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Gangliosides and Neutral Glycosphingolipids of Human Pancreas

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The gangliosides and neutral glycosphingolipids composition of adult human pancreas were quantitatively isolated and characterized in autopsy material obtained from persons who died in accidents. The concentrations of proteins were 34,9 and 37,1 mg/g fresh tissue, whereas the values for DNA were 3,2 and 2,7 mg per g wet weight. The total gangliosides (lipid-bound sialic acid) in pancreas were 286,1 and 230,4 nmol NeuAc per g fresh tissue. Gangliosides G_{M3} and G_{D3} were most abundant, but G_{M2} , G_{M1} , G_{D1a} , G_{D1b} , and G_{T1b} were also present. The molar ratio of protein - and lipid-bound sialic acid were 2,1 and 1,8. The concentrations of neutral glycosphingolipids were 38,3 and 44,4 nmol/g wet weight. The neutral glycosphingolipids consisted of ceramide mono-, di-, tri-, tetrahexosides, asialo G_{M1} and two compounds having more than four monosaccharides.

INTRODUCTION

Glycosphingolipids are present in the plasma membrane of a great number of different cell types. Their location and their extensive variability suggest prominent role for these compounds in the membrane-mediated cellular phenomena. Indeed, there is a great number of evidence indicating that these compounds play an important role in the cell behavior during differentiation, development, growth control and oncogenic transformation (3). The complex glycolipids serve as cell surface receptors for various hormones (4 - 9), but the involvement of the cell surface glycosphingolipids in the hormone action is complex. Saltiel et al. (10) demonstrated involvement of a target cell glycolipid in the action of insulin. These authors suggested that stimulation of an endogenous phospholipase C activity results in formation of a complex carbohydrate effector which may mediate some of insulins' actions. Insulin has important medical implications. Five percent of the population in developed countries have diabetes mellitus, and an equal number are likely to develop the disease. Diabetes mellitus is due to the insufficient action of insulin, owing either to its absence or to resistance to its action (11).

Whereas glycosphingolipids have been extensively studied in other organs (12), little information is available on these structures in the pancreas. For this reason we report the results from our examinations of the glycosphingolipids in human pancreas.

Key words: Gangliosides, glycosphingolipids (human pancreas)

MATERIALS AND METHODS

Tissue material. The pancreas were obtained from two subjects, who had died in accidents. The pancreas were checked by a trained pathologist and were found to be without macroscopical signs of disorder. Surrounding connective tissue and large blood vessels were removed, and the pancreas was rinsed several times with saline.

Abbreviations: NeuAc, N-acetylneuraminic acid; CMH, ceramide monohexoside; CDH, ceramide dihexoside; CTH, ceramide trihexoside; CTetH, ceramide tetrahexoside; G_{M1} , $GgOse_4Cer^+$; G_{M3} , $II^3NeuAc-LacCer^+$; L_{M1} , $IV^3NeuAc-LcOse_4Cer^+$; G_{M1} , $II^3NeuAc-GgOse_4Cer^+$; G_{D3} , $II^3(NeuAc)_2-LacCer^+$; G_{D1a} , $IV^3NeuAc-GgOse_4Cer^+$; G_{D1b} , $II^3(NeuAc)_2-GgOse_4Cer^+$; HPTLC, high performance thin-layer chromatography.

^{*} Nomenclature according to Svennerholm (1)

^{*} Nomenclature according to IUPAC-IUB recommendations (2).

Chemicals. Sephadex LH 20 and DEAE Sephadex A 25 were purchased from Pharmacia Fine Chemicals, Uppsala, Sweden. HPTLC plates were obtained from Merck A.G. Darmstadt, F.R.G. *Vibrio cholerae* neuraminidase E.C.3.2.1.18. was obtained from Behringwerke A.G., Marburg, F.R.G. All other chemicals and organic solvents used in these experiments were of the highest purity grade available, and were purchased from Kemika, Zagreb, Croatia. Standard gangliosides were a gift from Dr.I.Kračun. Neutral glycosphingolipids and sphingosine base were isolated in this laboratory and were obtained from Prof.M.Proštenik.

Isolation and separation of glycosphingolipids. Pancreas tissue (between 400 and 500 mg) was homogenized in a glass homogenizer with Teflon pestle in ice cold water. Glycosphingolipids were isolated with chloroform/methanol/ water mixture (13). After reextraction and centrifugation the quantity of protein-bound sialic acid was determined in the residue after hydrolysis with 0.1 M H_2SO_4 (14). Lipid extracts were applied to the column with 1.1 g DEAE Sephadex A 25 equilibrated with 100 ml chloroform /methanol/water 30:60:8 (15). After elution two fractions were collected: Fraction I which was eluted with 150 ml chloroform/methanol/water 30:60:8 contained neutral glycosphingolipids and Fraction II eluted with 100 ml chloroform/methanol/0.08 M NaOAc 30:60:8 which contained gangliosides. After mild alkaline hydrolysis and purification on Sephadex LH 20 column (13), the gangliosides were quantified with resorcinol/HCl reagent (16). Neutral glycosphingolipids were purified on magnesia-silica gel column (17). The amounts of neutral glycosphingolipids were measured by determination of the sphingosine content (18). The proportions of individual glycosphingolipids were quantified after separation on HPTLC plates at 515 nm (neutral glycosphingolipids) and 620 nm (gangliosides) using Camag densitometer. The developing systems were chloroform / methanol / 0.2% $CaCl_2$ 60:40:10 for gangliosides and chloroform/methanol/ water 65:25:4 for neutral glycosphingolipids. The spots were located with resorcinol reagent and with orcinol- H_2SO_4 reagent respectively. The gangliosides were also analysed by enzymatic hydrolysis (19).

A small amount of tissue (about 50 mg) was homogenized separately and quantity of proteins (20), and DNA (21) was determined.

RESULTS AND DISCUSSION

The concentrations of proteins in the samples of human pancreas were 34.9 (Case 1) and 37.1 (Case 2) mg per gram fresh tissue weight. The concentrations of DNA were 3.2 (Case 1) and 2.7 (Case 2) mg per g wet weight. The values for sialoglycoproteins were 477.7 (Case 1) and 509.3 nmol NeuAc/g tissue.

The total gangliosides determined in pancreas were 230.4 (Case 1) and 286.1 (Case 2) nmol per g fresh tissue. Gangliosides can be divided into several groups according to their biosynthetic pathway (22). Starting from the G_{M3} which is a key intermediate of ganglioside biosynthesis, the gangliosides G_{M2} , G_{M1} , and G_{D1a} are assigned to the a-series and G_{D3} , G_{D1b} and G_{T1b} are regarded as b-series products. The ganglioside pattern of human pancreas is shown in Fig. 1. For quantification, the bands were analysed by densitometry and the proportion of gangliosides was calculated. In Table 1 the percentage of gangliosides is presented for human pancreas. The a/b ratio of 0.7 which was calculated by dividing the percentage of sialic acid bound to G_{M2} , G_{M1} plus G_{D1a} by the percentage bound to G_{D3} , G_{D1b} plus

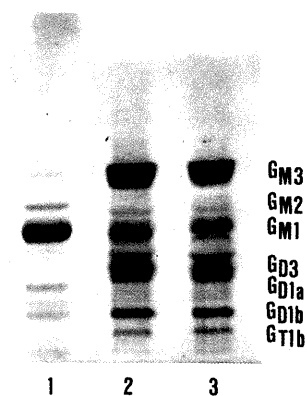


Figure 1:

Gangliosides from human pancreas.

Gangliosides from human pancreas were isolated and purified as described in Material and Methods, separated on HPTLC plates using chloroform/methanol/0.2% $CaCl_2$ 60:40:10, and visualised by spraying with resorcinol reagent. Lane 1: standard of gangliosides; Lane 2 and 3: gangliosides from human pancreas.

TABLE 1
Ganglioside composition in the human pancreas. (%)

Ganglioside	Case 1	Case 2
G _{M3}	43.2	61.7
G _{M2}	3.7	2.0
G _{M1}	16.7	9.2
G _{D3}	20.3	16.4
G _{D1a}	3.2	2.7
G _{D1b}	9.9	5.6
G _{T1b}	3.0	2.4
a/b ratio	0.7	0.7

The gangliosides are designated according to Svennerholm (1).
Results are expressed as percentage distribution of total lipid-bound sialic acid.

G_{T1b} showed preference for b-series gangliosides. It is well known that extraneural tissues contained predominantly lactose-containing gangliosides such as G_{M3} and G_{D3}, but they also contained polar gangliosides with ganglio-N-tetraose in significant concentrations (23).

The molar ratio of protein- and lipid-bound sialic acid which were 2.1 (Case 1) and 1.8 (Case 2) could point out the prominent role of sialoglycoproteins in the adult human pancreas.

The concentration of neutral glycosphingolipids was 38.3 (Case 1) and 44.4 (Case 2) nmol per g fresh tissue weight. The neutral glycosphingolipid fraction of human pancreas was significantly lower than the concentration of the ganglioside fraction. Thin-layer chromatography of neutral glycosphingolipids from human pancreas showed five orcinol positive double bands migrating at the position of standard ceramide monohexoside, ceramide dihexoside, ceramide trihexoside, ceramide tetrahexoside, asialo G_{M1} and two bands migrating at the position of more polar components (Fig. 2). After densitometry at 580 nm proportion of neutral glycosphingolipids was calculated. Table 2 presented the percentage distribution of neutral glycosphingolipids in human pancreas. The pattern of pancreatic neutral glycosphingolipids has not been previously described. In other extraneural tissues it ranged from predominantly glucosylceramide with only traces of more polar components (24), to a composition with predominantly polar glycosphingolipids (25). In the present study pancreatic neutral glycosphingolipids were found to consist of predominantly polar fractions.

Three systems, nervous, haematopoietic and digestive, are characterized by a wide molecular diversity of glycosphingolipids, that is, the molecular diversification of gangliosides in brain, gangliosides and neutral glycosphingolipids in haematopoietic system and neutral glycosphingolipids in intestinal tissues (26). Although we have only been able to determine the major glycosphingolipids of human pancreas, this study has shown the diversity in the composition of gangliosides and neutral glycosphingolipids.

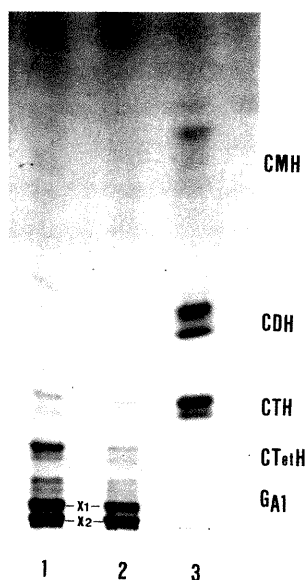


Figure 2:

Neutral glycosphingolipids from human pancreas.

Neutral glycosphingolipids from human pancreas were isolated and purified as described in Material and Methods. Samples were separated on HPTLC plates using chloroform/methanol/water 65:25:4, and the spots were located with orcinol- H_2SO_4 reagent.

Lane 1 and 2: neutral glycosphingolipids from human pancreas; Lane 3: standard of neutral glycosphingolipids.

TABLE 2.

Composition of neutral glycosphingolipids in human pancreas (%)

Glycosphingolipid	Case 1	Case 2
CMH	3.1	3.9
CDH	6.7	7.5
CTH	10.2	8.5
CTetH	21.1	13.5
G _{A1}	15.1	14.7
X1	22.3	26.0
X2	21.5	25.9

Results are expressed as percentage distribution of total sphingosine content.

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SAŽETAK

Gangliozidi i neutralni glikosfingolipidi pankreasa čovjeka

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Gangliozidi i neutralni glikosfingolipidi pankreasa čovjeka su kvantitativno izolirani i karakterizirani u materijalu dobivenim nakon autopsije osoba koje su poginule u nesrećama. Koncentracija proteina iznosila je 34.9 odnosno 37.1 mg/g svježeg tkiva, dok su vrijednosti za DNA bile 3.2 i 2.7 mg/g svježeg tkiva. Koncentracija ukupnih gangliozida (lipidno vezana sijalinska kiselina) iznosila je 286.1 odnosno 230.4 nmol NeuAc po gramu svježeg tkiva. Humani pankreas sadrži najviše G_{M3} i G_{D3} gangliozida, a prisutni su također G_{M2} , G_{M1} , G_{D1a} , G_{D1b} , G_{T1b} gangliozidi. Molarni odnos između proteinski i lipidno vezane sijalinske kiseline iznosio je 2.1 odnosno 1.8. Koncentracija neutralnih glikosfingolipida u pankreasu čovjeka bila je 38.3 odnosno 44.4 nmol/g svježeg tkiva. Metodom tankoslojne kromatografije ustanovljeno je da se neutralni glikosfingolipidi sastoje od ceramid mono-, di-, tri-, i tetrasaharida, od asialo G_{M1} i dvije frakcije koje sadrže više od četiri šećerna ostatka vezana na ceramidni dio.

Gangliosides and neutral glycosphingolipids of human liver during prenatal development

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The gangliosides and neutral glycosphingolipids from the fetal human liver at 16, 22, and 30 weeks of the gestation, as well as from newborn babies' livers were quantitatively isolated and characterized. The most striking developmental accretion of gangliosides was established between 16 and 22 weeks of the gestation characterized by an increase of the total gangliosides and of G_{M3} which represented between 76 and 83% of total lipid-bound sialic acid. Gangliosides L_{M1} , G_{M1} , G_{D3} , G_{D1a} , and G_{D1b} were also present. The concentrations of lipid bound sialic acid expressed in nmol NeuAc/g fresh tissue were 142 ± 12 (16 weeks), 260 ± 24 (22 weeks), 219 ± 22 (30 weeks), and 246 ± 20 in newborn babies. The NeuAc molar ratio between protein- and lipid-bound sialic acid decreased from 4.7 (16 weeks) to 1.4 (22 weeks). The neutral glycosphingolipid fraction was approximately as large as the ganglioside fraction at 16 weeks of the gestation and in newborn babies. At 22 and 30 weeks the concentrations of neutral glycosphingolipids were only 50% of the concentrations of the gangliosides. The major neutral glycosphingolipid was ceramide dihexoside which comprised 65-75% of total neutral glycosphingolipid fraction.

INTRODUCTION

Glycosphingolipids are present in the plasma membrane of a great number of different cell types. Their location and their extensive variability suggest prominent role for these compounds in the membrane-mediated cellular phenomena. Indeed, there is a great number of evidence indicating that these compounds play an important role in the cell behavior during differentiation, development, growth control and oncogenic transformation (3). The glycosphingolipids from human liver have been the subject of two comprehensive studies (4, 5). Glycosphingolipids of the human liver during prenatal development have not previously been studied. It

was of interest to characterize the glycosphingolipids of fetal human liver and compare it with the results for adult one. Therefore, changes in the composition of the glycosphingolipids of fetal human liver during gestation have been investigated.

Key words: Glycosphingolipids, Gangliosides (Human fetal liver)

MATERIALS AND METHODS

Tissue material. Fetal human livers at 16 (4), 22 (6), and 30 (4) weeks of the gestation were obtained from legal abortions. Six livers from newborn babies were included. The livers were checked by a trained pathologist and were found to be without macroscopical signs of disorder. Surrounding connective tissue and large blood vessels were removed, and the liver was rinsed several times with saline.

Abbreviations: NeuAc, N-acetylneuraminic acid; CMII, ceramide monohexoside; CDII, ceramide dihexoside; CIII, ceramide trihexoside; CTetII, ceramide tetrahexoside; NGSL, neutral glycosphingolipids; PL, phospholipids; G_{M3} , II^3 NeuAc-LacCer⁺; L_{M1} , IV^3 NeuAc-LcOse₄Cer⁺; G_{M1} , II^3 NeuAc-GgOse₄Cer⁺; G_{D3} , II^3 (NeuAc)₂-LacCer⁺; G_{D1a} , IV^3 NeuAc, II^3 NeuAc-GgOse₄Cer⁺; G_{D1b} , II^3 (NeuAc)₂-GgOse₄Cer⁺; HPTLC, high-performance thin-layer chromatography.

⁺ Nomenclature according to Svennerholm (1).

^{*} Nomenclature according to IUPAC-IUB recommendations (2).

Chemicals. Sephadex LH 20 and DEAE Sephadex A 25 were purchased from Pharmacia Fine Chemicals, Uppsala, Sweden. HPTLC plates were obtained from Merck A.G. Darmstadt, F.R.G. *Vibrio cholerae* neuraminidase E.C.3.2.1.18 was obtained from Behringwerke A.G., Marburg, F.R.G. All other chemicals and organic solvents used in these experiments were of the highest purity grade available, and were purchased from Kemika, Zagreb, Croatia. Standard gangliosides were a gift from Dr. I. Kračun. Neutral glycosphingolipids and sphingosine base were isolated in this laboratory and were obtained from Prof. M. Prošćenik.

Isolation and separation of glycosphingolipids. Liver tissue (between 400 and 500 mg) was homogenized in a glass homogenizer with Teflon pestle in ice cold water. Glycosphingolipids were isolated with chloroform/methanol/ water mixture (6). After reextraction and centrifugation the quantity of protein-bound sialic acid was determined in the residue after hydrolysis with 0.1 M H_2SO_4 (7). Lipid extracts were applied to the column with 1.1 g DEAE Sephadex A 25 equilibrated with 100 ml chloroform /methanol/water 30:60:8 (8). After elution two fractions were collected: Fraction I which was eluted with 150 ml chloroform/methanol/water 30:60:8 contained neutral glycosphingolipids and Fraction II eluted with 100 ml chloroform/methanol/0.08 M NaOAc 30:60:8 which contained gangliosides. After mild alkaline hydrolysis and purification on Sephadex LH 20 column (6), the gangliosides were quantified with resorcinol/HCl reagent (9). Neutral glycosphingolipids were purified on magnesia-silica gel column (10). The amounts of neutral glycosphingolipids were measured by determination of the sphingosine content (11). The proportions of individual glycosphingolipids were quantified after separation on HPTLC plates at 515 nm (neutral glycosphingolipids) and 620 nm (gangliosides) using Camag densitometer. The developing systems were chloroform/methanol/ 0.2% $CaCl_2$ 60:40:10 for gangliosides and chloroform/methanol/ water 65:25:4 for neutral glycosphingolipids. The spots were located with resorcinol reagent and with orcinol- H_2SO_4 reagent respectively. The gangliosides were also analysed by enzymatic hydrolysis (12).

A small amount of tissue (about 50 mg) was homogenized separately and quantity of proteins (13), DNA (14), and phospholipids (15) were determined.

Statistical evaluation. Statistical significance of the differences between groups was calculated by using a paired t-test; 0.05 was assumed as the minimum level of significance.

RESULTS

Proteins, DNA and phospholipids of human fetal liver

The changes in the concentration of proteins and DNA are given in Figure 1.

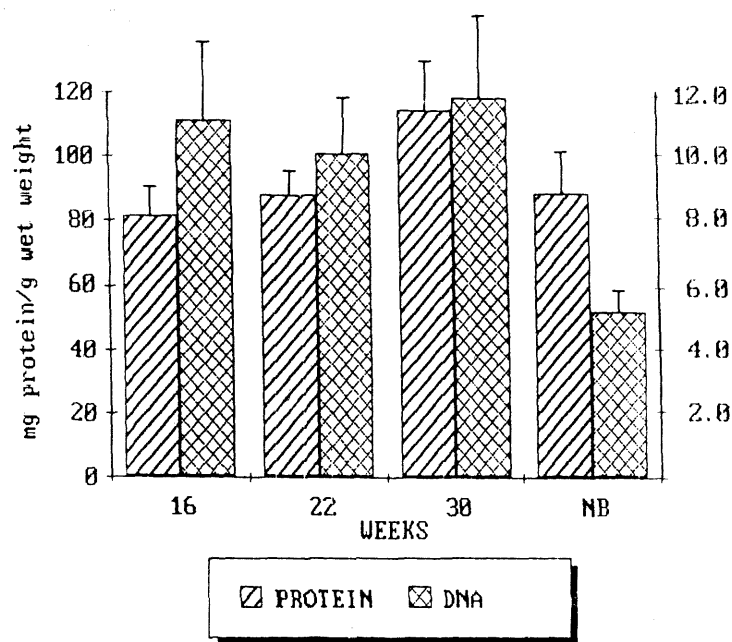


Fig.1:

Changes in the concentration of protein and DNA in human liver during prenatal development. Individual values were combined and results represent means $\pm$ SD of four (16 and 30 weeks of the gestation) and six livers (22 weeks of the gestation and newborn babies).

Values represent the mean value $\pm$ SD of four (16 and 30 weeks of the gestation) and six livers (22 weeks of the gestation and newborn babies). For proteins a statistically significant difference was found between 22 and 30 weeks of the gestation ($t = 3.47$, $p < 0.05$) and between 30 weeks of the gestation and newborn babies ($t = 2.57$, $p < 0.05$). For DNA a statistically significant difference was found only between 30 weeks of the gestation and newborn babies ($t = 6.19$, $p < 0.05$).

Figure 2 shows changes in the concentration of phospholipids during gestation. The concentration of phospholipids was fairly constant (between 8.05 and 9.65 μmol per gram fresh tissue weight), and no significant difference was observed.

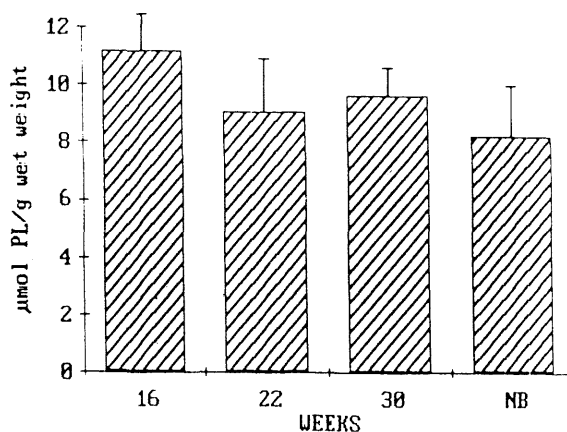


Fig.2:

Concentration of phospholipids in human fetal liver.

Results are expressed as μmol phospholipids/g wet weight and represent the mean value $\pm$ SD. Number of the samples were the same as in Figure 1.

Changes in the lipid- and protein-bound sialic acid of human liver during gestation

Changes in the ganglioside composition of human liver during prenatal development are given in Figure 3 and in Table 1. Total gangliosides determined in livers at 16 weeks of the gestation ranged from 105 to 197 nmol NeuAc/g; at 22 weeks from 217 to 280 nmol NeuAc/g; at 30 weeks from 182 to 259 nmol NeuAc/g; and at newborn babies from 213 to 265 nmol NeuAc per gram fresh tissue weight. The proportion of G_{M3} which was the major ganglioside in fetal human liver was exceedingly high, comprising between 76 and 83% of total lipid bound sialic acid. Between 16 and 22 weeks of the gestation statistically significant difference was found for total gangliosides ($t = 5.33$, $p < 0.05$) as well as for individual gangliosides; G_{M3} ($t = 4.23$, $p < 0.05$), G_{D3} ($t = 4.01$, $p < 0.05$), and G_{D1a} ($t = 3.15$, $p < 0.05$); between 22 and 30 weeks of the gestation for gangliosides L_{M1} ($t = 5.98$, $p < 0.05$), G_{M1} ($t = 11.59$, $p < 0.05$), G_{D3} ($t = 3.44$, $p < 0.05$), G_{D1a} ($t = 2.70$, $p < 0.05$), and G_{D1b} ($t = 3.85$, $p < 0.05$); between 30 weeks of the gestation and newborn babies for L_{M1} ($t = 2.47$, $p < 0.05$), G_{M1} ($t = 4.11$, $p < 0.05$) and G_{D3} ($t = 4.31$, $p < 0.05$).

The changes in the concentration of protein-bound sialic acid are given in Figure 3. Sialoglycoproteins decreased significantly from 16 to 22 weeks of the gestation ($t = 3.14$, $p < 0.05$). The amount of protein-bound sialic acid during

gestation expressed in nmol NeuAc per gram fresh tissue ranged between 529 and 841 (16 weeks); 300 and 401 (22 weeks); 210 and 390 (30 weeks); 348 and 461 (newborn babies). The molar ratios of protein- and lipid-bound sialic acid during gestation were 4.7 (16 weeks), 1.4 (22 weeks), 1.8 (30 weeks), and 1.9 (newborn babies).

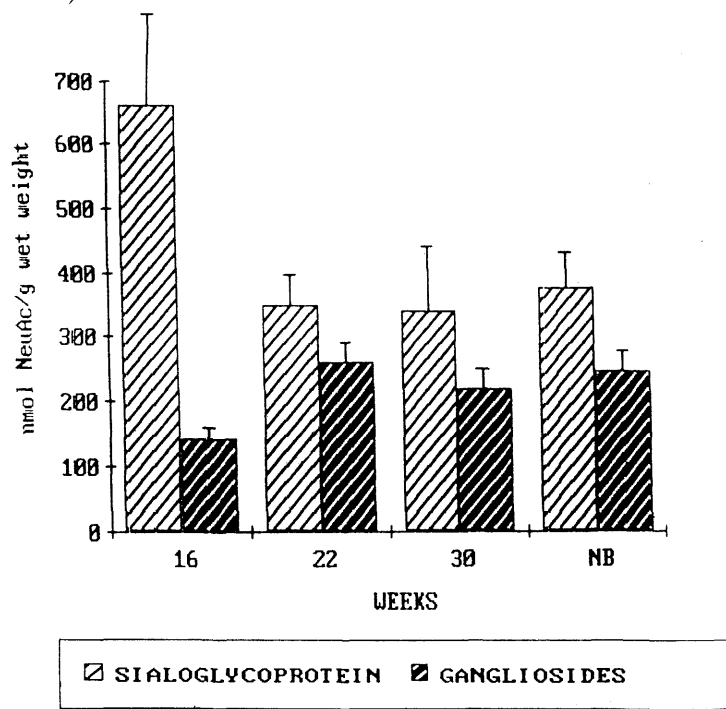


Fig.3:

Changes in the concentration of sialoglycoproteins and gangliosides in human liver during prenatal development.

Sialoglycoproteins and gangliosides were purified and quantified as described in Methods. Individual values were combined and means $\pm$ SD are shown for each gestational stage. Number of the samples were the same as in Table 1.

Changes in the neutral glycosphingolipids of human liver during gestation

Figure 4 and Table 2 represent the mean values $\pm$ SD of neutral glycosphingolipids of human liver during prenatal development. Neutral glycosphingolipids were

determined from sphingosine assay of the lipid extract of the magnesia-silica gel column. They ranged expressed in nmol per gram wet tissue between 137 and 195 (16 weeks); 101 and 174 (22 weeks); 121 and 169 (30 weeks); and between 205 and

TABLE 1.
Ganglioside composition in the human liver during prenatal development

Ganglioside	Age (weeks)			
	16	22	30	Newborn
G _{M3}	108.2 ± 24.4	218.2 ± 29.5	181.5 ± 13.1	204.3 ± 24.7
L _{M1}	3.5 ± 0.8	3.3 ± 0.5	6.1 ± 0.8	4.8 ± 0.4
G _{M1}	5.5 ± 0.5	4.9 ± 0.8	9.1 ± 0.7	7.3 ± 0.6
G _{D3}	10.1 ± 1.5	13.1 ± 1.2	10.4 ± 1.4	15.9 ± 1.8
G _{D1a}	6.7 ± 0.9	10.9 ± 1.3	7.7 ± 1.5	9.5 ± 1.5
G _{D1b}	7.9 ± 0.9	10.1 ± 1.6	5.5 ± 0.9	4.2 ± 1.0

The gangliosides are designated according to Svennerholm. Results are expressed as nmol NeuAc/g wet tissue and represent the mean value ± S.D. of four (16 and 30 weeks of the gestation) and six livers (22 weeks of the gestation and newborn babies).

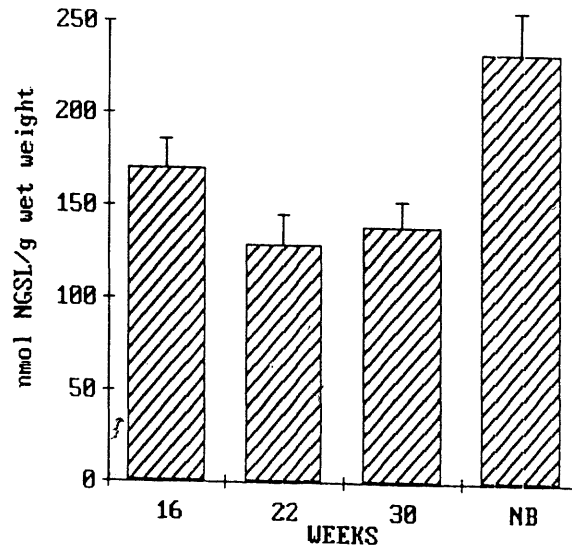


Fig. 4:
Changes in the concentration of hepatic neutral glycosphingolipids (NGSL) during prenatal development.

Neutral glycosphingolipids were purified and quantified as described in Methods. Results represent the mean value ± SD. Number of independent determinations were the same as in Table 1.

TABLE 2.
Neutral glycosphingolipids of human liver during prenatal development

Glycosphingolipid	Age (weeks)			
	16	22	30	Newborn
CMH	29.4 $\pm$ 11.6	22.8 $\pm$ 12.6	23.9 $\pm$ 1.6	20.4 $\pm$ 8.0
CDH	109.6 $\pm$ 21.6	87.1 $\pm$ 15.4	94.5 $\pm$ 21.0	172.8 $\pm$ 33.2
CTH	16.1 $\pm$ 5.4	13.0 $\pm$ 1.4	14.8 $\pm$ 0.7	25.8 $\pm$ 5.1
CTetH	9.0 $\pm$ 1.6	6.3 $\pm$ 2.6	5.9 $\pm$ 3.3	8.2 $\pm$ 3.2

Results are expressed as nmol sphingosine/g wet tissue and represent the mean value $\pm$ S.D. Number of the samples were the same as in Table 1.

275 at newborn babies. Statistically significant difference was found between 30 weeks of the gestation and newborn babies ($t = 2.88$, $p < 0.05$). In the fetal human liver the major fraction was ceramide dihexoside comprising between 76 and 88% of total neutral glycosphingolipids, while monohexosides constituted 10 to 22%. The concentration of higher neutral glycosphingolipids was negligible. Statistically significant difference was found only between 30 weeks of the gestation and newborn babies for ceramide dihexoside ($t = 4.97$, $p < 0.05$) and for ceramide trihexoside ($t = 2.49$, $p < 0.05$).

DISCUSSION

Changes in the structure and function of tissues and organs occur throughout all stages of life from the earliest embryo to the old age. Such changes require a continual breakdown and resynthesis of all tissue components. Protein is especially important because it is the macromolecule contributing most to the tissue mass. It comprises about 0.9 kg of an average pregnancy weight gain of 12.5 kg, and about 50% of this extra protein is deposited in fetus (16). Protein metabolism in individual tissues has been studied predominately in rats (17). Our data demonstrate that the total hepatic proteins decreased after 30 weeks of the gestation.

Cellular growth and multiplication are being examined by determination of DNA content of the liver, which decreased remarkably after 30 weeks of the gestation. Winich and Noble (18) studied the growth of the rat before and after birth and found that increase in the amount of DNA is indicative of cell multiplication and stops in various tissues of a characteristic time after birth, in most instances a few days postnatally. Davison and Dobbing (19) illustrated with regard to brain development that processes occurring after birth in one species may occur prenatally in another.

The concentration of hepatic phospholipids during gestation was fairly constant. Phospholipid synthesis in fetuses and newborns has been examined only sporadically and in no detail. Cholin, which is an essential precursor for phosphatidyl-choline, the predominant phospholipid in mammalian cell membranes is rapidly taken up by the perfused placenta, but only about 10% of the injected dose is recovered on

the fetal side (20). Most work deals with the rate of incorporation of a labeled fattyacid or phosphate into phospholipids of different tissues. In the rat, the rate of incorporation into both lung and liver phospholipids is at its highest immediately after birth (21).

The present paper shows that the total content and the qualitative pattern of liver gangliosides and neutral glycosphingolipids undergo changes during prenatal development.

The concentrations of gangliosides, sialoglycoproteins and neutral glycosphingolipids showed variations in the same time interval during gestation. Possible explanation could be genetic polymorphism as has been observed by Nakamura et al. (22), as well as influence of sex (23, 24, 25). In our study 11 females and 9 male fetuses were used. Although the number of samples in one particular interval was too small to make any conclusion, in general, the livers of a female fetus possessed a little higher concentration of protein- and lipid-bound sialic acid than those of a male one. It is in agreement with results of Phillips et al. (24), and Schütz-Henninger et al. (25) who observed a higher concentration of gangliosides in the female rat liver. Concentration of neutral glycosphingolipids did not show significant differences between female and male fetuses. Finally, we made a large effort to remove all large blood vessels and nerves from the liver tissue samples, but it cannot be excluded that a part of gangliosides, sialoglycoproteins and neutral glycosphingolipids were derived from nerve tissue and from blood.

In the fetal human liver the most striking developmental accretion of gangliosides was established between 16 and 22 weeks of the gestation, characterized by an increase of the total gangliosides and of G_{M3} which is the major ganglioside. The data presented show a moderate decrease of total lipid-bound sialic acid between 22 and 30 weeks of the gestation and an increase after 30 weeks of the gestation. The concentrations of individual gangliosides followed the changes in the concentration of total lipid-bound sialic acid except L_{MI} and G_{MI} which increased remarkably from 22 to 30 weeks and G_{D1b} which decreased constantly after 22 weeks of the gestation.

G_{M3} is the key intermediate of ganglioside biosynthesis. It constitutes a branch point to the monosialo (a)- and polysialo (b)-glycosphingolipid pathway (26, 27). The a/b ratio which was calculated by dividing the concentration of sialic acid bound to G_{MI} plus G_{D1a} by the concentration bound to G_{D3} plus G_{D1b} showed no preference for either "a" or "b" gangliosides during the first 22 weeks of the gestation. In contrast, after 22 weeks of the gestation predominance of the a-pathway gangliosides was found.

The amount of gangliosides in newborn babies was a little bit higher than it had been previously reported for the adult human liver. In our findings the concentration of gangliosides in newborn babies varied between 220 and 265 nmol/g with the mean value 246 nmol/g. Seyfried et al. (28) found that concentration of total lipid-bound sialic acid was 213 nmol/g, while Nilsson and Svennerholm (5) reported that its concentration ranged between 190 and 248 nmol/g with the mean value 214 nmol N-acetylneuraminic acid per gram fresh tissue weight.

The molar ratio of protein- and lipid-bound sialic acid decreased remarkably from 16 to 22 weeks of the gestation. These results could point out the prominent role of sialoglycoproteins in the earliest developmental stages of the human liver.

The neutral glycosphingolipid fraction of fetal human liver was approximately as large as ganglioside fraction at 16 weeks of the gestation and in newborn babies. At 22 and 30 weeks of the gestation the concentrations of neutral glycosphingolipids were almost twice lower than the concentration of the ganglioside fraction. The amount of neutral glycosphingolipids in newborn babies (233 nmol/g wet tissue) was remarkably higher than it had been previously reported for the adult human liver (178 nmol/g) (5). In the adult human liver the concentration of ceramide dihexoside was about 54 nmol/g fresh tissue (5). The concentration of ceramide dihexoside which is the major neutral glycosphingolipid during gestation ranged in newborn babies from 140 to 200 nmol/g. So great an increase of ceramide dihexoside was observed previously in tumor tissue only (29, 30).

Although we have only been able to determine the major glycosphingolipids, this study has shown the difference in the composition of glycosphingolipids between fetal and the adult human liver. For better understanding of their biological functions, further studies on fetal material should be carried out.

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SAŽETAK

Gangliozidi i neutralni glikosfingolipidi jetre čovjeka tijekom prenatalnog razvitka

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Gangliozidi i neutralni glikosfingolipidi fetalne jetre čovjeka u 16, 22 i 30 tjednu trudnoće, kao i kod novorođenčadi kvantitativno su izolirani i karakterizirani. Najizrazitiji porast koncentracije gangliozida utvrđen je između 16 i 22 tjedna trudnoće, a karakteriziran je porastom ukupnih gangliozida i gangliozida G_{M3} koji čini 76-83% ukupne lipidno vezane sijalinske kiseline. Gangliozidi L_{M1} , G_{M1} , G_{D3} , G_{D1a} i G_{D1b} su također izolirani. Koncentracija lipidno vezane sijalinske kiseline izražena kao nmol NeuAc/g svježeg tkiva u određenim razdobljima trudnoće kretala se od 142 ± 12 (16. tjedan), 260 ± 24 (22. tjedan), 219 ± 22 (30. tjedan) do 246 ± 20 kod novorođenčadi. Molarni omjer proteinski i lipidno vezane sijalinske kiseline opada s 4.7 (16. tjedan) na 1.4 (22. tjedan). Koncentracija neutralnih glikosfingolipida gotovo je jednaka onoj gangliozida u 16. tjednu trudnoće i kod novorođenčadi. U 22. i 30. tjednu trudnoće koncentracija neutralnih glikosfingolipida skoro je dva puta manja od koncentracije gangliozida. Najzastupljeniji neutralni glikosfingolipid je laktosilceramid koji čini 65 do 75%, dok cerebrozidi čine 10 do 22% ukupne frakcije neutralnih glikosfingolipida.

Copolymerization of Electron-Donor-Acceptor Monomers which form Charge-Transfer Complexes

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Two groups of comonomers which form charge-transfer complex with high complexation constant ($K = 0.22 - 0.40 \text{ L mol}^{-1}$) and low complexation constant ($K = 0.02 - 0.07 \text{ L mol}^{-1}$) respectively have been copolymerized gravimetrically via »point-by-point« method and in Calvet microcalorimeter in the presence of free radical initiators, preferably AIBN. On the basis of a large number of alternating copolymerizations it was found that for both groups of monomers the mechanism which best explains the copolymerization in the presence of CT-complex is concerted mechanism which consists of simultaneous propagation through free monomers and CT-complex monomers. However all phenomena related to the participation of CT-complexes were more pronounced in case of high concentration of complex in the feed.

Alternating copolymers are characterized by the fact that a nearly one-to-one molar ratio of comonomers is found in copolymers produced by chain reaction from different monomer-to-monomer ratios in the feed. Special interest in the free radical initiated copolymerization is centered around the precise mechanism of copolymerization of monomers which form charge-transfer complexes. A number of studies concerning the mechanism of this type of copolymerization have been published and several different models have been proposed. The following three mechanisms are prevailing.

The first mechanism, called »complex mechanism« is based on the assumption that copolymerization proceeds via the addition of stable monomer complex composed of electron donor-acceptor monomer. It is proposed that the complex has a higher reactivity toward the growing radicals than the uncomplexed free radicals.¹ According to the second mechanism, the alternating copolymerization proceeds via the crosspropagation of free monomers. The high degree of alternation is a natural consequence of the reactivity ratios of monomers and radicals derived from them. According to this mechanism, the electrostatic interaction between polarized monomers and radicals decreases the activation energy for the alternating chain propagation. This mechanism is called »crosspropagation mechanism«.² The third mechanism proposed by Shirota³ and Tsuchida⁴ respectively, and supported by Butler and coworkers,⁵⁻⁸ is based on the assumption that alternating copolymers are

formed by a simultaneous crosspropagation of free monomers and addition of charge-transfer complexes.

Neither of the three mechanisms is completely satisfactory, but the mechanism which considers the simultaneous propagation through free monomers and charge-transfer complex have gained favour. This mechanism is also supported by our work which will be reviewed in this article.

Many papers, several monographs and review articles have been published concerning the alternating copolymerization including the copolymerization in the presence of charge-transfer monomers. Worth mentioning is a recently published book »Alternating Copolymerization«, edited by Cowie.⁹

It is not the intention of this article to review comprehensively the work published so far on the alternating copolymerization. Instead, I wish to report a part of our work on the alternating copolymerization as a contribution to the mechanism of alternating copolymerization in the presence of charge-transfer complex monomers.

The following copolymers were prepared in our Laboratories:

Poly [phenylvinyl alkyl ether (or thioether)-co-maleic anhydride] ; alkyl = Me, Et, n-Pr, iso-Pr, n-Bu, sec-Bu, CH₂CH(Me)Et.

Poly [phenylvinyl alkyl ether-co-N-phenyl-(or N-methyl)-maleimide] alkyl = Me, Et, n-Pr, iso-Pr, n-Bu, sec-Bu, CH₂CH(Me)Et.

Poly (α-methylstyrene-co-maleic anhydride).

Poly (β-methylstyrene-co-maleic anhydride).

Poly (α-methylstyrene-co-N-substituted maleimides); N-substituent = Me, Et, n-Pr, iso-Pr, n-Bu, tert-Bu, Ph, benzo-15-crown-5.

The copolymerization was performed in bulk, in chloroform or in toluene solution in the presence of free radical initiators like benzoyl peroxide, lauroyl peroxide, oxygen, high energy radiation, but preferably with AIBN. For kinetic measurements the copolymers were prepared by »point-by-point« method up to the low conversion not exceeding 15%. The high conversion copolymerization was performed in Calvet microcalorimeter.

Complexation constants, K/L mol⁻¹, were determined by the transformed Benesi-Hildebrand continuous variation method¹⁰ using ¹H NMR or ¹³C NMR spectroscopy in deuterated chloroform at 25°C.¹¹ The electron acceptor was kept at a constant concentration, while concentration of electron donor varied from 0,5 to 3 mol L⁻¹. The complexation constants were calculated from the plot of reciprocal concentration of electron donor 1/[D_o] against reciprocal values of the difference between the chemical shifts of uncomplexed and complexed electron acceptor according to the equation proposed by Tsuchida and coworkers:¹²

$$1/[D_o] = \Delta_{\text{compl}} K / \Delta_{\text{obs}} - K$$

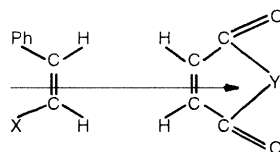
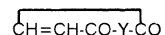
Numerical values of complexation constants of electron-donor-acceptor monomers studied in our laboratories are listed in Tables I and II respectively.

A series of poly(phenylvinyl alkyl ethers-co-maleic anhydride) (poly-PhCH=CH-OR) were prepared by copolymerization of PhCH=CHOR with MAn in bulk or in solution, initiated by free radical initiators like O₂,¹³ benzoyl or lauroyl peroxide,¹⁴ high energy radiation¹⁵ and preferably by AIBN. Copolymers listed in Tables I and

II were prepared in the presence of 0.3 - 0.4 wt% of AIBN, with the exception of poly(PhCH=CHOR-co-MAn) (R = Et, sec-Bu) prepared by radiation induced copolymerization.¹⁵

TABLE I

Properties of monomers and alternating copolymers of PhCH=CHX with



X	Y	K/Lmol ⁻¹		T _g /K	10 ³ M _n	10 ³ M _w	Lit
		¹ H NMR	¹³ C NMR				
OMe	0	0.22	0.26	520	15.3	57.1	14,16
OEt ^a	0	0.28	0.28	506	40.4	107.7	14,15 16,20
On-Pr	0	0.30	0.24	489	43.5	136.0	14,16
Oiso-Pr	0	0.20	-	521	12.3	28.3	16,37
On-Bu	0	0.20	-	481	47.2	123.9	14
Osec-Bu ^b	0	0.20	-	515	13.0	33.6	13-15 16,17,20
OCH ₂ CH(Me)Et	0	0.38	-	517	44.5	127.4	13,14
SMe	0	-	-	-	-	-	21,18
SEt	0	0.25	0.28	491	5.0	-	21
Sn-Pr	0	0.38	-	470	-	-	21
Siso-Pr	0	0.28	-	-	-	-	21
Sn-Bu	0	-	-	-	-	-	21,18
Ssec-Bu	0	0.40	0.42	498	4.6	5.5	21,18
SCH ₂ CH(Me)Et	0	-	-	-	-	-	21
S(CH ₂) ₂ CH(Me)Et	0	-	-	-	-	-	21
CH ₃	0	0.22	-	555	3 to 10	3 to 10	22
OMe	NPh	-	-	551	63.2	133.3	23
OEt	NPh	0.28	-	531	55.0	116.4	23
On-Pr	NPh	-	-	523	44.3	91.0	23
Oiso-Pr	NPh	-	-	544	17.2	36.0	23
On-Bu	NPh	-	-	513	39.0	82.0	23
Osec-Bu	NPh	0.20	-	535	11.0	23.6	23
OCH ₂ CH(Me)Et	NPh	-	-	540	31.6	67.4	23
O(CH ₂) ₂ CH(Me)Et	NPh	-	-	401	63.4	123.9	23
OMe	NMe	-	-	519	43.8	89.43	23
OEt	NMe	-	-	508	40.2	84.7	23
On-Pr	NMe	-	-	494	36.7	80.0	23
Oiso-Pr	NMe	-	-	514	25.3	48.8	23
On-Bu	NMe	-	-	478	34.0	72.0	23
Osec-Bu	NMe	-	-	496	10.4	21.5	23
OCH ₂ CH(Me)Et	NMe	-	-	505	56.0	102.4	23
O(CH ₂) ₂ CH(Me)Et	NMe	-	-	497	31.5	63.0	23

a) E_A = 91.0 kJ/mol; AIBN as initiator;²⁰ E_A = 15.5 kJ/mol; radiation-induced copolymerization.¹⁵
 b) E_A = 91.4 kJ/mol; AIBN as initiator;²⁰ E_A = 18.8 kJ/mol; radiation induced copolymerization.¹⁵

Copolymers listed in Table I were prepared in bulk or in chloroform or toluene solution. Chloroform was preferred solvent since it does not interfere with complex.²⁴ The properties of copolymers refer to the alternating products obtained from equimolar ratios of comonomers.

The alternating composition of copolymers was proved by elemental analysis, NMR spectroscopy or from the data of optical rotation, since it was found that there was no asymmetric induction in copolymerization of optically active electron donors, PhCH=CHOR^* with MAn. The chiral groups R^* were: CH(Me)Et , $\text{CH}_2\text{CH(Me)Et}$ and $(\text{CH}_2)_2\text{CH(Me)Et}$.²⁵ It is of interest to mention that in cationic homopolymerization of optically active phenylvinyl alkyl ethers there was a strong effect of asymmetric induction,²⁶ while no asymmetric induction was observed in homopolymerization of phenylvinyl alkyl thioethers.²⁷

In most cases the copolymers were prepared by »point-by-point« procedure up to the low conversion. It is evident from the data in Table I that equilibrium constants are not significantly dependent on steric factors, thus indicating that complexes are formed through vinyl bonds, and not through oxygen or phenyl groups.

The properties of another series of alternating copolymers prepared in our Laboratories are listed in Table II.

TABELE II

Properties of alternating copolymers of α -methylstyrene with N-substituted maleimides and maleic anhydride

X	K/Lmol ⁻¹ ¹ H NMR	T _g /K	10 ⁻³ M _n	10 ⁻³ M _w	Lit
O	0.07	518			28
N-H	0.03	567			29,49
N-Me	0.02	499	27.0	68.0	30,31
N-Et	0.05	462	22.9	52.7	32
N-n-Pr	0.03	454	19.0	41.2	32
N-iso-Pr	0.03	485	18.1	34.2	32
N-n-Bu	0.04	410	25.2	52.3	32
N-n-Hex	0.06	382	25.3	70.8	32
N-benzo-15-crown-5	0.07	459	4.8	7.8	33
N-Ph	0.02	518	-	-	31,34

The data in Table II show that equilibrium constants of the complexes of α -methylstyrene with N-substituted maleimides are relatively low in comparison with the data in Table I. Often the equilibrium constant values are within the limits of precision of measurement methods, although in all cases there was a distinct chan-

ge in chemical shifts when the ratio of concentration of electron-donor and electron-acceptor was changed. It is further evident that equilibrium constants are not significantly dependent on steric factors, what indicates that the complexes are also formed via the vinyl bonds.

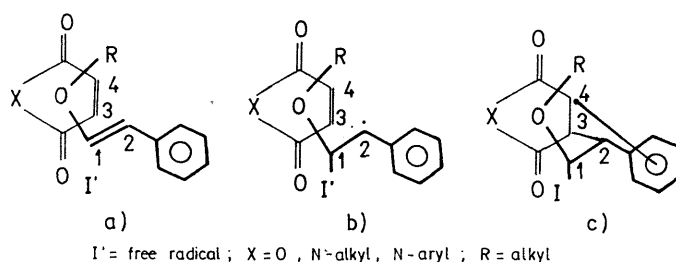


Fig.1. Stereochemistry of electron-donor-acceptor complex; a) non-bonding structure of the charge-transfer complex; b) free radical attaching to the complex; c) radical formation of the complex.

It is of interest to note that all one-to-one complexes of monomers listed in Tables I and II contain large electron aromatic rings as pendant group. Due to the presence of aromatic ring the probable structure of the complex is the one proposed by Tsuchida et al.¹² and by Butler and Olson³⁶ in which the overlap of the monomers in the complex is at a maximum (Fig. 1). In this conformation the planes of the components are parallel. Carbonyl oxygen is situated far from phenyl nucleus of electron donor since the electrostatic repulsion ($\delta^- \leftrightarrow \delta^-$) exists between carbonyl oxygen and phenyl nucleus. This conformation conforms with the one proposed by Tsuchida and coworkers¹² for the conformation of styrene with maleic anhydride. In the proposed conformation the free radical initiator or polymer radicals attack the C-1 carbon atom of electron donor, thus forming the free radical **b** in Figure 1. Since the carbon atoms 1 and 2 are close to each other in the complex, they readily form covalent bond under the formation of free radical on electron acceptor molecule **c** in Figure 1. The free radical on C-4 is stabilized by the charge transfer interaction with phenyl nucleus. This interaction causes the delayed termination what results in the increase of molecular weight in alternating copolymerization with conversion. This is exemplified in copolymerization of PhCH=CHOEt with MAu where it was found that in bulk radiation induced copolymerization the intrinsic viscosity more than doubles when conversion was increased from 10-60%.¹⁵ Similar results were also obtained in the bulk and solution copolymerization initiated by AIBN.¹³ It was established that the increase of molecular weight is due to the mechanism of delayed termination different than the Trommsdorf effect.¹³

From the data in Table I it is evident that the complexation constants of all listed electron-donor-acceptor monomers are relatively high, so that the complex

concentration in the feed is significant. On the other hand, the data in Table II show that the complexation constants of α -MeSt with N-substituted maleimides are very low. It was therefore of interest to compare the copolymerization mechanism of the monomers listed in Table I and Table II respectively.

While the complexation constants of monomers in Table I and II are not dependent on the steric factors, the rates of copolymerization are significantly dependent on steric factors, as shown in Fig. 2 in which are shown the initial copolymerization rates of N-PhMI with $\text{PhCH}=\text{CHR}$ ($\text{R} = \text{OMe}, \text{OEt}, \text{On-Pr}, \text{Oiso-Pr}, \text{Osec-Bu}$) and of N-RMI ($\text{R} = \text{Me}, \text{Et}, \text{n-Bu}, \text{n-Pr}, \text{iso-Pr}, \text{n-Hex}$) with α -MeSt.³⁷ The initial rates of copolymerization of $\text{PhCH}=\text{CHR}$ with N-PhMI were calculated from the data of gravimetric copolymerization in toluene at 70°C at different monomer-to-monomer ratios in the feed by »point-by-point« method, while the initial rates of copolymerization of N-RMI with α -MeSt were determined on the basis of conversion after 60 minutes in Calvet microcalorimeter in CHCl_3 at 60°C.^{32,37}

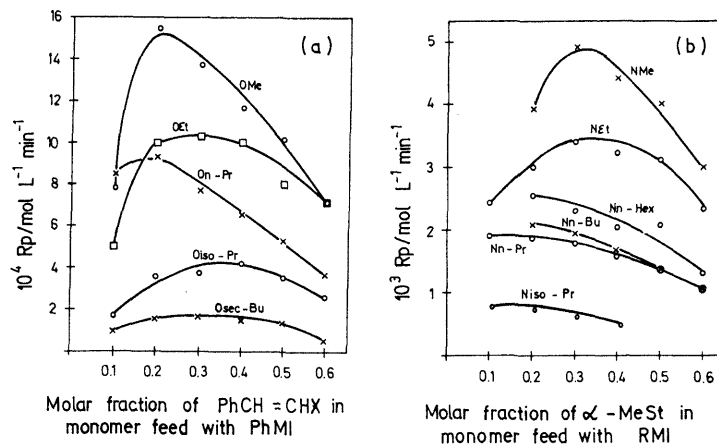


Fig. 2. The initial rates of copolymerization of a) NPhMI with $\text{PhCH}=\text{CHX}$ in toluene at 70°C,³⁷ and b) N-RMI with α -MeSt in CHCl_3 at 60°C; high conversion copolymerization in Calvet microcalorimeter with 0.3% AIBN.^{32,37}

The data in Fig. 2 indicate that in both systems the initial rates of copolymerization depend on steric factors and that initial rates do not maximize at equimolar ratios of comonomers where the concentration of complex is maximal.

The influence of steric factors on the rate of copolymerization of equimolar ratios of $\text{PhCH}=\text{CHX}$ with N-PhMI and α -MeSt with N-RMI respectively up to the low conversion by »point-by-point« method is presented in Fig. 3.^{19,37}

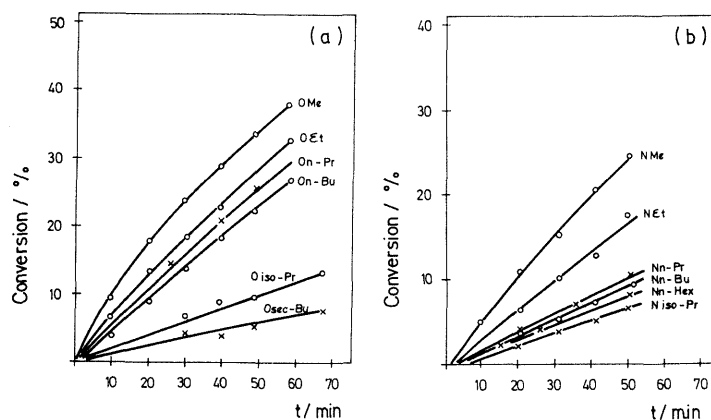


Fig. 3. Time - conversion curves in low conversion copolymerization of a) N-PhMI with PhCH=CHX in toluene at 70°C, 0.4% AIBN,¹⁹ and b) α-MeSt with RMI in CHCl₃ at 60°C, 0.3% AIBN.³⁷

The data in Fig. 2 and 3 indicate that the rates of conversion in both systems decrease with the increase of alkyl groups in electron donor molecules (PhCH=CH-X) or in electron acceptor monomers (N-RMI). Assuming that the concentration of complex is small in comparison with the concentration of monomers, and that at different monomer-to-monomer feed ratios the alternating copolymers are obtained, the reaction can be considered as a first-order reaction, so that the plot

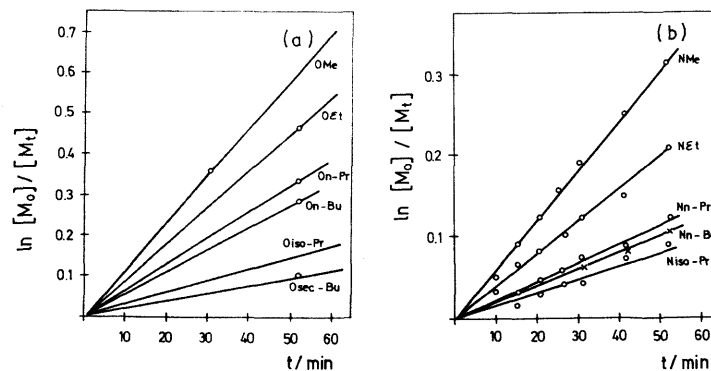


Fig. 4. First order rate plots in the copolymerization of a) N-PhMI with PhCH=CHX in toluene at 70°C, 0.4% AIBN,¹⁹ and b) α-MeSt with N-RMI in CHCl₃ at 60°C, 0.3% AIBN.³⁷

of $\ln[M_0]/[M_t]$ against time should be linear; $[M_0]$ is molar concentration of monomer at the beginning of reaction and $[M_t]$ is molar concentration of monomer at time t . The first order rate plots in the copolymerization of $\text{PhCH}=\text{C-IIX}$ with N-PhMI and $\alpha\text{-MeSt}$ with N-RMI respectively are shown in Fig. 4. From the slope of straight lines in Fig. 3 the rate constants, k/min^{-1} are calculated: a) $k_{\text{Me}} = 0.012$; $k_{\text{Et}} = 0.0092$; $k_{\text{n-Pr}} = 0.0066$; $k_{\text{n-Bu}} = 0.0056$; $k_{\text{sec-Bu}} = 0.002 \text{ min}^{-1}$; b) $k_{\text{Me}} = 0.006$; $k_{\text{Et}} = 0.0038$; $k_{\text{n-Pr}} = 0.0023$; $k_{\text{iso-Pr}} = 0.0015$; $k_{\text{n-Bu}} = 0.00222$; $k_{\text{terc-Bu}} = 0.00049$; $k_{\text{n-Hex}} = 0.0023 \text{ min}^{-1}$. As evident the k_R in both copolymerization systems decreases with the increase of effective bulkiness of alkyl group.

It is of interest to note that corrected Taft-Hancock parameters, E_s^c , calculated from the rate constants of esterification of alcohols, or hydrolysis of esters, can be applied to the free radical initiated copolymerization of N-PhMI with $\text{PhCH}=\text{CHOR}$ ($R = \text{Me, Et, n-Pr, n-Bu, iso-Pr, sec-Bu}$) (Fig. 5)¹⁹

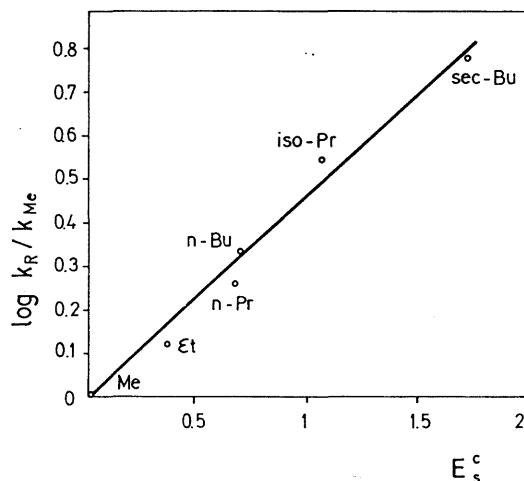


Fig. 5. Plot of $\log(k_R/k_{\text{Me}})$ and corrected Taft-Hancock steric factor E_s^c in the copolymerization of $\text{PhCH}=\text{CHOR}$ with N-PhMI in CHCl_3 , 0.3% AIBN.¹⁹

In the subsequent work it was found that the same is also true for the copolymerization of $\alpha\text{-MeSt}$ with $\text{N-substituted maleimides N-RMI}$ ($R = \text{Me, Et, n-Pr, n-Hex, iso-Pr, terc-Bu}$) (Fig. 6)^{37, 38}

It is further of interest to mention that besides the steric control of copolymerization, the thermal degradation of poly($\text{PhCH}=\text{CHOR-co-MAN}$) ($R = \text{Me, Et, n-Pr, iso-Pr, sec-Bu}$) (Fig. 7) and of poly($\text{PhCH}=\text{CHSR-co-N-PhMI}$) ($R = \text{Me, Et, n-Pr, iso-Pr, sec-Bu}$), are also sterically controlled reactions.¹⁹

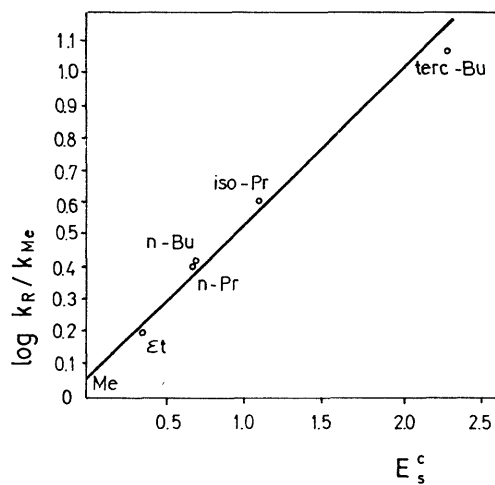


Fig. 6. Relationship between $\log (k_R/k_{Me})$ and corrected Taft-Hancock steric factor E_s^c in the copolymerization of α -MeSt with N-RMI in $CHCl_3$, 0.3 AIBN at $60^\circ C$.³⁷

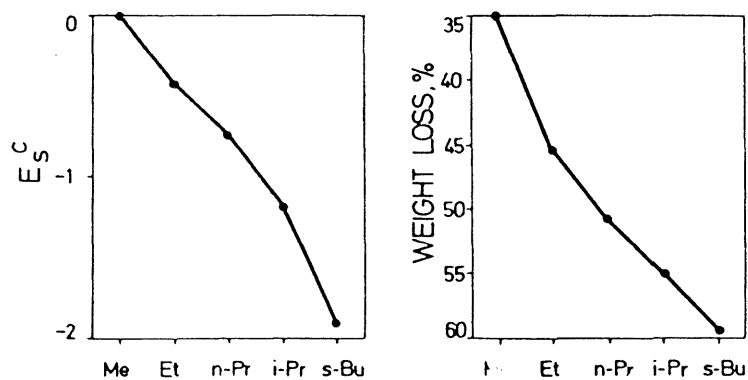


Fig. 7. A comparison of corrected Taft-Hancock steric constants E_s^c and the weight loss in thermogravimetric analysis of poly $(PhCH=CHOR-co-NPhMI)$.¹⁹

As mentioned in the introductory part, the alternating copolymerization with the participation of CT-complexes can be best explained as a concerted propagation through free monomers and charge-transfer complex:

$$R_p = R_p(f) + R_p(CT) \quad (1)$$

$$\text{where } R_p(f) = k_{12} [m_1^{\cdot}] [M_2] + k_{21} [m_2^{\cdot}] [M_1] \quad (2)$$

$$\text{and } R_p(CT) = 2(k_{1c} [m_1^{\cdot}] [CT] + k_{2c} [m_2^{\cdot}] [CT]) \quad (3)$$

$[M_1]$ and $[M_2]$ are molar concentrations of electron acceptor and electron donor monomers, respectively; $[m_1^{\cdot}]$ and $[m_2^{\cdot}]$ represent the molar concentrations of the corresponding free radicals with M_1 or M_2 ends, and $[CT]$ is molar concentration of charge-transfer complex $[M_1M_2]$ or $[M_2M_1]$.

The ratio of $R_p(CT)$ and $R_p(f)$ at steady state approximation is given by the equation (4):

$$\begin{aligned} R_p(CT)/R_p(f) &= (k_{1c}/k_{12} + k_{2c}/k_{21}X)K[M_1] \\ X &= [M_2]/[M_1] \end{aligned} \quad (4)$$

By applying the steady-state approximation to the copolymerization reaction it was shown by Shiota and coworkers^{3,4,43} that $R_p/[M_1]$ is a first order function with respect to $[M_1]$ according to the equation 5:

$$R_p/[M_1] = \Lambda(X)K(k_{1c}/k_{12} + k_{2c}/k_{21}X) [M_1] + \Lambda(X) \quad (5)$$

where K is the complexation constant, and $\Lambda(X)$ is given by equation (6):⁴¹

$$\Lambda(X) = \frac{2k_{21}k_{12}R_i^{1/2}X}{(k_{111}k_{21}^2 + 2k_{112}k_{21}k_{12}X + k_{122}k_{12}^2X^2)^{1/2}} \quad (6)$$

The same authors have also shown that

$$R_p(f) = \Lambda(X) [M_1] \quad (7)$$

In continuation of this review a comparison of the influence of CT-complex monomers to the mechanism of copolymerization of monomers with high complexation constants (Table I) and low complexation constants (Table II) will be illustrated in a few examples.

Figure 8 represents the plot of initial rates of copolymerization of a) PhCH=CHOMe with maleic anhydride ($K = 0.28 \text{ Lmol}^{-1}$)¹⁶ and b) $\alpha\text{-MeSt}$ with N-MeMI ($K=0.02 \text{ Lmol}^{-1}$).³⁰ Each curve represents a set of copolymerizations with different monomer feed ratios, but at a constant total monomer concentration in feed (a) 0.8, 2.0, and 4.0 molL^{-1} ; b) 1.0, 2.0, and 3.0 molL^{-1} .

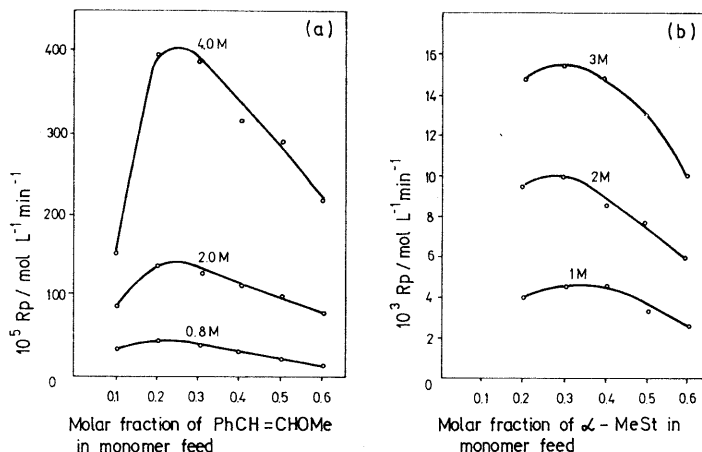


Fig. 8. Initial copolymerization rates versus monomer feed molar ratios in copolymerization of a) PhCH=CHOMe and MAn in C_6H_6 , 0.4% AIBN at $70^\circ C$; PhCH=CHOMe + MAn = 0.8; 2.0; 4.0 molL⁻¹,¹⁶ b) α -MeSt and N-MeMI in $CHCl_3$; $60^\circ C$, 0.3% AIBN; α -MeSt + N-MeMI = 1.0; 2.0; 3.0 molL⁻¹.³⁰

It is evident that in both systems plotted in Fig.8, the initial rate of copolymerization does not maximize at equimolar ratio of comonomers, where the concentration of CT-complex is maximal. The maximum of initial copolymerization rates is higher at higher electron acceptor concentration. This is inconsistent with the mechanism which is based on the homopolymerization of CT-complex, and suggests the participation of free monomers in the copolymerization reaction. The same is also shown in Figure 2 for the copolymerization of PhCH=CHIX with N-PhMI (X = Me, Et, n-Pr, iso-Pr, sec-Bu) and for the copolymerization of α -MeSt with N-RMI (R = Me, Et, n-Bu, n-Pr, iso-Pr, n-Hex).

On the other hand, if copolymerization proceeds only via the crosspropagation of free monomers, the rate of copolymerization should be proportional to the concentration of monomers. In fact the rates are always higher, thus indicating the participation of CT-complex monomers.

Assuming that the initial copolymerization rate R_p equals sum of the rate of copolymerization of free monomers, $R_p(f)$, and the rate of copolymerization of CT complex monomers, $R_p(CT)$, the $\Delta(X)$ from the equation 7 can be calculated as intercept of the straight lines of the plot of $R_p/[M_1]$ against $[M_1]$ as exemplified in Fig.9 for the copolymerization of PhCH=CHOMe with MAn¹⁶ and α -MeSt with N-MeMI.³⁰

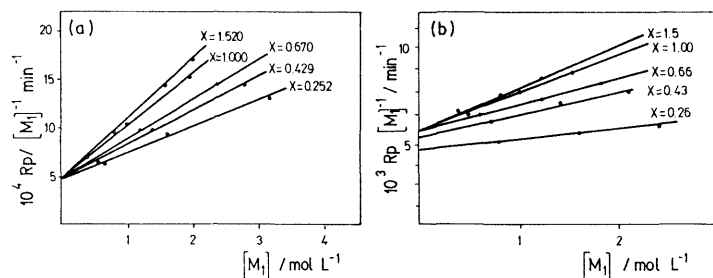


Fig. 9. Plot of $R_p/[M_1]$ against $[M_1]$ at different $X = [M_2]/[M_1]$ ratios in the copolymerization of a) PhCH=CHOMe with MAN in benzene; 70°C ; 0.4% AIBN ; b) $\alpha\text{-MeSt}$ with N-MeMI in CHCl_3 ; 60°C ; 0.3% AIBN .³⁰

The relative participation of $R_p(f)$ is calculated from the equation 7 by multiplying the molar concentration of electron acceptor ($[M_1]$) with the intercept of straight lines in Fig. 9. The $R_p(\text{CT})$ was calculated as difference between total R_p and $R_p(f)$ according to the equation 1.

An example which illustrates the relative participation of $R_p(f)$ and $R_p(\text{CT})$ in the copolymerization of PhCH=CHOMe with MAN and N-MeMI with $\alpha\text{-MeSt}$ respectively is shown in Fig. 10.

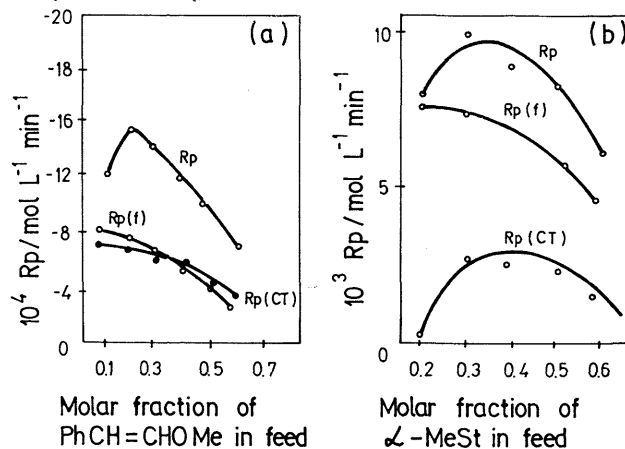


Fig. 10. Relative participation of free monomers and CT-complex in copolymerization at different monomer-to-monomer ratio in the feed at total monomer concentration of 2 mol L^{-1} : (a) PhCH=CHOMe with MAN ; in benzene with 0.4% AIBN at 70°C ; (b) $\alpha\text{-MeSt}$ with N-MeMI in CHCl_3 with 0.3% AIBN at 60°C .

The influence of steric factors, total monomer concentration and complexation constant in the copolymerization of electron-donor-acceptor monomers which form CT-complexes is presented in Figs 11 and 12.

Fig. 11 shows the influence of steric factors and total monomer concentration in the copolymerization of PhCH=CHOMe with MAn (Fig. 11a) and PhCH=CH-OisoPr with MAn (Fig. 11b) to the initial rate of copolymerization and to the participation of CT-complex monomers. In both systems the complexations are relatively high: $K = 0.22 \text{ Lmol}^{-1}$ for PhCH=CHOMe/MAn and $K = 0.20 \text{ Lmol}^{-1}$ for $\text{PhCH=CH-OisoPr/MAn}$.

It is evident from the data of Fig.11 that the participation of CT-complex increases with the increase of total monomer concentration and with the increase of bulkiness of alkyl group in electron donor monomer.¹⁶ Similar results were also obtained in copolymerization of N-MeMI with α -MeSt and N-isoPrMI with α -MeSt respectively, as shown in Fig.12. It is of interest to mention that complexation constants in these systems are very low: $K = 0.02 \text{ Lmol}^{-1}$ for N-MeMI/ α -MeSt, and $K = 0.03 \text{ Lmol}^{-1}$ for N-isoPrMI/ α -MeSt.

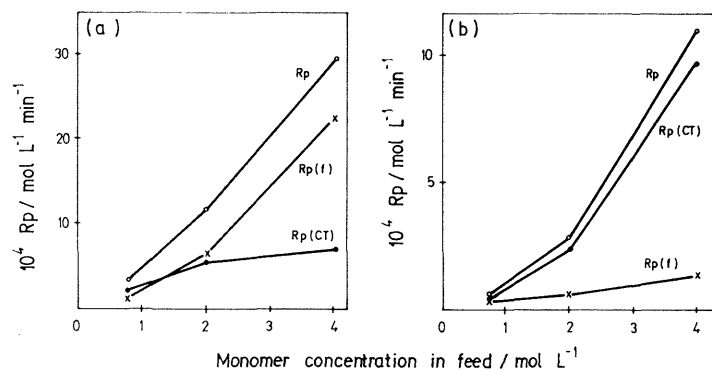


Fig. 11. Relative participation of free monomers and CT-complex monomer in the copolymerization at different total monomer concentration in benzene at 70°C; 0.4% AIBN: a) PhCH=CHOMe with MAn; b) PhCH=CH-OisoPr with MAn.

However although complexation constants of monomers in Fig. 11 and 12 are different, the mechanism of copolymerization is basically the same. The same was also proved for other monomers listed in Tables I and II.

It should however be mentioned that besides the steric factors and total monomer concentration in the feed, the rate of alternating copolymerization may be appreciably affected by the polarity of solvent. This is illustrated in Fig. 13 for

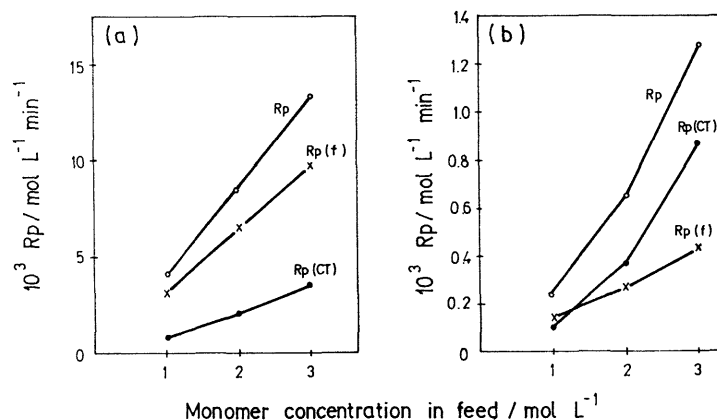


Fig. 12. Relative participation of free monomers and CT-complex monomers in the copolymerization at different total monomer concentration in CHCl_3 at 60°C ; 0.3% AIBN: a) N-MeMI with α -MeSt; b) N-iso-PrMI with α -MeSt.

the copolymerization of α -methylstyrene with maleic anhydride in benzene which does not interfere significantly with complex and in butanone which has a high dielectric constant and strongly interferes with monomers.²⁴ On the basis of equation 1, the relative participation of $R_p(f)$ and $R_p(CT)$ is calculated and graphically presented in Fig. 13.²⁸ It is evident that in benzene copolymerization

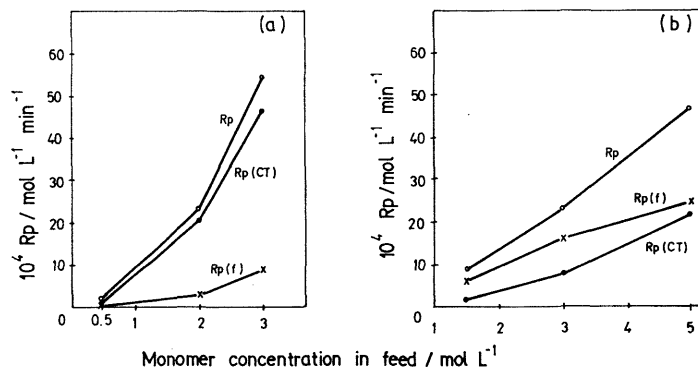


Fig. 13. Relative participation of free monomers and CT-complex monomers in the copolymerization of α -MeSt with MAn at different monomer concentrations in the feed; 60°C ; 0.3% AIBN. a) in benzene; b) in butanone.

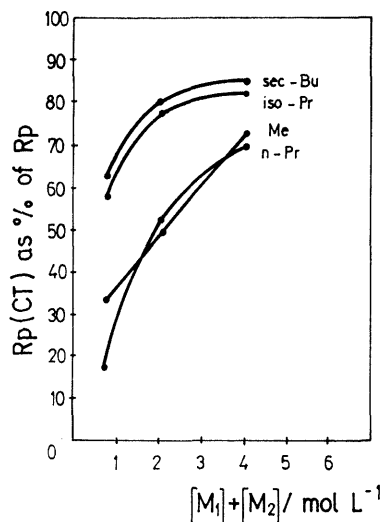


Fig. 14. Plot of $R_p(\text{CT})$ against molar concentration of monomers in the feed in copolymerization of $\text{PhCH}=\text{CHOR}$ with MAn in benzene.²⁸

proceeds predominantly via CT-complex, while in butanone, the participation of free monomers is prevailing.

The results similar to those presented in Fig.11 and 12 are obtained in a series of copolymerization of α -MeSt with N-alkylmaleimides (RMI: R = Me, Et, iso-Pr, Ph, benzo-15-crown-5)^{32,33}

The influence of steric factors and concentration of monomers in the feed to the participation of CT-complex in copolymerization of $\text{PhCH}=\text{CHOR}$ (R = Me, n-Pr, iso-Pr, sec-Bu) with MAn is presented in Fig.14, while in Fig.15 is shown the participation of CT-complex in the copolymerization of α -MeSt with N-substituted maleimides, RMI (R = H, Me, Et, iso-Pr, Ph, benzo-15-crown-5). In Fig.15 are also included the data which show the influence of solvent to the participation of CT-complex in the copolymerization of α -MeSt with MAn in benzene and in butanone respectively.

It is of interest to note that in the copolymerization of RMI with α -MeSt the participation of CT-complex prevails, thus indicating that the mechanism of propagation depends not only on steric factors, but that polar effects of electron acceptor monomer significantly contribute to the mechanism of propagation.²⁹

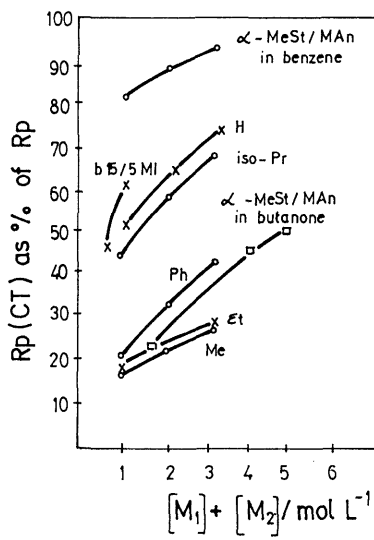


Fig. 15. Plot of $R_p(CT)$ against molar concentration of monomer in the copolymerization of RMI with α -MeSt in $CHCl_3$, and in copolymerization of α -MeSt with MAn in benzene or in butanone.

The key observation that the plot of $R_p/[M_1]$ vs. $[M_1]$ shows a good linearity for each given monomer feed molar ratio X having definite values of the intercept and the slope enables one to calculate and $R_p(CT)$ from the intercept, while the reactivity ratios can be calculated from the slope:

$$\Lambda(X)K(k_{1c}/k_{12} + k_{2c}/k_{21}X) \quad (8)$$

Since $\Lambda(X)$ is known for each given X , and K is equilibrium constant, the equation (9):

$$K(k_{1c}/k_{12} + k_{2c}/k_{21}) = F(X) \quad (9)$$

indicates that a plot of $F(X)$ against X (Fig.16) should be linear giving the values of k_{1c}/k_{12} and k_{2c}/k_{21} as the intercept and the slope respectively. The numerical values of reactivity ratios are listed in Table III.

By dividing the values of intercepts and slopes of the straight lines in Figure 16 the rates of formation of electron - acceptor polymer free radicals $k_{1c} \cdot k_{21}$ over rates of formation of electron-donor polymer free radical $k_{2c} \cdot k_{21}$ are obtained. The data

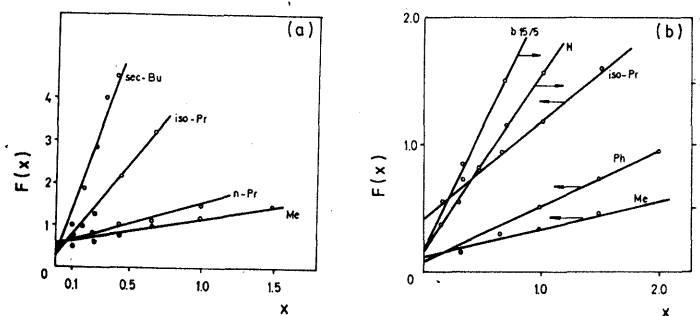


Fig. 16. Plot of $K(k_{1c}/k_{12} + k_{2c}/k_{21}X) = F(X)$ vs. X in the copolymerization of a) $(\text{PhCH}=\text{CHOR})$ with MAn ,^{13,16} b) $\alpha\text{-MeSt}$ with RMI ($\text{b15/5} = \text{benzo-15-crown-5}$).^{30,34,37}

TABLE III

Reactivity ratios of CT-complex monomers and free monomers
 $\text{PhCH}=\text{CHX}$ with MAn

X	Kk_{1c}/k_{12}	Kk_{2c}/k_{21}	$k_{1c}k_{21}/k_{2c}k_{12}$	Lit
OMe	0.51	0.58	0.88	16
OEt	0.06	1.25	0.048	13
On-Pr	0.45	1.03	0.44	16
Oiso-Pr	0.27	4.28	0.06	16
Osec-Bu	0.30	9.87	0.03	16
Me	0.20	1.06	0.23	22
RMI with $\alpha\text{-MeSt}$				
H	0.26	3.2	0.08	29
Me	0.11	0.25	0.42	30
Ph	0.08	0.40	0.20	34
iso-Pr	0.40	1.20	0.33	32
b15/5 ^a	0.60	4.17	0.14	37
$\text{PhCH}=\text{CHSR}$ with MAn				
Me	0.5	4.39	0.114	18
Et	1.6	3.5	0.45	13
n-Bu	0.7	5.05	0.139	18
sec-Bu	0.8	6.12	0.130	18

a) benzo-15-crown-5

of the ratio of k_{1c}/k_{12} to k_{2c}/k_{21} i.e. $k_{1c}k_{21}/k_{2c}k_{12}$ show in all cases the values lower than 1, thus indicating that the rate of formation of macroradicals $m_2\cdot$ (electron donor) are higher than the rate of formation of macrofree radicals $m_1\cdot$ (electron acceptor). But since in all cases the alternating copolymers are obtained, it indicates that

electron donors (α -MeSt, PhCH=CHX) preferably react via the complex addition, while electron acceptors (MAn, RMI) react through the crosspropagation of free monomers.

In summarizing the results of alternating copolymerization of monomers listed in Tables I and II it can be concluded that the same mechanism can be applied to monomers which form CT-complexes with relatively high complexation constants (Table I) and to monomers in Table II which form CT-complexes with very low complexation constants. In both cases the initial rates of copolymerization at different monomer-to-monomer concentration and at different total monomer concentration are not consistent with homopolymerization of complex nor with crosspropagation of free monomers. The mechanism is consistent with the concerted mechanism of simultaneous crosspropagation of monomer and addition of complex during the propagation reaction. But it is evident that although the mechanism of copolymerization is the same when equilibrium constants are high (Table I) or low as in Table II, all effects related to the participation of CT-complex are more pronounced in case of high values of complexation constant. Further proof of the participation of CT-complex in free radical initiated copolymerization is based on the fact that rate of formation of electron donor macrofree radicals is faster than the formation of electron acceptor macrofree radicals, although in all cases alternating copolymers are obtained.

Further contribution to the mechanism of alternating copolymerization in the presence of CT-complex is given by the high conversion copolymerization of electron-donor-acceptor monomers in which electron acceptors are homopolymerizable. The copolymerization was performed in Calvet microcalorimeter at different monomer-to-monomer ratios in the feed. As an example on the Fig.17 is presented the electric signal and conversion in the high conversion copolymerization of N-EtMI with α -MeSt in CHCl_3 in the presence of 0.3% of AIBN at 60°C.³³ Fig. 17a

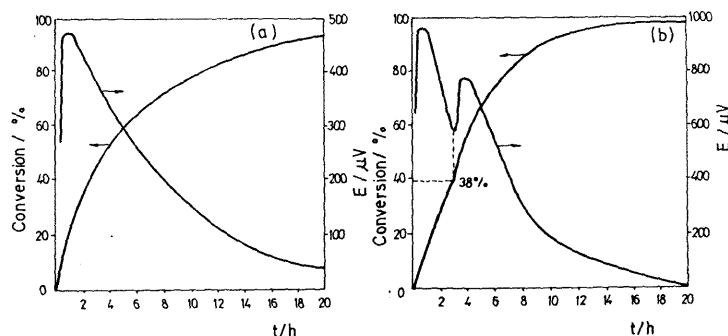


Fig. 17. Calvet microcalorimeter tracing and conversion against time in the copolymerization of α -MeSt with N-EtMI in CHCl_3 ; 0.3% AIBN: a) at equimolar ratio of comonomers; b) at molar ratio of 0.2 to 0.8.³³

shows the data obtained in copolymerization of N-EtMI with α -MeSt at equimolar ratio of comonomers, while Fig.17b gives the plot of electric signal of calorimeter and conversion against time in the copolymerization of N-EtMI with α -MeSt at a molar ratio of 0.8 to 0.2. It is evident that after the predetermined temperature of 60°C is reached, the tracing shows the expected pathway up to the point which corresponds to a conversion of 39.2% where a minimum is formed. After that the electric signal passes through another maximum and from this point the electromotive force decreases to zero. It is of interest to note that theoretical yield of alternating copolymer in the copolymerization of N-EtMI with α -MeSt at a molar ratio of 0.8 to 0.2 is 38%.

The same was also observed in the copolymerization of α -MeSt with RMI (R = Me, Et, n-Pr, n-Bu, n-Hex, Ph). In Table IV are summerized numerical data of theoretical and experimental yields of alternating copolymers of α -MeSt and RMI.

TABLE IV

Comparison of theoretical and experimental conversion of alternating copolymers of α -MeSt with RMI at 60°C in CHCl_3 ; 0.3% AIBN; total monomer concentration 2 molL⁻¹

Molar ratio		Conversion of		Molar ratio		Conversion of	
α -MeSt	RMI	alt - copolymer/%		α -MeSt	RMI	alt - copolymer/%	
		Theor.	Exptl.			Theor.	Exptl.
R = Me				R = n-Bu			
0.2	0.8	40.72	38.0	0.2	0.8	37.1	37.7
0.3	0.7	60.70	61.0	0.3	0.7	57.0	60.0
0.4	0.6	80.49	82.0	0.4	0.6	78.0	82.0 ^a
R = Et				R = n-Hex			
0.1	0.9	19.6	18.2				
0.2	0.8	39.2	38.0	0.2	0.8	35.5	35.0
0.3	0.7	59.3	57.8	0.3	0.7	53.3	61.0 ^a
0.4	0.6	79.5	84.5	0.4	0.6	76.8	84.0 ^a
R = n-Pr				R = Ph			
0.1	0.9	18.8	16.8	0.1	0.9	17.3	17.0
0.2	0.8	38.1	39.2	0.2	0.8	36.0	40.0
0.3	0.7	58.1	60.1	0.3	0.7	56.0	55.0
0.4	0.6	78.7	80.0	0.4	0.6	78.0	81.0
R = iso-Pr				0.5	0.5	100.0	100.0
				0.6	0.4	83.4	83.0
0.2	0.8	38.1	36.1	0.7	0.3	65.0	61.0
0.3	0.7	58.1	56.1				
0.4	0.6	78.7	84.0				

a) broad minimum in calorigram

The data in Table IV indicate that the same mechanism of initiation, propagation and termination is operable in the copolymerization of all studied copolymers of α -MeSt with N-substituted maleimides. In all cases there is a distinct

evidence that alternating copolymers are formed prior to homopolymerization of homopolymerizable RMI which in all polymerizations was used in excess - the homopolymerization starts only after the copolymerization is finished.

Although these results could be considered as a natural consequence of the reactivity ratios of monomers and free radicals derived from them, they nevertheless do not contradict the participation of CT-complex during the propagation step. In fact the discovery that the rate of formation of electron donor free radicals is much faster than the rate of formation of electron acceptor free radicals supports the concerted mechanism of crosspropagation and addition of CT-complex monomers.

It is of interest to mention that the mechanism of consecutive copolymerization and homopolymerization represents a convenient method for the preparation of polymer blends of alternating copolymers with predetermined amount of homopolymer directly from the calorimeter reactor without subsequent mixing of components. The miscibility behaviour of alternating poly (α -MeSt-alt-N-RMI) with RMI (R = Me or Ph) was studied on the basis of T_g 's of copolymer/homopolymer blends.²⁹ In all cases it was found that blends have T_g 's between electron acceptor homopolymer and alternating copolymer, and all mixtures have a single T_g . This indicates that blends of studied alternating copolymers and homopolymers are

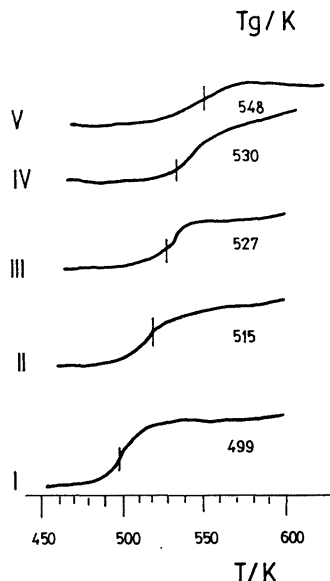


Fig. 18. T_g of blends of poly (α -MeSt-co-N-MeMI) (I) with poly (N-MeMI) (V); ratio of I to V in %: I, 100:0; II, 85.6:14.4; III, 64.6:35.4; IV, 43.3:56.7; V, 0:100.

miscible on molecular level if single T_g is taken as a criterion (Figs.18 and 19). Recently the same was also found for the mixtures of poly [N-4'- (benzo-15-crown-5) maleimide-alt- α -MeSt] and poly [N-4'- (benzo-15-crown-5) maleimide].³²

In conclusion it can be stated that basically the same mechanism describes the alternating copolymerization of electron-donor-acceptor monomers which form CT-complexes regardless whether the complexation constant is high or low. The examples described in this Review show that in the copolymerization of PhCH=CHOR with MAn as well as in the copolymerization of RMI with α -MeSt, the key observation is that the plot of initial rate of polymerization over monomer concentration against monomer concentration showed a good linearity for each given monomer feed molar ratio $[M_2]/[M_1]$. It was further shown that the intercepts of straight lines of the plot of $R_p/[M_1]$ against $[M_1]$ and the slopes provide an indication of the validity of proposed mechanism which involves the concerted propagation which consists of crosspropagation of free monomer and addition of CT-complex monomer. On the basis of these results it is possible to make quantitative separation of overall initial rates of copolymerization into the initial rates of crosspropagation

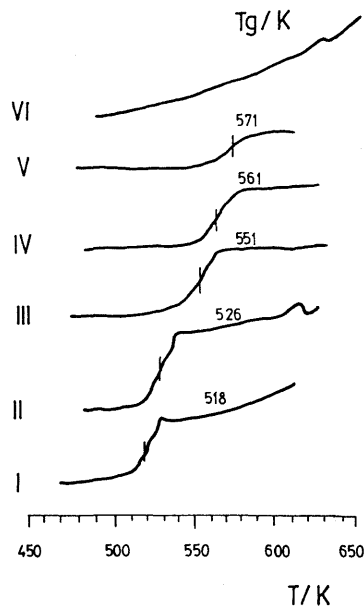


Fig. 19. T_g of blends of poly (α -MeSt-co-N-PhMI) (I) with poly (N-PhMI) (VI); ratio of I to VI in %: I, 100:0; II, 77.5:22.5; III, 55.93:44.07; IV, 35.9:64.1; V, 17.8:82.2; VI, 0:100.

of free monomers, $R_p(f)$, and of charge-transfer complex $R_p(CT)$ respectively.: $R_p = R_p(f) + R_p(CT)$.

However it should be emphasized that although the mechanism of free radical initiated copolymerization in the presence of high and low complexation constants is basically the same, all effects which are related to the participation of CT-complex monomers are less pronounced with monomers which form CT-complexes with low complexation constants.

It was further shown that in both studied systems the rate of formation of electron donor polymer free radicals is faster than the rate of formation of electron acceptor free radicals. In case of alternating copolymerization it indicates that electron donors preferably react via the addition of CT-complex monomers. The fact that in the high conversion copolymerization of the mixture of α -MeSt with an excess of homopolymerizable N-substituted maleimide, the homopolymerization starts after the alternating copolymerization is terminated, is also in agreement with concerted mechanism of copolymerization which involves the participation of CT complex.

The results presented in this article no doubt represent a contribution to the mechanism of propagation in the free radical initiated alternating copolymerization in the presence of CT-complex monomers. However there still remains a problem which is not yet solved. It is the mechanism and the role of CT-complex in the initiation of the alternating copolymerization.

Several authors consider that CT-complexes do not participate in the initiation of alternating copolymerization. Rätzsch and Seiner⁴⁴ found that in the copolymerization of styrene with MAn only free radicals of styrene or MAn and not of CT-complex participate in the initiation reaction. However Deb and Meyerhoff⁴⁵ consider that main features of this reaction can be explained by the "complex mechanism". Tirell and Jones have experimentally proved that the complex does not participate in the initiation of copolymerization of N-PhMI with styrene⁴⁶ or N-PhMI with chloroethylvinyl ether.^{47,48}

In order to get better insight into the role of CT-complex in the initiation step in free radical initiated copolymerization more work should be done, especially with CT-complexes of monomers which do not homopolymerize. The studies along this line are under way in our Laboratories.

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SAŽETAK

Kopolimerizacija elektron-donor-akceptor monomera koji formiraju komplekse uz prijenos naboja

Dragutin Fleš

Opisan je mehanizam alternirajuće kopolimerizacije dvije serije komonomera od kojih jedna formira komplekse uz prijenos naboja s velikom konstantom kompleksiranja ($K = 0,2-0,4 \text{ Lmol}^{-1}$), a druga formira komplekse s malom konstantom kompleksiranja ($K = 0,02-0,06 \text{ Lmol}^{-1}$). Prva serija obuhvaća slijedeće kopolimere: poli (fenilvinil alikil eter-co-MAN); (alkil = Me, Et, n-Pr, izo-Pr, n-Bu, sek-Bu, $\text{CH}_2\text{CH}(\text{Me})\text{Et}$, $(\text{CH}_2)_2\text{CH}(\text{Me})\text{Et}$); poli [fenilvinil alikil eter-co-fenilmaleimid]; (alkil = Me, Et, n-Pr, n-Bu, sek-Bu, $\text{CH}_2\text{CH}(\text{Me})\text{Et}$, $(\text{CH}_2)_2\text{CH}(\text{Me})\text{Et}$); poli (α -metilstiren-co-anhidrid meleinske kiseline). Druga skupina obuhvaća: poli (α -metilstiren-co-anhidrid meleinske kiseline), poli (α -metilstiren-co-N-supstituirane maleimide); (N-supstituent. = Me, Et, n-Pr, izo-Pr, n-Bu, tere-Bu, Ph, benzo-15-kruna-5). U obje serije komonomera utvrđeno je da konstante kompleksiranja ne ovise o stetičkim faktorima, što pokazuje da se kompleksi formiraju putem vinilnih veza, a ne putem etera ili karbonila u maleimidu. Kopolimerizacija je vršena uz radikalne inicijatore kao što su O_2 , benzoil peroksid, lauroil peroksid, ionizirajuće zračenje i AIBN postupkom »točka-po-točka, do konverzije od 15% ili do visoke konverzije u Calvet mikrokolorimetru. U svim kopolimerizacijama utvrđeno je da je inicijalna brzina kopolimerizacija jednaka sumi brzine kopolimerizacije putem slobodnih monomera i putem adicije CT-kompleksa: $R_p = R_p(f) + R_p(\text{CT})$.

Pokazano je osim toga da je u svim kopolimerizacijama brzina nastajanja makromolekula elektron donora veća nego brzina nastajanja elektron akceptor makroradikala, što također dokazuje sudjelovanje CT-kompleksa u alternirajućem rastu lanca. Kod kopolimerizacije do

visoke konverzije u Calvet mikrokolorimetru pokazano je da uz višak homopolimerizirajućeg elektron akceptora - (N-suptituirani maleimidi) - homopolimerizacija počinje tek nakon što je dovršena alternirajuća kopolimerizacija.

Rezultati opisani u ovom pregledu pokazuju da i u slučaju kada komonomeri formiraju CT-komplekse s velikom konstantom kompleksiranja, ili s malom konstantom kompleksiranja, CT-kompleks sudjeluje u rastu lanca kod radikalne polimerizacije. Međutim u svim slučajevima je zapaženo da je mehanizam povezan sa sudjelovanjem CT-kompleksa jače izražen u slučaju kada je koncentracija kompleksa u smjesi za polimerizaciju veća.

U zaključku je naglašeno da rezultati dobiveni u opisanim radovima dokazuju sudjelovanje CT-kompleksa pri propagaciji alternirajuće kopolimerizacije, ali da su potrebni dodatni radovi da bi se dokazalo dali CT-kompleks sudjeluje pri inicijaciji.

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