Whether such is also the case in the Scheie syndrome could not be determined, for precise determination of low activity of the factor is difficult. Alternatively, the Scheie individuals may have compensatory mechanisms which prevent accumulation of MPS in certain tissues in spite of the absence of factor. Or the loss of activity of the factor may be of late onset, after some critical stage in growth and mental development has been passed. Discrimination between these and yet other possibilities must await studies of the structure and function of the normal factor and of its presumably altered counterparts in the Scheie and Hurler syndromes.

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17. We thank Dr. V. McKusick for biopsies of the Scheie patients and Dr. J. Derge for identification of the radioactive MPS.
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Absence of Cystathionase in Human Fetal Liver: Is Cystine Essential?

Abstract. *Cystathionase activity is not measurable in the livers of 24 human fetuses and 3 premature infants, and the concentration of cystathionine in the liver is higher than that of the brain. The placenta does not subserve the transsulfuration function. Cystine (or cysteine) thus may be an essential amino acid in the immature human.*

In the normal adult human, about 90 percent of ingested methionine is converted to cystine or cysteine (*1*), a nonessential amino acid, via the transsulfuration pathway (Fig. 1). Cystathionine, a key intermediate on this pathway, is normally present in high concentrations in human brain (*2*), is virtually absent from the brain of patients with homocystinuria due to a deficiency of cystathionine synthase activity (*3*), and is present in barely measurable quantities in human liver (*3*).

We have examined the development of the transsulfuration pathway in the human, including in this study: 24 fetuses (2.5 to 20.5 cm, crown-rump length), obtained at therapeutic abortions; 3 premature infants (birth weights of 830, 1060, and 1260 g), who died during the first 24 hours of life; and 1 full-term newborn, who died after 4 hours of life because of an intraabdominal hemorrhage secondary to a catheter accident.

The activities of methionine-activating enzyme (ATP : L-methionine *S*-adenosyltransferase, E.C. 2.5.1.6), cystathionine synthase (*4*), and cystathionase (*4*) were measured by previously described methods (*5*). The concentration of cystathionine was measured by minor modifications of the method of Spackman, Moore, and Stein (*6*).

Results of these determinations in crude extracts of liver from the human fetus, premature infant, and full-term infant are contrasted (Table 1) to previously determined values (*5*) obtained from nine human adults and children (minimum age, 2½ years). The most striking finding is that cystathionase activity is not measurable in the liver of any of the 24 fetuses or 3 premature infants and is only barely measurable in a full-term infant less than 24 hours old. The use of 0 to 4 μmole of cystathionine in the assay (instead of the 2 μmole normally used) or the use of a range of concentrations of pyridoxal 5′-phosphate from 0 to 6.25 μmole (0.125 μmole used normally) failed to elicit a measurable activity of cystathionase in the extracts of fetal liver. The possibility that the lack of cystathionase activity was due to a soluble or loosely bound inhibitor in the crude extracts of fetal liver was tested by incubating these extracts with crude extracts of rat liver; no change in the cystathionase activity of the rat liver extract was found, ruling out the presence of such an inhibitor.

Cystathionine, the substrate of cystathionase, is barely measurable in mature human liver (*3*), but is present in fetal liver in relatively high concentrations (Table 1). The concentrations of cystathionine in the fetal liver are lower than those observed in the adult human brain (Table 2) (*2*, *3*) but are *higher* than in the corresponding immature brains (Table 2). Since there was no apparent relation between concentration of cystathionine in fetal liver or fetal brain and crown-rump length over the period of gestation examined (co-

Table 1. Enzymes of transsulfuration and concentration of cystathionine in developing human liver.

	Methionine-activating enzyme*	Cystathionine synthase*	Cystathionase*	Cystathionine concentration†
Fetus (24)	26 ± 3	21 ± 4	0	14 ± 2
Premature newborn (3)	32 ± 2	17 ± 8	0	13 ± 4
Full-term newborn (1)	10	32	9	32
Mature (9)	86 ± 16	98 ± 19	126 ± 12	0

* Enzymatic activities are expressed as the number of nanomoles of product formed per milligram of soluble protein per hour ± standard error. † Micromoles per 100 g, wet weight.

Table 2. Enzymes of transsulfuration and concentration of cystathionine in developing human brain.

Source	Methionine-activating enzyme*	Cystathionine synthase*	Cystathionase*	Cystathionine concentration†
Fetus (24)	6.2 ± 0.4†	12 ± 2	0	4.0 ± 0.6
Six-month-old infant				
Brainstem	12	39	59	
Cerebellum	15	46	48	211
Diencephalon plus basal ganglia	12	36	72	
Occipital lobe	19	18	48	41
Frontal lobe	4	7	38	59
Parietal lobe gray matter	1	37	56	
Parietal lobe white matter	14	11	47	

* Enzymatic activities are expressed as the number of nanomoles of product formed per milligram of soluble protein per hour ± standard error where indicated. † Micomoles per 100 g, wet weight.

efficients of correlation .23 and .12, respectively), the results are presented as the mean of the group ± standard error.

The activities of cystathionine synthase and of methionine-activating enzyme in crude extracts from the liver of the immature human during the period of gestation studied are one-quarter to one-third of those found in similar preparations from the mature human (Table 1). Neither of these enzymatic activities showed a correlation with crown-rump length (coefficient of correlation .29 and .07, respectively). Cystathionine synthase activity in crude extracts of brain behaved in a similar fashion (coefficient of correlation .29). Methionine-activating enzyme activity in crude extracts of brain showed a tendency to increase with the development of the fetus (coefficient of correlation .62; $.01 < P < .05$). Comparison with mature human brain is difficult, of course, for ideal controls are not possible. However, data from various areas of brain obtained at autopsy from a 6-month-old child with so-called hereditary tyrosinemia are presented for rough comparison (Table 2); they represent a minimum estimate since the activities of methionine-activating enzyme and of cystathionine synthase have been shown to be considerably reduced in crude extracts from the liver of this child and from that of another patient with this disease (*7*).

Crude extracts from placentas obtained with the same fetuses showed no measurable activity of any of these enzymes of transsulfuration and no measurable cystathionine. No cystathionine was detected in the blood or amniotic fluid from the same fetuses. Administration of massive doses of pyridoxine hydrochloride to three mothers (300 mg/day) for 2 weeks prior to interruption of pregnancy did not result in measurable activity of cystathionase in the liver of the fetus. Considerable cystathionase activity was present in the liver of the fetal rat as early as day 12 of gestation and in the liver of the fetal guinea pig, fetal gerbil, and fetal mouse near term.

Thus, in the developing human, cystathionase activity does not appear until after birth. In the absence of an alternative pathway for its production, cyst(e)ine, therefore, would appear to be an essential amino acid in the liver and brain of the immature human until sometime after birth, suggesting that premature infants in particular, may require a diet supplemented with cyst(e)ine. Human milk is known to be relatively low in protein, high in cystine, and low in methionine; whereas cow's milk, although containing far more protein, is low in cystine and high in methionine (*8*). Thus, our studies suggest that premature infants fed a cow's milk formula with a high protein content retain more nitrogen and grow faster than premature infants fed a cow's milk formula containing a lower concentration of protein (*9*)—closer to that found in human milk—because the amount of cystine, rather than the total nitrogen, may be a limiting factor for protein synthesis. These results may also afford an enzymatic explanation for the transient hypermethioninemia seen in infants on high protein diets (*10*).

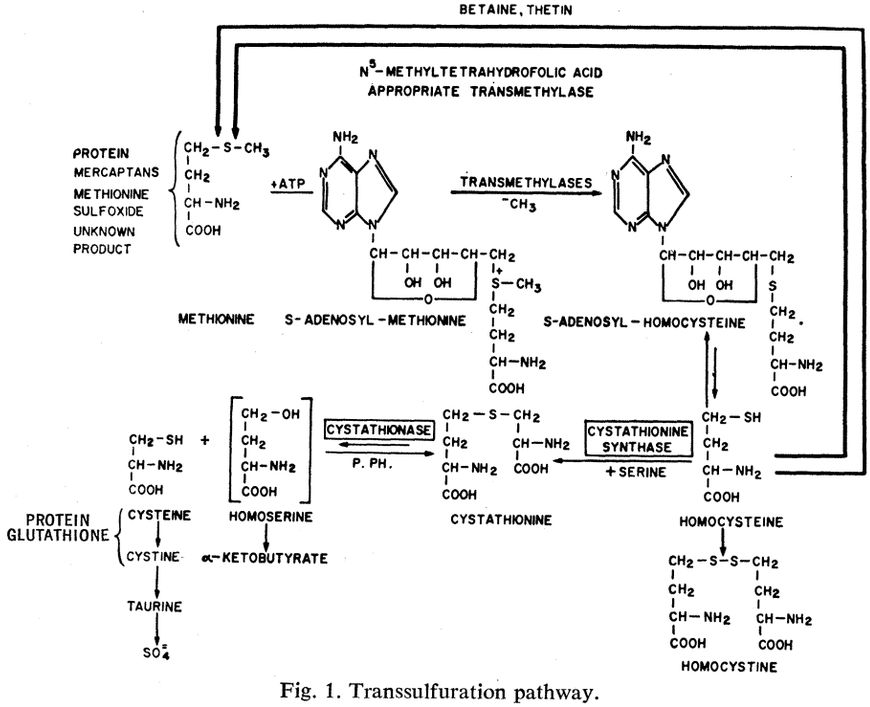

Fig. 1. Transsulfuration pathway.

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Ontogeny of the Estrogen Receptor during Early Uterine Development

Abstract. *The number of estrogen binding sites in uterine cytoplasm on a per cell basis reaches a maximum by day 10 of life in both intact and castrate female rats. After this peak is reached, the number of binding sites per cell decreases, and the ratio remains constant until days 22 to 23 of life. Thus, the ontogeny of the estrogen binding protein is not dependent upon estrogen from the ovary and is probably an autonomous property of uterine cells. Sedimentation values and dissociation constants of the protein when the animals are 5 to 10 days of age are similar to those of the 22-day-old animal, indicating that the same protein is present throughout postnatal development.*

The existence of a uterine cytoplasmic molecule which preferentially binds estrogen in the uterus and other target tissues is well established (*1*). The estrogen binding protein or estrogen receptor interacts with estrogen in the cytoplasm or at the cell membrane and appears to move as a complex to the nucleus of the uterine cell (*2*). Once translocated to the appropriate site or sites on or in the nucleus, the binding protein–estrogen complex may act as an effector of gene activation. Using a new assay technique developed by Clark and Gorski (*3*) for estimation of the number of binding sites, we have examined the rat uterus at different stages of development in both the intact and castrate animal. This was done in order to learn more about the relationship between the presence of the binding protein and uterine growth responses, and to evaluate whether or not the synthesis of the binding protein is dependent on the presence of the ovary.

The immature rats (Holtzman) used in this study were kept with mother rats until 21 days of age, and the litter size was held to six. The day that pups were found with the mother was designated as day 0, and rats were killed on days 1 or 2, 5 or 6, 9 or 10, 15, and 22 or 23 of age. We refer to these days as 1, 5, 10, 15, and 22, respectively. Rats were killed by decapitation, and the uteri were removed, cleaned of fat, and weighed. Uteri were then homogenized in cold 0.04*M* tris-HCl, 0.1*M* KCl, 0.001*M* $MgCl_2 \cdot 6H_2O$ (*p*H 7.2 at 25°C) in all-glass tissue grinders. The uterine homogenates were then centrifuged for 10 minutes at 2000 rev/min in an IEC refrigerated centrifuge. The supernatant fraction from the above centrifugation step was divided, and portions were analyzed for the number of estrogen binding sites (EBS) and binding affinity by the glass-binding method (*3*).

Table 1. Quantities of estrogen present in rat uteri during development. The required amount of estrogen is that which would be present in uteri if endogenous estrogen were binding receptors and were causing the observed low numbers of estrogen binding sites in 2- and 5-day-old animals. The sensitivity of the estrogen assay is between 50 and 1000 pg. Therefore, if estrogen is present, it is in amounts less than 50 pg per sample.

Age (days)	Uteri per sample	Estrogen concentration (pg)	
		Actual	Required
1	30	N.D.*	225
5	20	N.D.*	500
10	5	N.D.*	
23	8	200	

*N.D., not detectable.

The glass-binding assay consists of warming the supernatants of the uterine homogenates to 25°C for 30 minutes in the presence of [^{3}H]estradiol and ground glass (alumina pellets may also be used). The EBS-[^{3}H]estradiol complex binds to the pellets and the [^{3}H]-estradiol is extracted and counted in a scintillation counter. The amount of [^{3}H]estradiol bound to the pellets is proportional to the number of EBS in the supernatant. Cytoplasmic and nuclear fractions were also assayed for protein and DNA content (*4*).

The number of free EBS per 100 μg of DNA increases sharply between day 1 (0.14 pmole per 100 μg of DNA—approximately 5000 sites per cell) (*5*) and day 10 (0.56 pmole per 100 μg of DNA—approximately 20,000/cell) (Fig. 1). After day 10 the number of sites decreases to a value of approximately 16,000 per cell, and this ratio remains constant to day 22. The dramatic increase in EBS per cell observed prior to day 10 occurs at a time when the uterine growth rate is only one-half the growth rate between 10 and 20 days. It should be emphasized that we are measuring only free EBS and that any site which is bound to endogenous estrogen is not measured. One could argue that the lower number of sites observed in the 1- and 5-day-old rats compared to the 10-day-old animal was the result of the presence in the newborn rat of maternal estrogen which would bind EBS and therefore not be assayed. However, this does not appear to be the case. It is possible to calculate the amount of endogenous estrogen that would be required to bind EBS to the sites and thereby to exclude the estrogen-bound sites from measurement; for example, if the number of binding sites were actually 15,000 per cell in the 1-day-old rat uterus, instead of 5000 per cell, then the amount of estrogen per uterus would be equivalent to 10,000 molecules per cell or 7.5 pg of estrogen per uterus.

We have developed a new sensitive