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S-Adenosylhomocysteine Metabolism in Rat Hepatomas[1] (40339)

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Methionine metabolism in neoplasms may differ significantly from metabolism in normal tissue (Fig. 1). Changes in the rates of both synthesis of polyamines (1) and the transmethylation of macromolecules (2–7) indicate an increased requirement for *S*-adenosylmethionine. In turn, this implies a greater need for precursor methionine which could be achieved by an increase in homocysteine remethylation relative to transsulfuration (cystathionine synthesis).

In an earlier study of six rat hepatoma lines, we measured the tumor content of five enzymes of methionine metabolism (8). We found considerable variation between the tumor lines and between the tumors and liver. However, we did not observe any changes characteristic of neoplasia. Specifically, we did not define an enyzmatic basis for the presumed changes in methionine metabolism.

An alternative regulatory hypothesis focuses on *S*-adenosylhomocysteine. This metabolite, which is the product of all transmethylation reactions which utilize *S*-adenosylmethionine as the methyl donor (Fig. 1, reaction 2) is hydrolyzed by *S*-adenosylhomocysteine hydrolase (EC 3.3.1.1; Fig. 1, reaction 3)[2]—an enzyme present in virtually all mammalian tissues (10, 11). Adenosylhomocysteine possesses several interesting regulatory properties. It is a potent inhibitor of several classes of transmethylation reactions (12–16). Adenosylhomocysteine also inhibits both betaine-homocysteine methyltransferase (Fig. 1, reaction 7) (17) and 5-methyltetrahydrofolate-homocysteine methyltransferase (Fig. 1, reaction 8) (18)—the two enzymes which can conserve methionine. Conversely adenosylhomocysteine activates the competing cystathionine synthase reaction (Fig. 1, reaction 4) (17).

Thus, a decrease in the concentration of *S*-adenosylhomocysteine in neoplastic tissues could result in the metabolic alterations described in the first paragraph. However, the observation that *S*-adenosylhomocysteine hydrolase declines when chick embryo fibroblasts are transformed following infection with Rous sarcoma virus (19) would not be consistent with this formulation. For this reason we are reporting the results of direct assays of the adenosylhomocysteine enzyme in the six lines of rat hepatoma.

Since the hepatic content of adenosylhomocysteine hydrolase increases in animals fed a high-protein diet (10), we included studies to define whether the enzyme in hepatomas was subject to similar control. In addition, we measured the effect of the tumors both on the basal level of enzyme activity in the livers of host animals and on the regulation of the hepatic enzyme by changes in the dietary protein content.

Materials and methods. We studied a spectrum of transplantable hepatomas which ranged from the highly differentiated hepatoma 7787 which grew at 0.7 cm/month to the less-differentiated hepatomas 5123tc and 7777 with growth rates of 4.0 to 5.0 cm/month. The Morris hepatoma cells were inoculated into the thigh muscles of male Buffalo rats. The tumor-bearing and control animals received either a high-protein (55% casein) or low-protein diet (8% casein) for the 7 to 10 days prior to sacrifice. General Biochemicals Corporation (Chagrin Falls, Ohio) supplied the diets.

When the tumors attained a diameter of

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[2] As indicated, this enzyme is designated *S*-adenosyl-L-homocysteine hydrolase (EC 3.3.1.1) despite the fact that the thermodynamics of the reversible reaction favor the synthesis of adenosylhomocysteine (9). Since we measure enzyme activity in the direction of synthesis, we have chosen to use the term adenosylhomocysteine synthase when we discuss our results.

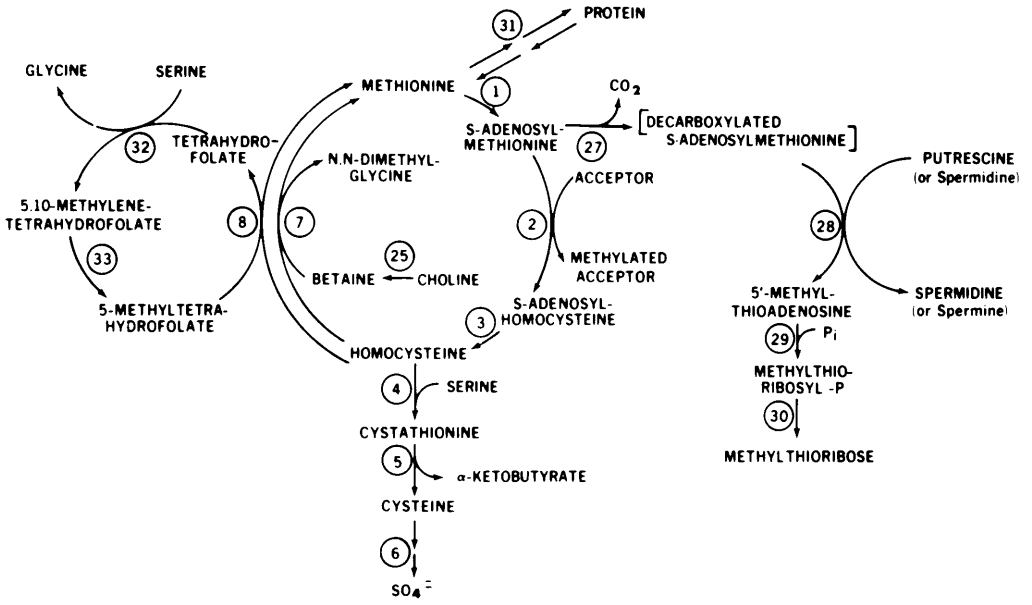

FIG. 1. Methionine metabolism in mammalian liver. Reactions 1 through 5 constitute the transsulfuration sequence. Reactions 1 through 3 together with either reaction 7 or 8 represent the methionine cycle.[3]

approximately 2 cm, we weighed the animals and sacrificed them by carotid exsanguination. Livers and tumors were removed rapidly and chilled. The tumors were dissected free of necrotic tissue. We prepared homogenates in 4 to 5 vol of 10 m*M* potassium phosphate, pH 7.4. The crude homogenate was centrifuged at 8000*g* at 4° for 15 min and the supernatant was stored at −70° until the time of assay. In preliminary studies, we had established that *S*-adenosylhomocysteine synthase is stable for at least 6 months under these conditions.

In all individual experiments the body weight of the tumor-bearing rats was comparable to that of the control animals fed the same diet. There were differences in body weights between studies of the various hepatomas since the slower growing tumors reached the designated size at a later time. Body weight and liver weight were lower in animals fed the low-protein ration. In general, dietary protein content had no effect on the weight of the tumors. Only with hepatoma 5123tc did we observe that the tumors hosted by animals fed the low-protein diet were smaller than the tumors in rats fed the high-protein ration.

Assay of adenosylhomocysteine synthase. We have published the details of our method (10). This is a specific and sensitive assay based on the synthesis of radioactive product from [8-^{14}C]adenosine. The reaction mixture contains 0.2 *M* potassium phosphate, pH 7.3; 2 m*M* L-homocysteine; 1 m*M* [8-^{14}C]adenosine (containing 10^5 dpm); and tissue extract in a final volume of 1.0 ml. Following a 15-min incubation, we stop the reaction with 0.1 ml of 30% perchloric acid and add *S*-[8-^{3}H]-adenosylhomocysteine. The neutralized supernatant is placed on a column of AG-50(H^+) × 4 (100–200 mesh), 0.9 × 3.0 cm. After washes with 1% thiodiglycol and 1 *N* HCl, we elute the adenosylhomocysteine with 3 *N* NH_4OH. By measuring the ratio $^{3}H/^{14}C$, we can calculate product formation from the [8-^{14}C]adenosine. Protein concentration was determined by the method of Lowry (20).

Expression of results. We have presented our results as specific activities in nanomoles of product per 15 min per milligram of protein. In these studies and in previous experiments, we found that the relative values in liver rarely change when we relate product formation to wet weight of tissue rather than to extractable protein. We used the *t* test for

[3] This figure was reproduced from the article by Mudd, S. H., and Poole, J. R., Metabolism **24**, 721 (1975) with the permission of the publisher.

unpaired samples of all statistical comparisons.

Results. The mean specific activity of *S*-adenosylhomocysteine synthase in the livers of control animals was 425 ± 65 units in rats fed the low-protein diet and was 517 ± 70 units in the high-protein group. Although the increase in specific activities was statistically significant in five of the six individual studies, it was less than the twofold increase observed in our earlier study with Sprague–Dawley rats (10).

The activity of *S*-adenosylhomocysteine synthase was comparable in the livers of host and control rats. We found no instance in which the presence of the hepatoma affected either the hepatic content of enzyme or the response to dietary protein.

Table I demonstrates that extracts from each tumor line contained enzyme activity. In contrast to the liver enzyme, the specific activity of *S*-adenosylhomocysteine synthase in the hepatomas did not increase significantly when the host rat ingested the high-protein diet. Indeed, the only statistically significant change induced by diet was the paradoxical increase in activity in hepatoma 7787 from animals fed the low-protein diet.

Discussion. The regulation of the tissue concentration of adenosylhomocysteine depends on the integrity of a metabolic sequence which includes *S*-adenosylhomocysteine hydrolase linked to enzymes with the capacity to catabolize adenosine and homocysteine. In the current study, we found that the hydrolase was present in six rat hepatoma lines. This is consistent with our previous report that these same tumors contained five other enzymes which are components of the pathway for methionine metabolism in mammalian liver (8). However, the various hepatoma lines differed in the pattern of enzyme activities. On that basis, we suggested that hepatomas 9633, 7800, and 5123tc might be incapable of conserving methionine by means of homocysteine remethylation. Conversely, hepatomas 7787, 7794A, and 7777 were relatively deficient in cystathionine synthase and might require an exogenous supply of cyst(e)ine.

TABLE I. ADENOSYLHOMOCYSTEINE SYNTHASE IN RAT HEPATOMAS.[a]

Hepatoma[b]	Specific activity (nmole/mg of protein/15 min)	
	LPD	HPD
7787	127 ± 16[c]	80 ± 14[d]
9633	101 ± 19	123 ± 18
7794A	132 ± 24	160 ± 49
7800	207 ± 43	151 ± 30
5123Tc	93 ± 25	101 ± 10
7777	48 ± 19	50 ± 20

[a] Each study of a specific hepatoma line included at least four animals fed the low-protien diet (LPD) and four fed the high-protein diet (HPD).

[b] The hepatoma lines are listed in the order of increasing growth rate.

[c] Mean ± SD.

[d] Statistical significance between diet groups: $P < 0.02$.

In contrast, the specific activity of *S*-adenosylhomocysteine synthase in these hepatomas was remarkably constant. When we expressed the results relative to the activities in host livers, the range was 25 to 56% in animals fed the 8% casein diet and was 12 to 28% in rats fed the 55% casein ration. These relative values are equivalent to, or greater than, the relative values obtained for the other five enzymes—with the exception of one study. In hepatoma 5123tc obtained from rats fed the low-protein diet, the relative specific activities were: methionine adenosyltransferase, 99%; 5-methyltetrahydrofolate-homocysteine methyltransferase, 139%; cystathionine synthase, 225%; and betaine-homocysteine methyltransferase, 37% (8). In this hepatoma, a value of 31% may indicate a relative deficiency of *S*-adenosylhomocysteine synthase.

Clearly the present study does not define a significant role for adenosylhomocysteine in the pathochemistry of oncogenesis. The data do not support the suggestion that a deficiency of adenosylhomocysteinase may be characteristic of neoplastic tissue (19). However, adenosylhomocysteine might be present in excess as a consequence of either augmented transmethylation or the failure to catabolize adenosine. Conversely, malignant cells may contain diminished concentrations of adenosylhomocysteine. Indeed, abnormal methylation is compatible with the release of the transmethylases from product inhibition. Obviously we require detailed studies of the adenosylhomocysteine concentration in tumors of known biological properties under controlled conditions of nutrition.

Summary. *S*-Adenosylhomocysteine syn-

thase was present in extracts prepared from six lines of rat hepatoma. There was no apparent correlation between the specific activity of this enzyme and any of the other biological properties of the tumors. The presence of the hepatoma did not affect the activity of adenosylhomocysteine synthase in livers of host animals. Hepatic enzyme activity in both host and control rats showed an adaptive increase to an increase in dietary protein. In contrast, dietary protein failed to affect the specific activity of adenosylhomocysteine synthase in five hepatomas. Paradoxically, enzyme activity in hepatoma 7787 declined when the host rats were fed a high-protein ration.

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