Occurrence of lead in placenta – important information for prenatal and postnatal development of child

Janka Foltinová [1], Viktor Foltin [2] & Eva Neu [3]

1. Institute of Histology and Embryology, Faculty of Medicine, Comenius University, Bratislava, Slovakia
2. Department of Experimental Physics - Division of Plasma Physics, Faculty of Mathematics, Physics and Informatics, Comenius University, Bratislava, Slovakia
3. Umweltmedizin Institut, Feucht bei Nürnberg, 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
EMAIL for physical questions: vfoltin@fmph.uniba.sk

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Abstract

This work points out consequences of lead on prenatal and postnatal development of child that have not been elaborated in such extent before. Our new method for proof of lead in placenta enabled us to show how lead is from mother's blood erythrocytes in the intervillous space released and received by the villous syncytiotrophoblast. This finding enriches relation between mother's erythrocytes, lead, calcium that is a lead carrier, and syncytiotrophoblast. Our finding of abundant thin terminal villi, that in some places form bunches observed in scanning electron microscope, points out deficiency of O_2 and CO_2 transport in placenta. This phenomenon is indirect evidence that periphery "starves" for oxygen that participates in maintaining conditions for intact development of child. Behaviour of fibrin deposit layer before the childbirth is also discussed. Attention is paid to possible rise of hyperkinetic syndrome of children as a consequence of mother's dwelling in environment polluted with lead. Presence of lead is verified by infrared spectroscopy.

INTRODUCTION

In recent years all over the world attention has been paid to studying the effect of heavy metals and their chemical compounds on the human organism from various points of view. As a special example may be mentioned Thimerosal (ethylmercury-containing compound) used in the USA during the period between the years 1994 and 2000 as the preservative level in vaccines. Application of such vaccines led to certain neurodevelopment disorders [6]. Works of recognized research teams bring new prospective results that help to reveal etiology [25,29], pathogenesis [19] and biochemistry of changes caused by these metals. This knowledge is a basis for early application of the aimed therapy that may be helpful for patient, his neighborhood, and society [7]. In patients diagnosed as having chronic mercury toxicity (CMT) removal of amalgam mercury fillings combined with appropriate treatment resulted in a significant symptom reduction to levels reported by healthy subjects [41].

In this work we have focused our attention on lead and its occurrence in human placenta. Function of placenta in reproduction has been known since blood circulation was revealed. Since that time investigation and revelation of new functions of this unique organ continues [1,4,13,17,22,26]. Research on interdisciplinary level succeeds to find new details or to explain more profoundly already known facts [11,12,16,30,33,38]. Intact structure and function of placenta is basic condition for maintaining homeostasis for optimal development of human fetus [5]. Contamination of placenta with heavy metals [10,17,31,43], but also with other chemical compounds occurring in environment, may have negative effect on the intrauterine development of individual. Results of [34], based on investigation carried out in certain district of India indicated that Pb levels were higher not only in those who experienced abnormal exposition but also in those who ate nonvegetarian diet. Developing fetus is very sensitive on unfavourable effects of many toxic substances [15,23,26], mainly due to high mitotic activity of the developing cells in tissues in organs when microscopic structures of cells and tissues are still immature. Nowadays we know that placenta does not form efficient barrier against penetration of harmful substances from the mother's blood into the blood of fetus [1,27,35]. Transport of many harmful substances, including remedies, and allergen through transplacental barrier to organism of fetus has been proven. Actual problem of smoking [15,20,37] will not remain without consequences on the development of the respiratory system of fetus. Decreased function of lungs of the exposed children may contribute to predisposition of individual to rise of asthma in the future. Mother's exposition to lead [4,11,12], as well as to polycyclic aromatic carbohydrates has unfavourable effect on the nervous system of fetus. Increased concentration of hazardous metals in placenta leads to impair of balance with essential elements – zinc, selenium, copper, because these elements are extruded by toxic elements [2]. Authors of that paper have found that placental barrier is more efficient for cadmium than for lead [2]. This agrees with our finding that calcium is a carrier for transport of lead, supported also by [21]. During pregnancy calcium is released from mother's bones and carries lead that was deposited there during her life. Bone and tooth are convenient terrain for deposition of lead in the mother's body, but also in the body of fetus. Neurotoxic effect of this heavy metal may be therefore manifested in a child also in the higher age [9,17,36,]. Occurrence of lead in placenta has been shown to be one information pointing out the postnatal rise of hyperkinetic syndrome in children (ADHD) [11,39].

In this work we present new results concerning relation of lead to erythrocytes of mother and fetus in placenta accompanied with changes in its microscopic structure. Release of lead from mother erythrocytes in placenta and acceptance of lead by syncytiotrophoblast of placental villus is evidenced [11]. The finding of abundant bunches of thin terminal villi in placenta with lead presence is also discussed. We consider this abundance of terminal villi as compensation mechanism for insufficient O_2 transport in placenta. Besides helping in the fundamental understanding of the transport of hazardous metals in placenta, this knowledge can, for example, be basis for developing placenta screening methods to inform parents about increased risk of their child to hyperkinetic syndrome due to lead deposition. Mothers, giving birth to children today, lived in environment polluted with lead added to the petrol. This did not remain without consequences to prenatal and postnatal development of their children. Early knowledge of the risks, education about the symptoms and about the support networks available may help the parents to provide high quality of care in the case the symptoms of this disease would appear during postnatal development. In case of early beginning of this care, lives of the affected children will be significantly improved. Several institutions are in search of the way how to help on the interdisciplinary levels to families also with so affected children, for example, the Institute of Family [40].

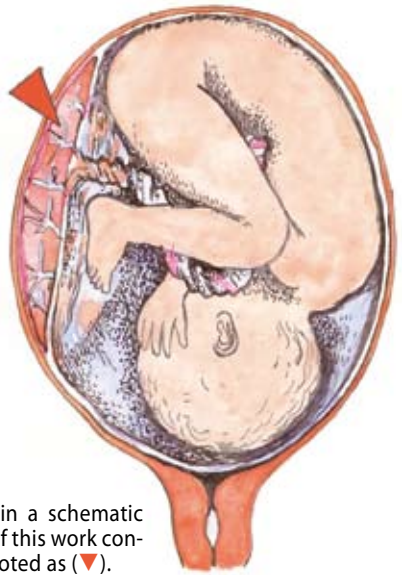

Figure 1. Placenta in a schematic picture. Results of this work concern the site denoted as (▼).

MATERIAL AND METHODS

In this work we prepared and evaluated sections from excisions of placentas (Figure 1) of 104 healthy patients.

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

- hematoxylin-eosin
- Lendrum "acid-picro Mallory"
- Una Tanzer "acid-picro, indigocarmin-orcein"
- method for the proof of calcium after Koss
- 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 prepared for the scanning electron microscope by double fixation with glutaraldehyde (200 mmol/L) and osmium tetroxide (OsO_4 40 mmol/L), which 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 disks and coated with a thin conducting layer of colloidal silver for further processing. The excisions on the disks were coated with a 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 [10].

The following microscopes were used for the evaluation:

- light microscope Reichart Polyvar (Germany) at magnifications 180×–1 500×;
- scanning electron microscope PHILIPS CM 20 (Holland) at magnifications 220×.

Concurrently we have studied identical samples from placenta by means of infrared spectroscopy using KBr pellet making technique [3,14,24]. Since we expected presence of fibrinogen in the placental tissue we used as a reference sample fibrinogen Haemocomplettan P (1 g, Centeon Pharma GmbH, Wien, Austria). The spectra of samples were compared with this pure fibrinogen spectrum. We used infrared spectrometer SPECORD M80, Carl Zeiss, Jena (Germany).

RESULTS AND DISCUSSION

We aimed at studying microscopic structure of placenta, its fibrin deposits, and correlation between the character of these structures and the environment. We performed this investigation in an interdisciplinary way by methods of ecomorphology, infrared spectrometry and scanning electron microscopy. In literature there are only modest and rather vague than clearly interpreted pieces of information about these deposits, about their origin and structure. In [30] there is syncytiotrophoblast determined as a locality of lead, but precise identification of microscopic structure and character of phagocytosis were not shown. This has been a challenge for us to reveal this mystery. Our results obtained from evaluation of many microscopic pictures of structure of placenta give the following evidence: during nidation of embryo into endometry of uterus due to action of hydrolytic enzymes there occurs damage of vessels and consequently a leak out of the mother's blood. It is known that early in development, the blood vessels of the villi become connected with vessels from the embryo [32]. We have found presence of calcinated fibrin deposits in the intervillous space and in the interstitium of placenta what leads to uteroplacental ischemia. Origin of this phenomenon can be explained in such a way that blood is squirting and not pouring into placenta. At high pressure of mother's blood the blood flows in the closed vessel system more quickly. It carries also the ions of calcium. In our opinion presence of lead in placenta is a consequence of its disengagement from bones and teeth together with calcium during pregnancy. This releasing of calcium and lead is facilitated by intervention of pregnancy to hormonal stability of the future mothers. Our histochemical finding points out that calcium (Figure 2) plays a role of lead carrier. By the above described methodical approach also small amounts of lead and positions of sites where lead is phagocyted within the syncytiotrophoblast and released from mother's blood erythrocytes in the intervillous space of placenta are clearly seen in Figure 3. In the microscopic structure of placenta calcium and lead show positivity and character of phagocytosis in the same places of the surface part of syncytiotrophoblast that is neighbouring to the flowing mother's blood. Namely this fact is important on the Figures 2 and 3, because this points out that calcium is a carrier of lead. This finding means a contribution to investigation of the way of cumulating lead in placenta and is relevant for the future development of a child.

By means of scanning electron microscope, which has advantage of high sharpness and depth of the obtained picture of microscopic structures, we gained important information about topography of free thin villi of placenta that were abundant and formed bunches (Figure 5). Such abundance of free villi contributes to enlargement of their surface. In the given circumstances this finding is important for transport of oxygen and carbon dioxide. Such finding points out that lead is involved in the process of expelling iron from hemoglobin of erythrocytes. Beside this, lead is shortening life time of erythrocytes. Periphery "starves" for oxygen and forming thin villi into bunches is a way how to achieve in the small intervillous space enlargement of villous surface by increasing number of thin free villi. In this way oxygen, that would be otherwise lacking, is gained for the life of fetus. These free villi occur in that part of placenta where the most intensive exchange of substances appears. Blood from the spiral twisted mother's arteries squirts under pressure to intervillous space so far as to chorial plate of placenta. Amount of blood and its flow through the intervillous space increase during gravidity. Arterial mother's blood is washing chorial villi. Its flow is slow in order that exchange of substances between mother's blood and blood of fetus might be realized through transplacental barrier. This offers sufficient time for transport of neurotoxic lead.

Results obtained by our new methodic approach for the proof of lead confronted with the results of scanning electron microscopy and results obtained with help of infrared spectroscopy [11] are significant. Infrared spectroscopy verified presence of lead in form of $Pb(NO_3)_2$ what may be the result of interaction of lead with $(NO)_x$. Moreover, by means of computer graphics our method can be enriched to such extent that even in case of trace occurrence of lead, when sites of lead are in pictures invisible for human eye, they can be converted to visible form by "Eyedropper Tool" and "Color Range" (Figure 4).

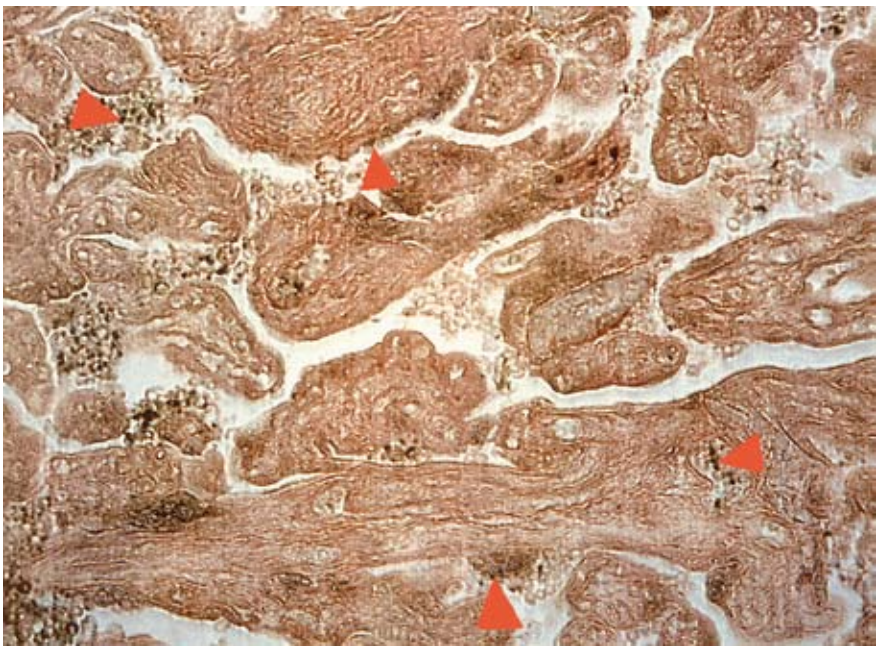

Figure 2. Placenta – proof of calcium. Method after Koss was used. Cumulation of calcium deposits (▲). Intravascular positivity in the chorium villus (◀). Positivity of calcium in the mother's blood and phagocyting by syncytiotrophoblast (▶). Magnified 320×.

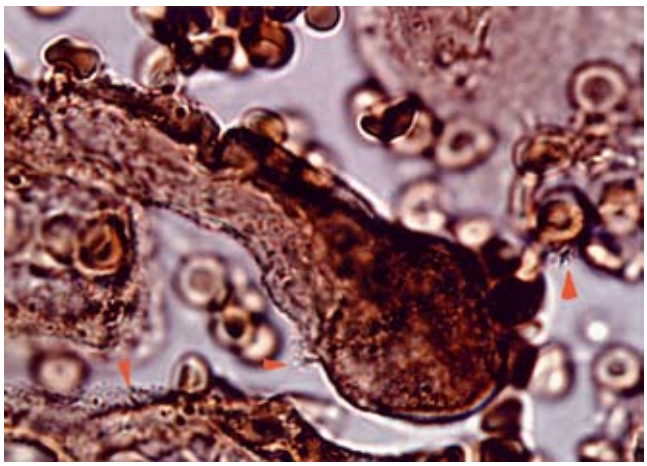

Figure 3. Placenta – proof of lead carried out by a new methodical approach after Foltinová. Phagocyting of lead by syncytiotrophoblast, entrance for transport of lead into umbilical vessels of fetus (▶). Syncytiotrophoblast, its apical surface (▼). Disengagement of lead from erythrocyte of mother's blood (▲). Magnified 1 500×.

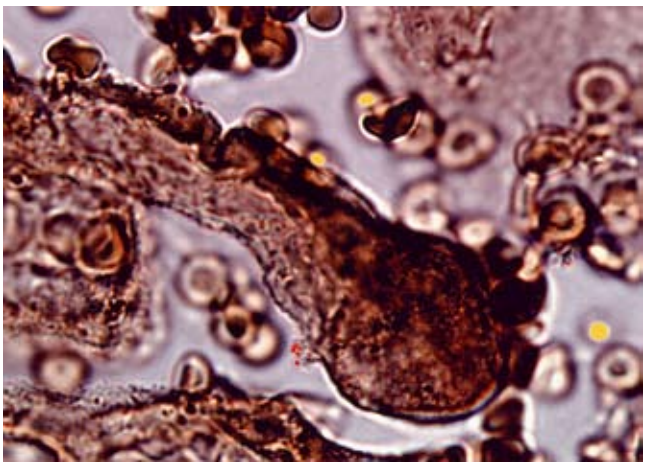

Figure 4. Placenta – demonstration of sites of positivity on trace occurrence of lead by means of computer graphic. As a first step correct definition of investigated colour (in our case turquoise green) is performed by "Eyedropper Tool". This colour is then pointed up against background by combination of graphic filters and tool "Colour Range". In our case conversion to red colour appeared to be most convenient. Red colour then means significant positivity on lead in the structure of placenta. In this way trace positivity on lead has been visualized. Magnified 1 500×.

The results are contribution for etiology and pathogenesis of consequences determining profile of hyperkinetic syndrome – ADHD (Attention-deficit hyperactivity disorder) in the postnatal development of children. It is a multifactorial disorder clinically characterized by inattentativeness, impulsivity, and hyperactivity. Occurrence of this disorder is between 3 and 6% of the children population and hyperactivity is suspected to be the result of alteration of the dopamine/noradrenalin levels [18]. Genetic background of the ADHD is discussed in [28]. In our opinion cellular malformation in development of the gray matter of brain cortex in the early stage of intrauterine development of the fetus caused by lead may appear and continue in development also in the postnatal stage and affect IQ of individual due to special affinity of lead to gray matter of brain cortex. This may happen when releasing of calcium (which is a lead carrier) from bones during growth of the child occurs. It is indicated by increasing occurrence of ADHD in suckling, pre-school, and pubescent stage of the child. Incorrect psychology in education of a child may act as triggering mechanism for ADHD. This work points out that investigation on the interdisciplinary level is contribution for medicine and for healthy development of a child.

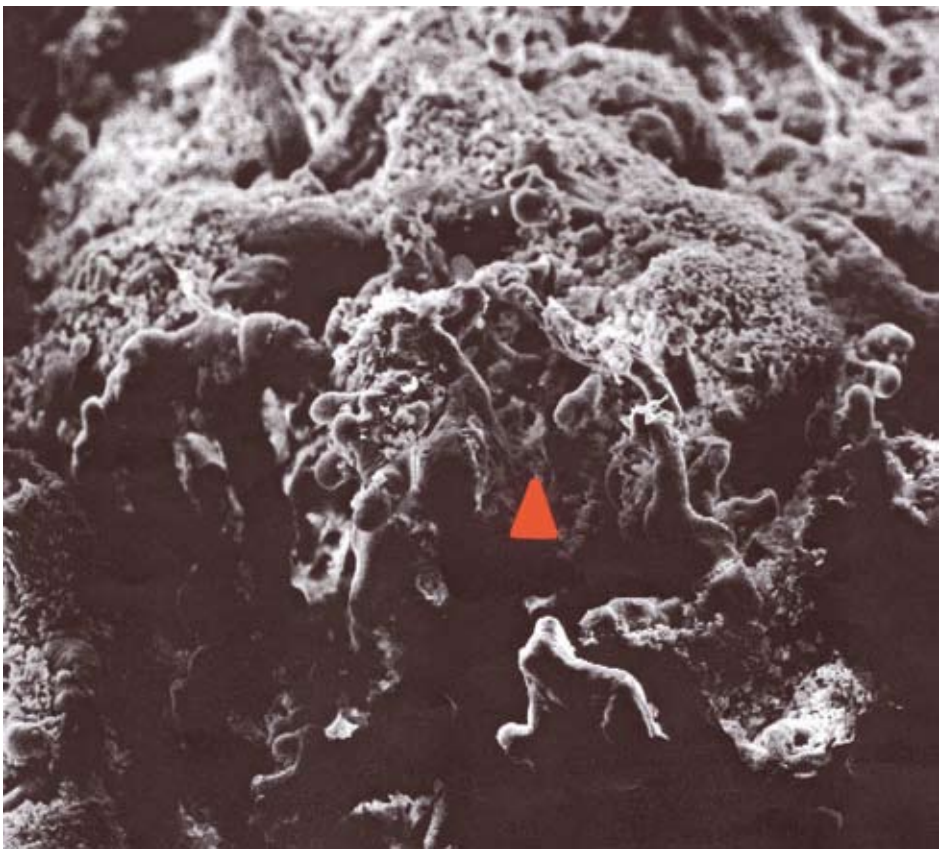

Figure 5. Placenta in scanning electron microscope. Presence of bunch of thin villi (▲). This is compensating effect in case of insufficient supply of oxygen to placenta (lead displaces iron from hemoglobin). Magnified 220×.

CONCLUSIONS

This work presents new methodic approach that we have suggested for the proof of lead in placenta with its application in practice. This method can be used also for the proof of lead in other organs. By means of this method we have shown relation of lead to the erythrocytes in placanta. Our histochemical approach combined with the computer graphics makes possible to visualize sites of lead that cannot be seen by human eye due to trace occurrence of lead that is, however, still capable to influence the development of a child. In the picture of scanning electron microscope we found in placenta abundance of thin free villi in form of bunches. We identify them as a compensating effect for the interference of lead with oxygen transport resulting from expelling iron from hemoglobin in erythrocytes. Presence of bunches enlarges the villous surface and facilitates basic functions of placenta inevitable for the life of fetus. Our findings are contribution for prevention of complications caused by lead that may appear in postnatal development of a child. One of them is hyperkinetic syndrome (ADHD) that is discussed in this work. We recommend that mother should get information about lead in placenta after the childbirth. This may help in maintaining conditions and performing education for avoiding development of ADHD. Such prevention will lead to decreasing occurrence of this disease in the world.

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