Amino acid acceptor activity and aminoacylation of tRNA during development of human placenta

^{14}C-Amino acid	Weeks of gestation Formation of placenta 8–12 n=3		Growth and differentiation 20 n=1		28 n=1		Fully developed placenta 39–41 n=4		Involution of placenta 43–45 n=2	
	I*	II*	I	II	I	II	I	II	I	II
Alanine	83.1	99	57.6	53	47.2	50	56.9	45	23.2	58
Arginine	37.0	98	21.9	67	27.3	76	46.2	60	18.2	43
Cysteine	27.2	82	41.2	40	30.5	49	39.6	74	17.0	25
Glutamic acid	12.5	98	8.1	55	3.8	58	8.6	62	4.3	46
Glycine	21.6	87	37.7	48	30.4	50	31.2	59	7.4	65
Histidine	10.8	86	18.0	79	16.8	45	23.9	49	4.6	60
Leucine	31.6	82	41.0	38	42.0	47	28.6	60	22.1	58
Phenylalanine	22.6	99	15.5	72	16.3	70	10.7	55	16.1	52
Proline	12.3	82	18.6	40	12.3	36	5.8	50	6.4	62
Tyrosine	12.0	62	15.8	80	16.0	49	16.3	60	4.1	48
Valine	34.6	66	49.9	82	46.0	55	43.5	80	41.8	48
Total amino acid acceptor activity	309.8		325.3		288.6		311.4		165.2	
Level of aminoacylation mean ± SD		86 ± 12		60 ± 16		52 ± 12		60 ± 10		52 ± 11

* Amino acid acceptor activity in picomoles of ^{14}C-amino acids/optical unit A_{260}. ** Level of aminoacylation of tRNA synthesized in vivo as percent of total tRNA i.e. percent of acceptor activity of tRNA left following periodate treatment as related to acceptor activity of the control. In the cases where n > 1, the data are mean values.

for 86% only in the 1st stage of the development, then were found to be 50–60% in next stages. The tRNA concentration/g of placentas of the different ages was similar. It seems likely that the vigorous metabolic processes as reflected by the tRNA activity, correlated with placental weight and birth weight, are restricted to early gestational age of the placenta. Similarly Baliga et al.[9] reported inferior charging activity of pH 5 fraction in the late placenta. This may be ascribed either to inefficiency of the synthetases or to a loss of amino acids during processing of the placenta. Moreover, the duration of labor, length of anoxia, anesthesia, medications etc. are unknown factors that should be taken into consideration if some contradicting data are obtained. This would be especially important when processing placentas from 18 to 32 weeks, obtained in general from pathological cases. In spite of only 2 placentas of such an age used in the present work, our results demonstrate certain trends in protein biosynthesis in the developing human placenta. Because of different structural features, such as unique modified nucleotides or incomplete modification, the mammalian tRNA may exist in many different conformations[3]. The concept of tRNA-controlled translation is a particularly attractive regulatory mechanism in eukaryotic cells where mRNA is relatively long-lived and control by transcription can function slowly[4]. Control of translation by the availability of tRNA may act both positively and restrictively. It seems likely that under hormonal influence in later stages of placental development tRNA can be diverted to ribosomal cellular sites where it is not being actively cycled in protein synthesis. Studies of complex rules that control placental metabolism, will hasten an understanding of the molecular basis of fetal physiopathology.

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Effect of vitamin E deficiency on lipid composition of CNS-myelin in the rat

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Summary. Vitamin E deficiency in the rat is accompanied by a decrease in total lipids and in cholesterol and an elevation in the lysolecithin content of CNS-myelin.

Vitamin E deficiency has often been associated with enhanced fragility of cellular membranes[3]. However, myelin membrane of central nervous system (CNS) has received scanty attention, especially with regard to its lipids in vitamin E deficiency. Since a number of inherent disorders (e.g. A-β-lipoproteinemia[4] with associated segmental demyelinating neuropathy in humans with low serum vitamin E levels) have been shown to have a primary or a secondary neurological manifestation attributable to abnormality in lipids[5], it was of interest to study the changes in

Table 1. Lipid composition of CNS-myelin and brain

	Group	Total lipids mg/mg protein	Phospholipids mg/mg protein	Cholesterol mg/mg protein	Cholesterol phospholipid molar ratio
Myelin	Control	4.40±0.25	1.20±0.13	0.74±0.06	1.60
	Vitamin E deficient	3.47±0.16[a]	1.10±0.14	0.57±0.04[b]	1.06
Brain	Control	1.40±0.17	0.27±0.03	0.17±0.09	-
	Vitamin E deficient	1.28±0.18	0.29±0.03	0.14±0.02	-

The values are averages of 5 independent estimations ± SEM. Significantly different from the control values at [a] $p<0.01$, [b] $p<0.02$.

Table 2. Percentage distribution of phospholipids in CNS-myelin

Group	Lysolecithin	Sphingomyelin	Phosphatidyl choline	Phosphatidyl serine	Phosphatidyl ethanolamine
Control	Nil	9.0±0.72	30.0±2.78	3.9±0.26	57.1±3.31
Vitamin E deficient	2.3±0.18*	8.4±0.63	26.4±2.07	3.8±0.18	59.1±4.55

The values are averages of 5 independent observations ± SEM. Significantly different from the control value at * $p<0.001$.

lipid composition, if any, of CNS-myelin in vitamin E deficiency, commonly involving neurological syndromes like muscular dystrophy. The present report details observations on the lipid profiles of the CNS-myelin in weanling rats reared on a vitamin E-deficient diet for 4–5 months.

Materials and methods. Weanling male albino rats of Wistar Strain weighing 35–40 g were maintained on a vitamin E-deficient diet[6] for a period of 4–5 months. The control rats were fed diets supplemented with 150 mg of α-tocopheryl acetate per kg of diet. Rats exhibiting 95% hemolysis of erythrocytes and showing a decrease of around 40% in testes weight were used for the experiment.

Isolation of CNS-myelin was accomplished following the method of Autilio et al[7]. Lipids from CNS-myelin and brain homogenates were extracted with chloroform:methanol (2:1, v/v) as described by Folch et al.[8]. Total lipids were measured by Bragdon's dichromate method[9]. Phospholipids and cholesterol were estimated, respectively, according to methods cited[10,11]. Individual phospholipids were separated by TLC using chloroform:methanol:water (65:25:4 v/v/v) as developing solvent[10]. Protein was estimated by the method of Lowry et al[13].

Results and discussion. The isolated myelin fraction was checked for purity and was found to have less than 5% contamination by brain subcellular fractions when assessed in terms of recovery of marker enzymes for mitochondria (succinic dehydrogenase), lysosomes (acid phosphatase) and nuclei (DNA and RNA).

In vitamin E deficiency, the brain weight remained unaltered as in case of human malnutrition[14] and in essential fatty acid deficiency[15] in the rat. The vitamin E content of the brain, however, was only 1.5±0.17 µg/g brain as compared to 121.00± 10.2 µg/g brain of the control rats.

There was a decrease of 21% in myelin total lipids which reflected in 23% reduction of cholesterol with a resultant decrease (34%) in the molar ratio of cholesterol to phospholipids (table 1). Probably this is indicative of a defective myelin sheath in the deficiency condition since it is known that myelinogenesis is normally accompanied by an elevation in the molar ratio of these lipids[16]. In this respect, however, studies with other species exhibiting marked muscular dystrophy (monkey) or encephalomalacia (chicks), are necessary for a better understanding of the role of vitamin E in myelination or demyelination.

Accumulation of certain specific phospholipids in myelin has been observed to be characteristic of some demyelinating diseases[17]. Observations on the percentage distribution of individual phospholipids of the CNS-myelin in vitamin E-deficient rat show that, except for an increase in lysolecithin, there was no alteration in phospholipid pattern. This increase in lysolecithin appears to be highly significant in view of the fact that many derangements in the structure-function of the cellular organelles can be brought about by this highly lytic substance[18]. In vitamin E deficiency, similar increase in lysolecithin levels was reported in rat testes[19] and also in rat liver mitochondria[20]. Thompson suggested that accumulation of lysolecithin may play a part in the etiology of demyelinating disorders[21]. It is interesting to speculate whether brain phospholipase[22] may be responsible for the elevation in lysolecithin and subsequent demyelination.

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