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Metalloproteins in human placenta and fetal membranes in non-smoking and smoking women

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The function of placenta and fetal membranes in protection of fetus against the influence of heavy metals is still under investigation [1, 2]. Metalloproteins play an important role in binding of both the essential and toxic metals [3, 4].

Metallothionein is a low-molecular protein of M_r about 7000, with a high cysteine content [5], and –SH groups acting as the ligands binding bivalent ions. It has been demonstrated that placenta and fetal membranes contain a metallothionein-type protein which is able to bind Cd and Zn *in vitro* [2, 6]. Recent studies have shown that perfusion of the Cd containing liquid through an isolated human placenta causes accumulation of that metal and an increase in metallothionein concentration [1].

In the present work, the content of Cd and low-molecular proteins binding the essential (Zn, Cu) and toxic (Cd) metals were studied in placentas and fetal membranes from women being either addict smokers or non-smokers.

The tissues were subjected to preliminary preparation according to Waalkes *et al.* [2]. The supernatant obtained was separated into two, II and III, metallothionein fractions by precipitation with 40 - 60% and 60 - 80% acetone, respectively.

It was found that, in the tissues of smokers, total protein concentration was increased; in placenta, protein concentration in the fractions II and III was increased by 60 and 50%, respectively, while in the chorion fractions by 50 and 30%, respectively. In amnion, concentration of protein in either fraction was increased by 30% (not shown).

The mean concentration of metallothionein (MT), as determined according to [7], in fraction III was significantly higher in the smoking group than in non-smokers (Fig. 1). In placenta, chorion and amnion of smoking women, MT concentration was 1.6, 1.8 and 2.2 times higher as compared to the respective tissues of non-smokers. Our results referring to placenta are in accordance with those of Waalkes *et al.* [2] and Clough *et al.* [8] but for fetal membranes they are lower than the values reported by those authors.

Consistently with the data on MT, accumulation of Cd was about twice as high in the tissues of smokers than in the tissues of non-smoking women. The highest accumulation of Cd was found in placenta, in which MT accumulation was also the highest. Our data on Cd concentrations in maternal tissues were somewhat lower than those of Chisolm & Handorf [9].

To study in more detail the metal binding protein fractions in maternal and fetal tissues of smoking and non-smoking women, fraction III was subjected to analysis in 7% polyacrylamide gel according to Davis [10]. The gels were cut into 3 mm thick fragments which were eluted with nitric acid; in the eluates the presence of protein and Cu, Zn and Cd was detected according to [11] and [12], respectively. Four metal binding protein fractions were found, of R_F 0.30, 0.46, 0.52 and 0.69. The bands of R_F 0.46 and 0.69 corresponded to the two MT isoforms, as proved with the appropriate standards from rabbit liver. In placenta of pregnant rats exposed to Cd, two forms of MT were also found that differed in the ability to bind Cd, in electrophoretic mobility and molecular mass [13, 15].

The fraction of R_F 0.46 binds all three metal ions studied while the fraction of R_F 0.30 seems

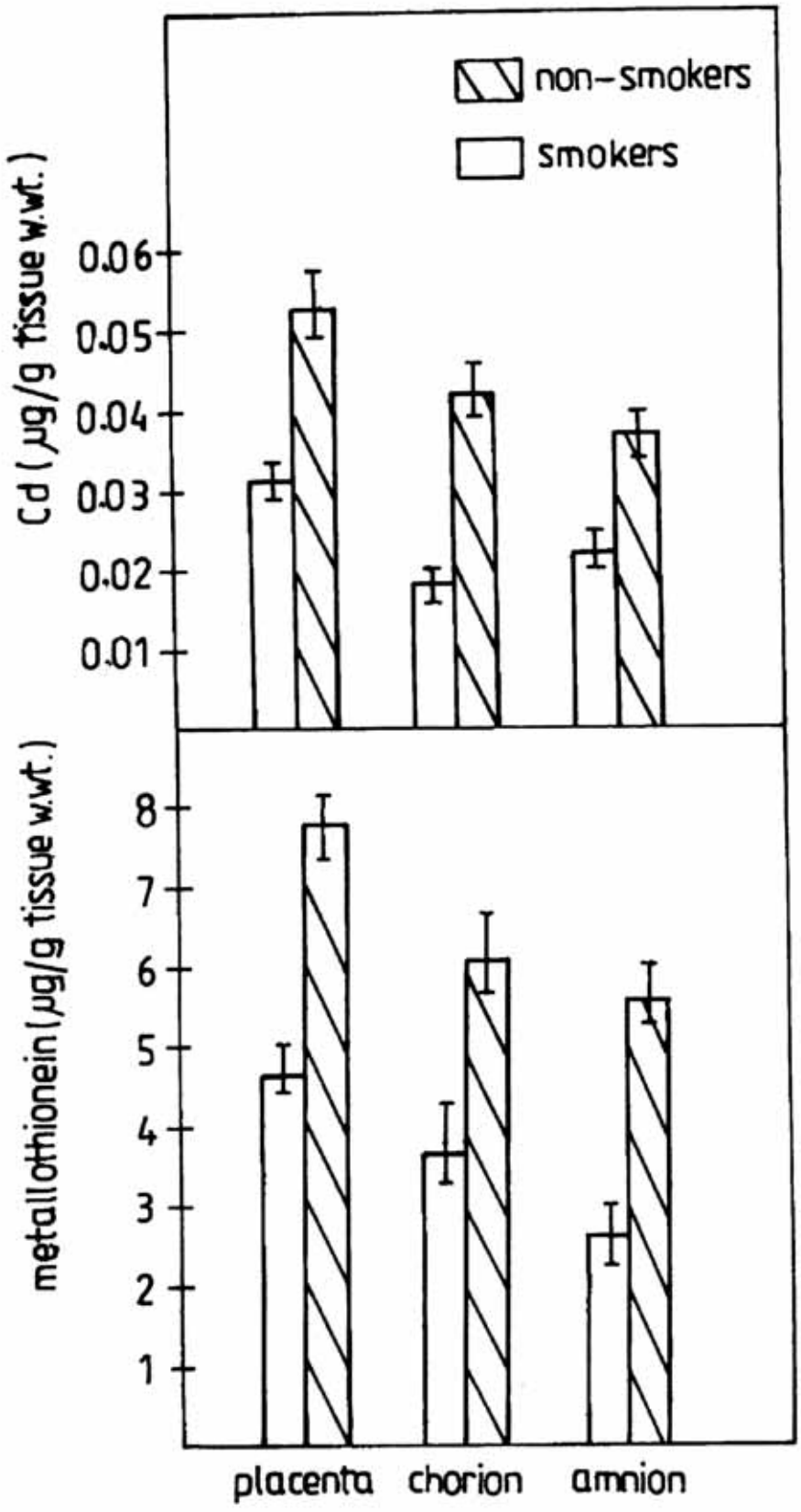

Fig. 1. Cd and metallothionein contents in pregnant women placenta, chorion and amnion.
Data refer to the fraction precipitated at 60 - 80% acetone concentration. The values are mean ± S.D. of 3 determinations

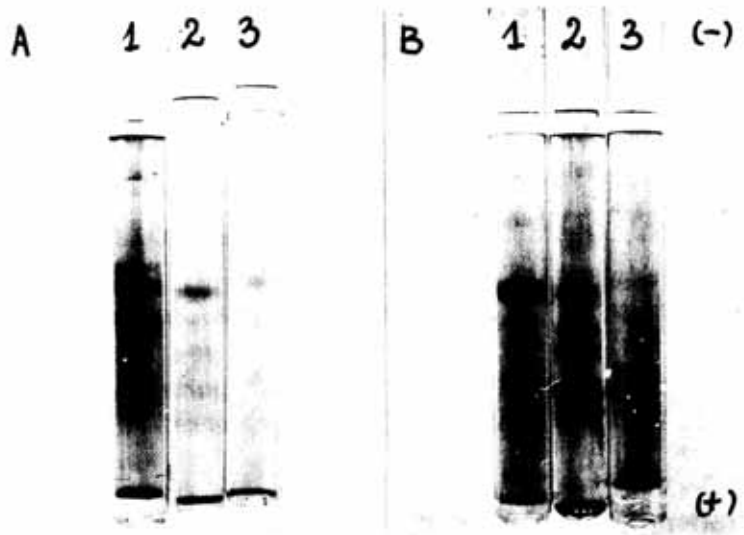

Fig. 2. Proteinograms of protein fractions precipitated with 80% acetone from: placenta (1), chorion (2) and amnion (3), of: A, non-smoking and B, smoking women

to exhibit mainly the ability to bind Cu. Out of the fractions analyzed, the protein of R_F 0.52 bound the smallest quantity of metals.

It has been found previously that, in placenta, significant amounts of Zn and Cu are bound by high molecular proteins [14, 15].

The comparison of proteinograms from the tissues of smoking and non-smoking women shows clearly that a greater amount of metal ions was bound to the metallothionein (R_F 0.46 and 0.69) of smoking women (Fig. 2).

The results obtained seem to confirm the hypothesis proposed by other authors on the significant function of placenta and fetal membranes in protecting fetus against toxic metals [16, 17] but, on the other hand, on accumulation of metals, Zn and Cu, essential for fetus development [18].

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