**Short Communication**

**METABOLISM OF 5-METHYLTETRAHYDROFOLATE BY RATS BEARING THE WALKER 256 CARCINOSARCOMA**

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The therapeutic success of anti-folates has prompted research into the metabolism of folates in malignant tumours. However, most studies have used folic acid as a tracer, though this compound is not naturally occurring, and is only assimilated by an unusual reaction of DHFR (Gready, 1979). We report the metabolism of 5-methyltetrahydrofolate (5MeTHF) a naturally occurring reduced folate, in rats bearing the Walker 256 carcinosarcoma. This tumour is of special interest as, in cell culture, it has been claimed to display methionine auxotrophy, while normal cells are able to survive substitution of methionine by homocysteine (Halpern et al., 1974). It has been suggested that other cell lines with similar properties are deficient in 5MeTHF, requiring methionine synthetase (Ashe et al., 1974).

This effect, however, is not found in all tumour cell lines (Magnum et al., 1969; Tisdale, 1979) showing that methionine auxotrophy is a poor indicator of malignancy (Kreis & Goodenow, 1978). With Walker 256, Hoffman & Erbe (1976) showed that although the line has a methionine requirement, both FA and 5MeTHF are equally effective in stimulating the folate-depleted cell to divide. The cells are also able to take up 5MeTHF normally and use it for methionine synthesis at a rate similar to or above normal cells (Hoffman & Erbe, 1976). Thus, the methionine auxotrophy of Walker 256 cells does not arise simply from methionine synthetase deficiency. However, it might result from some other difference in folate metabolism between the tumour and normal cells.

In these experiments rats bearing the Walker 256 tumour were dosed with 5[14C]MeTHF to observe one-carbon-group metabolism, and mixed label [2-14C] plus [3',5',7,9-3H] 5-MeTHF to observe the fate of the tetrahydrofolate moiety.

5-MeTHF (Mg salt) was obtained from Eprova Research Laboratories (Basle, Switzerland); 5[14C]MeTHF (Ba salt, 88 μCi/μmol), [2-14C] folic acid (55 μCi/μmol) and [3',5',7,9-3H] folic acid (500 μCi/μmol) from the Radiochemical Centre (Amersham, Bucks). Mixed label [2-14C] plus [3',5',7,9-3H] 5MeTHF, the natural diastereoisomer, was prepared by orally dosing rats with a mixture of similarly labelled folic acid and extracting the 5MeTHF excreted (see below) in the presence of 0.2% (w/v) sodium ascorbate. All other substances used were of "Analar" grade or equivalent.

Male Wistar rats (150–200 g) were obtained from the Chester Beatty Institute, London. The tumour-bearing rats had 10^6 cells of Walker 256 carcinosarcoma implanted s.c. on the right flank. The animals were used in experiments 7 days after implantation, when tumour mass represented up to 5% body mass.

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TABLE I.—Excreted and retained $^{14}$C following an oral dose of $5[^{14}$C]$\text{MeTHF}$ (80 µg/kg) in normal rats and those bearing the Walker 256 carcinosarcoma

| Day 1 Urine (mean ± s.e.) | % dose $^{14}$C |
|---------------------------|------------------|
| Normal rats (4)           | Walker 256-implemented (6) |
| 2                         | 50.9 ± 4.0        |
| 3                         | 1.9 ± 0.2         |
| 3                         | 1.2 ± 0.2         |
| Day 1 CO$_2$              | 2.0              |
| 2                         | 0.4              |
| 3                         | 0.4              |
| Day 1 Faeces (mean ± s.e.)| 2.3 ± 1.0        |
| 2                         | 0.1 ± 0.03       |
| 3                         | 0.02 ± 0.01      |
| Day 3 Tissues (mean of 2 values) |              |
| Liver                     | 0.1              |
| Kidney                    | 0.1              |
| Spleen                    | 0.01             |
| Muscle                    | 13.2             |
| Tumour                    | 0.6              |
| Total                     | 72.7             |
| * Calculated assuming muscle=40% body weight. |

Sephadex G15 gel filtration, DEAE-cellulose chromatography (Barford, et al., 1977) and paper chromatography (Connor et al., 1979) were performed as described previously.

The distribution and excretion of $^{14}$C activity following an oral dose of $5[^{14}$C]$\text{MeTHF}$ (80 µg/kg) are given in Table I. The tumour-bearing animals showed greater production of $^{14}$CO$_2$, greater $^{14}$C retention in the liver and less retention in muscle than normal animals. Chromatographic analysis of the urine samples showed the presence of 5MeTHF and the non-folate fraction (NFF; methionine + creatine) as described in Kennelly et al.

TABLE II.—Metabolites excreted in the urine of normal and W256 tumour-bearing rats dosed with $5[^{14}$C]$\text{MeTHF}$ (80 µg/kg)

| (% dose) | Normal | W 256 |
|----------|--------|--------|
| Day 1    |        |        |
| NFF      | 3.2    | 5.2    |
| 5MeTHF   | 47.8   | 34.3   |
| Day 2    |        |        |
| NFF      | 0.52   | 2.3    |
| 5MeTHF   | 1.1    | 1.5    |
| NFF—non-folate fraction.
(1979). No qualitative difference was observed between the 2 groups of animals, but the tumour-bearing animals showed an absolutely and proportionally greater excretion of NFF than normals and less 5-MeTHF excretion (Table II).

Normal and tumour-bearing animals were dosed orally with [2-¹⁴C]+[3′,5′,7,9-³H] 5MeTHF at 8 and 6 μg/kg respectively. The distribution of radioactivity recovered is given in Table III. Notably, excretion of radioactivity in urine and faeces was less in tumour-bearing rats than normals (P<0.001 for both ¹⁴C and ³H). The excreted urinary metabolites were qualitatively similar in the 2 groups; scission products, 10CHOFA, 5-MeTHF and an unidentified dual-labelled compound (“folate X” as reported in Saleh et al., 1981) (Table IV).

Table IV.—DEAE-cellulose fractionation of first-day urines from animals dosed orally with [2-¹⁴C] + [3′,5′,7,9-³H]-5MeTHF

| % dose | Normal (5) | W 256 (6) |
|--------|------------|------------|
|        | ¹⁴C         | ³H         | ¹⁴C         | ³H         |
| Urine (mean ± s.e.) | 74±2 · 8±2.4 | 92±24±12·8 | 28±5±2·9 | 31±5±3·2 |
| Faeces (mean ± s.e.) | 27±1·2±2·0 | 22±0±1·8 | 5±3±0·5 | 4±4±0·6 |

Table III.—Excreted (0–3) days and retained radioactivity from rats orally dosed with [2-¹⁴C]+[3′,5′,7,9-³H] 5MeTHF (normals 8 μg/kg; W256-implanted 6 μg/kg)

| % dose | Normals (5) | W 256 (6) |
|--------|------------|------------|
|        | ¹⁴C         | ³H         | ¹⁴C         | ³H         |
| Kidney | 0·8         | 0·3        | 1·1         | 1·3        |
| Liver  | 6·8         | ND         | 7·2         | 5·7        |
| Tumour | —           | —          | 5·1         | 5·1        |

G15 into 2 fractions, identified by paper chromatography (Connor et al., 1979) as p-acetamidobenzoylglutamate and p-acetamidobenzoate. Less of the dose was catabolized to scission products by the tumour-bearing animals, despite the retention of more radioactivity in the tissues.

The presence of an implanted Walker 256 carcinosarcoma imposes changes on the whole body metabolism of 5MeTHF in the rat, both of the methyl group and the tetrahydrofolate moiety. There is more rapid demethylation and a diversion of methyl groups from muscle. This is accompanied by retention of radioactivity in the tumour, increased retention in the liver and increased production of CO₂ (Table I). As this particular tumour displays an in vitro methionine requirement, the diversion of methyl groups from muscle may be due to the tumour’s demand for methionine, and its metabolism to CO₂.

Following a dose of mixed label [2-¹⁴C] + [3′,5′,7,9-³H]-5MeTHF, markedly less radioactivity was excreted in the urine and faeces of the tumour-bearing animals, indicating that the presence of the tumour increased the demand for folate. Also folate scission was reduced, both absolutely and as a proportion of the body burden of folate polyglutamates in the tumour-bearing animals. Similar observations have been made using radioactive folic acid as the tracer (Saleh et al., 1981); these data confirm the phenomenon with a naturally occurring reduced folate. The fact that similar results are obtained
with folic acid and 5MeTHF also suggests that the methionine auxotrophy of the Walker 256 carcinosarcoma is not due to inability to demethylate 5MeTHF.

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