units. Average aldolase value for normal liver, hepatoma, hepatoma-free liver, sarcoma and sarcoma-rat liver were 14,400, 12,400, 9,600, 7,700 and 9,100 units, respectively. Liver aldolase levels for tumor-bearing rats were about 35% lower than those for control rats. Aldolase values for necrotic areas of tumors were similar to serum aldolase values for respective rats.

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6-Methylmercaptopurine: Identification as Metabolite of 6-Mercaptopurine *in vivo* and Its Activity *in vitro*.*† (25088)

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6-Mercaptopurine possesses definite antitumor activity in animals(3) and is widely used clinically in leukemia(4). Previous studies demonstrated the thiol methylation of thiouracil(5) and its iodo analogue *in vivo* (6). It appeared of interest to determine whether 6-mercaptopurine can also undergo thiol methylation *in vivo* and to quantitate the extent of this reaction. 6-Mercaptopurine can inhibit incorporation of C^{14}-precursors into nucleic acid fractions of tumor cells *in vivo*(7) and *in vitro*(8). To determine whether thiol-methylation interferes with or increases this action, the inhibitory effects of 6-methylmercaptopurine (6-MeMP) and 6-mercaptopurine (6-MP) on incorporation of formate $-C^{14}$ and glycine $-C^{14}$ into nucleic acid and protein fractions of ascites tumor cells were compared.

Methods. 6-MP-S^{35}, unlabeled 6-MeMP, and 6-MeMP-S^{35} used were prepared by methods of Elion *et al.*(9,10) and their purity determined chromatographically prior to use. *Identification and quantitation of 6-MeMP in urine.* Sprague-Dawley rats weighing 250-300 g were injected subcutaneously with 48.5 mg of either unlabeled 6-MP or 6-MP-S^{35} (0.62 mc/mM) dissolved in slightly alkaline aqueous solution. The animals were placed in individual metabolism cages and urine was collected for 24 hours under toluene. Urine from animals injected with unlabeled 6-MP was lyophilized to dryness and extracted with absolute ethanol. Aliquots of alcohol extracts were then chromatographed on Whatman No. 40 paper, alongside authentic 6-MeMP, by the descending method. 6-MeMP was located by its vivid yellow fluorescence under ultraviolet light (Mineralite lamp). R_f values of authentic and suspected 6-MeMP corresponded exactly when these fractions were eluted and rechromatographed in 3 different solvent systems. The solvent systems used were n-butanol:acetic acid:water (35.5:9.6:16.0); n-butanol saturated with 2N NH_4OH; and 70% ethanol. R_f values obtained were 0.84, 0.52 and 0.81 respectively. The yellow fluorescent areas were eluted from the paper with 0.1 N HCl and their absorption spectra were compared in a Beckman U.V. spectrophotometer. Spectra of authentic and sus-

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† Preliminary reports have been presented(1,2).

pected 6-MeMP at pH 1 were identical, with the same maxima at 224 and 295 mμ. To quantitate urinary excretion of 6-MeMP, unlabeled carrier 6-MeMP was added to urine collected from animals injected with 6-MP-S^{35}. Aliquots of this urine were chromatographed on paper and the 6-MeMP was located by methods described above. To obtain the relative per cent of excreted 6-MeMP, paper chromatograms were passed under an automatically recording scanner (Actigraph) to locate the S^{35} components. S^{35} was determined by cutting out each radioactive area of the paper and counting on aluminum planchets in a gas flow counter. In 2 experiments, 0.5-1.0% of excreted S^{35} was 6-MeMP in urine collected during 24 hours after administration of 6-MP-S^{35}.

Incorporation of glycine-2-C^{14} and formate-C^{14} into nucleic acid and protein fractions of ascites tumor cells in vitro. Formate-C^{14} (2mc/mM) and glycine-2-C^{14} (1 mc/mM) were obtained from Nuclear-Chicago; 6-MP, from Burroughs Wellcome and Co.; 6-MeMP was synthesized by methods described above. Ascites tumor cells were obtained from Swiss mice inoculated intraperitoneally 6-7 days previously with Sarcoma 180 ascites tumor cells. The cells were withdrawn under light ether anesthesia, centrifuged, and resuspended in 4 volumes of clot-free ascitic fluid. To each 25 ml Erlenmeyer flask was added 1 ml of cell suspension; 1 ml of isotonic saline: 0.1 M Na_2HPO_4 buffer mixture at pH 7.4 containing 80 mg% glucose and 5 μc of either formate-C^{14} or glycine-2-C^{14}; and 0.4 ml of slightly alkaline saline containing either 6-MP or 6-MeMP, to produce a final concentration of thiopurine inhibitor of 2.5 mM. To isolate the combined nucleic acid and protein fractions, 3 ml of 95% ethanol was added to each flask at end of incubation period. The entire contents of flasks, together with 1 ml ethanol rinse, were transferred to centrifuge tubes. These were centrifuged and precipitates obtained washed successively with absolute ethanol-ether mixture and ether, then air dried. The dry precipitates were extracted with 10% NaCl at 100°C for 30 min, then centrifuged. Supernatants contained combined sodium nucleate fraction, and insoluble precipitates contained the protein fraction. Sodium nucleates were precipitated from supernatants with 2.5 volumes of absolute ethanol, redissolved in 1 ml distilled water and reprecipitated with ethanol, washed successively with absolute ethanol and ether, then air dried. Protein precipitates were washed successively with hot 10% NaCl, absolute ethanol, and ether, then air dried. C^{14}-activity of combined sodium nucleate fractions and protein fractions was determined by suspending the dried fractions in aqueous solution and plating on aluminum planchets. The planchets were dried, counted in end-window gas flow counter, and corrected for self-absorption.

The *results* shown in Table I reveal that, on an equimolar basis, 6-MeMP inhibited incorporation of either formate-C^{14} or glycine-C^{14} into total nucleic acid and protein fractions of Sarcoma-180 ascites tumor cells to a considerably greater extent than did 6-MP. It is also observed that the effect of 6-MP on formate and glycine incorporation into proteins is considerably less than its effect on incorporation into nucleic acids. This differential effect is not seen with 6-MeMP.

Metabolism and chemical stability of 6-MP and 6-MeMP in vitro. To ascertain whether tumor cells can metabolize the thio-purine inhibitors *in vitro,* ascites tumor cells were incubated with those agents under our conditions, with the exceptions that 6-MP-S^{35} and 6-MeMP-S^{35} were substituted for their unlabeled analogues and the formate-C^{14} or glycine-2-C^{14} was omitted. Stability of each inhibitor purine was determined by simultaneously incubating control flasks containing 6-MP-S^{35} or 6-MeMP-S^{35} with ascites tumor cells previously killed by boiling. At end of incubation period, 0.2 ml of 26% perchloric acid was added to each flask and the precipitated material transferred to tubes and centrifuged. Precipitates were reextracted with cold 2% perchloric acid and recentrifuged. Combined perchloric acid supernatants were neutralized in an ice bath with 0.5 N KOH and the precipitates formed were separated by centrifugation. Aliquots of clear supernatants were chromatographed on paper in 2 solvent systems (n-butanol : acetic acid : water,

TABLE I. Comparative Effects of 6-Mercaptopurine and 6-Methylmercaptopurine on Incorporation of C^{14} into Components of Sarcoma 180 Ascites Cells *In Vitro*.

Labeled compound	Inhibitor*	Protein, cpm/mg	Inhibition, %	Combined nucleic acids, cpm/mg	Inhibition, %
Formate-C^{14}	Control	22,800		2174	
"	6-MP	16,750	26.5	1159	46.6
"	6-MeMP	9,725	57.3	706	68.0
Glycine-2-C^{14}	Control	37,000		1149	
"	6-MP	33,400	9.7	871	24.0
"	6-MeMP	14,200	61.6	381	66.8

* Inhibitor conc. 2.5 mM. Values are means of quadruplicate determinations.

35.5 : 9.6 : 16.0, and n-butanol saturated with 2N NH_4OH).

Examination of chromatograms of acid soluble extracts derived from both types of incubations by scanning with the Actigraph did not reveal presence of any S^{35} compounds other than 6-MP-S^{35} and the 6-MeMP-S^{35} originally added.

Discussion. Identification of 6-MeMP as a metabolite of 6-MP and previously reported evidence for biological methylation of thiol compounds, suggest that methylation may be a generalized reaction for bio-transformation of thiol drugs *in vivo*. It would be of interest to know whether 6-thioguanine, structurally closely related to 6-MP and reported as a potent inhibitor of Sarcoma-180(11), can also undergo thio-methylation *in vivo*.

The small quantity of 6-MeMP in 24 hour urine collections appears to indicate that the magnitude of purine thiol methylation occurring *in vivo* is small. The significance of the S-methylation reaction is unknown. It may represent a detoxication mechanism, unrelated to tumor inhibitory effects *in vivo*.

That 6-MP-S^{35} did not undergo spontaneous decomposition and that it was not metabolized during incubation with ascites cells *in vitro*, indicates that its lesser effectiveness in inhibiting incorporation of formate-C^{14} and glycine-C^{14} into Sarcoma-180 tumor fractions, when compared to equimolar concentration of 6-MeMP, cannot be attributed to these factors. Methylation of a small fraction of 6-MP by ascites cells during incubation *in vitro* cannot be excluded by the analytical technics used. Therefore, it is possible that inhibitory activity of 6-MP observed, might be dependent on its conversion to 6-MeMP. However, the converse hypothesis, that activity of 6-MeMP might be due to demethylation of a small fraction to 6-MP is considered very improbable based on data shown in Table I.

From the standpoint of a structure-activity relationship, these *in vitro* data are consistent with the conclusion that inhibitory activity can be exerted in the presence of the methyl thioether linkage (CH_3-S-) and is not dependent on a free sulfhydryl group. Similar evidence has been presented by Skinner *et al.* (12). In their experiments, using inhibition of regeneration of hydra tentacles *in vitro* as assay of cell division, increase in aliphatic chain length of 6-substituted-thio-purines caused a definite increase in activity, when compared to 6-MP.

Several reports demonstrated that 6-MeMP, while possessing some degree of *in vivo* activity, is not as active as 6-MP against several mouse tumors and human leukemia(11,13,14). Two possible explanations for the apparent discrepancies between inhibitory effects of 6-MeMP *in vitro* and *in vivo* are: a. Inhibition of glycine-C^{14} and formate-C^{14} incorporation into Sarcoma-180 ascites tumor cells *in vitro* may bear no relation to antitumor effect *in vivo*. b. Differences in metabolic fates for 6-MP and 6-MeMP may exist *in vivo*. In this regard, Clarke *et al.*(11) suggested that 6-MeMP may have a metabolic fate different from that of 6-MP, due to its lack of oxidation by xanthine oxidase.

Conclusions. 1. 6-methylmercaptopurine was identified as a urinary metabolite of 6-mercaptopurine in normal Sprague-Dawley rats. 2. In *in vitro* experiments, 6-MeMP exerted greater inhibition of both formate and glycine incorporation into the combined nucleic acid and protein fractions of Sarcoma 180 ascites tumor cells than did 6-MP.

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Implantation of Normal Blood-Forming Tissue in Genetically Anemic Mice, Without X-irradiation of Host.* (25089)

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Numerous experiments(1-7) have demonstrated that blood-forming tissue may be successfully transplanted into heavily X-irradiated normal mice, but this phenomenon has never been demonstrated with adult untreated hosts. Transfers of blood-forming tissue between normal donors and genetically anemic hosts, reported here, indicate that, at least under certain circumstances, implantation of blood-forming tissue from another individual may be achieved without X-irradiation of recipient. The successfully implanted hosts include not only adult anemic mice similar to those in which implantation of isologous normal hematopoietic cells following mild (200r) whole-body radiation has already been reported(8,9), but also juvenile hosts of this genotype (WW^v) and of the lethally anemic genotype (WW). The anemia encountered in both of these genotypes has been reported (10) as a normochromic, macrocytic type. Although experiments using heavily irradiated normal hosts have demonstrated successful implantation of both homologous and isologous hematopoietic cells, homologous normal blood-forming cells have not been effectively implanted in adult anemic hosts after 200 r and much heavier radiation doses(11). These mice are extremely radiosensitive with median lethal doses in range of 200 r to 300 r(8). The low frequency of spontaneous survival to adulthood observed both in WW^v mice (25-31%) and in WW mice (0-10%)(12) make it especially doubtful whether juvenile animals of these genotypes could withstand heavy doses of radiation even as a prelude to tissue implantation. Thus, radiation to induce acceptance of blood-forming tissue appears unsuitable as therapy for these genetically anemic mice. It seemed advisable, therefore, to test for the possibility of implantation of isologous normal cells without radiation of the host. A critical test for success of implantation is possible in these genetically anemic hosts, since erythrocytes derived from normal blood-forming tissues, isologous with

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