The importance of S-adenosylhomocysteine as an inhibitor of biological transmethylations has been suggested previously,[10,11] and the recent findings involving biogenic amine[2] and transfer RNA[12] methylation, together with the present work, tend to support this hypothesis. The hydrolysis of SAM to adenosine and homocysteine serves to regulate the activity of methylases in a brain homogenate.[2] More stable analogs of SAH would be useful as inhibitors of methylation reactions, and the first series of such analogs has recently been synthesized in this laboratory.[13]

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Inhibition of purine biosynthesis *de novo* and of Ehrlich ascites tumor cell growth by 6-methylmercaptopurine ribonucleoside*

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6-METHYLMERCAPTOPURINE ribonucleoside (6 MeMPR) is metabolized to its 5'-phosphate,[1–4] and inhibits purine biosynthesis *de novo* in Ehrlich ascites tumor cells ,[5,6] H. Ep. No. 2 cells,[1] leukemia L1210 cells,[1] adenocarcinoma 755 cells,[7] and in normal human fibroblasts[8–10] and several mouse tissues.[11] The first enzyme in this pathway, PP-ribose-P amidotransferase (EC 2.4.1.14), is strongly inhibited by 6 MeMPR-phosphate.[12,13] The idea that such inhibition is the basis of the growth-inhibitory activity of 6 MeMPR is very attractive, but as Mandel[14] has pointed out, such a conclusion cannot be fully accepted without being subjected to a number of tests. This is particularly relevant because recent studies have shown that 6 MeMPR does have other biochemical effects, including inhibition of the conversion of purine bases to nucleotides in cultured adenocarcinoma 755 cells.[15]

The most conclusive evidence presented to date to support inhibition of purine biosynthesis *de novo* as the mechanism of action of 6 MeMPR was the demonstration by Bennett and Adamson[7] that hypoxanthine and aminoimidazole carboxamide could completely reverse the growth-inhibitory effects of 3·5 μM 6 MeMPR, using cultured adenocarcinoma 755 cells. Similar observations have been made using cultured lymphoma L5178Y cells.†

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† A. R. P. Paterson, personal communication.

Ehrlich ascites tumor cells can be cultured in Fischer's medium and 20 per cent horse serum, under which conditions they increase in number 5- to 6-fold in 72 hr (to between 24 and 32 $\times$ 10^4 cells/ml). Cell growth was completely inhibited by 1 μM 6 MeMPR, but addition of 30 μM hypoxanthine effected 90–98 per cent reversal of the drug effect. Adenine, 10 μM, produced 50–60 per cent reversal; higher concentrations of adenine were themselves inhibitory. These results therefore confirm those of Bennett and Adamson[7] in a different line of cells.

The principal concern of this study was to investigate the relationship between growth inhibition, inhibition of purine biosynthesis *de novo*, and 6 MeMPR-phosphate concentrations in Ehrlich ascites tumor cells. Ha/ICR mice were implanted with 1–2 $\times$ 10^6 tumor cells, and treated daily four times with various doses of 6 MeMPR, starting 1 day after implantation. Growth inhibition was calculated by measuring packed tumor cell volumes 3·5 hr after the fourth treatment. To calculate inhibition of purine biosynthesis *de novo*, the accumulation of [^{14}C]phosphoribosyl-formylglycineamide (FGAR) was measured in azaserine-treated mice injected with [^{14}C]glycine.[16,17] 6 MeMPR-phosphate concentrations were determined using [^{3}H]6 MeMPR; after 3·5 hr, cells were extracted and the extracts added to 1 $\times$ 4 cm columns of DEAE-Sephadex A-25 acetate. 6 MeMPR was eluted with 50 ml H_2O, and the phosphate was eluted with 40 ml of 0·5 M triethylammonium acetate. At this time (3·5 hr) after drug administration, 99 per cent of the radioactivity was in 6 MeMPR-phosphate; virtually no free ribonucleoside was detected.

Figure 1(a) shows that there was a correlation between inhibition of tumor growth, inhibition of purine biosynthesis *de novo*, and the amount of 6 MeMPR-phosphate present in the cells. However these results do not indicate whether inhibition of purine biosynthesis was a cause or merely a con-

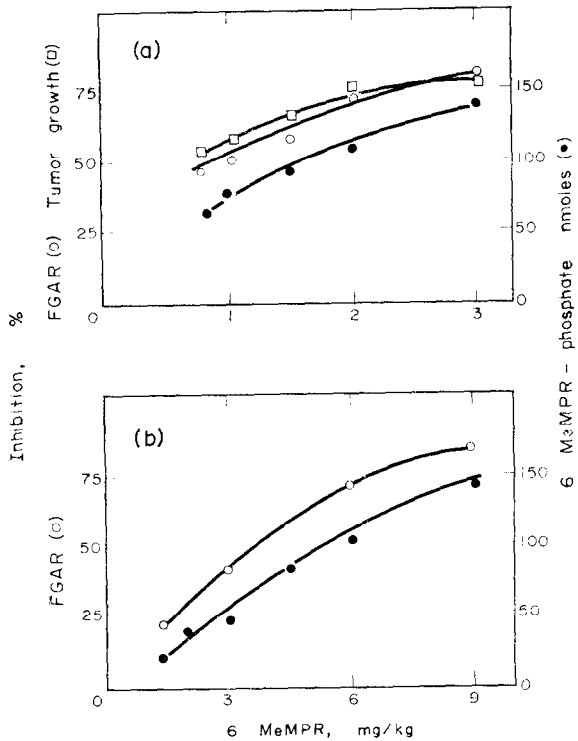

FIG. 1. Metabolism of 6 MeMPR and its effect on purine biosynthesis and tumor cell growth. (a) Mice implanted with 1–2 $\times$ 10^6 Ehrlich ascites tumor cells were treated with various doses of 6 MeMPR daily for 4 days beginning 24 hr after implantation. Two hr after the last drug treatment, 10 μg azaserine was injected i.p., and 30 min later, 2 μmoles [^{14}C]glycine (2 μc). One hr after injection of [^{14}C]glycine tumor cells were removed, packed cell volumes were determined, and [^{14}C]FGAR contents of perchloric acid extracts were measured. In separate experiments, [^{3}H]6 MeMPR (8·2 μc/μmole) was used at the same schedule and doses; 3·5 hr after the fourth daily injection, tumor cells were removed and [^{3}H]6 MeMPR-phosphate was measured. Each point represents an average of six to nine determinations in two experiments. (b) Mice bearing 4-day ascites tumor cells were treated with a single i.p. injection of various doses of 6 MeMPR or [^{3}H]6 MeMPR. [^{14}C]FGAR and [^{3}H]-6 MPR-phosphate were then determined as described above.

sequence of diminished tumor growth, and numerous difficulties arise when comparisons are attempted among mice containing wide variations in tumor cell mass. In an attempt to avoid these difficulties, inhibition of purine biosynthesis *de novo* and 6 MeMPR-phosphate concentrations were also measured in tumor-bearing mice which were treated with 6 MeMPR only once, on the fourth day after transplantation. Figure 1b shows that there was again a correlation between concentration of 6 MeMPR-phosphate and inhibition of purine biosynthesis *de novo*.

The degrees of inhibition of purine biosynthesis measured in cells after four daily treatments with 6 MeMPR were approximately the same as those after single injections of 3-fold larger amounts of 6 MeMPR. This was not unexpected, because 6 MeMPR-phosphate is known to accumulate in cells given multiple daily injections of 6 MeMPR. Figure 2 shows that, in fact, the relationship between inhibition of purine biosynthesis and concentration of 6 MeMPR-phosphate was the same regardless of treatment schedule and dose. This result suggests that the data of Fig. 1(a) indicate a causal relationship between inhibition of purine biosynthesis and inhibition of tumor growth.

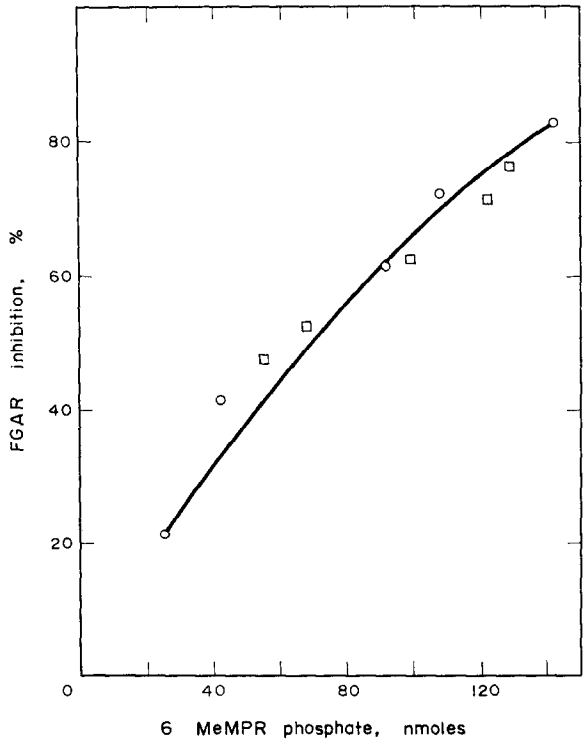

FIG. 2. 6 MeMPR-phosphate and inhibition of purine biosynthesis. Data from Fig. 1 are replotted to show the relationship between inhibition of [^{14}C]FGAR formation and concentration of [^{3}H]6 MeMPR-phosphate when mice were given four daily doses (□) or a single (○) dose of 6 MeMPR.

Although these results do not prove conclusively that 6 MeMPR inhibits tumor cell growth because it inhibits purine biosynthesis *de novo*, they certainly strengthen this view, and are in accord with other evidence on this point.[18]

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Effect of experimentally induced seizures on some amino acids and ammonia in rat brain

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IN A PREVIOUS investigation, we studied the effects of electroconvulsive shock, leptazol and sound induced seizures on glucose metabolism in the rat brain.[1] The results of this study showed that there were differences between the changes associated with seizures induced by these different stimuli. It therefore seemed worthwhile to see if any differences occurred involving those amino acids which are metabolically connected with the citric acid cycle and which are also putative transmitter substances in the brain. Brain ammonia was also studied because there is evidence that this compound is a sensitive index of cerebral excitability.[2]

Methods

Albino rats (female; 100–120 g) of the Wistar strain were used. Convulsions were induced in groups of at least six animals by leptazol (100 mg/kg i.p.), high intensity sound or electroshock using the methods described in the previous study.[1]

The rats were killed by total immersion in liquid nitrogen. All determinations were made on the acid soluble extracts of whole brain. After centrifugation (800 *g* for 15 min), aliquots of the supernatant fraction were taken for the determination of ammonia,[3] glutamine,[4] glutamate,[5] tyrosine[6] and total free amino nitrogen.[7] γ-Amino-*n*-butyric acid (γABA) was estimated in the acidified ethanol extract of crushed frozen brain by paper chromatography.[8] Glutamate decarboxylase was estimated in homogenates of unfrozen brain by the monometric method of Roberts and Frankel.[9] In most cases the assays were made on brain extracts of animals killed during the full tonic phase of the seizure. A detailed description of the seizure phases induced by these stimuli is given in the previous publication.[1]