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Enzymes of Methionine Metabolism in Regenerating Rat Liver (40906)[1]

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Abstract. Significant changes in the activities of the enzymes of methionine metabolism occur in the regenerating rat liver. We found that the levels of methionine adenosyltransferase and cystathionine synthase increased immediately after partial hepatectomy. A secondary peak of activity was noted on the eighth postoperative day. In contrast, activities of both 5-methyltetrahydrofolate homocysteine methyltransferase and betaine–homocysteine methyltransferase declined. This pattern of changes suggests that transsulfuration, rather than methionine conservation, is a major metabolic response in hepatic regeneration.

The major metabolic functions of methionine are utilization for protein synthesis; conversion to adenosylmethionine, which is both the primary biological methyl donor and an essential reactant in the synthesis of the higher polyamines; and conversion to cystathionine and cysteine by means of the transsulfuration pathway. Figure 1 depicts these reactions. In view of the numerous competing pathways, it is not surprising that the metabolism of methionine appears to be modulated by several complex mechanisms (1). A major regulatory locus is the distribution of available methionine between the synthesis of protein (Fig. 1, reaction 31) and the formation of *S*-adenosylmethionine (Fig. 1, reaction 1). The reactions which utilize homocysteine provide a second control point. At that junction homocysteine may be converted to cystathionine (Fig. 1, reaction 4) which irreversibly commits the compound to the transsulfuration pathway. Alternatively there are two enzymes which may remethylate homocysteine and thus conserve methionine. Based on both experimental data and theoretical considerations (1, 2), it is likely that 5-methyltetrahydrofolate homocysteine methyltransferase (Fig. 1, reaction 8) rather than betaine homocysteine methyltransferase (Fig. 1, reaction 7) is the primary methionine-conserving reaction.

The rapid regenerative phase, immediately following partial resection of the liver, is characterized by an increase in reactions and pathways which require methionine and its metabolic derivatives. Consequently we have studied the effect of partial hepatectomy on the hepatic activities of the enzymes which participate in the regulation of methionine metabolism.

Methods. We used male Sprague–Dawley rats weighing 250–300 g. Wayne Lab Blox was the routine laboratory diet. Partial hepatectomy was performed by the method of Higgins and Anderson (3) under light ether anesthesia. Laparotomy with manipulation of the liver was the control, "sham" procedure. Following surgery the control animals were pair-fed the measured intake of the hepatectomized animals. This level was virtually zero for the first 36 hr postoperatively. During this period we provided 5% glucose as the drinking solution.

Animals were stunned and sacrificed by carotid transection. We used published methods for the preparation of the extracts and for the assays of methionine adenosyltransferase (EC 2.5.1.6) (4); cystathionine β-synthase (EC 4.21.22) (4); γ-cystathionase (EC 4.4.1.1) (4); 5-methyltetrahydrofolate homocysteine methyltransferase (EC 2.1.1.13) (2); betaine–homocysteine methyltransferase (EC 2.1.1.5) (5); and adenosylhomocysteinase (EC 3.3.1.1) (6).

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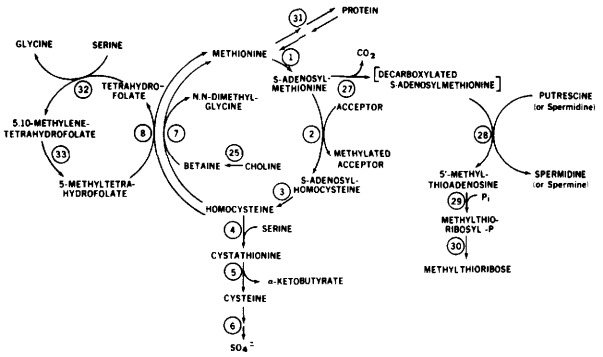

FIG. 1. Methionine metabolism in mammalian liver. Reactions 1 through 5 constitute the transsulfuration pathway. Reactions 1 through 3 together with either reaction 7 or 8 represent the methionine cycle. Reproduced, with permission, from S. H. Mudd and J. R. Poole, Metabolism **24**, 721 (1975).

Protein was determined by the Lowry method (7).

We analyzed our results expressed as enzyme concentration (units/g liver), hepatic content (units/liver), and specific activity (units/mg protein). Student's *t* test for unpaired samples was the method for statistical comparisons.

Results. Table 1 summarizes the data obtained from studies during the first 2 weeks following hepatectomy. The responses of the enzymes were not coordinate and no two enzymes followed the same pattern. The specific activity of methionine adenosyltransferase increased immediately after surgery and reached a peak of 170% of the control value on the second postoperative day. A second, equivalent peak in the activity of this enzyme occurred on the eighth day. This biphasic pattern also characterized the response of betaine–homocysteine methyltransferase. However, the magnitude of the maximum activities was less with this enzyme. Conversely the nadir on the fourth day was 58% for the betaine enzyme and 73% for methionine adenosyltransferase. The specific activity of cystathionine synthase showed only a single peak on the second day. Thereafter the value declined to the control levels. During the first 10 days the specific activity of methyltetrahydrofolate homocysteine methyltransferase was invariably less than the control activity. The relative value of 132% on Day 15 was not significant statistically.

When we expressed our results as units per gram of liver, these patterns changed. The primary difference was on the second day and resulted from the significant decrease in soluble protein at that time (Table 1). As a consequence the values for methionine adenosyltransferase and cystathionine synthase approximated the control level while the values for the two methyltransferases were decreased significantly.

We did not assay the activities of adenosylhomocysteinase and cystathionase in all studies. One day after hepatectomy both the specific activity and the hepatic concentration of adenosylhomocysteinase were increased to 117 and 139% ($P < 0.001$), respectively. These values were at the control levels when tested 8 days following surgery. We found no significant changes in hepatic cystathionase activity on the second and third postoperative days.

Discussion. Changes in the pattern of methionine metabolism must occur in the regenerating rat liver since previous studies have demonstrated enhanced activity in at least three pathways which require this amino acid. Clearly the rate of protein synthesis is augmented. In addition, both methylation of nucleic acids and histones (8, 9) as well as the synthesis of polyamines (10, 11) are increased. The latter two processes utilize adenosylmethionine as a donor of either a methyl group (transmethylation) or of a propylamine moiety (spermidine and spermine synthesis). In-

TABLE I. SPECIFIC ACTIVITIES OF ENZYMES IN REGENERATING RAT LIVER[a]

Day	(No.)[b]	Liver weight	Protein	MAT	Cyst. Syn.	mTHF Enz	BH Enz
1	(17/15)	51	106	127 ± 25[c]	114 ± 16[d]	67 ± 27[c]	80 ± 14[d]
2	(12/10)	60	65	169 ± 46[c]	173 ± 60[d]	84 ± 29	113 ± 17
4	(13/10)	73	98	73 ± 18[d]	116 ± 16[e]	87 ± 13	58 ± 10[c]
6	(5/5)	75	92	131 ± 23[e]	96 ± 7	92 ± 19	86 ± 10
7	(6/5)	86	100	132 ± 28[e]	89 ± 22	75 ± 25	120 ± 11[d]
8	(6/5)	74	100	167 ± 55[e]	113 ± 46	81 ± 9	119 ± 30
10	(5/5)	87	113	97 ± 31	95 ± 33	70 ± 28	89 ± 13
15	(6/5)	89	98	108 ± 17	88 ± 9	132 ± 31	122 ± 14

[a] Results for liver weight, protein concentration, and enzyme specific activities are expressed as a percentage of the mean values for the sham-operated animals which we studied simultaneously. Abbreviations: MAT, methionine adenosyltransferase; Cyst. Syn., cystathionine-β-synthase; mTHF Enz, methyltetrahydrofolate homocysteine methyltransferase; BH Enz, betaine–homocysteine methyltransferase.

[b] Number of hepatectomized animals/control animals.

[c–e] P values relative to control.

[c] $P < 0.001$.

[d] $P < 0.01$.

[e] $P < 0.05$.

deed the level of adenosylmethionine decarboxylase (Fig. 1, reaction 27) increases by approximately 300% within the first 2 days following partial hepatectomy (11, 12).

Based on these considerations we anticipated that the enzyme pattern in the regenerating liver, similar to that in fetal and growing liver, would reflect the need to conserve methionine by limiting the synthesis of cystathionine (1, 13, 14). The present data indicate the contrary. During the first 10 days of regeneration the ratio of cystathionine synthase/methyltetrahydrofolate homocysteine methyltransferase invariably exceeds that found in the livers of the control rats. We observed values greater than twice normal on Days 1 and 2. Nor is the level of the second homocysteine methylase increased during the acute postsurgical period. The concentration of this betaine enzyme is decreased significantly until the end of the first week. Consequently the pattern of enzymes suggests that sustained synthesis of adenosylmethionine and maintenance of the transsulfuration pathway characterize the regenerating liver. This conclusion generates several interesting questions. An immediate problem is the source of methionine during the initial 24–36 hr when the rat is neither eating nor apparently conserving the amino acid. The plasma concentration of methionine increases substantially following partial hepatectomy (15, 16). Mobilization from skeletal muscle and other extrahepatic sources is a likely explanation.

A second question is the teleological basis for the observed maintenance of the transsulfuration sequence. This pathway provides cysteine and several significant metabolites including taurine and glutathione. We are tempted to speculate that one, or more, of these compounds is essential for the regenerative process.

Finally we may seek a nutritional or hormonal mechanism which initiates and coordinates the changes in the hepatic content of the enzymes. Morley *et al.* noted marked increases in serum glucagon following partial hepatectomy (17). Insulin levels were constant while growth hormone and thyroxine declined. However, our previous studies (2, 5, 18) do not allow a simple correlation between the changes in hepatic enzymes and either these alterations in the levels of hormones or the increase in plasma methionine.

1. Finkelstein, J. D., *in* "Transmethylation" (E. Usdin, R. T. Borcardt, and C. R. Creveling, eds.), p. 49. Elsevier/North Holland, New York (1978).
2. Finkelstein, J. D., Kyle, W. E., and Harris, B. J., Arch. Biochem. Biophys. **146**, 84 (1971).
3. Higgins, G. M., and Anderson, R. M., Arch. Pathol. **12**, 186 (1931).

4. Mudd, S. H., Finkelstein, J. D., Irreverre, F., and Laster, L., J. Biol. Chem. **240**, 4382 (1965).
5. Finkelstein, J. D., and Mudd, S. H., J. Biol. Chem. **242**, 873 (1967).
6. Finkelstein, J. D., and Harris, B. J., Arch. Biochem. Biophys. **159**, 160 (1973).
7. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193**, 265 (1951).
8. Morris, N. R., and Pih, K. D., Cancer Res. **31**, 433 (1971).
9. Lee, H. W., and Paik, W. K., Biochim. Biophys. Acta **277**, 107 (1972).
10. Russell, D. H., Medina, V. J., and Snyder, S. H., J. Biol. Chem. **245**, 6732 (1970).
11. Russell, D. H., and Lombardini, J. B., Biochim. Biophys. Acta **240**, 273 (1971).
12. Hannonen, P., Raina, A., and Janne, J., Biochim. Biophys. Acta **273**, 84 (1972).
13. Volpe, J. J., and Laster, L., Biol. Neonate **20**, 385 (1972).
14. Sturman, J. A., Gaull, G. E., and Niemann, W. H., J. Neurochem. **27**, 425 (1976).
15. Ferris, G. M., and Clark, J. B., Biochim. Biophys. Acta **273**, 73 (1972).
16. Ord, M. G., and Stocken, L. A., Biochem. J. **129**, 175 (1972).
17. Morley, C. G. D., Kuku, S., Rubenstein, A. H., and Boyer, J. L., Biochem. Biophys. Res. Commun. **67**, 653 (1975).
18. Finkelstein, J. D., Arch. Biochem. Biophys. **122**, 583 (1967).

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