# SYNTHESIS OF SERUM PROTEINS IN THE HUMAN PLACENTA IN VITRO

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It has been shown by immunodiffusion analysis after preliminary incorporation of  $C^{14}$ -phenylalanine that human placental tissue in vitro synthesizes only one of the serum proteins of the  $\beta_1$ -fraction in detectable amounts, presumably the  $\beta_1$ -C component. Neither  $\gamma$ -globulin nor  $\beta$ -fetoprotein is synthesized.

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The problem of the importance of the placenta in fetal protein metabolism occupies a considerable place in the proteinology of embryogenesis. Investigations [8] by the immunoelectrophoresis method using antiserum against serum have shown that washed placental tissue contains serum albumin, transferrin,  $\gamma$ -globulin (Igg),  $\beta_{2-M}$  (IgM), and  $\beta_{2-A}$  (IgA) globulins. Labeled lipoprotein was absent from the placenta. However, these observations were made without introduction of labeled amino acid into the tissue, and the presence of particular proteins in the tissue thus gave only indirect evidence of the production of these proteins by the placenta.

In other investigations [4] synthesis of serum protein in the placenta was studied by incubation of fragments of placental tissue in a medium with C<sup>14</sup>-labeled amino acids followed by electrophoresis in a starch block, precipitation of eluates from individual fractions by specific antisera in tubes, and measurement of radioactivity of the immunoprecipitate.

The authors cited above concluded that the mature placenta is capable of producing proteins in the  $\alpha$ -and  $\beta$ -globulin zones. Evidence of synthesis of  $\gamma$ -globulin was not precise.

The two investigations cited above were marked by technical errors, and as a result, the problem of protein synthesis by the placenta remains unsolved.

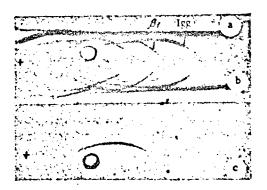


Fig. 1. Immunoelectrophoresis of placental tissue homogenate (a) and adult human serum (b) development by antiserum against adult human serum; autoradiograph of the same preparation after exposure of 7 days (c). No radioactivity was found in the  $\gamma$ -globulin arc ( $I_{CC}$ ).

The object of the present investigation was to study synthesis of serum proteins by the human placenta in vitro.

# EXPERIMENTAL METHOD

A combined method [5] was used, embracing the following techniques: 1) isotope labeling of protein synthesized in vitro, 2) immunodiffusion analysis [3], and 3) autoradiography of the blocks after immunoelectrophoresis.

The tissue of a full-term placenta was cut up with scissors into pieces 1-2 mm in diameter, and mixed with Eagle's medium in the proportion of 3:1.  $C^{14}$ -phenylalanine with a specific radioactivity of 0.2  $\mu$ Ci/mg was added to the medium in a dose of 10  $\mu$ Ci/ml.

Incubation was carried out in special vessels [2, 7]. After incubation for 24 h, the liquid from the upper part of the reservoir containing proteins was subjected to immuno-electrophoresis [3]. Antiserum against serum and also against  $\beta$ -fetoprotein was used for development. The pa-

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rameters of electrophoresis which were used are described in earlier publications [1]. Washing in salt solution continued for 3-4 days, and after drying the specimens were subjected to autoradiography. PF-3 film was used and the exposures were 7, 14, and 150 days.

# EXPERIMENTAL RESULTS

The results of immunoelectrophoresis, using antiserum against adult human serum as developer, with subsequent autoradiography gave blackening only in the arc located in the  $\beta_1$ -zone, evidently the arc of the  $\beta_1$ -C component (Fig. 1).

The blackening of other arcs was observed. An increase of exposure to 14, or even to 150 days, while sharply increasing the intensity of the components previously observed, did not reveal radioactivity in the other arcs (Fig. 1b, c). No radioactivity likewise was found in the  $\gamma$ -globulin (Igg) arc.

Antiserum against  $\beta$ -fetoprotein clearly revealed a  $\beta$ -fetoprotein arc during electrophoresis of the placenta. However, this protein had evidently been introduced with the blood, because no radioactivity was found in it even after very long exposures.

It may therefore be concluded from these experiments that the placenta synthesizes only one of the serum proteins of the  $\beta_1$ -fraction in detectable amounts, presumably the  $\beta_1$ -C component. Neither  $\gamma$ -globulin nor  $\beta$ -fetoprotein is synthesized.

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