Placenta and umbilical cord blood deserve attention

Janka Foltinová [1], Viktor Foltin [2], Marcela Morvová [3], Eva Neu [4], Michal Šimera [1]

1 Institute of Histology and Embryology, Faculty of Medicine, Comenius University, Bratislava, Slovakia.
2 Institute of Natural Sciences, Humanities and Social Sciences, Faculty of Mechanical Engineering, Slovak University of Technology (SjF-STU), Bratislava, Slovakia.
3 Department of Astronomy, Earth Sciences, and Meteorology, Faculty of Mathematics, Physics and Informatics, Comenius University Bratislava, Slovakia.
4 Umweltmedizin Institut, Feucht bei Nűrnberg, Germany.

Correspondence to: Assoc. Prof. Janka Foltinová, MD., PhD.
Institute of Histology and Embryology, Faculty of Medicine, Comenius University, Sasinkova 4, SK-81108 Bratislava, Slovakia.
PHONE: +421-2-59357540
EMAIL: foltin@naex.sk; viktor.foltin@stuba.sk; morvova@fmph.uniba.sk

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Abstract

OBJECTIVES: This work follows up with our already published results concerning consequences of lead on prenatal and postnatal development of child in connection with the rise of hyperkinetic syndrome (ADHD). This disease has in children increasing tendency all over the world.
METHODS: In our work we used a set of histological and histochemical methods, method of scanning electron microscopy, infrared spectroscopy and statistical evaluation.
RESULTS: Our new method for proof of lead in placenta enabled us to show how lead is cumulated in syncytiotrophoblast. We have found release of lead from mother's erythrocytes in the intervillous space and receipt of lead by erythrocytes of fetus in the vessels of the villi of placenta. This finding enriches knowledge about relation between mother's erythrocytes, lead, calcium that is lead carrier, syncytiotrophoblast, and erythrocytes of fetus in the vessels of placental villi. We have proved that syncytiotrophoblast is the most frequent place for cumulation of lead deposits. We verified our ecomorphologic results by means of infrared spectroscopy in cooperation with physicists and statistically evaluated occurrence of ADHD in particular age categories what helps to fill gaps in knowledge of ADHD etiology.
CONCLUSIONS: Our finding of lead in umbilical cord blood immediately after the child birth is forewarning against the possible rise of the ADHD. This finding facilitates early diagnostics and means preventing step against the rise, development and consequences of this disease. The obtained results give evidence about the serious influence of mother's dwelling in environment polluted with neurotoxic metal – lead on the prenatal and postnatal development of child.

ORIGINAL ARTICLE

INTRODUCTION

For a long time placenta was considered biologically worthless material that was after the child birth liquidated as a biological waste. In 1974 scientists for the first time pointed out that placenta may be a suitable indicator of environmental pollution. In eighties, after recognizing that also in umbilical cord blood and in placenta sufficient amount of blood forming cells occur, banks for international exchange of umbilical cord blood were established. There has been gathered umbilical cord blood of voluntary donors that is used for transplantates. Scientific studies aimed at investigation of placenta (Baglan *et al.* 1997; Foltinová *et al.* 2000; Foltinová *et al.* 2006; Foltinová *et al.* 2007; Goyer, 1990; Lafond *et al.* 2004; Reichrtová *et al.* 1998) show that placenta is in fact valuable source of information for the further research. In the same way as growth rings of a trunk reveal history of a tree, placenta reveals history of pregnancy. Influence of polluted environment may cause changes in the microscopic structure of placenta. Physicians in the USA do not recommend pregnant women to dwell in peasant houses built in the beginning of the previous century since in that time lead had been frequently added to paints for decorating interiors. In our environment lead occurs almost everywhere. It can be detected in air, dust, and its levels are strictly checked also in the drinking water. Lead is deposited in teeth, long bones, but it may be found also in hair and nails. During pregnancy growth of the fetus requires release of mother's calcium and together with calcium lead is transported since calcium is a lead carrier (Foltinová *et al.* 2007). It has not passed long time from days when lead was added to petrol that was used as a fuel for vehicles (Bailey *et al.* 2002). That is why nowadays cumulation of lead and its conseque×nces occur in generation of children whose 25–30 year old mothers lived in conditions when leadless petrol was not generally used. Heavy metals are characterized by very slow release from the organism. Occurrence of lead in the villi of placenta deserves attention because thickness of transplacental barrier changes during the development of placenta from approximately 0.025 mm to mere 0.002 mm in the fully mature placenta. This is very important for the transfer of lead and investigation on the interdisciplinary level is therefore advisable. This is a task on which our team works already for several years. In this paper we present our new findings.

MATERIAL AND METHODS

In this work we prepared and evaluated sections from excisions of placentas of 104 healthy patients. Concurrently we examined umbilical cord blood of 50 patients by means of infrared spectroscopy using KBr pellet making technique. Moreover, from 3000 child patients hospitalized at Clinic of Child Psychiatry of Child Faculty Hospital with Polyclinic in Bratislava during years 2003–2006 were 120 patients with hyperkinetic syndrome. We investigated this set of patients. In our investigation we abode rules of medical ethics.

Excisions from placenta were fixed in AFO – alcohol:formol:acetic acid in the ratio 12:6:1. On the 7μm thick paraffin sections we carried out the following histological staining methods:

- hematoxylin-eosin
- Lendrum "acid-picro Mallory"
- Una Tanzer "acid-picro, indigocarmin-orcein"
- new methodical approach after Foltinová, which is combination of Mallory and Parker method for proof of lead with the software program Imago Pro Plus 45 Media Cybernetics Inc. assisting to microscope Olympus BX-50 with Sony three CCD. Positivity on lead is manifested by turquoise green colour.

Excisions from placenta were for the scanning electron microscope prepared by double fixation with glutaraldehyde (200 mmol/L) and osmium tetroxide (OsO_4 40 mmol/L) that were buffered by phosphates with pH 7.25. The specimens were dehydrated by alcohols and dried at the critical point of CO_2. The excisions were placed on aluminum discs and coated with a thin conducting layer of colloidal silver for further processing. The excisions on the discs were coated with an 18 nm thick layer of pure gold in a coating device (Balzers Union, Balzers, Lichtenstein) in argon atmosphere. The method for evaluation of excisions in scanning electron microscopy was described in our previous paper (Foltinová *et al.* 2007).

The following microscopes were used for the evaluation:

- light microscope Reichart Polyvar (Germany) at magnifications 180× – 1500×;
- scanning electron microscope PHILIPS CM 20 (Holland) at magnifications 365×.

For concurrent investigation of umbilical cord blood the smears were stained by method after May Grűnwald-Giems Romanovsky and for their evaluation by infrared spectroscopy we used infrared spectrometer SPECORD M80, Carl Zeiss, Jena (Germany).

RESULTS AND DISCUSSION

By means of our new histochemical method we have succeeded to show releasing of lead from the mother's erythrocyte in placenta and its receipt by the erythrocyte of fetus in the vessel of placental villus (***Fig. 1***). This way of visualization can be utilized for fast diagnostics. Furthermore we have shown that syncytiotrophoblast is the place with the most frequent cumulation of lead (***Figs. 1, 2, 3, 4, 5***). We have pointed out importance of phagocytosis of apical surface of syncytiotrophoblast in forming deposits of this heavy metal (***Fig. 5***). In our recent work we paid attention to formation of deposits of calcium that is a lead carrier (Foltinová *et al.* 2007). Lead comes out from the mother's erythrocytes and through transplacental barrier (Goyer, 1990; Needleman and Bellinger, 1991) enters erythrocytes of fetus and directly attacks developing fetus. For investigation of subtle details of transplacental barrier we utilized method of scanning electron microscopy because of its excellent distinction ability (***Fig.6***). This barrier suffers changes during the development of the fetus. Placenta matures and number of branching villi increases. Diameter of villi decreases from 170 μm in early pregnancy to 40 μm in time of the child birth. Part of fetal blood vessels becomes more eccentric. Layer of the syncytiotrophoblast becomes thicker what results in decreasing of the distance between mother's and fetal blood vessels.

Importance of our study consists also in the fact that lead transport from mother to fetus may cause serious consequences in the postnatal development of a child. When effect of lead on brain is concerned it should be stressed that premature endothelial cells forming capillaries of the developing brain are less resisting to the effect of lead than capillaries of the mature brain. They leak cations, including lead, into astrocytes and neurons. Primary mechanism for transplacental transfer of lead is probably a simple diffusion (Derelanko and Hollinger, 2001; De Silva, 1981). Concentration of lead in developing fetal tissues, including brain, concerns blood lead and increases with increasing size of the organ. Blood-brain barrier is immature, what makes entering of lead into brain easier. These facts mean warning against possible danger for human embryo and fetus even at low levels of lead in the mother's system (Derelanko and Hollinger, 2001).

Neurotoxic metal – lead may cause the disease "Attention Deficit Hyperactivity Disorder" (ADHD). Occurrence of this disease has increasing tendency all over the world. Hyperkinetic defect belongs to the most frequent reasons of psychiatric treatment in the child age (Drtílková, 2007). Exposition to lead during intrauterine or postnatal development may cause neurocognitive damage (Foltinová *et al.* 2006; Reichrtová *et al.* 1998). Basal gan-

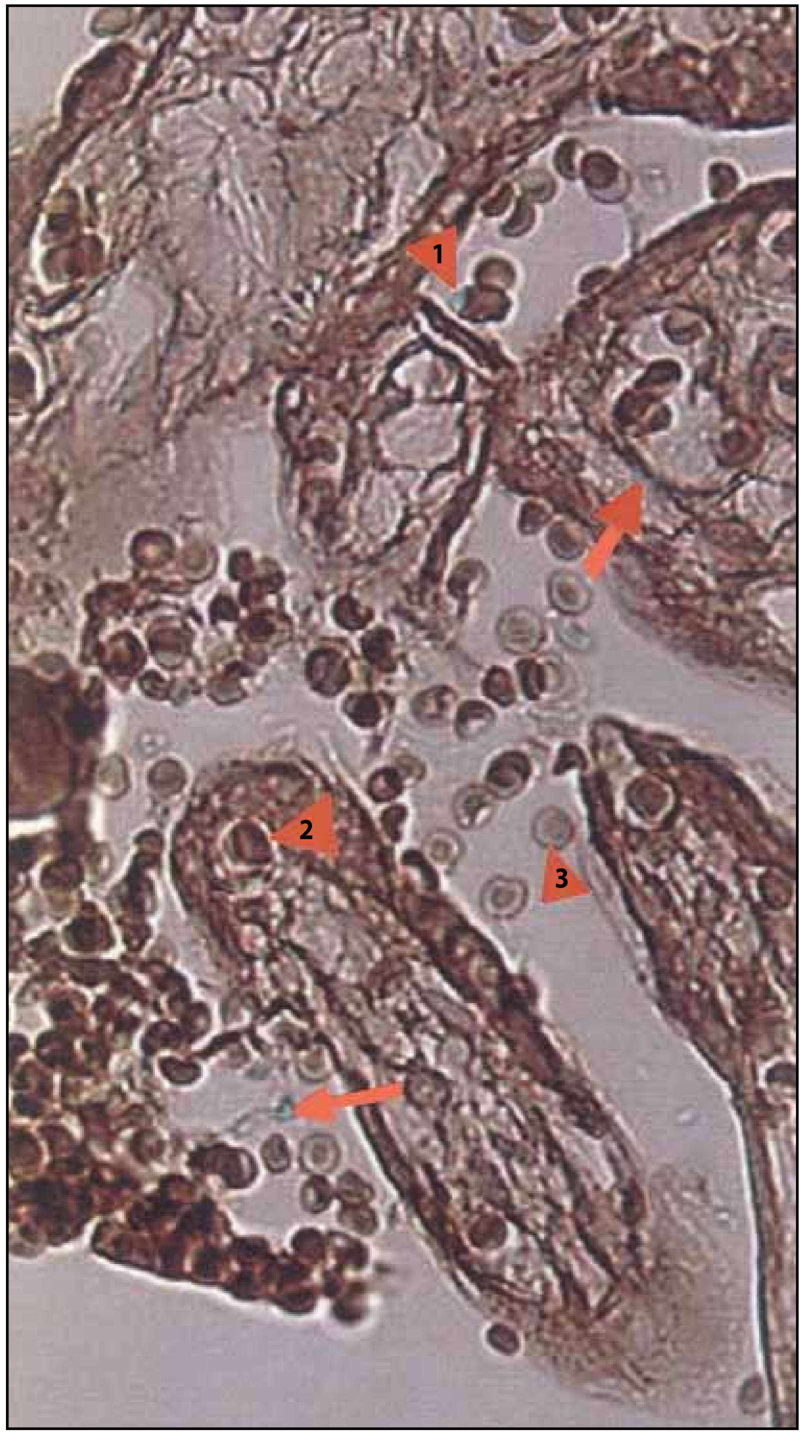

Fig.1: Placenta - 7μm thick section stained by new method for proof of lead after Foltinová. Cross section of placental villi and intervillous space. Magnified 650 ×. Findings:

- release of lead from mother's erythrocyte (1▼);
- lead on the surface of capillary (➔);
- receipt of lead by fetal erythrocyte with entrance veil of this heavy metal (2◀);
- decaying lead deposit of syncytiotrophoblast (🡐)
- lead in erythrocyte (3▲).

Fig.2

Fig.2: Placenta - 7μm thick section stained by Una-Tanzer method. Villus of placenta, its interstitium and vessels (↓). Magnified 180 ×.

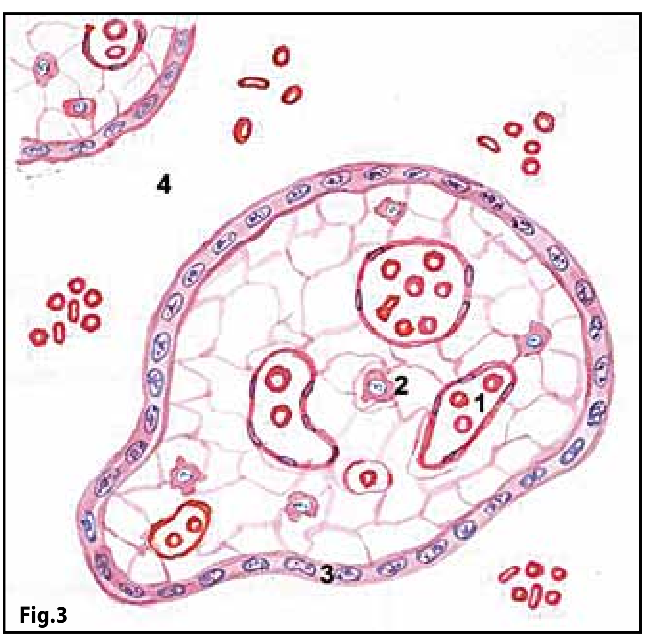

Fig.3

Fig.3: Placenta – schematic picture in the cross section of the villus of mature placenta (vessel with erythrocytes 1; Hofbauer cell 2; syncytiotrophoblast 3; intervillous space 4).

glia, mainly striatum (Bressler, 1999; Grandjean, 1978), are very sensitive to hypoxy and change of metabolism katecholamins, what plays important role in patophysiology of hyperkinetic defect. We have found that problems connected with hyperkinetic defect occurs in 5–8% of children, mainly in boys (***Fig.7A***). Approximately a third part of children suffering from this disease do not finish their education, 50% of them inclines to alcoholism and taking drugs, almost 70% of them say that they do not have friends at all, or a just very few ones. More than 30% suffers from states of anxiety and depressions. In our set of 3000 children hospitalized in last 15 years, 23% of them suffered from hyperactivity with the defect of attention (ADHD). Almost half of the children with ADHD (***Fig.7B***) have in their clinical picture hazardous course of pregnancy caused by premature peeling off placenta what opens the way for

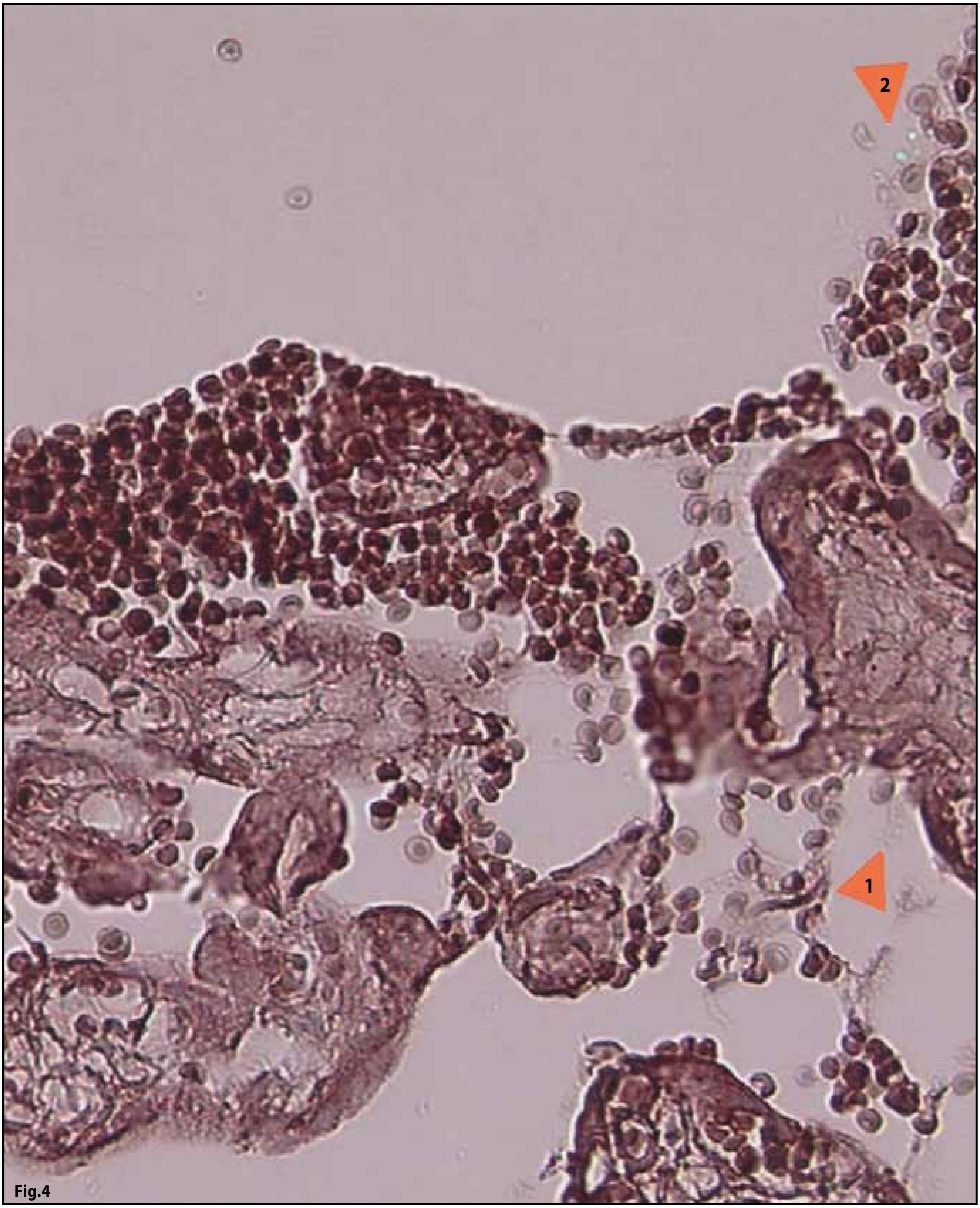

Fig.4: Placenta - 7µm thick section stained by new method for proof of lead after Foltinová. Positivity of lead in the released syncytiotrophoblast (1▲); in erythrocytes and their vicinity(2▼). Turquoise colour informs of positivity. Magnified 320 ×.

Fig.5: Placenta – Proof for lead. Phagocytosis of lead by syncytiotrophoblast (1▶). Cumulation of lead on the surface of syncytiotrophoblast (2▼). Release of lead from erythrocyte of mother's blood (3▲). Stained by histochemical method for proof of lead after Foltinová. Magnified 1500 ×.

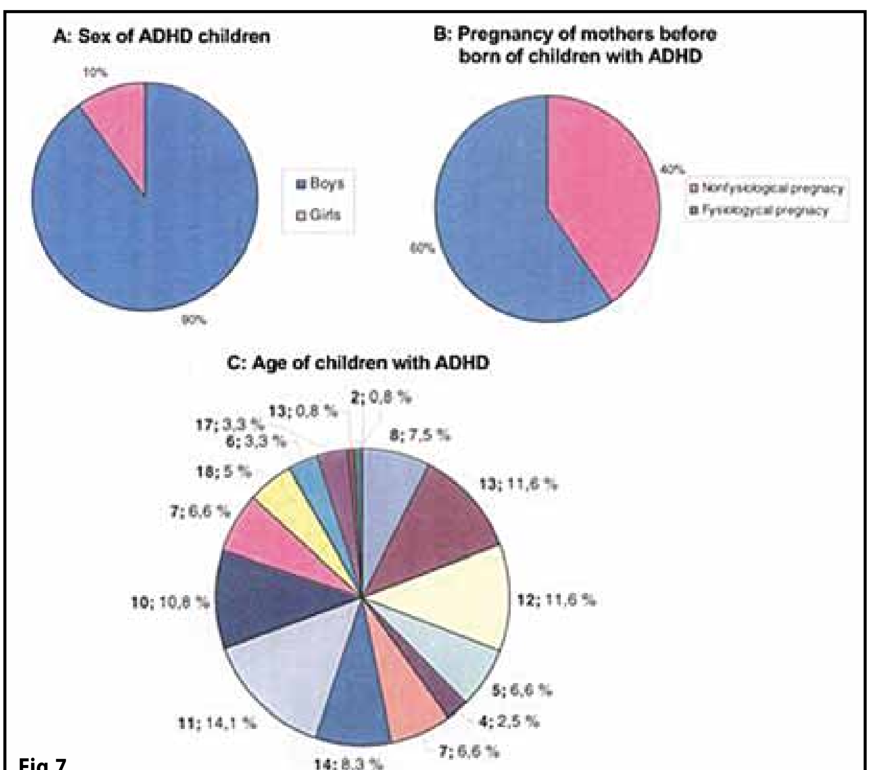

Fig.7: Statistical diagrams of the ADHD occurrence
A) Gender of children suffering from ADHD: boys 90%; girls 10%
B) Character of pregnancy of mothers of children suffering from ADHD: 40% nonphysiological; 60% physiological
C) Age of children suffering from ADHD

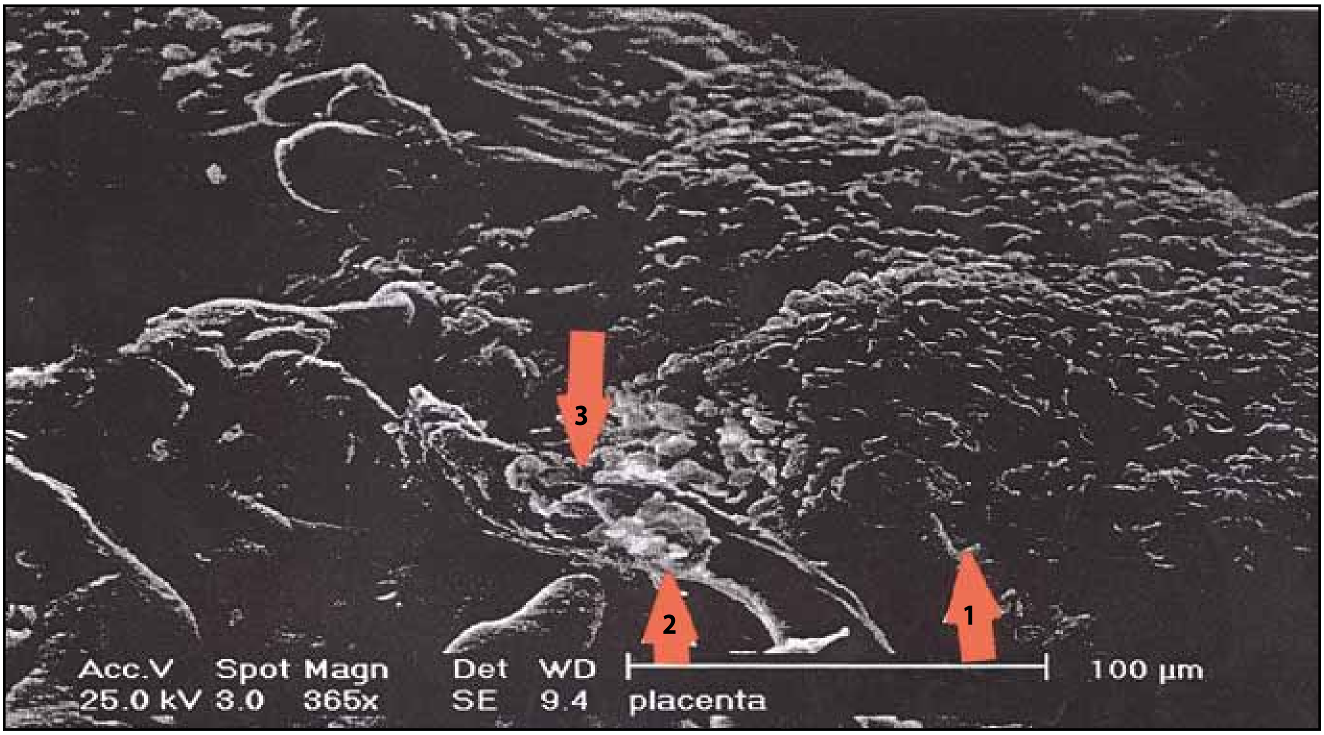

Fig.6: Placenta – picture of scanning electron microscope, transplacental barrier in cut placental villus: part of the terminal villus (1↖); fetal erythrocytes (2↑); syncytiotrophoblast with mother's erythrocytes (3↓).Magnified 365×.

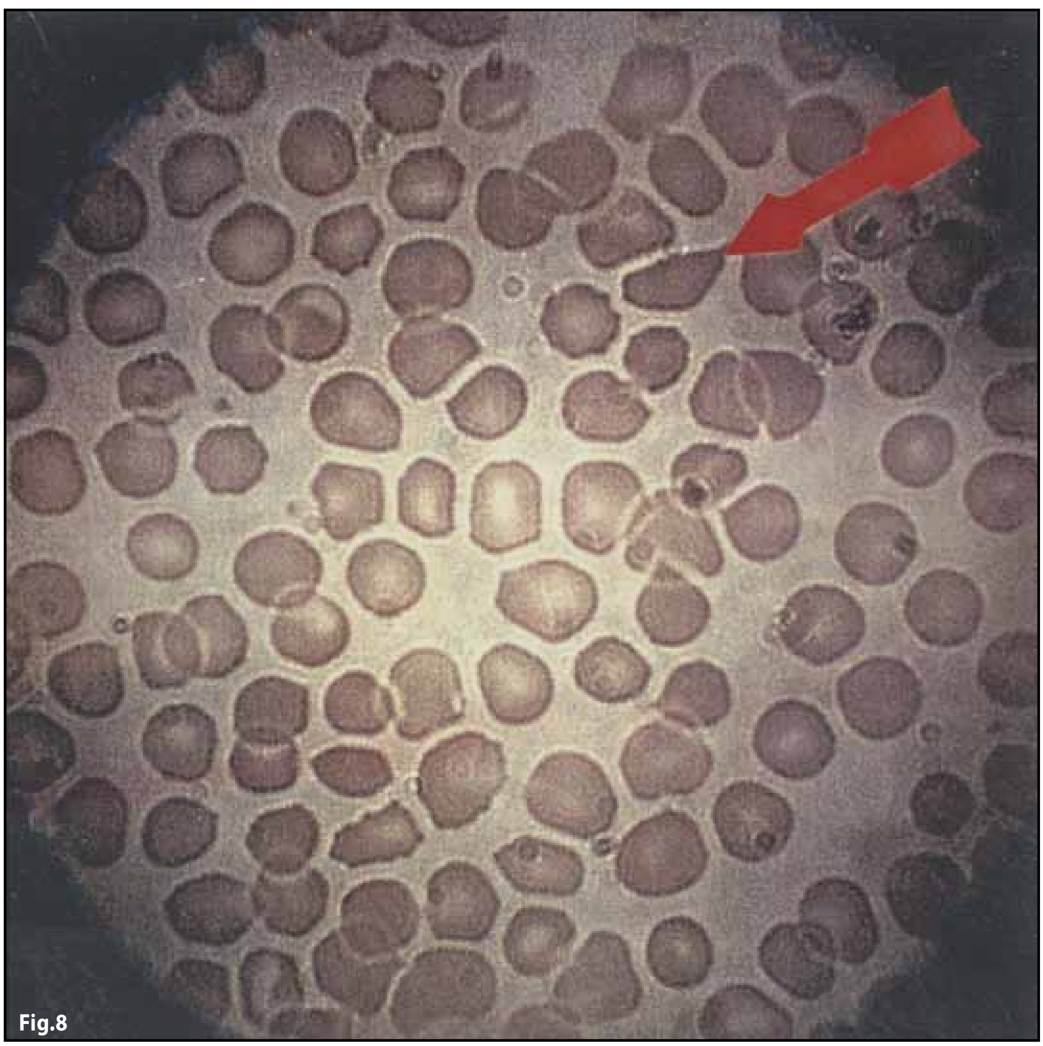

Fig.8: Umbilical cord blood – smear stained by May Grűnwald-Giems Romanovsky. At the end of pregnancy number of fetal erythrocytes is reduced; erythrocyte changes its shape (↙), decays and lead may leave the space of erythrocyte. Magnified 1000 ×.

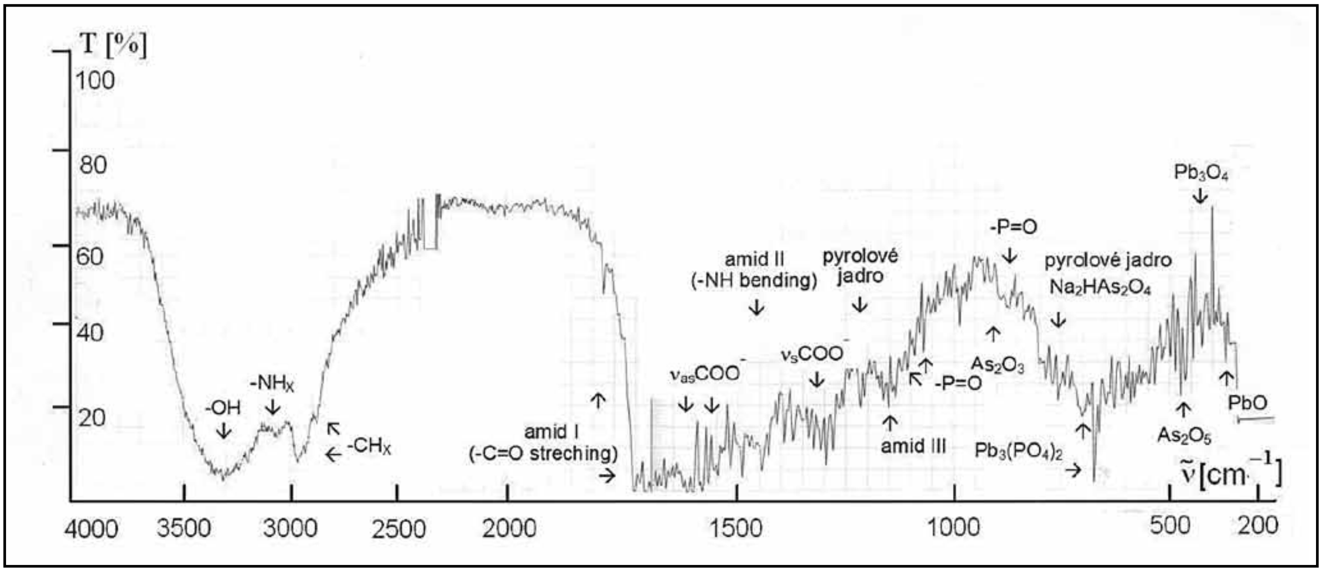

Fig.9: Umbilical cord blood of the child of mother born in 1981 (child birth in 2005) – infrared spectroscopy. Graphical record of spectrum; positivity on lead in chemical compounds $Pb_3(PO_4)_2$, Pb_3O_4, and PbO. Presence of As_2O_3 and As_2O_5 deserves attention and will be analyzed in the future. Expression „pyrolove jadro" is pyrolle ring.

possible penetration of lead into the blood circulation of the fetus. ***Fig.7A*** shows that disease occurs mainly in boys, what can be explained by the relation of lead towards testosterone. Hyperactivity occurs in children of various ages. The most often occurrence is in case of children from the 6th year of their age up to the period of puberty (***Fig.7C***). However, it is not easy to determine the beginning of this disease since children are hospitalized as late as symptoms exceed certain level of tolerability for their families. By means of modern visualizing methods of 3D projection of magnetic resonance there was found reduction of total brain volume of 3–8% and reduction of gray and white brain mass of children with ADHD in comparison with the healthy children (Drtíková, 2007). These pieces of knowledge emphasize importance of our original findings concerning ADHD and its relation towards lead.

Our findings unambiguously show that lead is transported by blood. The proof of this knowledge consists in the fact that decay of erythrocytes in fetal blood before the child birth (***Fig.8***) rises conditions for the appearance of the following chemical compounds: $Pb_3(PO_4)$, Pb_3O_4, and PbO in the infrared spectrum of umbilical cord blood (***Fig.9***).

CONCLUSIONS

This work presents findings giving evidence about the way of lead transport from mother's blood into the blood of fetus. We have shown how lead is released from the mother's erythrocyte and received by the erythrocyte of fetus what can be utilized for fast diagnostics. Our findings enrich knowledge about the relation between mother's erythrocytes, lead, syncytiotrophoblast, and erythrocytes of fetus in the vessels of placental villi. Our finding of lead in umbilical cord blood is forewarning against the rise of complications caused by lead that may appear in postnatal development of child. Positivity of lead in the umbilical cord blood found immediately after the child birth will focus attention to prevention and therapy of the ADHD that belongs to the most frequent reasons of psychiatric treatment in the child age. Knowledge of etiology and pathogenesis of the disease is a guarantee of successful treatment what is a goal of our work. Early knowledge of the risks, education about the symptoms and about the support networks available may help parents to make lives of the affected children significantly improved. Several institutions are in search of the way how to help to families with so affected children, for example, the Institute of Family (Vojtko, 2005). Nowadays importance of interdisciplinary research concerning various aspects of science grows. One of significant rapidly developing scientific branches is potential utilization of stem cells from umbilical cord blood for purposes of transplantation and regeneration in medicine. From the point of view of security of an acceptor it is important to evaluate all aspects that may unfavourably influence prognosis after transplantation. Results of our work concerning occurrence of lead in umbilical cord blood are contribution for avoiding potential risk ensuing from the presence of lead.

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