sRNA Methylase Activity of Embryonic Liver[1]

ATP[2]:L-methionine S-adenosyltransferase catalyzes the reaction: ATP + L-methionine → SAM + PP + P (CANTONI[3]). SAM is utilized by sRNA methylase to methylate precursor sRNA by the following reaction: SAM + $sRNA^p$ → $sRNA^m$ + SAH (FLEISSNER and BOREK[4]). In a previous study on the quantitative changes of S-adenosyltransferase activity in mouse liver, large differences were found in livers from mice of different ages (HANCOCK[5,6]). In near term fetal liver only trace activities were present, but 1-day-old mice had significant activity which increased linearly until puberty. Thereafter, the activity declined until 35 days of age and remained constant for the rest of the lifetime. Similar studies with the rabbit showed the same changes from the embryo to the adult. Investigations began on a related enzymatic reaction, namely the methylation of sRNA with SAM, it became apparent that embryonic tissues in general contained high amounts of sRNA methylase activity as compared with the adult counterpart. This report is a more detailed study of the effect of age upon the quantity of sRNA methylase in the liver.

C57BL/6J female mice and strain III rabbits were procured from The Jackson Laboratory. *E. coli* K 12 sRNA was purchased from General Biochemicals, Inc., Chagrin Falls, Ohio and SAM-$^{14}CH_3$ (50.2 mC/mmole) from the New England Nuclear Corp., Boston, Mass. The sRNA methylase activity was assayed according to the methods of SRINIVASAN and BOREK[7].

Supernatant fraction (100,000 *g*) of prenatal and postpartum C57BL/6J female mouse and strain III rabbit livers were assayed for their ability to incorporate methyl groups from SAM-$^{14}CH_3$ to sRNA. Mouse liver from the prenatal gestation age of 18 days to the postpartum age of 18 months was studied (Table). The fetal liver (18 days old) had 296% more activity than 10-day-old liver and an approximately tenfold increase in activity as compared with mouse liver from animals 4 months or older.

Amount of endogenous[a] and *E. coli* K12 sRNA-dependent methylase activity of 100,000 *g* supernatant fractions of livers from animals of varying ages[b]

Mouse (C57BL/6J)			Rabbit (strain III)		
	cpm/100 mg protein			cpm/100 mg protein	
Age (days)	− sRNA	+ sRNA	Age (days)	− sRNA	+ sRNA
− 3	806	3440	− 10	975	1988
− 2	500	1950	− 6	600	1800
1	315	1990	− 4	559	1395
5	304	1760	1	264	276
10	194	1160	8	282	370
12	179	1520	15	215	325
32	91	1200			
54	185	1147			
120	92	805			
150	49	163			
240	46	138			
330	74	269			
540	130	62			

[a] sRNA methylase activity upon sRNA molecules present in 100,000 *g* supernatant fraction without the addition of *E. coli* sRNA. [b] Tissues of fetal to 12 days of age represent pooled tissues from both sexes. All points thereafter represent individual female mice. Each rabbit value represents the average of 2 individuals of a given age except for the − 10 day value which represents pooled liver from 5 individuals.

Rabbit liver was investigated because it allowed studies of earlier fetal liver. Liver from prenatal gestation age of 21 days to postpartum age of 15 days was assayed. Fetal liver (21 days old) proved to be 536% more active than 8-day-old liver. These percentages are based on the methylation of sRNA which was dependent upon added *E. coli* sRNA. However, it is interesting that the endogenous activity is increased also in fetal preparations when compared to those from adult sources.

Both species (mouse and rabbit) clearly demonstrate a high prenatal activity followed by an immediate decline after birth. Fetal liver cells have been shown to have extremely low amounts of ATP: L-methionine S-adenosyltransferase activity when compared to adult liver, yet they have much greater sRNA methylase activity. The low S-adenosyltransferase activity present must be

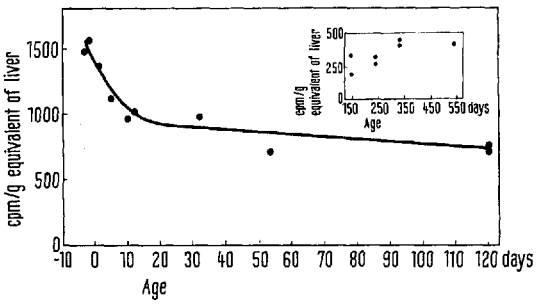

Fig. 1. sRNA methylase activity in livers from C57BL/6J mice of varying ages. Inset shows values obtained for older mice. See Table for the specific source of liver.

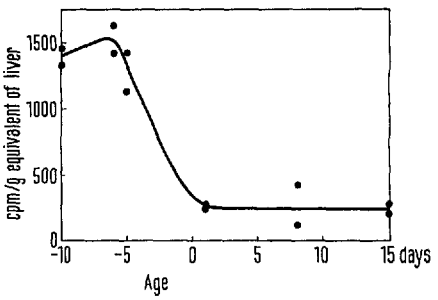

Fig. 2. sRNA methylase activity in livers from strain III rabbits of varying ages. Each value represents individual male or female rabbits except for the two 10-day values which are 2 assays on a pooled source of 5 livers.

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[2] The following abbreviations are used: ATP, adenosine triphosphate; sRNA, soluble ribonucleic acid; $sRNA^m$, methylated soluble ribonucleic acid; $sRNA^p$, precursor sRNA (sRNA prior to methylation); SAM, S-adenosyl-L-methionine; SAH, S-adenosyl-L-homocysteine; PP, pyrophosphate and P, monophosphate.

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adequate for the sRNA methylation of the growing fetal cell, if methylation is mandatory for normal protein synthesis. Perhaps the fetal cells have the ability to obtain methyl groups from another source – either maternal or via another pathway. It is quite conceivable that the sRNA methylase(s) of fetal liver is more diverse in its ability to methylate different sites on the sRNA molecule. Thus the increase in potential nucleotide sites is reflected in the increased efficiency and rate of methylation. The average sRNA molecule from embryonic liver may have more methyl groups than sRNA from adult liver. SIMON and GLASKY[8] have recently reported an increase in sRNA methylase activity in developing mammalian brain. RODEH et al.[9] showed that no increase in extent of methylation occurred using extracts of newborn rat liver. Our studies on the extent of methylation using fetal mouse liver as the source of sRNA methylase have shown a multifold increase in extent of methylation of *E. coli* sRNA by fetal liver preparations as compared with the adult liver fraction.

With the use of SAM-$^{14}CH_3$ it has been determined that fetal liver has large amounts of sRNA methylase activity in contrast to adult liver.

Résumé. Utilisant SAM-$^{14}CH_3$ il a été démontré que le foie foetal a plus d'activité de sRNA methylase que le foie adulte.

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The Influence of Antidepressant Drugs on Akinesia Produced in Mice by Intracisternally Administered Noradrenaline, Dopamine and Noradnamine

We have previously proposed that malfunction of catechol-O-methyl transferase (COMT) could be the biochemical disturbance responsible for endogenous depressive reactions, and have suggested an in vivo synthetic route whereby the excess deaminated metabolites of noradrenaline likely to occur under these conditions could be converted to noradnamine, a dibenzocycloheptatriene[1]. Subsequently it was pointed out that dopamine might also be able to produce this postulated depressive catabolite[2].

Reserpine induced sedation is antagonized by antidepressant drugs of both the monoamine oxidase (MAO) inhibitory and the iminodibenzyl groups[3–6], yet paradoxically catecholamines themselves cause loss of spontaneous motor activity when administered centrally to animals[7–9]. Since reserpine causes depletion of brain catecholamines predominantly via MAO[10] it seemed possible that the akinesia induced by reserpine and by the catecholamines could be mediated via a common deaminated metabolite such as our postulated noradnamine. If this were so, then noradrenaline, dopamine and noradnamine should all produce akinesia antagonizable by iminodibenzyl antidepressants; on the other hand only the loss of activity resulting from injections of noradrenaline and dopamine should be antagonized by MAO inhibitors. The present study is a test of this hypothesis.

Male albino mice (18–22 g) were placed individually into a corner of 1 of 4 boxes (55 × 33 cm) marked out into areas 11 × 11 cm and the number of lines the mice crossed in 5 min was recorded. Each mouse was then placed in a second box for 5 min and the mean of the 2 counts was used as the control activity. The mice were then given the drug under test by intracisternal injection under ether anaesthesia[11], and after 15 min rest in a neutral cage were again tested for periods of 5 min in the third and fourth boxes. The mean of these 2 counts gave the experimental activity of the mouse. The experiments were repeated in the presence of a MAO inhibitor (nialamide, 50 mg/kg, s.c. 18 h before the experiment) and a tricyclic antidepressant (amitriptyline, 3 × 50 mg/kg, orally 36, 24 and 12 h before the experiment).

Results were measured for each mouse as a percentage change in activity following the intracisternal injection and for each dose level the mean percentage change of 10 mice was calculated. These mean figures were plotted against the logarithm of the dose of injected amine and the calculated regression lines were subjected to a statistical analysis of variance.

The results are summarized in the Table. Pretreatment with amitriptyline, but not with nialamide, caused a highly significant ($p = 0.0001$) 33.75% reduction in activity.

All 3 amines induced akinesia in mice, but noradnamine was only half as potent as the equipotent dopamine and noradrenaline; in each case the loss of spontaneous motor activity was antagonized by both amitriptyline and nialamide. It must therefore be concluded that catecholamine induced akinesia in mice is not mediated via the formation of noradnamine. Several points of interest arise from our observations however. For example, the paradox that akinesia is produced both by the cate-

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