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S-Adenosylmethionine-synthesizing Activity of Normal and Neoplastic Mouse Tissues[1]

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Summary

An enzyme which synthesizes *S*-adenosylmethionine in mouse liver has been examined and found to possess the same characteristics as that described by others for pig and rabbit liver. Although liver has large quantities of the enzyme activity, a mouse hepatoma had no measurable activity. It was hypothesized that trace amounts of the enzyme in hepatoma cells might be sufficient for sRNA methylation. Estrogen was found to depress the activity of liver *S*-adenosyltransferase in castrate female mice.

Introduction

Mice utilize L-methionine as the major source of methyl groups for a variety of methylating reactions. The terminal *S*-methyl function of L-methionine is activated by the formation of a sulfonium group through the following reaction:

ATP[2] + L-methionine → *S*-adenosylmethionine + PPi + Pi.

The enzyme catalyzing this reaction (ATP: L-methionine *S*-adenosyltransferase E.C. 2.4.2.13, hereafter called *S*-adenosyltransferase) was first described for rabbit liver (4). One of the substances methylated by cells is sRNA (8). sRNA derived from mammary adenocarcinoma and HeLa cells has been found to be highly methylated as compared with normal tissues (2, 3). In view of the increased methylation of RNA in tumors, it was of interest to establish the amount of *S*-adenosyltransferase activity of normal and neoplastic mouse tissues. Also included were studies on age, sex, hormone, and drug effects on the enzyme activity.

Materials and Methods

Yeast inorganic pyrophosphatase was purchased from Nutritional Biochemical Co. (Cleveland, Ohio). Nucleotides were obtained either from Pabst Laboratories (Milwaukee, Wis.), or Sigma, Inc. (St. Louis, Mo.). D-Methionine and D and L-ethionine were from K and K Laboratories, Inc. (Jamaica, N. Y.). L-Methionine, Tris, and estradiol-17β were purchased from Sigma, Inc.

The enzyme was assayed essentially as described by Cantoni and Durell (5). L-Methionine-dependent release of inorganic phosphorus from ATP was determined by the method of Fiske and SubbaRow (7). Paper and column chromatography of *S*-adenosylmethionine were according to the methods of Schlenk and DePalma (14, 15). Protein was determined by the method of Warburg and Christian (17). A millimolar extinction coefficient of 15 was used for *S*-adenosylmethionine at 256 mμ (14). A model DB Beckman spectrophotometer was used to determine visible and ultraviolet absorption specta. The pH was determined on reaction mixtures with a radiometer pH meter just before stopping the reaction. Probability (*P*) values were calculated by a nonparametric method (Mann-Whitney U-test) (10).

Results

CHARACTERISTICS OF MOUSE *S*-ADENOSYLTRANSFERASE. C57BL/Ks female mouse livers which had been stored for several days at −10°C were homogenized in 2 volumes of 0.01 M acetic acid and centrifuged at 10,000 × *g* for 30 min. Supernatant fractions obtained after centrifugation were active in forming *S*-adenosylmethionine as shown by the L-methionine-dependent release of 0.019 μmole of inorganic phosphate/mg protein/30 min at 37°C. An ammonium sulfate preparation (Step 2 of Cantoni and Durrell's method) (5) released 0.76 μmole of phosphate/mg protein, and the isoelectric precipitated protein (Step 3) (5) released 2.1 μmoles phosphate/mg protein. A comparison of fresh and frozen liver from DBA/2J female mice, however, indicated considerable loss of activity upon freezing. Crude supernatants from fresh preparations produced 0.63 μmole inorganic phosphate/mg protein and the enzyme from Step 2 produced 2.66 μmoles. Frozen liver from female DBA/2J mice, if stored for 1 week at −14°C, lost about 50% of its activity.

When the ammonium sulfate fraction was used as the source of the enzyme there was a linear time curve over a 30-min period. A 3-fold increase in the rate was obtained when yeast inorganic pyrophosphatase was added. The increased rate using pyrophosphatase with the ammonium sulfate fraction is believed to be due to the removal of the major portion of liver inorganic pyrophosphatase during the purification step, since a 10,000 × *g* supernatant fraction gave a linear time curve up to 90 min without the addition of inorganic pyrophosphatase. The rate was optimal

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[2] The following abbreviations are used: AMP, adenosine monophosphate; ADP, adenosine diphosphate; ATP, adenosine triphosphate; dATP, deoxyadenosine triphosphate; CTP, cytidine triphosphate; GTP, guanosine triphosphate; UTP, uridine triphosphate; ITP, inosine triphosphate; GSH, glutathione; sRNA, soluble ribonucleic acid; PPi, inorganic pyrophosphate; Pi, inorganic phosphate.

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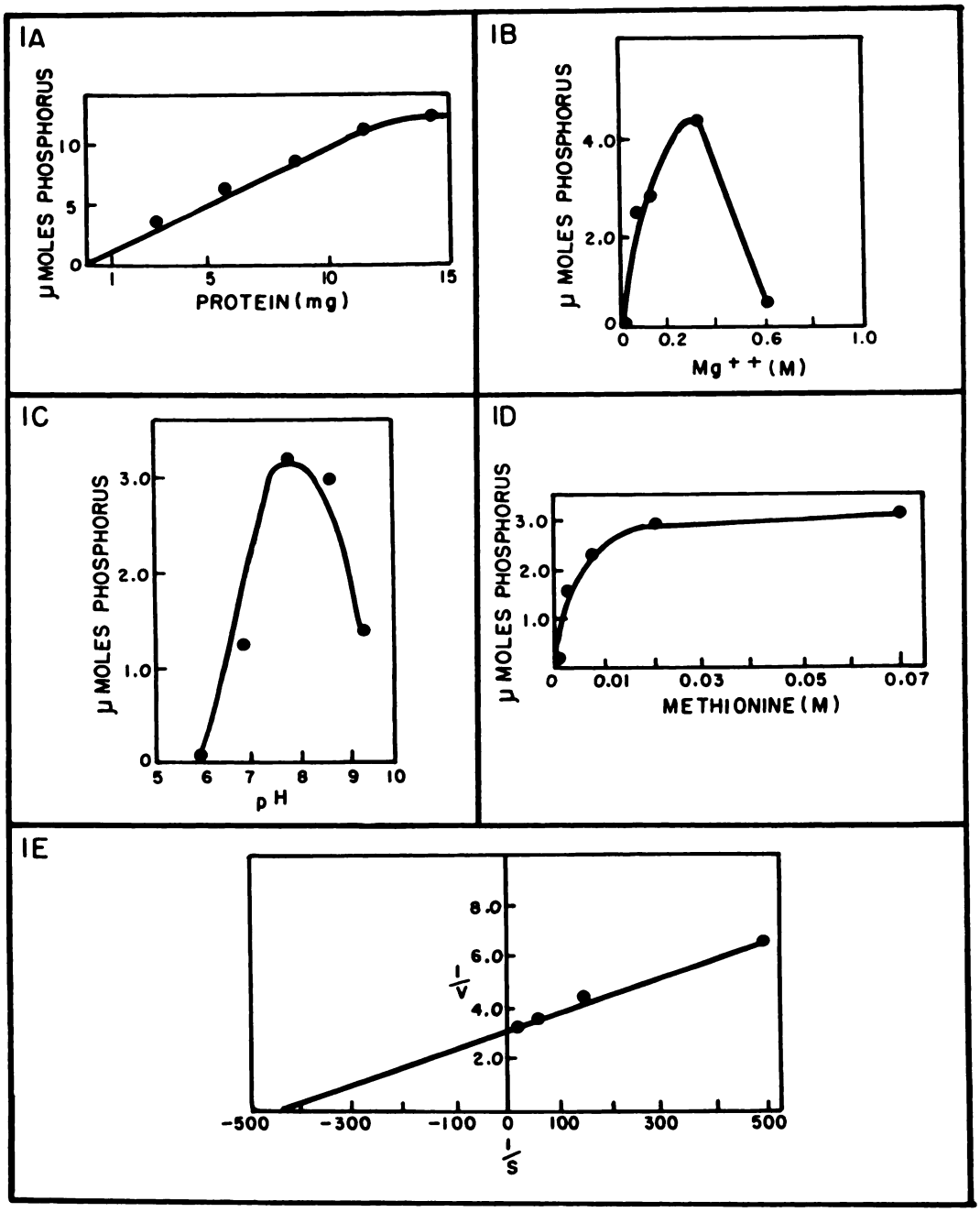

CHART 1. *A*, enzyme concentration curve. The reaction mixture contained 20 μmoles of L-methionine, 20 μmoles of ATP, 8 μmoles of GSH, 300 μmoles of $MgCl_2$, 130 μmoles of Tris buffer, pH 7.6, and varying amounts of a 30–50% ammonium sulfate-fractioned protein, in a total volume of 1 ml. Incubation was for 30 min at 37°C. Blanks contained no L-methionine. *B*, Magnesium concentration curve. The reaction mixture was the same as for *A*, except that 5 mg of protein and varying amounts of magnesium chloride were used. The 0.6 M reaction mixture was somewhat turbid. *C*, pH curve. The reaction mixture was the same as in *A*, except that 5 mg of enzyme preparation and 1810 μmoles of Tris buffers of varying pH were used. *D*, Substrate concentration curve. The reaction mixture was the same as in *A*, with the exception that 5 mg of enzyme preparation were used and the quantity of L-methionine was varied. *E*, Lineweaver-Burk reciprocal plot using L-methionine as substrate.

when L-methionine was present at a concentration of 0.02 M (Chart 1*D*) and the K_m (L-methionine) was 2.2×10^{-3} M (Chart 1*E*). K_m (L-methionine) values reported for the enzyme isolated from rabbit liver and yeast has a value of 2.2×10^{-3} M and 2.6×10^{-3} M respectively (11). *S*-Adenosylmethionine was demonstrated to be a reaction product by paper chromatography, column chromatography, by its ultraviolet absorbance spectrum and by the lability of the glycosyl bond under alkaline conditions (13). There was an absolute requirement for Mg and the rate of the reaction was optimal only when Mg was present at a concen-

tration of 0.3 M (Chart 1*B*). No potassium requirement was demonstrable as shown for the yeast enzyme (4), because potassium bicarbonate was used to neutralize the ATP and GSH solutions which were added to the reaction mixture. Omission of GSH resulted in a 40% loss of activity. The pH optimum was found to be 7.5 (Chart 1*C*), a value similar to that reported for yeast enzyme (11). Under optimal conditions the reaction rate was linear with respect to the concentration of the ammonium sulfate precipitate over a range of 2–12 mg of protein (Chart 1*A*).

The rate of phosphate to *S*-adenosylmethionine produced was 2.94 when the 10,000 × *g* supernatant fraction of fresh C57BL/6J female liver was used as enzyme. Preparations containing inorganic pyrophosphatase activity should give a value of 3.0 since the pyrophosphate released would be hydrolyzed. Approximately 90% of the activity of the whole liver homogenate was recovered in the supernatant fraction after recentrifugation of the 10,000 × *g* supernatant fraction at 100,000 × *g* for 1 hr.

The enzyme was specific for the L configuration of methionine and ethionine since neither D-methionine nor D-ethionine were utilized. AMP, ADP, dATP, ITP, GTP, CTP, could not be substituted for ATP in the reaction. In the presence of UTP the activity of the enzyme was only 4% of that found with ATP.

TABLE 1

DIFFERENCE IN LIVER *S*-ADENOSYLTRANSFERASE ACTIVITY IN ANIMALS OF DIFFERENT SPECIES, STRAINS, AND GENOTYPES

Species	Strain	Genotype	Sex	No. of animals	Mean activity (in units)[a]	S.E.	*P* value
Mouse	DBA/2J		F	5	65.4	1.9	
			M	5	27.7	1.7	0.008
Rabbit	Race III		F	7	51.0	3.4	0.26
			M	7	59.2	7.0	
Mouse	C57BL/6J		F	10	66.1	3.4	<0.002
			M	10	31.2	2.8	
Mouse	BALB/cJ		F	9	71.0	1.9	>0.1
			M	9	60.6	4.8	
Mouse	WB/B6	(W/W^v)	F	9	80.9	4.0	<0.002
		(+/+)	F	9	51.1	2.9	

[a] 1 unit = μmole of L-methionine-dependent formation of inorganic phosphorus per 30 min/gm wet weight of tissue.

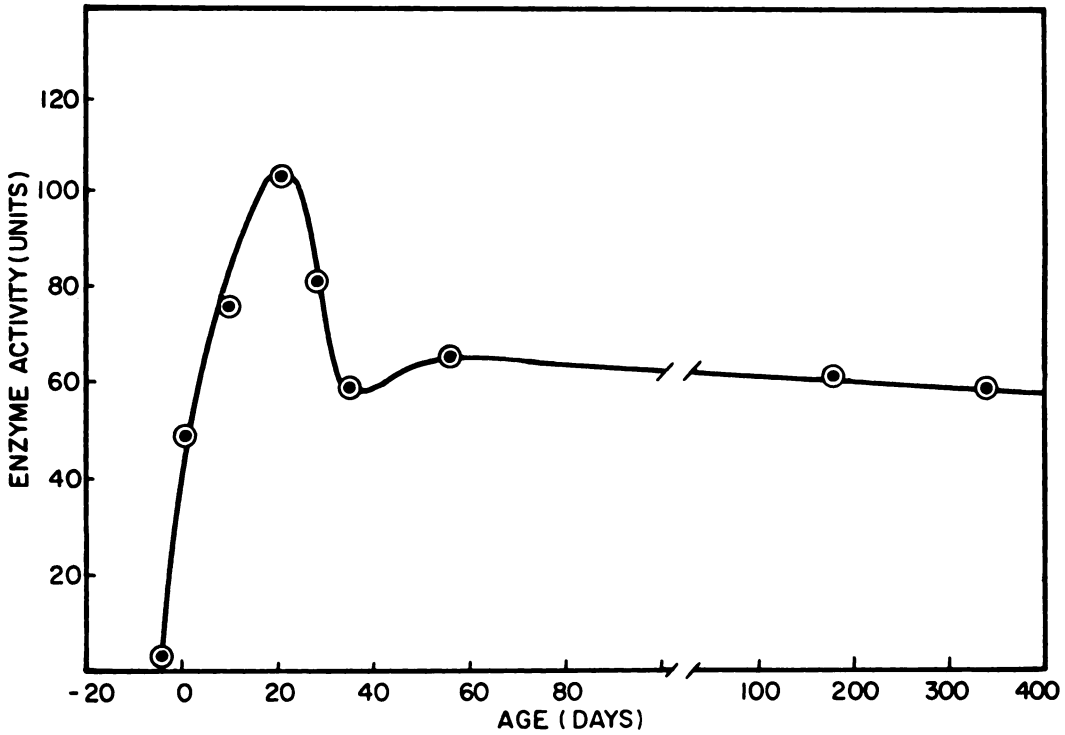

CHART 2. Variation of *S*-adenosyltransferase activity in C57BL/6J female mice livers of different ages. For fetal, newborn, and 10-day-old mice, livers were pooled from several individuals in order to obtain 500–1000 mg of tissue per assay. Other *points* are averages of 4 mice for 20 and 4 for 30 days, 5 mice for 34 and 5 for 48 days, 5 mice for 60 days, 2 mice for 180 days, and 1 mouse for 340 days.

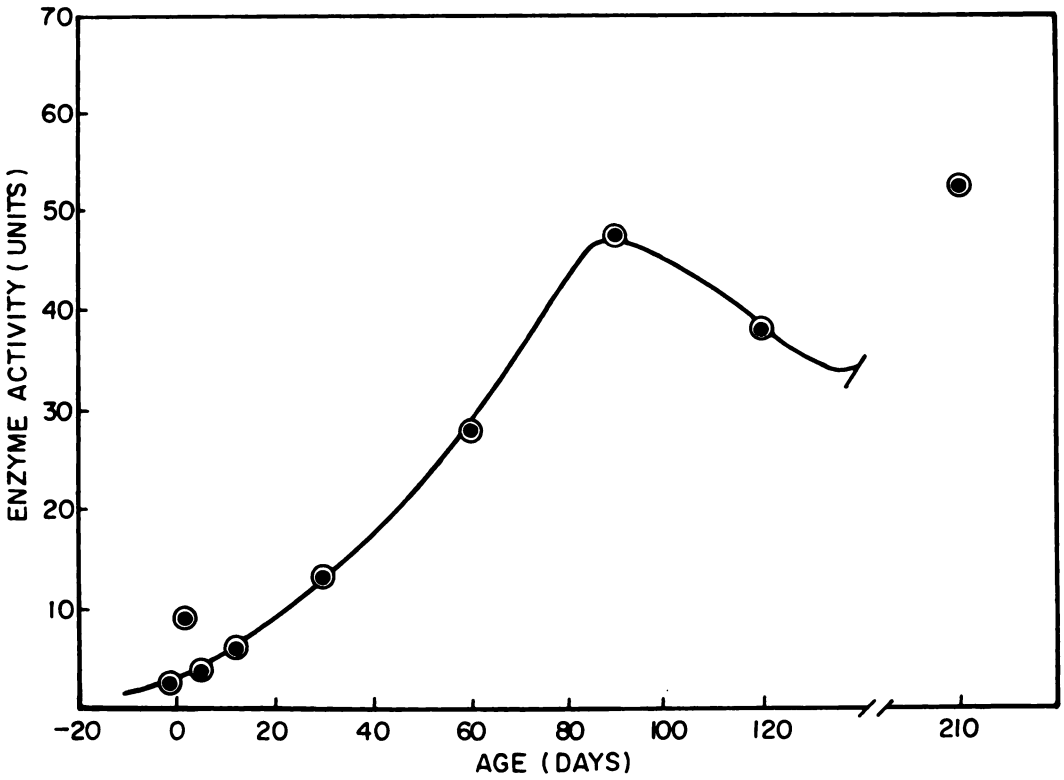

CHART 3. Variation of *S*-adenosyltransferase activity in Race III rabbit livers of different ages. Each *point* represents a value for an individual.

L-Ethionine was utilized at a rate which ranged between 22–35% of that found when L-methionine was the substrate.

SEX AND AGE DIFFERENCES IN LEVELS OF ENZYME ACTIVITY. Female rat liver was reported to have nearly twice the *S*-adenosyltransferase activity of male liver (12). In the present studies female DBA/2J mouse liver was also found to have over twice the activity of male liver. However, in contrast to this, Race III rabbits had a female:male ratio of only 0.9. Further data obtained on 2 selected strains, C57BL/6J and BALB/cJ, showed that C57BL/6J mice have a female:male ratio of *S*-adenosyltransferase activity of 2.2, while the ratio in BALB/cJ strain was only 1.2 (Table 1).

A striking change in the activity of the enzyme occurs with age (Chart 2). Only trace activity was found in the near-term fetal liver but the 1-day-old newborn had appreciable activity which continued to rise linearly until 21 days of age and then rapidly decreased until 35 days of age. Thereafter there is no appreciable change in the activity for the rest of the lifespan of the mouse. It should be noted that in all other experiments, mice of over 35 days (usually 8 weeks of age) were used. *S*-Adenosylmethionine (2 mg daily) administered for 10 days to newborn mice did not suppress the appearance of the enzyme activity. Studies with rabbits (Race III) showed a slower, but linear, rise in enzyme activity until 80 days of age (Chart 3).

S-ADENOSYLTRANSFERASE ACTIVITY OF VARIOUS NORMAL AND NEOPLASTIC TISSUES. Except for a small amount of activity in spleen, the enzyme was found in measurable quantities only in liver tissue. Ovary, kidney, adrenal, muscle, thymus, lung, heart, uterus, brain, and testis, assayed as fresh tissue, showed either none or only trace amounts of activity. Similarly a, variety of mouse neoplasms (lymphatic leukemia P1534, myeloid leukemia C1498, testicular interstitial cell tumor H10119, adrenal cortical adenocarcinoma E12529, ovarian granuloma H4929, spindle cell sarcoma SaI, mammary adenocarcinoma BW10232, and mammary adenocarcinoma CaD2 including a hepatic adenocarcinoma BW7756) had less than 5 units of activity. Two male mice bearing a purportedly estrogen-secreting granulosa cell tumor for 4 weeks showed no change in liver *S*-adenosyltransferase activity. Conversely 2 female BALB/cJ mice bearing interstitial cell tumors (at one time shown to be androgen secreting) which had been present for a 4-week period also showed no change in the *S*-adenosyltransferase activity of their host livers. Experiments with livers from partially hepatectomized mice, 6 and 14 days prior to assay, indicated no change in *S*-adenosyltransferase activity in the remaining regenerating lobes, as compared with those of sham-operated mice.

Trace amounts of activity can be demonstrated by using a 30–50% ammonium sulfate fraction. Thus, 5-gm equivalents of tissue based on wet weight, per tube instead of the standard 250-mg amount, allows a lower limit of measurement of 0.15 unit/gm of liver, whereas the standard assay has a lower limit of 3.0 units/gm.[3] Using this procedure no activity could be demonstrated in the hepatoma indicating that the amount of activity was less than 0.15 unit. This is in contrast to normal liver which has an activity of approximately 60 units.

[3] This is based upon a reading accuracy of 0.01 O.D. between 0.1 and 0.5 O.D.

EFFECTS OF HORMONES AND DRUGS ON LEVEL *S*-ADENOSYLTRANSFERASE ACTIVITY. A difference in the amount of enzyme activity was found between females of the viable dominant spotting mutant W/W^v on a WB/B6 background as compared with the normal (+/+). Females and males heterozygous for the W and W^v genes ($W/+$, $W^v/+$) and homozygous males (+/+) all had similar amounts of *S*-adenosyltransferase activities. However, W/W^v females which have very few primary egg follicles (6), which disappear with age, had elevated amounts of enzyme activity (Table 1). The theca interna cells of ovarian follicles are believed to be the site for synthesis of estradiol-17β from cholesterol (16). From these facts, one might expect to find estrogen as a depressant of *S*-adenosyltransferase. The following experiments have indicated that estrogen does depress the amount of enzyme activity. Castration in female mice elevated the enzyme activity (Table 2). Testosterone did not depress the enzyme in castrated female mice, but estrogen did depress the levels of activity to within normal limits. No *in vitro* effects of estrogen on the activity of the enzyme have been demonstrated using mashed, homogenate, or supernatant preparations.

Control mice anesthetized with sodium pentobarbital and sham-operated showed a marked increase in the level of *S*-adenosyltransferase activity within 5 days after the operation. As shown in Table 3 the increase was due to sodium pentobarbital and was induced when the only treatment was the injection of the drug.

TABLE 2

HORMONAL EFFECTS UPON *S*-ADENOSYLTRANSFERASE

C57BL/6J♀ mice were bilaterally oophorectomized and given either 0.05 ml sesame oil every 2 days (castrated), 100 μg estradiol-17β in 0.05 ml sesame oil s.c. every 2 days (estrogen treated), or 20 μg testosterone in 0.05 ml sesame oil every 2 days (testosterone treated). Mice were killed after the effect of sodium pentobarbital had disappeared (see Table 3) between 21 and 42 days postoperatively.

Treatment	No. of mice	Mean activity (in units)	S.E.	*P*[a] value
Castrate and no steroid	10	77.1	3.3	
Castrate and testosterone	8	85.1	5.1	>0.1
Castrate and estrogen	9	58.6	2.6	0.02
Nontreated	10	66.1	3.4	<0.05

[a] Compared with "castrate and no steroid" group.

TABLE 3

EFFECT OF PENTOBARBITAL ON *S*-ADENOSYLTRANSFERASE ACTIVITY

Twenty-one mice (129/RrJ♀) were given 1.5 mg of sodium pentobarbital and sacrificed at varying time intervals thereafter.

Postinjection time (days)	No. of mice	Mean activity (in units)	S.E.
0[a]	3	72.7	7.4
1	3	75.7	3.8
3	4	85.3	3.3
5	4	100.0	2.3
8	4	51.8	3.7
13	3	70.0	5.9

[a] No treatment.

Discussion

Mouse liver contains *S*-adenosylmethionine-synthesizing enzyme with the same properties as those first described by Cantoni for pig and rabbit liver. The occurrence of twice the amount of *S*-adenosyltransferase activity in the liver of female as opposed to male rat liver has been described by Natori (12). Rabbit liver did not show such a difference. However, 1 strain of mice (BALB/cJ) also showed little sex difference in the amount of enzyme activity. No explanation for these findings can be offered at this time.

The apparent absence of *S*-adenosyltransferase in hepatoma BW7756 is somewhat surprising since the turnover of sRNA is probably as great as in liver. However, the level of labile methyl groups produced by very low levels of *S*-adenosyltransferase may be adequate for methylation of sRNA. The capacity for *S*-adenosylmethionine formation in liver (in the range of μmoles/gm liver/hr) is large with respect to the amounts of *S*-adenosylmethionine present in liver (mμmoles/gm liver (1)). Other methylating reactions also occur, but any "excess" capacity of *S*-adenosylmethionine formation may function as a reserve for detoxification processes.

It appears that estrogen and testosterone (13) decrease *S*-adenosyltransferase in the female and male respectively, whereas there is an increase in RNA polymerase activity with estrogen (18) and testosterone (9). Depression of the levels of enzyme activity occurred in the mouse and possibly in the rabbit at their respective times of puberty and again suggests a hormonal control. The lack of enzyme activity in the fetal liver may be related to the maternal supply of *S*-adenosylmethionine. The continued rise in the amount of activity immediately after birth might be in response to the loss of the maternal source.

The finding of a barbital induced increase in *S*-adenosyltransferase may be explained if an *N*-methylation occurs on the barbital ring. The resultant depletion of the cellular "pool" of *S*-adenosylmethionine might in turn initiate an increase in *S*-adenosyltransferase synthesis.

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References

1. Baldessarini, R., and Kopin, I. Assay of Tissue Levels of S-Adenosylmethionine. Anal. Biochem., *6:* 289–92, 1963.
2. Bergquist, P., and Matthews, R. Occurrence and Distribution of Methylated Purines in the Ribonucleic Acids of Subcellular Fractions. Biochem. J., *85:* 305–12, 1962.
3. Brown, G., and Attardi, G. Methylation of Nucleic Acids in HeLa Cells. Federation Proc., *24:* 292, 1965.
4. Cantoni, G. Activation of Methionine for Transmethylation. J. Biol. Chem., *189:* 745–54, 1951.
5. Cantoni, G., and Durell, J. Activation of Methionine for Transmethylation. II. The Methionine-Activating Enzyme Studies on the Mechanism of the Reaction. *Ibid.*, *225:* 1033–48, 1957.
6. Coulombre, J., and Russell, E. Analysis of the Pleiotropism

at the *W*-locus in the Mouse. The Effects of *W* and W^v Substitution upon Postnatal Development of Germ Cells. J. Exptl. Zool., *126:* 277–96, 1954.
7. Fiske, G., and SubbaRow, Y. The Colorimetric Determination of Phosphorus. J. Biol. Chem., *66:* 375–400, 1925.
8. Fleissner, E., and Borek, E. A New Enzyme of RNA Synthesis: RNA Methylase. Proc. Nat. Acad. Sci. U. S., *48:* 1199–1203, 1962.
9. Hancock, R. L., Zelis, R., Shaw, M., and Williams-Ashman, H. G. Incorporation of Ribonucleoside Triphosphates into Ribonucleic Acid by Nuclei of the Prostate Gland. Biochim. Biophys. Acta, *55:* 257–60, 1962.
10. Mann, H., and Whitney, D. On a Test of Whether One or Two Random Variables is Stochastically Larger than the Other. Ann. Math. Statist., *18:* 52–54, 1947.
11. Mudd, S., and Cantoni, G. Activation of Methionine for Transmethylation. III. The Methionine-activating Enzyme of Bakers' Yeast. J. Biol. Chem., *231:* 481–91, 1958.
12. Natori, Y. Studies on Ethionine. VI. Sex-dependent Behavior of Methionine and Ethionine in Rats. *Ibid.*, *238:* 2075–80, 1963.
13. Parks, L., and Schlenk, F. The Stability and Hydrolysis of S-Adenosylmethionine: Isolation of S-Ribosylmethionine. *Ibid.*, *230:* 295–305, 1958.
14. Schlenk, F., and DePalma, R. The Formation of S-Adenosylmethionine in Yeast. *Ibid.*, *229:* 1037–50, 1957.
15. ———. The Preparation of S-Adenosylmethionine. *Ibid.*, *229:* 1051–57, 1957.
16. Short, R. Ovarian Steroid Synthesis and Secretion *in Vivo*. Recent Prog. Hormone Res., *20:* 303–40, 1964.
17. Warburg, O., and Christian, W. Isolation and Crystallization of Enolase. Biochem. Z., *310:* 384–421, 1942.
18. Weill, J., Bush, S., Chambon, P., and Mandel, P. The Effect of Estradiol Injections upon Chicken Liver Nuclei Ribonucleic Acid Polymerase. Biochem. Biophys. Res. Commun., *10:* 132–26, 1963.

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