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Original Research

Applying Plant Lectins to Assay the Effect of Environmental Pollution on the Glycosylation of Human Placenta

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Abstract

Our study was designed to establish whether air pollution in urbanized industrial centers of southern Poland affects the process of glycosylation in a full-term human placenta. This process of glycosylation was analyzed by the quantitative determination of the binding of WGA and LCA lectins to placental villi. The study was performed on human placentas collected in 1990-91 and 2000-01 in regions of southern Poland differing in their degree of environmental pollution: the highly polluted areas of Upper Silesia and Cracow agglomeration. The Bieszczady area with low pollution was considered the control. The concentrations of nitrogen and sulfur oxides and the concentration of aerosols were used as markers of the degree of air pollution.

The direct immunofluorescence reaction of the placenta tissues with fluorescein-labeled (FITC) lectins was used. The staining of the placenta tissues was examined under a fluorescence microscope linked to an analysis system. A microdensytometric method was used to assay the amount of tissue-bound lectins. The results showed no significant effect of the three main air pollutants in the study areas in southern Poland, i.e. nitrogen and sulfur oxides and high level of aerosols, on the structure of WGA- and LCA-specific glycoconjugates in human placenta. However, the marked quantitative changes in the degree of lectin binding to placental cellular structures were noted within the last 10-year period in all studied regions.

Keywords: Lectins, glycoconjugates, placenta, human, environmental pollution

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Introduction

Lectins have become an important tool in the studies of biological structures and processes in various organisms since the biological role of glycoconjugates has been understood and recognized. Immune properties and the high specificity of lectins in forming unstable bonds with certain sugar structures has let them be commonly used in histochemical studies [19]. The use of lectins in the study of the structure and distribution of glycoconjugates of the human placenta in comparison with the placentas of animals closely related to humans [2, 3] and the nature of immune interactions in the maternal-fetal contact zone [11] was noted. Moreover, the role of glycoconjugates in blastocyst implantation [11] and the significance of their structural modifications in different phases of placenta development [4] were studied with lectins.

Lectins of different sugar specificity were useful in localizing the structures containing α-D-mannose and *N*-acetyl-D-glucosamine in full-term human placenta and showing the lack of external fucosyl residues. The presence of sialic acid residues was observed in the superficial plasmalemma of syncytiotrophoblast and on the surface of the fetal capillary endothelium. *N*-acetyl-lactosaminyl residues were found on the cell surface of the amniotic endothelium, while sugar compounds binding mannose-specific PSA and LCA lectins were detected on basement membranes of the amniotic epithelium [6]. Moreover, pathological placental tissues showed a lesser amount or lack of this sugar residues in comparison with normal placenta what suggests serious functional consequences of changes in glycosylation. Moreover, it might contribute to restricted placenta growth and development and a reduction in the efficiency of maternal-fetal exchanges of gases and metabolites [10].

The degenerative effect of environmental pollution on the development of human placenta, leading to morphological and biochemical changes, are commonly known. The changes in placenta are in proportion to the concentration of xenobiotics and are associated with the microinfarcts (inactivation) of individual terminal villi and the fibrosis of the intervillous space [12, 13, 14]. The elevation of the content of neutral polysaccharides, acid mucopolysaccharides and collagen fibers [13], the formation of mineral deposits and an increase of fluorine compounds and heavy metals concentration [7] are other effects of environmental pollution. A decrease of the activity of oxidative enzymes, including cytochrome C oxidase, resulted in adaptive changes, i.e. the proliferation of tiny terminal villi (forced villi), increasing the area of diffusive exchange between maternal and fetal circulation [8, 9, 15] associated with a constriction of intervillous space hindering maternal blood flow [16]. Aerobic oxidation insufficiency resulted in a rise in the intensity of anaerobic oxidation (increased lactate dehydrogenase activity) [7, 9] and a decrease in the activity of enzymes of energy metabolism, such as pyruvate kinase [1, 7, 9]. Hence, long-term exposure to the chemical pollutants present in the environment resulting in placental insufficiency may lead to fetal hypoxia, lowered birth weight and retardation of the psychomotor development of a child [7, 16].

Despite extensive studies of the consequences of exposure to environmental stimuli and their influence on morphological and functional changes in the human placenta, data on the effect of xenobiotics on glycosylation processes in the placenta were limited. The changes of binding of the selected lectins to placental structures reflected changes in the glycosylation of defined sugar residues and may be a very sensitive indicator of biochemical transformations.

The present study was designed to establish whether selected environmental factors, nitrogen and sulfur oxides and high concentrations of aerosols affect the binding of WGA and LCA lectins to structures of normal mature (full-term) human placenta.

Materials and Methods

Lectins

The two lectins of plant origin, linked to fluorescein isothiocyanate (FITC) at a concentration of 25 µg/ml were used:

- WGA – from wheat sprouts (*Triticum vulgare*), showing an affinity for oligosaccharides containing branched *N*-acetylglucosamine chains (GlcNAcβ1-4)$_5$, (GlcNAcβ1-4)$_4$, (GlcNAcβ1-4)$_3$ and sialic acids (NeuNAc).
  The lectin WGA-FITC (5 mg/ml) was purchased from Vector Laboratories and dissolved with phosphate buffered saline (PBS) supplemented with 1% bovine serum albumin (PBS-BSA).
- LCA – from lentil seeds (*Lens culinaris*) exhibiting an affinity for oligosaccharides containing mannose, glucose, *N*-acetylglucosamine linked by α 1-6 bond to fucose.
  LCA lectin was isolated by affinity chromatography and FITC conjugated according to the Goldmann method (Department of Cell Biology, University of d'Auvergne, Clermont-Ferrand in France).

The lectins bind to different tissues of the placental villi. WGA reacts mostly with syncytiotrophoblast as well as to a slightly lesser degree with the mesenchymal tissue of the villus stroma, whereas LCA binds only to mesenchymal tissue and blood vessel walls.

Tissues

The study was performed on 68 human placentas collected from gynaecological departments in regions of southern Poland characterized by high levels of environmental pollution (Chorzów in Upper Silesia and the Cracow agglomeration) in two different periods: 1990-

91 (30 placentas) and 2000-01 (38 placentas). Placentas collected in the Bieszczady region in the same periods were used as the control group (low environmental pollution). Only full-term placentas from healthy women with neither nicotine nor alcohol addictions, residing permanently in the given area were included in the study. Placental specimens were fixed in AFA solution (70% ethanol:formalin:glacial acetic acid = 90:5:5). After dehydration, the samples were embedded in paraffin and cut on Leica RM 2145 microtome. All placenta fixed tissues were obtained from the Department of Cytobiology and Histology, Faculty of Pharmacy, Medical College of Jagiellonian University.

The following parts of the villous zone of placenta were analyzed: the area directly adjacent to the basal plate, the middle area and the area close to amniotic epithelium.

Histochemical Method

The method was based on direct fluorescence after incubation of tissue with a lectins-FITC (working dilution). Paraffin-embedded sections 4 mm thick were deparaffined, rehydrated, covered with lectin-FITC solution and incubated in a humid and dark chamber for 1 hour. Thereafter, they were washed with PBS-BSA, closed with cover glass and stored in darkness at 4^{o}C for no longer than 24 hours. The tissues were examined under a fluorescence microscope with an Olympus BX61 image analysis system.

Microdensytometric Method

The microdensytometric method was used to quantify the amount of lectin bound to the tissues (expressed as fluorescence intensity). The picture covering the complete microscopic field analyzed in standard conditions (10 X microscopic magnification, which corresponds to 100 X magnification of the picture) was taken with AnalySIS-Pro program (Soft Imaging System GmbH). Fluorescence intensity was quantified after transforming the obtained colored pictures into black-and-white images. The results are presented on the basis of a scale from 1-256 as the program can distinguish 256 gray levels, i.e. optical density levels. Statistical significance was deermined by Anova test and differences were regarded as significant when $p < 0.05$.

Analysis of Air Pollution in the Given Regions

The Provincial Institutes of Environmental Protection in Rzeszów, Kraków and Katowice provided the data of air pollution for these regions. We analyzed three major air pollutants: sulfur, nitrogen oxides and aerosols.

Results

The obtained results were comparatively analyzed in relation to the degree of WGA and LCA lectin binding in:

a. different areas of the placental villi,
b. placentas derived from different regions and
c. respective structures of placentas collected in 1990-91 and 2000-01.

Characterization of WGA and LCA Lectin Binding in Different Areas of the Placenta

Three different areas of the villuos zone were analyzed:

a. the area directly adjacent to the basal plate, the so-called anchoring villi,
b. the central area – tightly packed villi with small intervillous spaces,
c. the periepithelial area – located below amniotic epithelium, containing tiny villi with large intervillous spaces.

The mean fluorescence intensity of binding of WGA and LCA lectins in different areas of the villous zone does not show any significant variability. LCA binding was slightly higher than WGA binding in all tested areas. These differences between the binding of both lectins to the tested placenta regions resulted only from the tissue structure.

Characterization of WGA and LCA Lectin Binding in Relation to Region of Placenta Origin

The fluorescence intensity of structures composing villi from placentas collected in three different regions of southern Poland: Bieszczady, Upper Silesia (Chorzów) and Cracow was studied. The mean WGA and LCA fluorescence intensity of placental tissues derived from the given regions of Poland are shown in Figs. 1 and 3. It was noted that the values for the individual regions for the same time periods were similar. Only the placental tissues from the rural region of Bieszczady showed a somewhat higher value for both the WGA and LCA lectins in 1990-91 and 2000-01, but these differences did not reach statistical significance.

Comparison of WGA and LCA Lectin Binding to the Villi of Placentas

The comparison of the fluorescence intensity of villous structures of placentas derived from different periods is presented in Figs. 1 and 3. Marked quantitative differences in the intensity of both lectins binding to the villous structures of the placentas collected at an interval of 10 years were observed. Unexpected, the binding capacity of both lectins was significantly lower in 2000-01 than

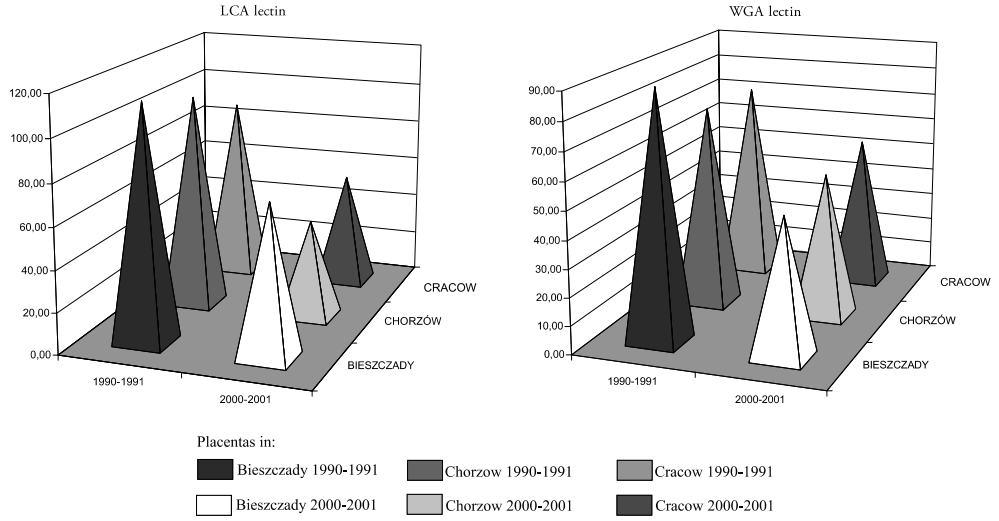

Fig. 1 Comparison of WGA and LCA lectin binding to villi of placentas collected in different regions of southern Poland throughout the last 10 years (1990-91 and 2000-01).

in 1990-91 characterized with much higher pollution. In 2000-01, the mean fluorescence intensity of the tissues labeled with LCA lectin was 58.10, while in 1990-91 it was 125.03. The respective values for the WGA lectin were 51.75 in 2000-01 and 77.4 in 1990-91. The differences were particularly visible for the LCA lectin and for placentas collected in the Bieszczady ($p<0.0008$) in comparison with placentas derived from Chorzów and Cracow ($p<0.0218$ and $p<0.064$ respectively).

Analysis of Air Pollution in the Studied Areas

The results are presented in Fig 2. The data collected in Upper Silesia and Cracow in 1990-91 showed the concentrations of aerosols, nitrogen and sulfur oxides significantly exceeding the permissible limit. In Upper Silesia, the aerosol concentration ranged from 237-219 μg/m^3 (for a permissible dose of 50 μg/m^3), the concentration of nitrogen oxides was 127-92 μg/m^3 and was over three times higher than the permissible limit. In Cracow, the concentration of sulfur oxide was twice the permissible amount. In Bieszczady, the respective values in 1990-91 were slightly lower than limits. Considering only these three pollutants, the Bieszczady region can be regarded as relatively unpolluted.

In 2000-01 a major improvement in the quality of air was noted in the studied regions. Concentrations of aerosols, and nitrogen and sulfur oxides dropped to permissible limits. However, in Upper Silesia, the concentration of sulfur oxide in 2000 and the aerosol concentration in 2001 slightly exceeded the limits. Air quality in the Bieszczady region also improved (a 2.5-fold decrease in aerosol concentration and a 5-fold decrease in sulfur oxide content, obtaining the low level of 0.9 μg/m^3 in 2001).

Discussion

The impact of air pollution in urban industrial regions on the morphology and enzymatic activity of the human

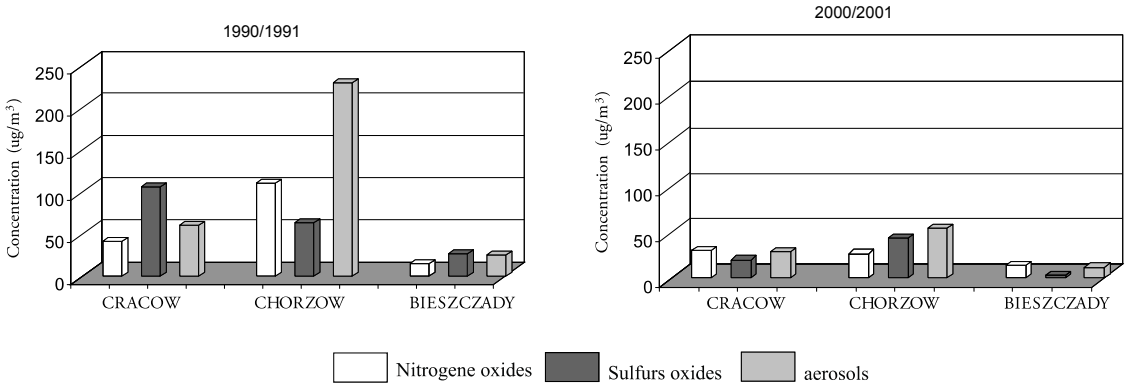

Fig. 2. The mean concentration of nitrogen and sulfur oxides and aerosols in regions of Upper Silesia (Chorzów) and Cracow agglomeration in comparison with the Carpathian Mountains (Bieszczady) in 1990-91 and 2000-01.

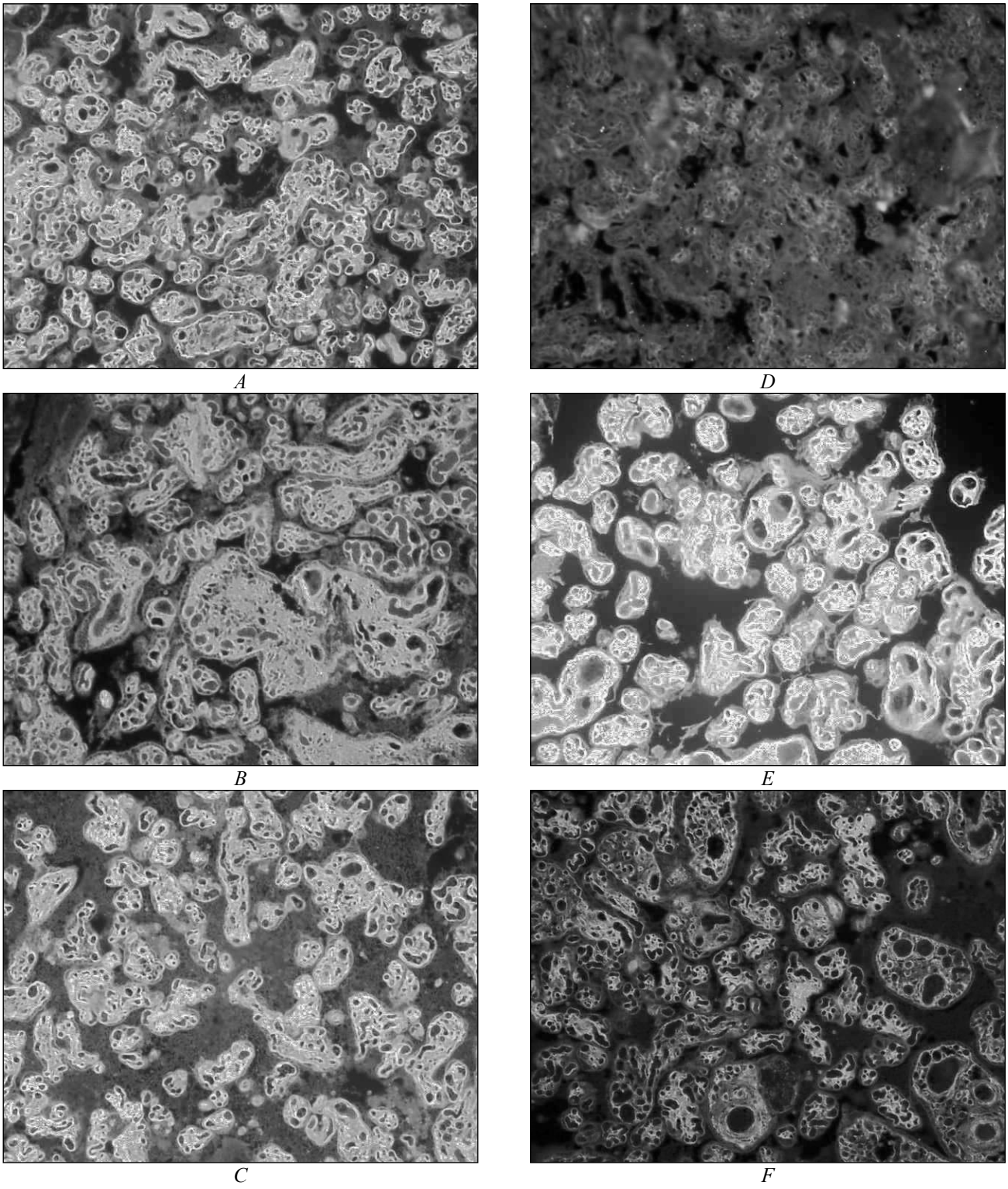

Fig. 3. Human placentas (central area of villous syncytiotrophoblast) collected in the Bieszczady region (A, B), Chorzów (C, D), and Cracow agglomeration (E, F) in two different periods: 1990-91 (A, C, E) and 2000-01 (B, D, F). Reaction with LCA-FITC.

placenta has been analyzed previously (1985-1996) and described [5, 7, 15, 16, 17]. The studies demonstrated significant progressive degenerative changes in placentas collected in Upper Silesia and Cracow in comparison with placenta derived from the Bieszczady region (Carpathian Mountains). These degenerative changes included a decrease in placenta weight, followed by lowered birth weights.

The results of the present study show that three main air pollutants, i.e. nitrogen and sulfur oxides and high

concentrations of aerosols, did not significantly affect the structure of WGA- and LCA-specific placental glycoconjugates. Despite the marked difference in the degree of air pollution between the Upper Silesia and Cracow agglomeration and the Bieszczady region, WGA and LCA lectin binding to the cellular structures of human placenta were comparable. However, the studies were focused only on some oligosaccharide structures, capable of specific binding with WGA and LCA lectins, i.e. sugar structures containing mostly *N*-acetylglucosamine, mannose and glucose. It cannot be excluded that the analyzed factors responsible for air pollution could influence glycoconjugates of a different structure. Moreover, the studied air pollution factors were selected because of permanent monitoring in Poland since 1990.

However, the changes in the degree of lectins' binding to the cellular structures of placentas have been observed clearly over the last ten years. In 2000-01, as opposed to 1990-91, the binding of LCA and WGA lectins to placental cellular structures diminished more than two-fold. This suggests the changes in the sugar structure of the analyzed glycoconjugates but as the effect of factors not associated with studied air pollutants. The nature and degree of these changes is not known. However, they are sufficient enough for the sugar structures of the glycoconjugates to be unrecognizable for the specific lectin. The lack of data on the influence of environmental factors on the process of glycosylation in the human placenta make difficult the interpretation of the observed phenomenon. Sgambati et al. [10] have observed a reduction in the quantity of sugar residues of various structures of placenta associated with the ability for lectin binding. These observations included binding of WGA lectin to structures of pathological placentas (intrauterine growth retardation based on absent or reversed diastolic flow). In the presented study a phenomenon of a similar nature has been observed in relation to normal placentas derived from full-term, correctly developed pregnancies. Therefore, the explanation of the observed phenomenon might be related to socio-economic and cultural factors and technological development noted in Poland during the last ten years. This hypothesis includes changes in the technology of production and preservation of food, detergents, cosmetics and medicines as examples of modern technologies introduced into this country. Besides the decrease of air pollution, new feeding habits and new chemicals in close contact with the skin of pregnant women might be associated with the function of placentas in human pregnancy. This problem is complex and requires wide research, including specialized and detailed analysis of many aspects of environmental changes in recent years.

References

1. GUMIŃSKA M., KĘDRYNA T., ZAMORSKA L. Pyruvate kinase in placenta of women living in polluted environments 21st Conference of the Polich Biochemical Society Warszawa **1995**
2. JONES C.J.P., KOOB B., STODDART R.W., HOFFMANN B., LEISER R. Lectin histochemical analysis of glycans in ovine and bovine near-term placenta binucleate cells. Cell Tissue Res. **278**, 601, **1994**.
3. JONES C.J.P., DANTZER V., LEISER R., KREBS Ch. Localization of glycans in the placenta, a comparative study of epitheliochorial, endotheliochorial and haemomonochorial placentation. Microsc. Res. Tech. **38**, 100, **1997**
4. JONES C.J.P., WOODING F.B, DANTZER V., LEISER R., STODDART R.W. A lectin binding analysis of glycosylation patterns during development of the equine placenta. Placenta **20**, 45, **1999**.
5. KASZNIA-KOCOT J., ZAMORSKA L., ŻOLNIEREK M., NIWELIŃSKI J. The condition of Chorzów hypotrophic and born-at-term infants in relation to the morphochemical features of the placentae. Polish J. Environ Stud. **2**, 29, **1993**.
6. KOŃSKA G., ZAMORSKA L., PITUCH-NOWOROLSKA A., SZMACIARZ M., GUILLOT J. Application of fluorescein-labelled lectins with different glycan-binding specificities to the studies of cellular glycoconjugates in human full-term placenta. Folia Histochem. et Cytobiol. **41**, 155, **2003**
7. NIWELIŃSKI J., ZAMORSKA L. Monitoring of air pollution in southern Poland using a picture of human placentas. Systematization and analysis of results obtained in southern Poland in the period between 1986-1990. In, Godzik S. [ed.], Condition and Threat to Natural Environment in the Silesian-Kraków Region **62**, 149, [Ed]. SGGW-AR, Warszawa, **1990**
8. NIWELIŃSKI J., ZAMORSKA L., KACZMARSKI F., PAWLICKI R. Enzyme histochemistry and microstructure of the human placenta as indicators of environmental pollution. Arch. Ochrony Środowiska **3-4**, 53, **1990**
9. NIWELIŃSKI J., ZAMORSKA L. The human placenta as an indicator of environmental pollution. Arch. Ochrony Środowiska **2**, 143,**1995**.
10. SGAMBATI E., BIAGIOTTI R., MARINI M., BRIZZI E. Lectin histochemistry in the human placenta of pregnancies complicated by intrauterine growth retardation based on absent or reversed diastolic flow. Placenta **23** (6), 503, **2002**
11. STEIN B.A., SHAW T.J., TURNER V.F., MURPHY C.R. Increased lectin binding capacity of trophoblastic cells of late day 5 rat blastocysts. J. Anat. **185**, 669, **1994**.
12. WIERZBA W., RADOWICKI S., PAWIŃSKI R. The microscopic evaluation of placenta from women in ecologic threatened area (Płock) I. Ginekologia Polska **68**, 109, **1997**
13. WIERZBA W., RADOWICKI S. The microscopic evaluation of placenta from women in ecologic threatened area (Płock) II. Ginekologia Polska **68**, 114, **1997**.
14 WIERZBA W., RADOWICKI S. The histochemical characteristic womens placentas from ecologic threatened area (Płock) Ginekologia Polska **68**, 118, **1997**.
15. ZADROŻNA M. Toxic effect of the chemical pollution in Lower and Upper Silesia on morphochemical differentiation of human placentae. Folia Medica Cracow. **38**, 117, **1997**.
16. ZAMORSKA L., KASZNIA-KOCOT J., ŻOŁNIEREK M., BUJAK P., SOCHACKA B., ZADROŻNA M., NIWELIŃSKI J. Physical development of neonantes in

chemically polluted environmental in southern regions of Poland. Ulga w cierpieniu **2**, 69, **1993**.

17. ZAMORSKA L. The newest results of studies of human placentas in a monitoring of biological value of environment in southern Poland. Report of meetings of the Scientific Commissions of the Polish Academy of Sciences in Kraków, **38**, 100. [Ed] Wydawnictwo i Drukarnia "Secesja" Kraków. **1995**.
18. ZAMORSKA L., ŻOŁNIEREK M., MENASZEK E. The villous cytotrophoblast of human placenta 7th Intern. Symposium "Molecular and physiological aspects of regulatory processes of the organism" Cracow **1998**
19. ŻEROMSKI J. Application of lectins in histochemistry**.** In, Zabel M.-Immunocytochemia [Ed.] Wydawnictwo Naukowe PWN, Warszawa, pp. 181-197, **1999**.