## DETECTION OF COMPONENTS OF THE EXTRACELLULAR MATRIX IN CYTOTROPHOBLASTIC COLUMNS OF THE HUMAN PLACENTA

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Besides its supporting and trophic function, an important role in tissue differentiation also is ascribed to the extracellular matrix (EM). However, information on the composition of EM of so functionally active and continuously developing an organ as the placenta is rather scanty and is concerned mainly with the chorionic villi [3, 7]. Recently plasma [10] and fetal fibronectin [5], and also type IV collagen and laminin [4] were found in cytotrophoblastic columns (CTC).

The aim of this investigation was to locate the principal types of collagen and fibronectin in CTC of the human placenta in the early and final stages of pregnancy.

### **EXPERIMENTAL METHOD**

Altogether 12 placentas (9-12 weeks), obtained at medical abortions, and eight placentas (39-40 weeks), at normal full-term pregnancy, were studied. Tissue fragments were frozen in liquid nitrogen. Serial frozen sections 5  $\mu$  thick were fixed for 3 min in cold acetone and processed by the indirect immunofluorescence method [9]. Polyclonal rabbit antibodies to collagen of types I, III, IV, and V and to human plasma fibronectin, and FITC-labeled goat antibodies to rabbit  $\gamma$ -globulin, were used: the method of obtaining them and their immunologic and immunomorphologic characteristics were described previously [1, 8]. Some sections were stained with hematoxylin-eosin and picrofuchsine. The sections were examined and photographed in an "Orthoplan" microscope (Leitz).

#### **EXPERIMENTAL RESULTS**

The distribution of the desired components of EM and CTC varied in character from case to case, irrespective of the stage of pregnancy, but with persistence of a general tendency toward small and discrete deposits of collagen of types I and III and accumulation of collagen of types IV and V and also, in particular, of fibronectin. Collagen of types I and III was distributed in the form of small and large granules and also of separate conglomerates (Fig. 1a, b). Conversely, collagen of types IV and V and fibronectin were distributed throughout the area of CTC, and gave off intensive specific fluorescence with small round dark areas at the sites of the cells (Fig. 1c, d, e). Single large cells with distinct circular fluorescence, resembling the characteristic basement membrane, were found at the periphery of CTC.

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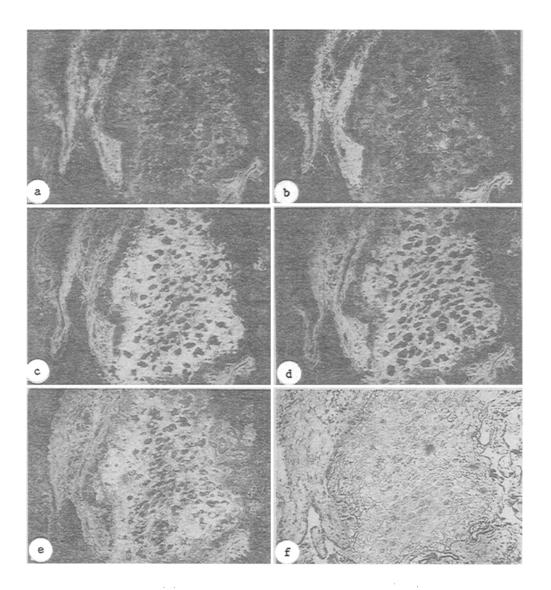


Fig. 1. Localization of components of extracellular matrix in cytotrophoblastic column of human placenta. Area of columnar villus, 40 weeks of pregnancy, shown on left. Small and large granules, small conglomerates of collagen of types I and III, and homogeneous masses of collagen of types IV and V and of fibronectin located among mainly dark cells. Serial frozen sections. Indirect fluorescent antibodies method. a-d) Collagen of types I, III, IV, and V; e) fibronectin, f) stained with picrofuchsine.  $160\times$ .

In histological sections the extracellular deposits had the appearance of fibrinoid masses. The typical fibrinoid, in the absence of cells, contained only fibronectin.

Our findings thus not only confirm previous observations of fibronectin and type IV collagen in CTC of the human placenta [4, 5, 10], but prove the presence of interstitial types of collagen in these structures. The immunofluorescence method, while not strictly quantitative, nevertheless enables it to be concluded with some degree of probability that the concentration of collagen of types IV and V and of fibronectin in CTC is higher than that of collagen of types I and III. It is logical to suggest that peripheral trophoblasts are involved in the formation of the

macromolecules of EM. In fact, amniotic epithelium, which is a derivative of trophoblasts, can synthesize types III, IV, and V of collagen in tissue culture [2]. Both in vitro and in vivo trophoblasts secrete fibronectin, type IV collagen, and laminin [4, 5]. It is not yet clear which of the known populations of trophoblasts [11] are responsible for the production of the various components of EM.

The cells evidently also determine the heterogeneous composition of fibrinoid. In their absence the fibrinoid masses are of predominantly hematogenous origin. On the other hand, the presence of the principal types of collagen in fibrinoid of the CTC even in the early stages of pregnancy raises the question of the existence of a physiological prototype of sclerosis in addition to the typical sclerosis of dying chorionic villi [6].

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# MORPHOLOGIC CHANGES IN THE CANINE CENTRAL NERVOUS SYSTEM IN UNILATERAL CAROTID ARTERIAL LESIONS

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Occlusive lesions of the main vessels of the brain are currently of great interest to clinicians because the problem of cerebral ischemia is closely linked with lesions of the carotid artery. In addition, surgeons are tackling the problem of preserving brain tissue when pathological changes are present in the vessels themselves and also after their surgical correction [2].

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