R_F in solvent system 1: 0·24, 0·38, 0·42 and 0·49; amount of radioactivity as a percentage of total urinary ^{14}C: 26·6, 37·2, 4·6 and 30·6 respectively) were folates: the compound chromatographing at R_F 0·38 may have been 2-amino-4-hydroxy-6-hydroxymethyl-[2-^{14}C]pteridine.

A similar experiment was done using 30 μg 10CHO—[2-^{14}C]PGA/kg body-weight administered to animals which had been starved for 72 h before administration of the compound. The higher ^{14}C recovery (133% of dose) was probably the result of inaccuracies in faecal analyses as it proved difficult to obtain truly homogenous

Table 2. *Amounts of metabolites in the urine, faeces, liver and kidney, and tissue radioactivity at intervals after oral administration of 30 μg labelled folate/kg body-weight to rats*

(Mean values and standard deviations; no. of animals in parentheses)

	10CHO—[2-^{14}C]PGA		10CHO—[2-^{14}C]PGA*		5CHO—[2-^{14}C]THF		5CHO—[2-^{14}C]THF†	
Urine	Mean	SD	Mean	SD	Mean	SD	Mean	SD
(% dose)							63·3	13·3 (8) (6 h)
Day 1	11·1	3·95 (17)	27·44	2·0 (5)	21·3	4·0 (8)	8·5	3·7 (8) (24 h)
2	1·8	0·37 (17)	21·88	7·4 (5)	2·3	1·0 (8)	2·1	0·6 (8)
3	1·2	0·58 (17)		—	1·1	0·6 (8)	1·5	0·5 (8)
4		—		—		—	0·3	0·1 (4)
5		—		—		—	0·5	0·1 (4)
6		—		—		—	0·3	0·1 (4)
Faeces (% dose)								
Day 1	36·8	9·4 (17)	45·5	10·8 (5)	32·6	12·5 (8)	15·3	5·4 (8)
2	5·8	2·0 (17)	8·3	4·5 (4)	4·4	1·9 (8)	5·3	1·8 (8)
3	2·8	0·6 (17)		—	2·0	2·0 (8)	2·0	0·8 (8)
Total	45·4	9·7 (17)	53·8	14·2 (4)	39·0	14·5 (8)	22·6	4·5 (8)
Liver								
3 d (nCi/g)	78·5	29·8 (17)	85·4	12·9 (4)	23·9	11·0 (8)	15·9	1·3 (4)
(% dose)	22·5	5·3 (17)	26·1	5·0 (4)	8·3	3·5 (8)	6·1	0·8 (4)
6 d (nCi/g)		—		—		—	17·7	2·8 (4)
(% dose)		—		—		—	6·1	0·7 (4)
Kidney								
3 d (nCi/g)	50·7	23·2 (17)	75·7	3·3 (4)	15·7	3·3 (8)	13·3	1·5 (4)
(% dose)	3·3	1·1 (17)	4·1	0·2 (4)	1·3	0·3 (8)	1·0	0·1 (4)
6 d (nCi/g)		—		—		—	10·2	1·6 (4)
(% dose)		—		—		—	0·5	0·1 (4)
Total ^{14}C accounted for (% dose)	85·3		133·3		73·3		106·2	

powders from the dried faeces and standard deviations for mean faecal values were, therefore, quite high. Urine values were considered to be accurate. The pattern of urinary metabolites was the same as that in the fed rats, but urinary excretion of ^{14}C was higher on day 1 in the starved animals than in the fed animals, and the difference was even greater on day 2 (Table 2).

Faeces and livers were extracted in the experiments with fed animals given 30 μg 10CHO—[2-^{14}C]PGA/kg body-weight. Faecal extracts contained one labelled compound, 10CHO—[2-^{14}C]PGA. The results obtained for liver extracts were different at different intervals after administration of the folate. After 3 d, two compounds were present; 10CHO—[2-^{14}C]PGA formed 80% of the total hepatic ^{14}C and the other compound was not identified, but was not $5CH_3$—[2-^{14}C]THF. After 6 d, [2-^{14}C]PGA and $5CH_3$—[2-^{14}C]THF were found in approximately equal amounts, and the total amounts of radioactivity found after 3 and 6 d were similar. Retention of radioactivity in the liver and kidney was higher after administration of 10CHO—[2-^{14}C]PGA than after [2-^{14}C]PGA (Blair & Dransfield, 1971), 5CHO—[2-^{14}C]THF or 5,10CH=[2-^{14}C]THF (Tables 1 and 2).

When two rats were given 150 μg 10CHO—[2-^{14}C]PGA/kg body-weight (results not given in the tables), $5CH_3$—[2-^{14}C]THF was not found in urine, but 10CHO—

Table 2 (*cont.*)

Urine (% dose)	5,10CH=[2-^{14}C]THF		10CHO—[2-^{14}C]THF		[5^{14}CH$_3$]-THF		5CH$_3$—[2-^{14}C]THF‡	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Day 1	10·2	3·0 (15)	6·9	1·4 (8)	15·2	6·4 (4)	13·0	3·1 (8)
2	2·1	0·8 (15)	2·3	0·4 (8)	2·8	0·6 (4)	1·8	0·5 (3)
3	1·2	0·7 (15)	1·2	0·5 (8)	1·3	1·0 (4)	1·9	1·6 (3)
4		—	1·1	0·2 (4)		—		—
5		—	1·0	0·4 (4)		—	2·2	0·1 (3)
6		—				—		—
Faeces (% dose)								
Day 1	49·8	6·9 (15)	54·9	18·6 (8)	30·7	6·4 (4)		—
2	2·5	1·5 (15)	3·9	2·7 (8)	5·2	2·5 (4)		—
3		—	3·9	2·1 (8)	1·8	0·6 (4)		—
Total	52·3	6·8 (15)	62·7	17·5 (8)	37·7	4·8 (4)	53·1	12·6 (5)
Liver								
3 d (nCi/g)	24·7	7·3 (15)	24·4	6·9 (4)	17·3	5·3 (4)		—
(% dose)	11·2	2·2 (15)	11·6	3·5 (4)	9·4	1·1 (4)	9·9	3·8 (8)
6 d (nCi/g)		—	30·9	15·7 (4)		—		—
(% dose)		—	10·9	6·1 (4)		—		—
Kidney								
3 d (nCi/g)	9·8	4·5 (15)	43·1	14·3 (4)	79·6	20·3 (4)		—
(% dose)	0·8	0·3 (15)	3·0	1·3 (4)	6·3	1·6 (4)	2·1	0·5 (8)
6 d (nCi/g)		—	26·5	5·3 (4)		—		—
(% dose)		—	1·8	0·5 (4)		—		—
Total ^{14}C accounted for (% dose)	77·8		89·8		74·6§		84·0	

10CHO—PGA, 10-formylpteroylmonoglutamic acid; 5CHO—THF, 5-formyltetrahydrofolic acid; 5,10CH=THF, 5,10-methylidynetetrahydrofolic acid; 10CHO—THF, 10-formyltetrahydrofolic acid; 5CH$_3$—THF, 5-methyltetrahydrofolic acid.

* Rats starved for 72 h before administration of the compound.

† Intravenously administered.

‡ L(−)-isomer, 18·8 μg/kg body-weight.

§ Includes exhaled $^{14}CO_2$: (Mean, SD) 1·5 ± 0·7 % on day 1, 0·4 ± 0·1 % on day 2 (four animals).

[2-^{14}C]PGA was present, together with two unidentified compounds. Urinary excretion on day 1 was 33 % of the dose.

The absence of 5CH$_3$—[2-^{14}C]THF in the urine and liver after 3 d suggested that 10CHO—PGA entered the folate pool slowly, and that this was unaffected by prior starvation for 72 h or a fiftyfold increase in the dose. Compounds which were metabolized rapidly would be expected to produce significant quantities of 5CH$_3$—THF in urine in the first 24 h and this compound would be expected to be found in liver also: but it appears that the conversion is very slow, and it is possible that 10CHO—PGA is not very important nutritionally.

10CHO—[2-^{14}C]THF. Oral administration of 30 μg 10CHO—[2-^{14}C]THF/kg body-weight produced two compounds in day-1 urine (R_F in solvent system 1: 0·38 and 0·55; amount of radioactivity as a percentage of urinary ^{14}C: 80 and 20 respectively). One compound (R_F 0·38) was identified as 5CH$_3$—[2-^{14}C]THF, the other compound was probably 10CHO—[2-^{14}C]THF. Radioactive pteridines, dihydrofolates (DHF) or tetrahydrofolates (THF) were not detected.

Extracts of dried liver from rats killed 3 d after administration of the folate contained 5CH_3—[2-^{14}C]THF as the main folate (90% of total hepatic radioactivity), and one other unidentified compound: after 6 d, only 5CH_3—[2-^{14}C]THF was found.

10CHO—[2-^{14}C]THF entered the folate pool rapidly, and produced a greater proportion of 5CH_3—[2-^{14}C]THF than any of the other folates studied. The levels of radioactivity in the liver after 3 and 6 d were similar; this may indicate some storage.

As the results differed from those for 5,10CHO=[2-^{14}C]THF, cyclization in the stomach (Beavon & Blair, 1972) was presumably negligible, and administration in weakly alkaline buffer may have altered gastric pH enough to prevent cyclization.

5CHO—[2-^{14}C]THF. After oral administration of 30 μg 5CHO—[2-^{14}C]THF/kg body-weight, five compounds were found in day-1 urine (R_F in solvent system 1: 0·16, 0·27, 0·36, 0·41 and 0·04; amount of radioactivity as a percentage of total urinary ^{14}C: 64, 10, 9·5, 9·5 and 6 respectively), which were identified as 5,10CH=[2-^{14}C]THF, unidentified, 5CHO—[2-^{14}C]THF, 10CHO—[2-^{14}C]PGA and 5CH_3—[2-^{14}C]THF respectively. PGA, DHF, THF and pteridines were not detected.

Three compounds were identified in the faeces: 5CHO—[2-^{14}C]THF, 10CHO—[2-^{14}C]PGA and 5CH_3—[2-^{14}C]THF, and these compounds formed 85, 10 and 5% of the total faecal ^{14}C respectively. Radioactive folates identified in the liver after 3 d were: 5,10CH=[2-^{14}C]THF, 10CHO—[2-^{14}C]PGA, 5CHO—[2-^{14}C]THF and 5CH_3—[2-^{14}C]THF, which formed 40, 15, 30 and 15% of the total hepatic radioactivity respectively.

After oral administration of 3 μg 5CHO—[2-^{14}C]THF/kg body-weight, day-1 urine contained four compounds: 5,10CH=[2-^{14}C]THF, 5CHO—[2-^{14}C]THF, 10CHO—[2-^{14}C]PGA, and 5CH_3—[2-^{14}C]THF, which formed 69·2, 12·4, 16·2 and 2% of the total urinary ^{14}C respectively. Faecal and hepatic folates were not analysed.

Urine collected in the first 6 h after intravenous administration of 30 μg 5CHO—[2-^{14}C]THF/kg body-weight, contained two compounds, 5CHO—[2-^{14}C]THF and 5,10CH=[2-^{14}C]THF, forming 70 and 30% of the total urinary ^{14}C respectively; 5CH_3—[2-^{14}C]THF was not found. After a further 18 h the urinary folates identified were 5CHO—[2-^{14}C]THF and 5CH_3—[2-^{14}C]THF, forming 90 and 10% of the total urinary ^{14}C respectively. Extracts of dried liver from rats killed after 3 d, contained two compounds: 5CHO—[2-^{14}C]THF and 5CH_3—[2-^{14}C]THF, forming 80 and 20% of the total hepatic ^{14}C respectively; after 6 d the same two compounds were present in approximately equal amounts. Faecal folates were not analysed.

The small extent of the conversion of 5CHO—[2-^{14}C]THF into 5CH_3—[2-^{14}C]THF suggested a slow entry into the folate pool; intravenous administration of 5CHO—[2-^{14}C]THF produced very little urinary 5CH_3—[2-^{14}C]THF during the first 24 h, which also supported the idea of a slow entry.

Under these conditions 5CHO—[2-^{14}C]THF did not appear to undergo extensive cyclization in the stomach, as the pattern of urinary metabolites differed from that found after administration of 5,10CH—[2-^{14}C]THF at the same dose.

5,10CH=[2-^{14}C]THF. At doses of 3 and 30 μg 5,10CH=[2-^{14}C]THF/kg body-weight, four compounds were excreted in day-1 urine, although the relative amounts of each compound differed widely. After administration of 30 μg/kg, 5,10CH=

[2-^{14}C]THF, 10CHO—[2-^{14}C]PGA and 5CH_3—[2-^{14}C]THF and an unidentified compound (forming 39, 15·5, 20 and 25·5% of the total urinary ^{14}C respectively) were excreted: corresponding amounts of these compounds in day-1 urine after a dose of 3 μg/kg were 7·9, 78·5, 5·4 and 8·2% of the total urinary ^{14}C respectively. The unidentified compound was qualitatively the same in both instances it was neither oxygen-labile nor sensitive to pH changes.

Faecal extracts (after administration of 30 μg/kg) contained four compounds: 5,10CH=[2-^{14}C]THF, 5CHO—[2-^{14}C]THF, 10CHO—[2-^{14}C]PGA and probably 5CH_3—[2-^{14}C]THF, which formed 10, 10, 70 and 10% of the total faecal ^{14}C respectively. The liver extract from rats given 30 μg/kg contained two compounds after 3 d, which were not thoroughly characterized: one was probably 5,10CH=[2-^{14}C]THF or 5CHO—[2-^{14}C]THF and the other was probably 5CH_3—[2-^{14}C]THF, these formed 90 and 10% of the total hepatic ^{14}C respectively.

A greater proportion of 5,10CH=[2-^{14}C]THF than of 5CHO—[2-^{14}C]THF was apparently able to enter the folate pool. This may have been due to the conversion of the former compound to 10CHO—[2-^{14}C]THF in the jejunum, interconversions between the various formylfolates being indicated by the complex faecal folate content.

The large proportion of 10CHO—[2-^{14}C]PGA found after a dose of 3 μg 5,10CH=[2-^{14}C]THF/kg body-weight may be explained as hydrolysis of 5,10CH=THF in the jejunum to give 5CHO—THF (as shown by Beavon & Blair (1972) in the presence of solid support, such as cellulosic plant materials) and 10CHO—THF. If 5CHO—THF is the main component, it will be absorbed, will remain largely unmetabolized and will produce 5,10CH=THF in the collection flask. This compound will then produce 10CHO—THF during subsequent handling in alkali, and then 10CHO—PGA by oxidation. The proportion of 10CHO—PGA increases with lower doses because the hydrolysis of 5,10CH=THF becomes more complete as the concentration falls (Beavon & Blair, 1972). The 5CH_3—[2-^{14}C]THF found in the urine was derived largely from the metabolism of 10CHO—[2-^{14}C]THF produced in the jejunum, plus a small amount from the metabolism of 5CHO—[2-^{14}C]THF. Oxidation of 10CHO—[2-^{14}C]THF in the jejunum cannot explain the results, as 10CHO—[2-^{14}C]PGA was not found in urine after administration of low doses of 10CHO—[2-^{14}C]PGA; also, the jejunum is fairly anaerobic (Maggi, Brue, Brousolle, Bensimon & Pérès, 1970).

5$^{14}CH_3$—*THF*. After oral administration of 30 μg 5$^{14}CH_3$—THF/kg body-weight, three unidentified radioactive compounds were found in day-1 urine, which were not identified as any of the major folates, serine or methionine; 5$^{14}CH_3$—THF was absent. A similar situation was found in liver after 3 d. Excretion of radioactivity as $^{14}CO_2$ (expressed as a percentage of administered radioactivity excreted) was small (1·52 ± 0·67 on day 1, 0·35 ± 0·10 on day 2).

5CH_3—[2-^{14}C]THF (isolated from urine of rats given [2-^{14}C]PGA, and therefore presumed to be the L(−)-isomer) gave principally 5CH_3—[2-^{14}C]THF, though other unidentified compounds were present (Dransfield, 1972).

The 5-N methyl group was, therefore, rapidly lost (or was in rapid equilibrium with unlabelled methyl) and was rapidly diluted over the animal's body.

Interpretation of the relative proportions of the various formyltetrahydrofolates in terms of the metabolism of the animal was hampered by the complexity of the changes which these compounds underwent when the pH was changed. Urine was collected, throughout the experiment, in concentrated hydrochloric acid, to facilitate comparison with earlier work (Blair & Dransfield, 1971; Dransfield, 1972); the subsequent manipulations would inevitably affect the distribution of formyltetrahydrofolates. It was not possible to devise conditions which would leave the formylfolates completely unchanged; but this problem did not apply to the main folate metabolite, $5CH_3$—THF, which in the presence of suitable antioxidants was unaffected by pH changes. The over-all pattern of folate metabolism was, therefore, not changed by the extraction and purification procedures.

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