- all other stages: [8]
$p=0.886$; $K_R=2.75$ $K_0-0.58$.

It is noticable that these equations differ by their slopes but not their origin. The natural Hill coefficients (n_0) are lower than 1 in larvae and pre-nymphs, and higher than 1 in foraging adults (figure 3). In this case, the general correlation with inhibition-resultant values (namely n_R) would be:

$$p=0.849;\ n_R=0.81 \quad n_0-0.09 \qquad [9]$$

but both pre-nymphs (N_0) and foraging adults (Ab) on one hand, and all other stages on the other, can be gathered on two curves, again with distinct slopes and same origin:

- in N_0 and Ab:
$p=0.990$; $n_R=0.82$ $n_0+0.02$ [10]

- in other stages:
$p=0.85$; $n_R=0.93$ $n_0+0.02$. [11]

The equations [5]-[8] indicate that the inhibition mechanism is rather of the 'K' type over the whole development range, but less marked at the N_0 stage; then, equations [9]-[11] show a general tendency towars the 'n-type' inhibition, especially in pre-nymphs and adults, in which the Hill coefficient are more significantly decreased than at other stages. It thus appears that for pre-nymphs, the 'n-type' effect protects the enzyme substrate affinity under inhibition conditions: the regression coefficient for K_R/K_0 is the lower as the coefficient for n_R/n_0 is itself decreased.

These correlations suggest that the molecular mechanism of haemolymph α-glucosidase activity might proceed from a common general molecular event, inflected in some cases by physiological factors probably related to developmental requirements (metamorphosis metabolism, and adults nutrition for instance).

In natural conditions, the α-glucosidase activity partly results from the equilibrium between the substrate (α-glucoside, mainly trehalose) and the reaction-product (D. glucose as inhibitor). The wide range of the observed values of the resultant Hill coefficients suggests that it might exist different specific coefficients: n_s and n_i respectively for substrate and inhibitor binding, in parallel to their respective affinities for the enzyme: K_s and K_i.

Our purpose is now to determine these parameters, in order to clarify the molecular mechanism of the observed modifications of α-glucosidase kinetics, taking into account both the competitive inhibition-factor: $f_i=1+(I/I_{50})^{ni}$, in agreement with Chou[8] and the 'n-type' effect described by Bounias[3]. This seems the more interesting as another (slightly specific) α-glucosidase purified from whole honeybees by Huber and Mathison[9] did not appear to be controlled by glucose.

1 M. Bounias, 25e Congr. Int. Apic., Grenoble *25*, 304 (1975).
2 M. Bounias, C.r. Acad. Sci. (Paris) *285D*, 261 (1977).
3 M. Bounias, C.r. Acad. Sci. (Paris) *285D*, 1263 (1977).
4 M. Sinha, Experientia *32*, 341 (1976).
5 P.E. Giebel and A. Domnas, Insect Biochem. *6*, 303 (1976).
6 M. Bounias, Comp. Biochem., in press (1980).
7 M. Bounias, Comp. Biochem. Physiol. *61A*, 13 (1978).
8 T.C. Chou, J. theor. Biol. *65*, 345 (1977).
9 R.E. Huber and R.D. Mathison, Can. J. Biochem. *54*, 153 (1976).

tRNA in developing human placenta

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Summary. Amino acid acceptor activity of tRNA in the human placenta as measured throughout gestation was found to be the lowest in post-term placenta. Aminoacylation of tRNA proceeded with maximum activity in the stage of formation of the placenta.

The placenta is probably the most complex mammalian tissue. It is characterized inter alia by the rapid growth, that is the vigorous protein biosynthesis, not observed in other organs, and bearing some resemblance to malignant growth. The magnitude of protein biosynthesis in cell-free placental extracts is known to be correlated with gestational age, DNA content, placental weight, birth weight[1], and RNA[2], respectively. In view of changes in human tRNA during normal and abnormal cellular development[3], and its function in regulation and control[4], it seemed worthwhile to detect changes in amino acid acceptor activity and aminoacylation of tRNA of human placenta from different gestational ages. This is reported in the present paper.

Materials and methods. Human placentas were obtained from the Institute of Obstetrics and Gynecology immediately after delivery. The placenta was washed with cold water from adherent blood, and was separated from fetal membranes. Uniformly ^{14}C-labeled amino acids were from UVVVR Czechoslovakia. tRNA and aminoacyl-tRNA synthetases were isolated from the placental tissue[5,6], the procedures being carried out at 4 °C. ^{14}C-Amino acid acceptor activity of the tRNA was measured by the method of Yang and Novelli[7] at individual Mg^{2+} concentrations for particular amino acids[5]. The periodate procedure was applied to placental tRNA to compare the relative proportions of tRNA charged with amino acids. Periodate treatment was carried out according to Kędzierski and Pawełkiewicz[8], the oxidation mixture containing 200 mM sodium acetate pH 5.0, 20 mM $NaIO_4$ and 5 mg tRNA. Acceptor activities of periodate treated tRNAs were compared to activities of control tRNA preparations subjected to all the procedure, except that $NaIO_4$ was omitted in the oxidation mixture.

Results and discussion. In the Table results of amino acid acceptor activity determinations are shown as well as those of aminoacylation of tRNA from human placentas of different gestational ages. As can be seen, total amino acid acceptor activities of tRNA from post-mature placentas were about 50% lower than those of developmental and term placentas. Levels of tRNA aminoacylation accounted

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.

1 M.E. Laga, S.G. Driscoll and H.N. Munro, Biologia Neonat. *23*, 260 (1973).
2 M. Mori, Am.J.Obstet. Gynec. *93*, 1164 (1965).
3 M.P.J.S. Anandaraj and B.A.Roe, Biochemistry *14*, 5068 (1975).
4 D.W.E. Smith, Science *190*, 529 (1975).
5 M. Woźniak and W. Dobryszycka, Acta biochim. pol. *23*, 171 (1976).
6 N.S. Penneys and K.H. Muench, Biochemistry *13*, 560 (1974).
7 W.K.Yang and S.D.Novelli, in Methods in Enzymology, vol. 20 c, p.35. Ed.S.P.Colowick and N.O.Kaplan. Academic Press, New York 1971.
8 W. Kędzierski and J. Pawełkiewicz, Phytochemistry *16*, 503 (1977).
9 B.S. Baliga, C. Hubert, A. Murphy, F. Meadow, P. Dourmashkin and H.N.Munro, Can.J.Biochem. *54*, 609 (1976).

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