α- and β-subunits of the peripheral insulin receptors of the liver and blood vessels, respectively, proteins localized with the aid of AS-3 corresponded more closely in their immunocytochemical properties to ILGF-1 than to insulin receptors.

By contrast with concepts put forward previously on the effect of insulin on functional activity of the neurosecretory cells of the median eminence through the intermediary of collaterals from a special type of insulin-sensitive neurons to central formations of the hypothalamus [13], the results of the present experiments are evidence of direct interaction of the peptides insulin and ILGF-1 with neurosecretory cells and ependymal elements of the median eminence of the hypothalamus, located on axon endings, and central receptors for ILGF-1. The results support the hypothesis that insulin and ILGF-1 peptides play a regulatory role in the CNS.

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DISTRIBUTION OF COLLAGEN OF TYPES III AND IV IN VILLI OF THE HUMAN PLACENTA

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Despite intensive biochemical reseach on the human placenta, little is still known about the distribution of the main components of the intercellular matrix in its structures. It was found previously that type IV collagen is located not only in the basement membrane, but also in the stroma of the villi of the human placenta [2]. To verify and clarify this observation an investigation was carried out with the use of immunofluorescence and immunoelectron-microscopic methods.

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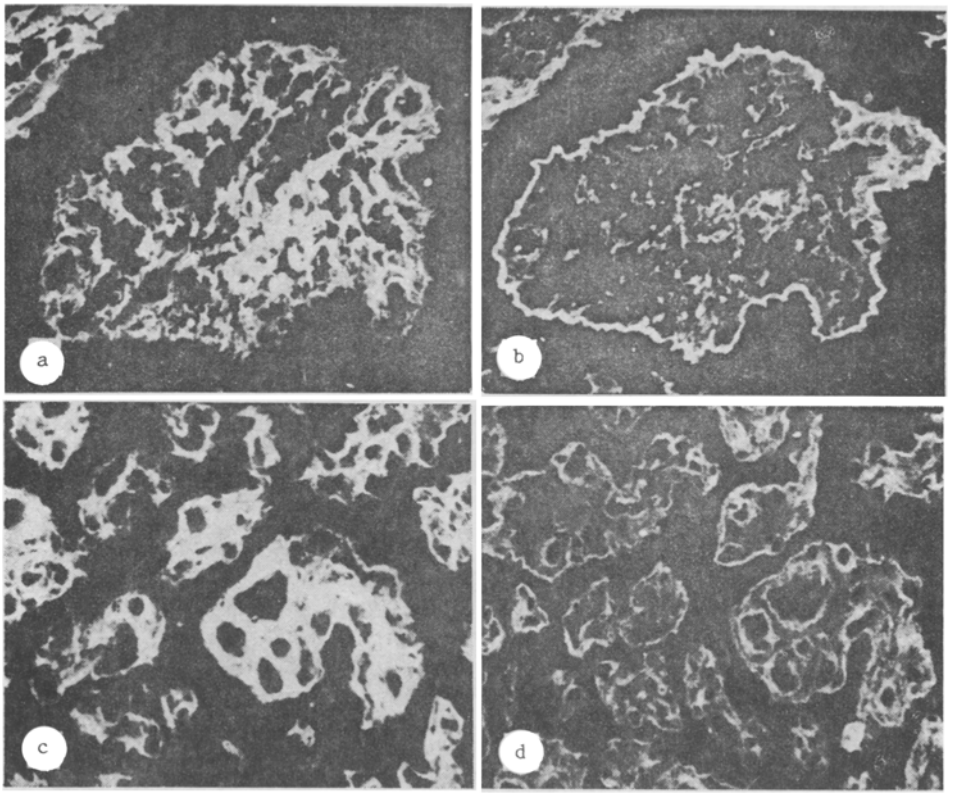

Fig. 1. Collagen in villi of immature and mature human placenta. 128 ×. a, b) Immunofluorescence of collagen of types III and IV in immature placenta, serial frozen sections (a – type III collagen in stroma of villi; b – type IV collagen in basement membrane of trophoblast and in stroma of villi; c, d – immunofluorescence of collagen of types III and IV in mature placenta, serial frozen sections (c – dense distribution of type III collagen in interstices of villi; d – localization of type IV collagen in basement membranes of endothelium of blood vessels and trophoblast and in stroma of villi).

EXPERIMENTAL METHOD

Immature placentas at 7-10 weeks of pregnancy obtained after medical abortions and mature placentas at the 39th-40th weeks of pregnancy obtained during normal deliveries were studied. Polyclonal rabbit antibodies to human collagen of types III and IV were used in a concentration of 0.05 mg/ml; the method of obtaining and the characteristics of these antibodies were described previously [1]. Affinity-purified goat antibodies to rabbit IgG, labeled with fluorescein isothiocyanate, were used for the indirect immunofluorescence investigation. Goat antibodies to rabbit IgG, labeled with colloidal gold, were used for the immunoelectron-microscopic study. Colloidal gold granules 5 nm in diameter were obtained [6]. The indirect immunofluorescence study was carried out on serial frozen sections 5 μm thick, fixed for 3 min in cold acetone. Pieces of tissue for immunoelectron-microscopic study were fixed in 2% paraformaldehyde with 0.1 M phosphate buffer (pH 7.2) for 2 h at room temperature, and then transferred for 39 min into 50 mM NH_4Cl in phosphate-buffered saline (PBS). To block aldehyde groups the material was washed in PBS for about 15-18 h at +4°C, then dehydrated in alcohls of increasing concentration at a temperature of –20°C: 50° alcohol for 15 min, 70° for 1 h, 90° for 1 h, and 100° for 30 min. Water-soluble LR-Gold resin (Polysciences), in various dilutions in alcohol, was then introduced into polyethylene capsules containing fragments of placental tissue at –20°C: 100° alcohol + LR-Gold (1:1) for 30 min; 100° alcohol + LR-Gold (3:7), LR-Gold for 1 h; LR-Gold + 0.5% catalyst for 1 h; LR-Gold + 0.5% catalyst overnight. Polymerization of the LR-Gold resin was carried out for 24 h at –20°C, using an ultraviolet lamp (wavelength 360 nm). Ultrathin sections, silvery yellow in color, were placed on nickel grids (300 mesh) with a formvar backing. The whole procedure of washing and incubation of the sections with antibodies was carried out at room temperature in a drop of solutions, with the

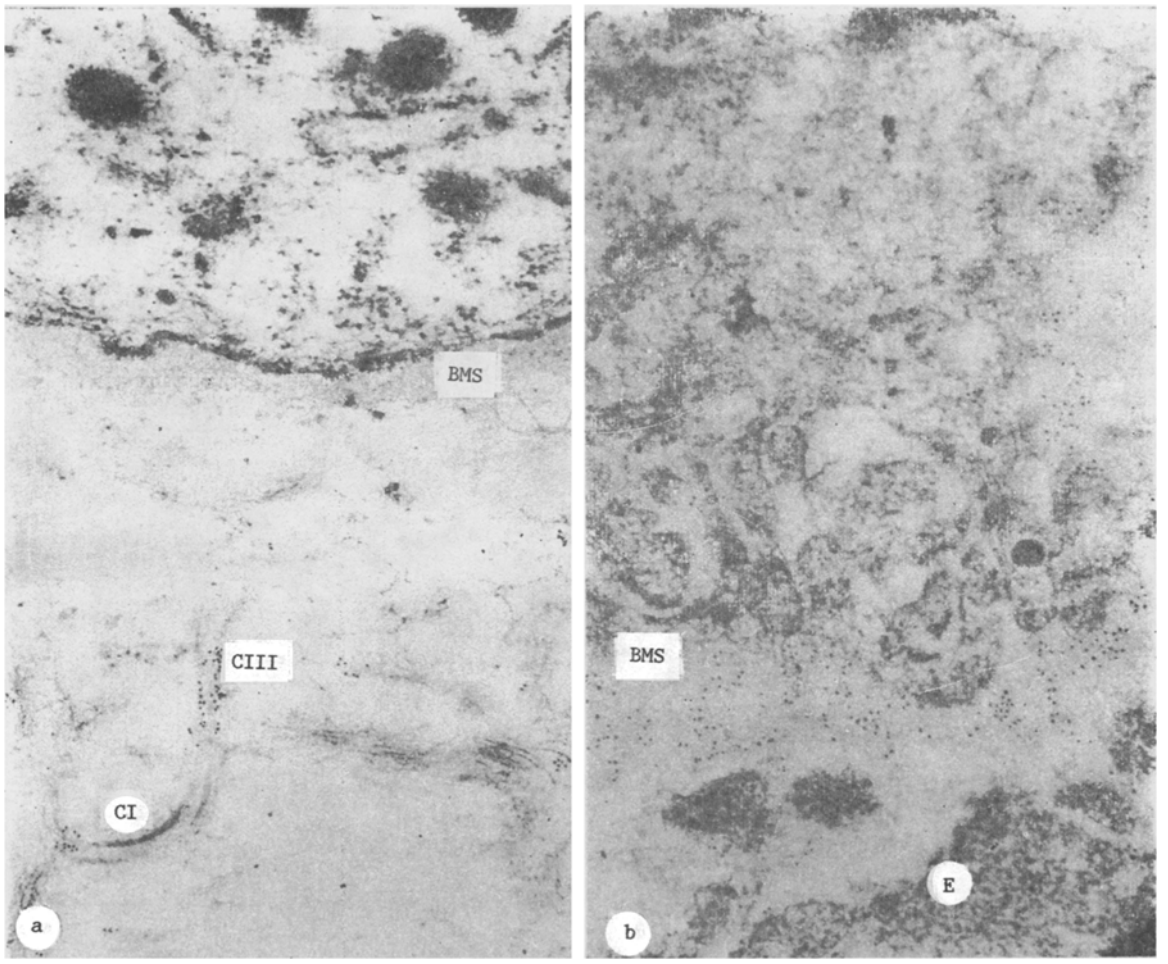

Fig. 2. Electron micrograph of a villus of immature human placenta. Collagen of types III and IV. Sections incubated with antibodies labeled with colloidal gold. a) Type III collagen, loose, poorly developed network of collagen fibers. Granules of colloidal gold distributed on type III collagen fibers. BMS) Basement membrane of syncytiotrophoblast; CIII) type III collagen, CI) type I collagen. 40,000 ×; b) type IV collagen. Granules of colloidal gold distributed throughout thickness of BMS. E) Endothelium of a blood vessel. 60,000 ×.

sections below. Initially the sections were treated in a drop of 50 mM NH_4Cl – PBS for 10 min, then for 10 min in Tris-buffer + 1% bovine serum albumin (BSA), then for 5 min in Tris-buffer, and incubated with the secondary antibodies, labeled with colloidal gold, for 1.5 h. The sections were again washed in Tris-buffer + 1% BSA for 10 min and in bidistilled water for 5 min. The sections were stained with 2% uranyl acetate for 15 min and with lead citrate. Ultrathin sections were examined and photographed in the JEM-100CX electron microscope (Japan).

EXPERIMENTAL RESULTS

The immunofluorescence study revealed type III collagen in the immature placenta on the side of the villi, in the form of a very loose network of thin fibers, at times closely adjacent to the zone of the basement membrane of the trophoblast (Fig. 1a). Type IV collagen was located in the basement membrane of the trophoblast of the villi and, to a lesser degree compared with type III collagen, in the stroma of the villi (Fig. 1b). Type III collagen was represented in the mature placenta in the stroma of the villi as numerous coarse, densely packed fibers and conglomerates (Fig. 1c). The localization of type IV collagen was not confined to thickened basement membranes of the trophoblast and endothelium of the blood vessels, but some collagen could also be detected in the stroma of the villi (Fig. 1d). On immunoelectron-microscopic study the stroma of the villi of the immature placenta consisted of a very loose fibrous network with only a few collagen fibers (Fig. 2a). On treatment with antibodies to type III collagen, granules of colloidal gold were localized in a linear manner

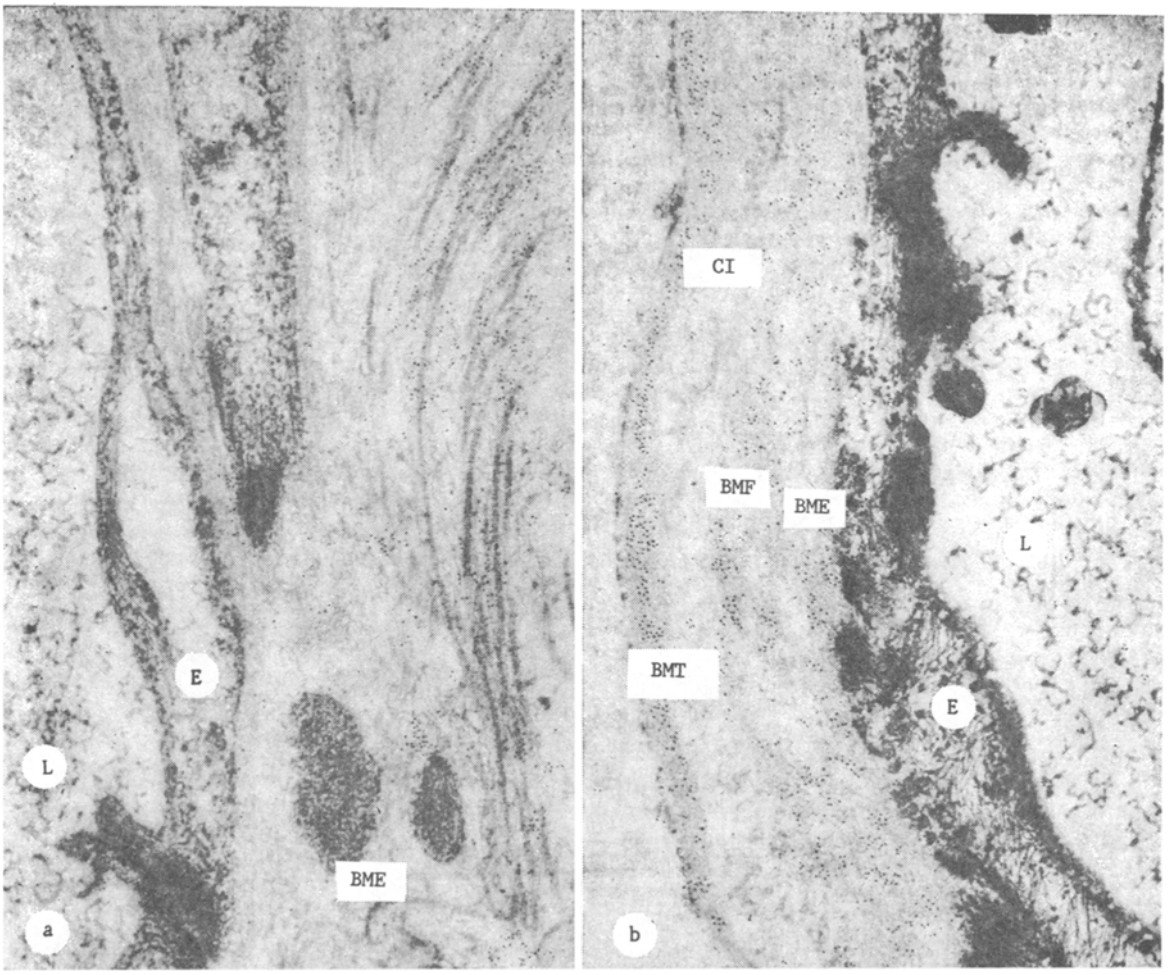

Fig. 3. Electron micrograph of a villus of mature human placenta. Collagen of types III and IV. Sections incubated with antibodies labeled with colloidal gold. 40,000 ×. a) Type III collagen. Gold particles on type III collagen fibers, running longitudinally and transversely in type I collagen fibers. E) Endothelium of a blood vessel, L) lumen of vessel, BME) basement membrane of endothelium of vessel; b) type IV collagen. Gold granules on basement membranes of endothelium of blood vessel and trophoblast of villus. BMT) Basement membrane of trophoblast, BMF) fragments of basement membrane, KI) type I collagen.

along the course of thin collagen fibers, which were arranged longitudinally and transversely in thick type I collagen fibers, with the characteristic periodic cross-striation. The basement membrane of the epithelium of the villi was free from the colloidal gold label. On treatment with antibodies to type IV collagen, gold particles were distributed throughout the thickness of the basement membrane of the syncytiotrophoblast (Fig. 2). Moreover, the electron-dense homogeneous basement membrane could not be found around the endothelium of the capillaries and, correspondingly, gold label also was absent in this zone. In regions corresponding to the structurally poor loose stroma, gold label for type IV collagen was found extremely rarely.

Villi of the mature placenta were characterized by a well defined network of collagen fibers in the stroma (Fig. 3a). Specific gold labeling was preserved only on thin collagen fibers of type III, which formed a network with thick type I collagen fibers. A fully formed basement membrane of the endothelium of the capillaries was present, with no gold particles. Interstitial collagen was located in close promixity to the basement membranes of the trophoblast and capillary endothelium.

The localization of type IV collagen in the basement membranes of blood vessels and, in particular, of the trophoblast of the mature placenta was very demonstrative and informative (Fig. 3b). Long twisted homogeneous structures, similar in density and homogeneity to basement membranes, and also containing gold granules, were often observed between these membranes

in the stroma among the fibers of interstitial collagen. Antibodies to type IV collagen were located mainly in the lamina densa in the basement membrane of the endothelium and trophoblast.

The poorly defined stroma of the villi of the immature placenta which was observed at the ultrastructural level and, conversely, the well developed stroma of the villi of the mature placenta reflect successive stages of placental development. Type III collagen fibers are thin, up to 10 nm in diameter, and interwining closely with thick type I collagen fibers, up to 30-35 nm in diameter, they formed a dense network, which was very often in close contact with the basement membranes. By means of immunoelectron microscopy, which confirmed the typical localization of type III collagen in the stroma of the villi and of type IV collagen in the basement membranes, fragments of type IV collagen could be reliably localized among interstitial collagens only in the stroma of the villi of the placenta in a pregnancy going on to full term. A picture of this kind was observed most frequently in the interstices between the membranes of the trophoblast and endothelium. In a recent, and so far the only, immunoelectron-microscopic study of the mature placenta, with peroxidase labeling of antibodies [3], the authors cited also found type IV collage in the stroma of the villi, and regard it as remnants of the basement membranes of capillaries. This view in our opinion is questionable, for in the early stages of histogenesis of the villi basement membranes are completely absent in the vascular spaces: specific gold labeling was absent in the zone of the early fetal vessels on treatment with antibodies to type IV collagen. Formation of the basement membrane of the epithelium of the early villi takes place much earlier than development of the capillary membranes, in agreement with data in the literature [4, 5]. The reason is probably that fetal capillaries arise through local angiogenesis in the stroma of villi already formed.

The impression is obtained that gold particles – markers of type IV collagen – were distributed in the early placenta throughout the thickness of the basement membrane of the syncytiotrophoblast, whereas in the mature placenta they were located mainly in the lamina densa of the basement membrane of the syncytiotrophoblast and of the fetal capillaries, reflecting processes of differentiation of basement membranes.

The use of an indirect immunofluorescence and immunoelectron-microscopic method with labeling of antibodies by colloidal gold thus demonstrated the distinctive distribution of interstitial (type III) and membrane (type IV) collagens in the villi of the early and mature human placenta. The solution to the problems raised requires further study, using the immunoelectron-microscopic method; in particular, the sources of formation of the type IV collagen in the stroma of the villi must be discovered and details of interaction of the components of the basement membranes of the fetal capillaries and syncytiotrophoblast must be clariclarified at the stages of histogenesis of the placenta.

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