Transsulfuration in Mammals: Fetal and Early Development of Methionine-Activating Enzyme and its Relation to Hormonal Influences

H. PETER CHASE, JOSEPH J. VOLPE, and LEONARD LASTER

From the Section on Gastroenterology, Metabolic Diseases Branch, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland 20014

ABSTRACT The development of activity of methionine-activating enzyme was studied in four organs of the rat. Three different patterns were observed: (*a*) in the liver, specific activity began to increase in late fetal life and reached a maximum 2 days after birth; (*b*) in the small intestine, specific activity began to rise in the 2nd wk after birth and reached a maximum at age 18 days; and (*c*) in the brain and kidney, specific activity did not change markedly from the earliest stage of fetal development studied to adult life. Hydrocortisone increased hepatic methionine-activating enzyme activity as much as 55% in the young rat. However, adrenalectomy in the newborn rat did not prevent the postnatal rise in hepatic methionine-activating enzyme activity, nor did adrenalectomy at age 10 days prevent the developmental rise of intestinal activity at age 18 days. Conjugated estrogens partially inhibited both the neonatal rise in hepatic methionine-activating enzyme activity and the rise in activity after adrenal steroid injection. Injection of L-methionine did not increase hepatic methionine-activating enzyme activity in the developing or adult rat.

This work was published in part in Abstracts of Society for Pediatric Research, 28–29 April 1967.

Drs. H. Peter Chase and Joseph J. Volpe were Research Associates with the National Institute of Child Health and Human Development, working in the National Institute of Arthritis and Metabolic Diseases during the course of this study.

Address requests for reprints to Leonard Laster, Clinical Center 8N-240, National Institutes of Health, Bethesda, Md. 20014.

Received for publication 20 March 1968 and in revised form 29 May 1968.

INTRODUCTION

Methionine-activating enzyme (*S*-adenosylmethionine synthase; methionine adenosyltransferase; adenosine triphosphate: L-methionine *S*-adenosyltransferase, EC 2.5.1.6.) catalyzes reaction 1.

$$\text{Methionine} + \text{ATP} \rightarrow S\text{-adenosylmethionine} + \text{PP}_i + \text{P}_i \quad (1)$$

The reaction, which generates *S*-adenosylmethionine, a major donor of methyl groups for transmethylation, is the first step in the transsulfuration pathway through which methionine gives rise to cysteine. The essential irreversibility of the reaction suggests the possibility that it might be an important site of action for regulatory factors related to this pathway. In the present study, we examined the fetal and neonatal development of activity of methionine-activating enzyme in rat liver, intestine, kidney, and brain. Hormonal and nutritional influences on the development of the enzyme activity were also investigated.

Results from two other laboratories bear on the present investigations. In a limited study of the development of hepatic methionine-activating enzyme in the mouse, Hancock (1) found a rapid rise after birth from trace activities in the near-term fetus to a peak value at age 21 days. Recently Finkelstein (2) reported significantly higher hepatic methionine-activating enzyme activity in the

suckling and weanling rat than in the adult. He did not, however, delineate the developmental pattern in the fetus or in the animal during the 1st wk of life.

Other studies of postnatal changes in activity of enzymes associated with amino acid metabolism were concerned with hepatic enzymes involved in degradation of phenylalanine (3–5) and tryptophan (6) in the rat. Phenylalanine hydroxylase activity increases between the 2nd and 4th days after birth (3), tyrosine transaminase activity increases during the first 12 hr (4) and *p*-hydroxyphenylpyruvic acid oxidase activity does not increase until after the 1st wk (5). Tryptophan pyrrolase activity begins to rise at 16 days after birth and reaches a peak at 24 days in the rat (6); the rise starts at an earlier age in the guinea pig and rabbit (6). In the rat, the rise in tyrosine transaminase activity depends on the presence of an intact adrenal gland (4), but the rise in tryptophan pyrrolase activity does not (7).

We found that the specific activity of methionine-activating enzyme in liver and intestine increases after birth, whereas in kidney and brain it does not. Adrenalectomy did not prevent the postnatal rise in liver and intestine, although injection of adrenal corticosteroid increased hepatic activity of methionine-activating enzyme in the young rat.

METHODS

Procedures. Rats of the Sprague-Dawley strain were used in all experiments except one in which white Swiss mice were used. The term "adults" refers to 200–300 g male animals. In all studies other than those in which the developmental patterns were determined, single litters were used with litter mates serving as control animals. Adrenalectomies were performed with animals under ether anesthesia. A longitudinal 2–3-cm dorsal skin incision was made over the vertebral column, and the adrenal glands were removed through bilateral 1–2-cm intraperitoneal incisions at the costovertebral angles. Control litter mates were subjected to similar surgery without removal of the adrenal glands. In experiments involving administration of compounds to fetal animals, intraperitoneal incisions were performed with the mother under ether anesthesia. Injections were made through the uterine wall into fetal subcutaneous tissue, and the animals were delivered by cesarian section 6 or 24 hr after injection. Injections of hormones and methionine to postnatal animals were subcutaneous (unless otherwise stated) and control litter mates were similarly injected with 0.15 M NaCl solution. Animals were killed by decapitation and tissues were removed for immediate analysis.

Tissues were homogenized in ice cold 0.03 M potassium phosphate buffer (pH 6.9). Ratios of buffer to tissue were 13 ml/g for liver, 6 ml/g for kidney and intestine, and 1.5 ml/g for brain. Homogenization was performed at 3°C with a Potter-Elvehjem homogenizer fitted with a Teflon pestle. Whole homogenate was used in the studies of liver methionine-activating enzyme activity and the supernatant solution obtained by centrifugation at 10,000 *g* for 30 min was used to study activity in the brain, kidney, and intestine. With each of these tissues, methionine-activating enzyme was recovered completely in the supernatant solution. The entire small intestine was used in the experiments that established the developmental pattern; in other experiments only the proximal third of the small intestine was used. In all experiments, the intestinal lumen was washed with ice cold 0.03 M potassium phosphate buffer (pH 6.9) before homogenization. All layers of the intestinal wall were used, except in one experiment in which activities of mucosal scrapings and remaining layers of intestinal wall were studied individually.

The standard assay mixture (8, 9) contained, in μmoles: Tris-HCl buffer (pH 7.6), 69.5; KCl, 490; $MgCl_2$, 120; ATP, 7.2; neutralized glutathione, 1.6; (−)-*S*-adenosyl-L-methionine, 0.12; L-methionine-$^{14}CH_3$, 0.061, containing 200–220 × 10^3 cpm; and tissue extract, all in a total volume of 0.5 ml. For the studies of liver extracts the amount of L-methionine was increased to 0.176 μmole. Incubations were carried out at 37°C, in air, for 30 min. Reactions were stopped and radioactive *S*-adenosylmethionine was isolated as previously described (8). It was dissolved in 0.5 ml of water, the standard counting solution (8) was added, and radioactivity was determined with a Packard Tri-Carb liquid scintillation spectrometer. A blank value for an incubation with heat-inactivated enzyme was determined routinely and subtracted from the experimental value. For each tissue studied it was shown that *S*-adenosylmethionine formation was a linear function of incubation time and of the amount of tissue added under conditions of the standard assay.

A unit of activity is defined as that amount of enzyme needed to catalyze the formation of 1 mμmole of *S*-adenosylmethionine in 30 min under the standard assay conditions. Specific activity is defined as the units per milligram of nitrogen. Nitrogen was determined by the Kjeldahl method (10). In a limited number of experiments, methionine-activating enzyme activity was also determined by a spectrophotometric assay (11) to verify results obtained with the radioactive assay. Alkaline phosphatase was assayed according to King and Armstrong (12), except that liberated inorganic phosphate was measured by the method of Chen, Toribara, and Warner (13).

Materials. L-Methionine-$^{14}CH_3$,[1] L-methionine,[2]

[1] Nuclear-Chicago Corp., Des Plaines, Ill.

[2] Mann Research Laboratories, Inc., N. Y.

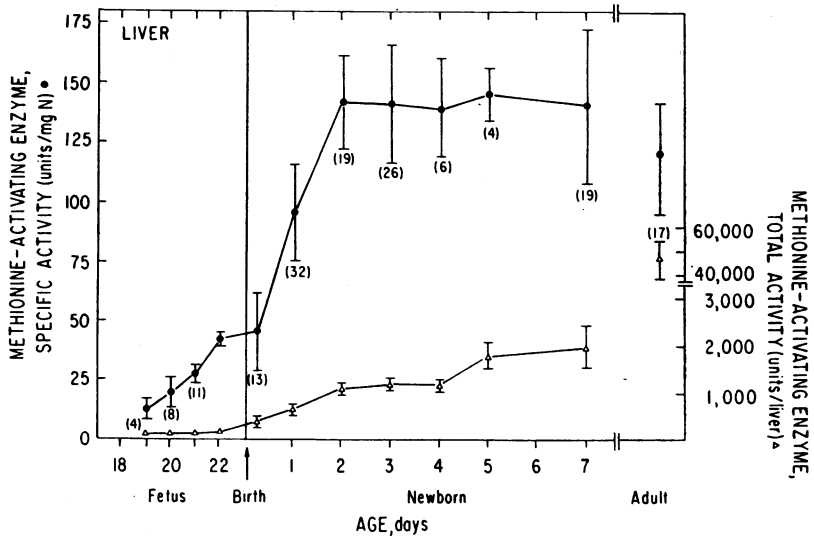

FIGURE 1 Activity of methionine-activating enzyme in fetal, newborn, and adult rat liver, expressed as specific activity, ●——●, and as total activity/liver, △——△. Vertical bars represent 1 SD, and number of animals studied at each age is shown in parentheses. Because accurate gestational ages of fetuses were not known, the fetus from each mother was weighed at delivery, and the age in days for each fetal weight was extrapolated from published tables (14).

S-adenosylmethionine,[3] and ATP [4] were obtained commercially, as were most of the hormones: hydrocortisone sodium succinate (Solu-Cortef);[5] corticosterone, estrone-3-sulfate;[4] conjugated estrogens (Premarin);[6] testosterone (Oreton);[7] deoxycorticosterone acetate,[8] and progesterone.[9] Triiodothyronine and growth hormone were gifts from Dr. Hans J. Cahnmann and the National Institutes of Health Endocrinology Study Section, respectively.

RESULTS

Development of methionine-activating enzyme activity in the liver, small intestine, kidney, and brain. The patterns of development of methionine-activating enzyme activity in the liver, small intestine, kidney, and brain are shown in Figs. 1 and 2. In the fetus, the specific activity of hepatic methionine-activating enzyme is low, but it increases progressively until birth. After birth, the rate of increase rises sharply so that there is a 3- to 4-fold change during the first 48 hr of postnatal life. The specific activity then remains constant at least until age 30 days, but by adulthood it decreases slightly. In contrast to specific activity, the total activity in the liver continues to increase with liver growth until adult life. Studies in which the spectrophotometric assay for methionine-activating enzyme was used confirmed the postnatal rise in hepatic activity as demonstrated by the radioactive assay.

The specific activity of intestinal methionine-activating enzyme (Fig. 2) is low in the fetus and neonatal animal, but it begins to rise at age 7 days and by 18 days it increases 4- to 5-fold. The specific activity in the adult small intestine is reduced to one-half that of the 18–20 day old animal. The reduction is probably not attributable to differences in intestinal protein concentration between the young and adult animals, since the determined values for milligrams of nitrogen per unit wet weight of intestine were similar. The total enzyme activity in the small intestine is very low in the newborn animal and increases 100-fold during the first 3 wk of postnatal life. The increase continues into adult life, but at a slower rate.

[3] CalBiochem, Los Angeles, Calif.
[4] Sigma Chemical Co., St. Louis, Mo.
[5] Upjohn Company, Kalamazoo, Mich.
[6] Ayerst Laboratories, N. Y.
[7] Schering Corp., Bloomfield, N. J.
[8] Nutritional Biochemicals Corp., Cleveland, Ohio.
[9] Eli Lilly and Company, Indianapolis, Ind.

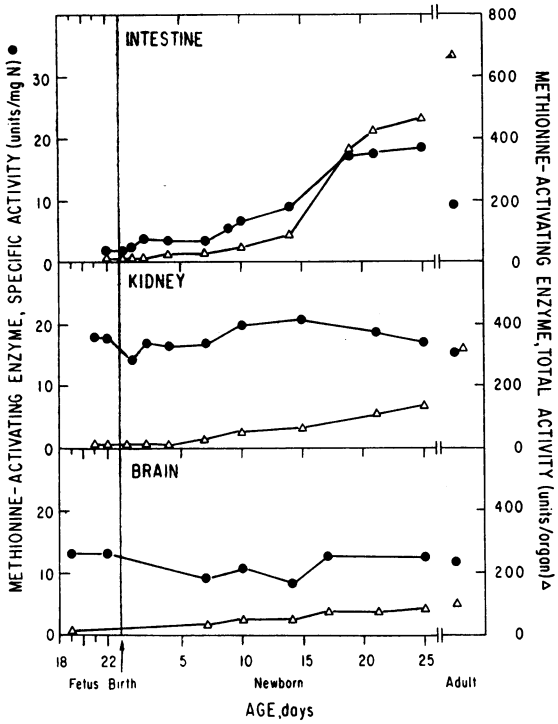

FIGURE 2 Activity of methionine-activating enzyme in fetal, newborn, and adult rat intestine, kidney and brain, expressed as specific activity, ●——●, and as total activity/organ, △——△. Each point represents the mean of values obtained from studies of two to seven animals. Fetal age was determined as described in the legend to Fig. 1. Since the enzyme preparation (10,000 *g* supernatant of tissue homogenate) and the conditions of the enzyme assay (0.061 μmole of L-methionine) we used for these three tissues differed from those used for liver (whole homogenate; 0.174 μmole of L-methionine), the results cannot be strictly compared with those in Fig. 1.

In the fetus, at the earliest time studied, the specific activity of methionine-activating enzyme in kidney and brain was about four times higher than that of intestine. No marked postnatal changes in specific activity were detected in either organ, but total activity increased postnatally in both due to growth of these organs. Total activity in kidney increased 60-fold from neonatal to adult life. Total activity in the brain increased 4-fold between birth and age 18 days, the period of rapid brain growth in the rat, but then it remained constant into adult life.

The gastrointestinal tract and brain were selected for more detailed comparisons of methionine-activating enzyme activity in young and adult animals (Table I). The specific activity throughout the gastrointestinal tract is uniformly lower in the adult than in the 20 day old rat. At both ages, the large intestine has the highest activity. In the small intestine, the activity in the mucosa is lower than in the remaining layers of the bowel wall. In the brain of the 20 day old animal, the cerebellum has greater activity than other regions, but this is not true of the adult. Other brain regions have similar levels of activity at the two ages.

Effect of adrenal corticoids on methionine-activating enzyme. Since adrenal corticoids have been shown to exert significant influences on the development of other enzymes involved in amino acid metabolism, we investigated possible relations between adrenal gland function and patterns of development of methionine-activating enzyme activity. The normal rise in hepatic methionine-

TABLE I

Methionine-Activating Enzyme Activity in Regions of Gastrointestinal Tract and Brain of Young and Adult Rats

Organ and region*	Methionine-activating enzyme	
	20-day animal	adult
	units/mg nitrogen	
Gastrointestinal tract		
Esophagus (full thickness, entire length)	7.7	3.2
Stomach (full thickness, entire length)	16.6	8.1
Small intestine (entire length)		
Mucosal scrapings	10.0	4.3
Remainder of bowel wall	14.0	9.4
Small intestine (full thickness)		
Proximal third	20.0	8.8
Middle third	17.8	8.1
Distal third	16.2	8.3
Large intestine (full thickness, entire length)	33.0	16.4
Cecal contents		none detected
Brain		
Entire organ	12.5	11.0
Cerebral gray matter	12.5	10.0
Cerebral white matter	8.9	8.2
Cerebellum	19.7	11.6
Brain stem	10.0	10.8

* Assays were performed on tissues from the same animal except for small intestine, entire length, and brain, entire organ, for which tissues from a different animal were used.

activating enzyme activity during the first day after birth was unaffected by adrenalectomy in the 1st hr, or by administration of an inhibitor of adrenal gland steroid biosynthesis (15), amphenone (0.5 mg/animal per injection in single or repeated injections), in utero and during the first day of life. The rise in methionine-activating enzyme activity in the small intestine between 7 and 18 days was unaffected by adrenalectomy at age 10 days.

The effect of adrenalectomy was studied in adult rats to learn whether adrenal secretions contribute to maintenance of activity of methionine-activating enzyme in the fully developed animal. 2 days after adrenalectomy, hepatic methionine-activating enzyme activity was slightly lower in the experimental animals than in sham-operated control animals, but by 3 days hepatic activity increased toward control values. Intestinal activity was not affected.

The parenteral administration of hydrocortisone did not alter fetal or adult hepatic methionine-activating enzyme activity, but it did produce a significant stimulation in animals between 1 and 12 days of age (Table II). Preliminary experiments revealed that in the developing rat the maximum response to hydrocortisone occurs 24 hr after injection and decreases by 48 hr. We did not determine when this responsiveness of the young animal to hydrocortisone disappears.

To compare the effects of two different adrenal corticoids, and of different routes of administration, and to evaluate the responsiveness of tissues other than the liver, we injected 10-day old animals as shown in Table III. A higher dose of

TABLE II

*Hepatic Methionine-Activating Enzyme Activity after Hydrocortisone Administration**

Age of rat at sacrifice	Hepatic methionine-activating enzyme‡			
	Control (a)	Experimental (b)	Significance of difference	Stimulation $\frac{(b) - (a)}{(a)} \times 100$
days	*units/mg nitrogen*		*P*	%
Fetus (5 g)	27 ± 2 (4)	25 ± 3 (4)		none
1	67 ± 5 (4)	89 ± 10 (4)	<0.01	33
2	164 ± 5 (4)	206 ± 12 (4)	<0.005	26
4	115 ± 7 (3)	178 ± 2 (3)	<0.001	55
8	122 ± 6 (4)	176 ± 5 (3)	<0.001	44
12	99 ± 8 (3)	132 ± 6 (3)	<0.02	33
Adult	96 ± 15 (5)	108 ± 3 (3)		none
Adult	103 ± 12 (3)	106 ± 4 (3)		none

* Hydrocortisone sodium succinate was injected subcutaneously 24 hr before sacrifice in a dose of 0.5 mg/animal in young animals and 30 mg/kg (approximately 6 mg/animal) in adult animals. The second set of data for adult animals was obtained after intraperitoneal injection of the hydrocortisone.

‡ Values represent the mean ± 1 SD. The number of animals studied is shown in parentheses. Significance was determined by Student's *t* test (16).

TABLE III

Methionine-Activating Enzyme Activity in Liver, Intestine, Kidney, and Brain after Injection of Different Preparations of Adrenal Steroids in Rats and Mice

Animal, age 10 days	Adrenal steroid* and route of injection	Time after steroid injection	Methionine-activating enzyme							
			Liver		Intestine		Kidney		Brain	
			Control	Experiment	Control	Experiment	Control	Experiment	Control	Experiment
		hr	*units/mg nitrogen*							
Rat	Hydrocortisone sodium succinate, subcutaneous	24	158, 162	292, 296	7, 7	7, 7	20, 20	20, 20	11, 11	10, 11
Rat	Hydrocortisone sodium succinate, intraperitoneal	24	150, 156, 162	200, 235, 237	9, 10, 10	11, 11, 12	18, 19, 19	19, 20, 20	10, 10, 11	10, 10, 10
Rat	Corticosterone, subcutaneous	24	126, 142, 146	190, 204, 230	6, 9, 13	10, 10, 12	21, 24, 25	24, 26, 32	10, 11, 14	8, 10, 11
Mouse	Hydrocortisone acetate, intraperitoneal	24 48	212, 223 236	384, 388 449, 473	8, 10, 10	7, 8 8, 9	16, 19, 26	34, 41 30, 32	9, 10, 10	10, 10 8, 10

* Experimental animals were injected with 0.5 mg adrenal steroid/5 g body weight (approximately 2.5 mg/animal), and control litter mates were injected with a similar volume of 0.15 M NaCl solution.

adrenal corticoids was used in these experiments than in those referred to in Table II. It was possible to stimulate hepatic methionine-activating enzyme activity by as much as 95% (see mouse, 48 hr, Table III). The stimulation by hydrocortisone of rat hepatic methionine-activating enzyme activity appeared to be somewhat greater when the steroid was administered subcutaneously rather than intraperitoneally. The stimulation by corticosterone was somewhat less than that by hydrocortisone. The other rat tissues studied did not respond to hydrocortisone, regardless of the route of injection, or to corticosterone.

Since it is known that administration of 0.5 mg/5 g of body weight of hydrocortisone acetate to the 10 day old mouse can induce a premature rise in the alkaline phosphatase activity of the duodenum (17), we studied this animal (Table III). After hydrocortisone acetate injection, methionine-activating enzyme activity in the small intestine did not increase, even though intestinal phosphatase activity increased approximately 50% by 2 days after the steroid injection. Methionine-activating enzyme activity was stimulated in mouse liver and kidney, but not in brain.

Studies of other hormones. In investigations of the effects of growth hormone, progesterone, triiodothyronine, and testosterone on fetal and young rats, we found no significant change in hepatic methionine-activating enzyme activity. Growth hormone (1.0 mg/animal) was administered to the 3 g fetus, and the newborn and 4-day old animals, and the livers were studied 5 and 24 hr later. When the same dose was given daily for the first 3 days of life, there was still no effect. Single doses of triiodothyronine (0.05 mg to the 4 g fetus and 5- and 7-day old animals), of testosterone (1.0 mg to the 5 g fetus and newborn and 5-day old animals), or of progesterone (0.5 mg to the 4 g fetus and 5-day old animal) were found to have produced no significant effect 24, 48, or 72 hr after injection.

Investigations of possible inhibitors of hepatic methionine-activating enzyme activity in fetal and newborn rats. The failure of adrenalectomy to influence the early neonatal rise in hepatic methionine-activating enzyme activity suggested that factors other than adrenal gland secretions contributed to this developmental change. To determine whether fetal liver contains a dissociable inhibitor of methionine-activating enzyme activity accounting for the low activity, homogenates of fetal and adult liver were assayed separately and in combination. The results were additive, and no inhibition by fetal liver homogenate was detected. Similar studies were performed with homogenates of liver from fetal and 2-day old animals, and no evidence was found to suggest the presence of a dissociable factor in the 2 day postpartum liver that stimulated methionine-activating enzyme activity.

The rapidity of the rise in hepatic methionine-activating enzyme activity immediately after birth raises the question whether fetal liver is exposed in utero to an inhibitory factor. Several observations tend to implicate estrogens as a possible inhibitory factor. The hepatic methionine-activating enzyme activity of the adult female rat 48 hr postpartum (Table IV, experiment 1, control column) is about twice that of other adult animals. When mothers were injected with conjugated estrogens immediately after delivery and again 24 hr later, hepatic methionine-activating enzyme activity was reduced 50% by 48 hr after delivery (Table IV, experiment 1).

Fetal liver is constantly perfused by placental estrogens which are immediately conjugated by the liver (18). With delivery, the exposure to placental estrogens and their conjugates subsides, and because of the possibility that this abrupt change could account for the rise in hepatic methionine-activating enzyme activity, we studied the effect of large doses of conjugated estrogens on the newborn rat (Table IV). Both conjugated estrogens as a group (experiments 2 and 3) and the higher dose (experiment 5) of estrone sulfate, the major constituent of the preparation of mixed conjugated estrogens, partially inhibited. However, the effect of conjugated estrogens was not of such magnitude as to suggest a major role in the regulation of fetal hepatic methionine-activating enzyme activity. Daily administration of conjugated estrogens for 5 days exerted no major effect on the newborn's hepatic methionine-activating enzyme activity (experiment 6). A more marked action of conjugated estrogens was seen when we examined the effect of a large dose on the stimulation of hepatic methionine-activating enzyme activity induced by hydrocortisone (ex-

TABLE IV

Methionine-Activiting Enzyme Activity after Subcutaneous Injection of Conjugated Estrogens

Experiment	Age of rat at sacrifice	Hormone and dose	Interval between injections and sacrifice	Methionine-activating enzyme*		Significance of difference
				Control	Experimental	
		mg/animal	*hr*	*units/mg nitrogen*		*P*
1	Adult female‡	Conjugated estrogens 0.8	24 and 48	267±26 (5)	123±35 (6)	<0.005
2	10 hr	Conjugated estrogens 0.2	10 and 16§	87±10 (3)	60±9 (5)	<0.02
3	10 hr	Conjugated estrogens 0.4	10 and 16	116±7 (3)	97±2 (4)	<0.005
4	10 hr	Estrone sulfate 0.5	10 and 16	70±5 (4)	72±8 (5)	
5	10 hr	Estrone sulfate 1.0	10 and 16	102±16 (4)	85±5 (4)	>0.05
6	5 days	Conjugated estrogens 0.5	24, 48, 72, 96, 120	174±10 (3)	159±14 (3)	>0.05
7	4 days‖	Conjugated estrogens 0.5	24	116, 119	82, 97, 107	
		Hydrocortisone 0.5	24		145, 173	
		Conjugated estrogen 0.5 plus hydrocortisone 0.5	24		103, 116, 117	

* Values represent the mean ± 1 SD. The number of animals studied is shown in parentheses.

‡ Adult female rats were 48 hr postpartum, and were injected immediately after delivery and 24 hr later. Control mothers were pair-fed and injected with 0.15 M NaCl.

§ Because of the late fetal rise in activity, animals were injected 6 hr before and immediately after delivery by cesarian section.

‖ Litter mate animals were used in this study. When both hydrocortisone and conjugated estrogens were given to the same animal, separate injection sites were used.

periment 7). The stimulation by hydrocortisone was diminished.

Estrone sulfate and the mixture of conjugated estrogens were tested in vitro at final concentrations of 10^{-6} and 10^{-4} mole/liter, and were not found to inhibit hepatic methionine-activating enzyme.

Nutrition and development of methionine-activating enzyme activity. When rats were fasted from birth, no effect on hepatic methionine-activating enzyme activity was seen at 24 hr, but by 48 hr the activity in the fasted animals was markedly lower than that of the control animals.

The parenteral injection of L-methionine (1.5 mg/g body weight 24 hr before sacrifice) failed to exert a consistent marked influence on hepatic methionine-activating enzyme activity in fetuses, neonatal animals, or adults. Animals fed cow's milk between 7 and 9 days had the same methionine-activating enzyme activity in liver and small intestine as did animals allowed to nurse with their own mother; supplementation of the cow's milk with L-methionine (5 mg/g body weight per day) did not change these results.

To examine the possibility that the rise in intestinal methionine-activating enzyme activity did not begin immediately after birth because mother's milk contains an inhibitory factor during the postpartum period, we transferred half of a litter of 10-day old animals to a mother who was 24 hr postpartum and left the remaining animals with the original mother. At age 18 days, the litter mates nursed by the two different mothers had the same activity of methionine-activating enzyme in the small intestine.

DISCUSSION

The specific activity of methionine-activating enzyme in the rat exhibits three different developmental patterns in the four organs studied. In liver, specific activity begins to increase late in fetal life and reaches a peak 2 days after birth; in small intestine, activity does not begin to climb until the 2nd wk after birth, and reaches a peak at age 18 days; whereas in brain and kidney, activity does not change markedly from the earliest fetal age studied to adult life. Finkelstein's (2) studies of brain and kidney from suckling to adult rats also revealed no marked change in specific activity of methionine-activating enzyme. Specific activity in liver increases just before the period of the animal's most rapid rate of growth. Since methio-

nine activation results in the formation of *S*-adenosylmethionine, a major substrate for the body's methylation reactions, and since the transsulfuration pathway generates cysteine for protein synthesis, the rise in liver specific activity would appear to be occurring at an appropriate time in postnatal development. This rise occurs slightly earlier than the developmental rise of hepatic phenylalanine hydroxylase activity (1), which takes place 2–4 days after birth, and much earlier than the rise in hepatic tryptophan pyrrolase activity (4), which occurs 15 to 24 days after birth in the rat. In the mouse, hepatic methionine-activating enzyme activity rises rapidly after birth from trace amounts in the near-term fetus to a peak at 21 days (1).

The increase of methionine-activating enzyme activity in small intestine during the 2nd and 3rd wk after birth, parallels the rise of intestinal activities for alkaline phosphatase (17) and α-disaccharidases (19, 20). The increase in intestinal methionine-activating enzyme activity occurs just before the time of weaning, after which there is a great increase in methionine intake.

Although specific activity in kidney and brain does not change significantly from the fetal to the adult animal, total brain activity increases 4-fold from birth to age 18 days, and total kidney activity increases 60-fold from fetal life to adulthood, thus greatly altering the capacity of these organs to metabolize methionine.

The mechanism of regulation of the developmental increase in specific activity of methionine-activating enzyme in the liver and small intestine remains uncertain. It has been shown (4) that the adrenal gland is important in the neonatal development of hepatic tyrosine transaminase activity but is not necessary for the development of hepatic tryptophan pyrrolase activity (7). Our studies did not demonstrate a need for an intact adrenal gland for the developmental rise of methionine-activating enzyme activity in either liver or intestine.

Under the conditions of the present study, administration of hydrocortisone to the young rat significantly increases activity of hepatic methionine-activating enzyme, but has no effect in the fetal or adult rat. No change in activity of methionine-activating enzyme in intestine, kidney, or brain was seen after injection of hydrocortisone into young rats. The use of different routes of injection, or of different preparations of adrenal corticoids, including corticosterone, the major adrenal steroid produced in the rat (21), all failed to induce a rise in these three organs, but did stimulate hepatic methionine-activating enzyme activity. The explanation for the differences in hepatic responsiveness to hydrocortisone at different stages of development, or for the differences in responsiveness of the several tissues studied is not known. Possibly isoenzymes for methionine-activating enzyme exist and are in some way related to the different responses. Intestinal alkaline phosphatase has been shown to be present in a form in the young mouse different from that of older mice (22), and mouse kidney alkaline phosphatase has different properties from mouse intestine phosphatase (23).

We also observed species differences in responsiveness to hydrocortisone. In contrast to the rat where no change occurred, mouse kidney methionine-activating enzyme activity doubled after hydrocortisone injection. Liver and intestine in the mouse responded in the same way they did in the rat.

In considering factors responsible for the increase in hepatic methionine-activating enzyme activity in the newborn rat, conjugated estrogens were studied as possible intrauterine inhibitors of methionine-activating enzyme activity. The placenta produces large quantities of estrogens, these are immediately converted by fetal liver to conjugated derivatives (18), and the disappearance of estrogens near the time of delivery coincides with the period of rise of hepatic methionine-activating enzyme activity in late fetal and early neonatal life. When large doses of conjugated estrogens were injected into fetal and newborn rats, partial, but not complete, inhibition of the neonatal development of hepatic activity consistently resulted. A high dose of conjugated estrogens also prevented the increase in hepatic methionine-activating enzyme activity produced by hydrocortisone in the 4 day old rat. Apparently conjugated estrogens could contribute to the regulation of hepatic methionine-activating enzyme activity in the fetal and newborn rat but other factors are certainly involved.

The mechanism through which conjugated estrogens might act has not been elucidated. They have

been shown to inhibit in vitro the activity of adult rat kidney kynurenine transaminase (24). The mechanism of inhibition has been suggested to involve competition for apoenzyme between the estrogens and the coenzyme, pyridoxal phosphate. Pyridoxal phosphate is not a cofactor for methionine-activating enzyme, and we could not demonstate inhibition in vitro by conjugated estrogens.

In the present study, a single injection of L-methionine into rats of various ages did not induce a rise in hepatic methionine-activating enzyme activity. In a previous study (9) of young adult rats fed a low sulfur diet, 3 days of L-methionine injections were followed by only small increases in hepatic methionine-activating enzyme activity, back up to levels observed before institution of the diet. The failure of hepatic methionine-activating enzyme activity to increase after an injection of L-methionine contrasts with results of studies of the effects of substrate on tyrosine transaminase (25) and tryptophan pyrrolase (26) activities. The feeding of L-methionine to rats before they were weaned failed to induce a premature rise in intestinal activity of methionine-activating enzyme. Moreover, it is doubtful that the neonatal pattern of development of methionine-activating enzyme activity in rat liver is related to methionine intake, since the rise still occurred in animals fasted from birth to age 24 hr. The fall in activity after the animal was fasted for 48 hr from birth probably is attributable to a lack of adequate precursors for protein synthesis. Thus, methionine-activating enzyme appears to respond to hormone induction, but not to substrate induction. Studies of tryptophan pyrrolase activity (7) have suggested that the mechanism of response of that enzyme to adrenal corticoids is dependent on ribonucleic acid synthesis, whereas its response to excess substrate may involve a reaction closer to protein synthesis than formation of messenger ribonucleic acid. Perhaps similar considerations apply to the hormonal stimulation of methionine-activating enzyme.

ACKNOWLEDGMENT

The authors wish to thank Dr. W. C. Alford, Laboratory of Chemistry, Nataional Institute of Arthritis and Metabolic Diseases, for performing the nitrogen determinations.

REFERENCES

1. Hancock, R. L. 1966. S-Adenosylmethionine-synthesizing activity of normal and neoplastic mouse tissue. *Cancer Res.* **26**: 2425.
2. Finkelstein, J. D. 1967. Methionine metabolism in mammals. Effects of age, diet, and hormones on three enzymes of the pathway in rat tissues. *Arch. Biochem. Biophys.* **122**: 583.
3. Reem, G. H., and N. Kretchmer. 1957. Development of phenylalanine hydroxylase in liver of the rat. *Proc. Soc. Exptl. Biol. Med.* **96**: 458.
4. Sereni, F., F. T. Kenney, and K. Kretchmer. 1959. Factors influencing the development of tyrosine-α-ketoglutarate transaminase activity in rat liver. *J. Biol. Chem.* **234**: 609.
5. Goswami, M. N. D., and W. E. Knox. 1961. Developmental changes of p-hydroxyphenylpyruvate-oxidase activity in mammalian liver. *Biochim. Biophys. Acta.* **50**: 35.
6. Nemeth, A. M. 1959. Mechanisms controlling changes in tryptophan peroxidase activity in developing mammalian liver. *J. Biol. Chem.* **234**: 2921.
7. Greengard, O. 1963. The role of coenzymes, cortisone and RNA in the control of liver enzyme levels. *In* Advances in Enzyme Regulation. G. Weber, editor. Pergamon Press, New York. **1**: 61.
8. Mudd, S. H., J. D. Finkelstein, F. Irreverre, and L. Laster. 1965. Transsulfuration in mammals. Microassays and tissue distributions of three enzymes of the pathway. *J. Biol. Chem.* **240**: 4382.
9. Finkelstein, J. D., and S. H. Mudd. 1967. Transsulfuration in mammals. The methionine-sparing effect of cystine. *J. Biol. Chem.* **242**: 873.
10. Ma, T. S., and G. Zuazaga. 1942. Micro Kjeldahl determination of nitrogen. A new indicator and an improved rapid method. *Ind. Eng. Chem.* **14**: 280.
11. Cantoni, G. L., and J. Durell. 1957. Activation of methionine for transmethylation. II. The methionine-activating enzyme: studies of the mechanism of the reaction. *J. Biol. Chem.* **225**: 1033.
12. King, E. J., and A. R. Armstrong. 1934. A convenient method for determining serum and bile phosphatase activity. *Can. Med. Assoc. J.* **31**: 376.
13. Chen, P. S., Jr., T. Y. Toribara, and H. Warner. 1956. Microdetermination of phosphorus. *Anal. Chem.* **28**: 1756.
14. Donaldson, H. H. 1924. The Rat, Data and Reference Tables. The Wistar Institute of Anatomy and Biology, Philadelphia. 173.
15. Peterson, R. E., R. Hertz, and H. A. Lubs. 1957. Suppression of biosynthesis of adrenal cortical steroids in man by amphenone. *Proc. Soc. Exptl. Biol. Med.* **94**: 421.
16. Snedecor, G. W. 1956. Statistical Methods Applied to Experiments in Agriculture and Biology. Iowa State College Press, Ames. 5th edition. 45.
17. Moog, F., and E. R. Thomas. 1955. The influence of various adrenal and gonadal steroids on the accumula-

tion of alkaline phosphatase in the duodenum of the suckling mouse. *Endocrinology.* **56:** 187.
18. Levitz, M., G. P. Gordon, and J. Dancis. 1961. Sulfurylation of estrogens by the human fetus. *Endocrinology.* **68:** 825.
19. Doell, R. G.. and N. Kretchmer. 1964. Intestinal invertase: precocious development of activity after injection of hydrocortisone. *Science.* **143:** 42.
20. Rubino, A., F. Zimbalatti, and S. Auricchio. 1964. Intestinal disaccharidase activities in adult and suckling rats. *Biochim. Biophys. Acta.* **92:** 305.
21. Bush, I. E. 1953. Species differences in adrenocortical secretion. *J. Endocrinol.* **9:** 95.
22. Etzler, M. E., and F. Moog. 1966. Inactive alkaline phosphatase in duodenum of nursling mouse: Immunological evidence. *Science.* **154:** 1037.
23. Moog, F. 1965. Acceleration of the normal and corticoid-induced increase of alkaline phosphatase in the duodenum of the nursling mouse by Actinomycin D, puromycin, colchicine, and ethionine. *In* Advances in Enzyme Regulation. G. Weber, editor. Pergamon Press, New York. **3:** 221.
24. Mason, M., and E. H. Gullekson. 1960. Estrogen-enzyme interactions: inhibition and protection of kynurenine transaminase by the sulfate esters of diethylstilbesterol, estradiol, and estrone. *J. Biol. Chem.* **235:** 1312.
25. Lin, E. C. C., and W. E. Knox. 1957. Adaptation of the rat liver tyrosine-α-ketoglutarate transaminase. *Biochim. Biophys. Acta.* **26:** 85.
26. Feigelson, P., and O. Greengard. 1962. Immunochemical evidence for increased titers of liver tryptophan pyrrolase during substrate and hormonal enzyme induction. *J. Biol. Chem.* **237:** 3714.