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Alterations in collagen of umbilical cord arteries in patients with EPH-gestosis

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It is well known that the state of the vascular system of mother and placenta exerts an important influence on intrauterinal development of fetus [1–4]. EPH-gestosis (EPH = Edema, Proteinuria, Hypertension) is the most common pathological syndrome connected with pregnancy. It is accompanied by significant morphological changes in the arterial walls of the uterus and placenta. Contraction of small arteries, hypertrophia of media, edema of intima, and degenerative changes in arterial walls have been observed [5–8]. Omental arteries of women with pre-eclampsia have a significantly smaller internal diameter than those of normotensive pregnant women [9].

Collagen is a group of at least 14 genetically distinct proteins [10]. The arterial walls contain mainly type I, III, IV and V collagens. It is well known that both collagen content and proportional relationship between collagens of various types are altered in some pathological conditions [11]. We have decided to compare the collagen content, solubility and molecular polymorphism of this protein in normal umbilical cord arteries and in those from newborns of mothers with EPH-gestosis.

Studies were performed on umbilical cord arteries taken from 30 control and 30 investigated newborns. The control material was taken from newborns, delivered by healthy mothers, aged 18–35, with normal blood pressure (systol. 100–120 mm Hg, diastol. 70–90 mm Hg), without edema or any symptoms of renal failure. The babies were born between the 38th and 41st week of gestation by physiological delivery (23 cases) or by cesarean section (7 cases). Their mean body weight was 3672 ± 438 g. The investigated babies were born to mothers with EPH-gestosis, diagnosed according to the criteria accepted by the Organisation Gestosis [12]. Severe and medium cases of polysymptomatic gestosis (4 to 11 points on the gestosis index scale) were investigated. Most of the women had all the symptoms of gestosis: edema, proteinuria and hypertension, the remainder demonstrated various combinations of two symptoms. Their babies with a mean body weight of 3531 ± 528 g were born between the 38th and 41st week of gestation. Fragments of arteries 20 cm long were excised beginning at their placental end.

Collagen contents in individual samples of arteries taken from 10 newborns of each group were measured by the assay of hydroxyproline as described by Prockop & Udenfriend [13]. Since hydroxyproline constitutes about 1/8 of collagen weight, the approximate amount of the latter protein in arterial tissue was calculated by multiplication of hydroxyproline content by 8.

Fractionation of collagen was performed by the method described by Murata *et al.* [14] and obtained fractions were submitted to electrophoresis on sodium dodecyl sulphate/8% polyacrylamide gel (SDS/PAGE) as described by Laemmli [15]. The amounts of particular collagen types were expressed as percentage of the total collagen content.

Collagen solubility and its susceptibility to depolymerizing action of EDTA were measured as previously described [16].

It was found that the walls of normal umbilical cord arteries contained about 0.13 mg of collagen per 1 mg of dry weight, whereas those taken from newborns of patients with EPH-gestosis contained more than twice that amount of collagen (Fig. 1).

The solubility of collagen from the investigated arteries was slightly lower in comparison to that of control vessels. Both solutions of sodium chloride (1 M) and of citrate (0.15 M, pH 3.7) extracted about 18% of total collagen from normal cord arteries and about 13% from those of newborns of patients with EPH-gestosis (Fig. 2). A part of polymeric (insoluble) collagen depolymerized in 4% EDTA and became soluble in 0.2 M acetic acid. As can be seen in Fig. 2, the collagen of investigated arteries was less susceptible to the action of EDTA than the control material.

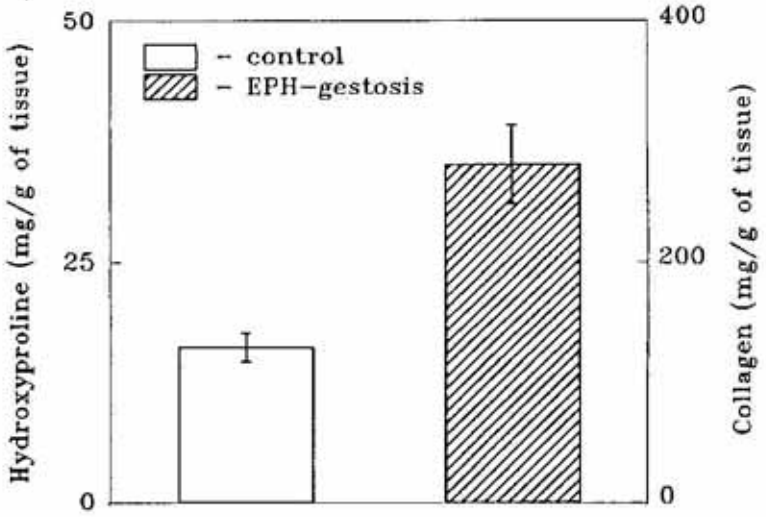

*Fig. 1. Collagen content in the walls of normal umbilical cord arteries and in those of newborns of mothers with EPH-gestosis (*P < 0.01).*
Mean values from 10 assays ± S.D.

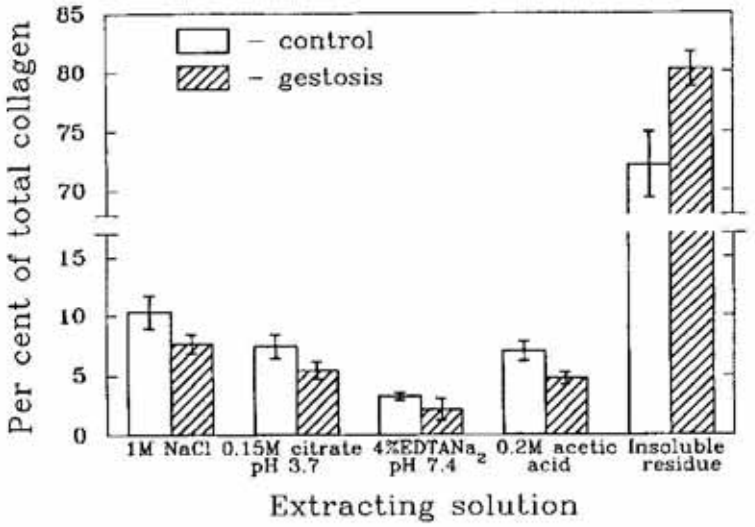

Fig. 2. Solubility of collagen from normal umbilical cord arteries and those of newborns delivered by mothers with EPH-gestosis.
Mean values from 4 experiments ± S.D.

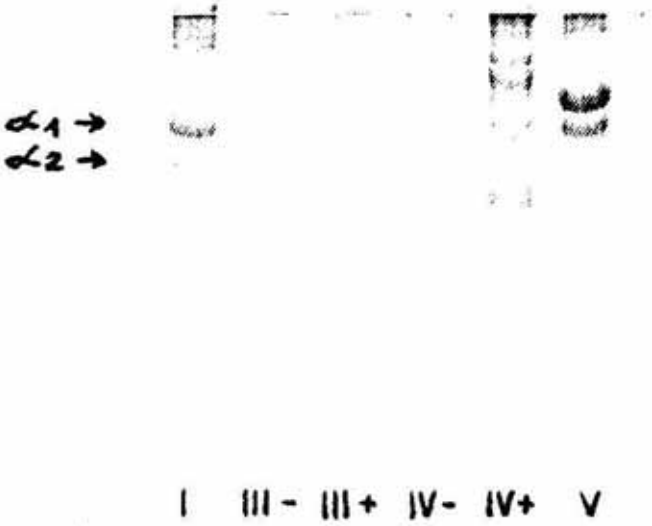

Fig. 3. SDS/PAGE of various collagen types isolated from umbilical cord arteries.
The same electrophoretic patterns were obtained both in the case of control and investigated material. Types of collagen are marked at the bottom. The position of α_1(I) and α_2(I) subunits are marked on the left margin. Nonreduced type III collagen does not penetrate the gel. Only trace amount of α_1(III) is visible. Reduction of disulphide bonds results in the appearance of this subunit. A similar situation was observed in the case of type IV collagen. Type V collagen subunits: α_1(V) and α_2(V) demonstrate lower electrophoretic mobility due to their higher molecular mass. (–) Without reduction, (+) reduced with dithiothreitol.

When the homogenates of cord arteries were digested with pepsin in acetic acid, total collagen was solubilized. The pepsin extracts submitted subsequently to specific fractionation yielded five collagen fractions. Four of them were isolated in a pure form. Their electrophoretic mobilities were typical of type I, III, IV and V collagens, respectively (Fig. 3). The type VI collagen containing two globular domains which are sensitive to digestion with proteinases [17], was partially degraded by pepsin during the isolation procedure. Furthermore, this fraction was contaminated by other, noncollagenous proteins which co-precipitated with type VI collagen. Several nonspecific bands were observed in the gel (not shown). No differences between the control and investigated material were observed.

Among the umbilical cord arterial collagens type I and type III were found to be the most abundant. In the control arteries they constituted about 63.5% and 25.5% of total collagen, respectively. The content of type IV, V and VI collagens was distinctly lower. They constituted 3.0%, 3.6% and 4.4% of total collagen, respectively. The walls of arteries taken from newborns of patients with EPH-gestosis contained a significantly higher percentage of type III collagen (35%) than the control material. At the same time the percentage of type I collagen was distinctly lower, namely 50.4% (Fig. 4).

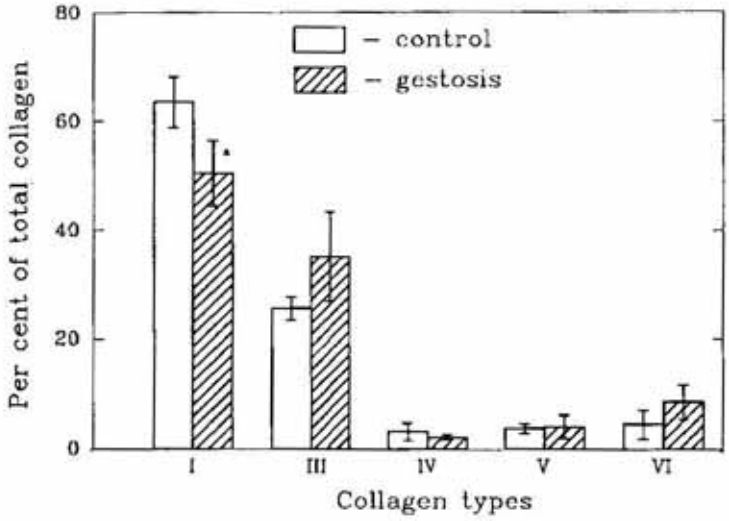

*Fig. 4. Relative content of different collagen types in the walls of normal umbilical cord arteries and in those of newborns delivered by mothers with EPH-gestosis (*P < 0.05).*
Mean values from 4 experiments ± S.D.

Also our histological studies (not shown) demonstrated that the walls of umbilical cord arteries taken from newborns of patients with EPH-gestosis contained more extracellular components of connective tissue, and the arrangement of these components differed from the pattern found in normal material. We have found that a normal umbilical cord artery contains about 0.12 mg of collagen per 1 mg of dry weight. This value is similar to the collagen content in the intima of human aorta reported by Morton & Barnes [18]. The amount of collagen found in EPH-gestosis arteries was over twice that found in controls. This could be due either to enhanced biosynthesis or decreased degradation of this protein.

It is known that type I collagen is the predominant species of this protein in normal adult human aorta [11]. A similar conclusion can be drawn from our studies. Type I collagen constitutes about 63% of total collagen content in normal umbilical cord arteries. The arteries of newborns of mothers with EPH-gestosis contained relatively less type I collagen and more type III collagen. Type III collagen biosynthesis seemed to be increased to a much higher degree than biosynthesis of other collagen types.

As can be seen from the presented results the increase in collagen content in the umbilical cord arteries was accompanied by a decrease of its solubility. This phenomenon is probably connected with an increase in the degree of collagen polymerization. It is known that polymeric collagen becomes insoluble in neutral salts and in acidic solutions. The decrease of solubility may be also evoked by a relative increase of the type III collagen content in umbilical cord arteries of the investigated newborns. It is well known that collagen of this type is less soluble than type I collagen [19].

It seems that the above described changes in the umbilical cord arteries may be responsible for the decreased blood flow in the fetus of women with EPH-gestosis [3].

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