A COMPARATIVE IMMUNOELECTROPHORETIC STUDY OF THE PROTEINS OF THE PLACENTA AND OF THE FETAL BLOOD SERUM

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An important aspect of the problem of the mutual relationship between mother and fetus is the comparative analysis of the fetal blood proteins and the proteins of the connecting organ – the placenta. The question of whether proteins may be synthesized independently in the placenta and subsequently transferred to the fetus is one which has given rise to discussion at the present time.

Immunological methods have shown [3, 4, 6, 7, 10] that a specific protein is present in the fetal serum which is not found in the serum of the adult person, but which can be detected in the placenta. Meanwhile, immunochemical investigations have given convincing evidence [9] of the nonidentity of the two components of the γ-globulin fraction of the umbilical blood and placenta and of the identity of one component of the γ-globulin fraction.

Zapp [12] studied the immunoelectrophoregram of the placental proteins and observed that their most marked component was present in the γ-globulin fraction (75% of the total placental protein). In this way, Zapp explained the increased concentration of γ-globulins in the blood of the new-born infant, by postulating that the placenta is an independent site of synthesis of γ-globulins, from which the reserves of γ-globulins obtained by the fetus are formed.

In the present research we compared the proteins of the placenta and umbilical blood by means of the methods of immunoelectrophoresis, described by Grabar and Williams [2], and of stereoimmunoelectrophoresis, described by Vasileiskii [1], both based on Ouchterlony's principle [7].

EXPERIMENTAL METHOD

The fully grown placenta was used. A placental homogenate was prepared after carefully washing the placenta free from blood by agitation in a shaker 16 times, for 5 min each time (for control purposes immunoelectrophoresis was carried out every time on a homogenate prepared from the washed material with antiserum against human blood). To make the homogenate, minced placenta was ground with two volumes of veronal buffer (0.03 M, pH 8.6). The homogenate was centrifuged at 7000 rpm. Electrophoresis of the extract was carried out in a 2% agar gel, prepared in 0.03 M veronal buffer (pH 8.6), at a potential gradient of 6.3 V/cm, for 2 h in a refrigerator at 4°. The thickness of the layer of agar was 3 mm. Antiserum was obtained from rabbits immunized with placental extract with a protein concentration of 2 mg/ml, in doses of 0.1 ml injected 9 times over a period of 3 weeks, and then reimmunized 30 days later by injection of 0.5 ml of extract with a protein concentration of 2 mg/ml, and 5 months after that by 1 ml of extract with a protein concentration of 2 mg/ml.

We used the method of Grabard and Williams [2], Vasileiskii's method of comparing pairs of immunoelectrophoregrams [1], and the method of development using a shortened gutter [5, 11].

EXPERIMENTAL RESULTS

The electrophoregrams of the placental extracts, when stained without the use of an immune developer, showed one weak component in the albumin and one in the α-globulin region, and a bright stain in the region of the β_2-globulins or, more accurately, between β_1 and β_2 (Fig. 1). Without the use of an immune developer, the staining in the region of the γ-globulins was weak.

Immune development by antiserum against placental extract revealed 7 arcs: a very intensive arc corresponding

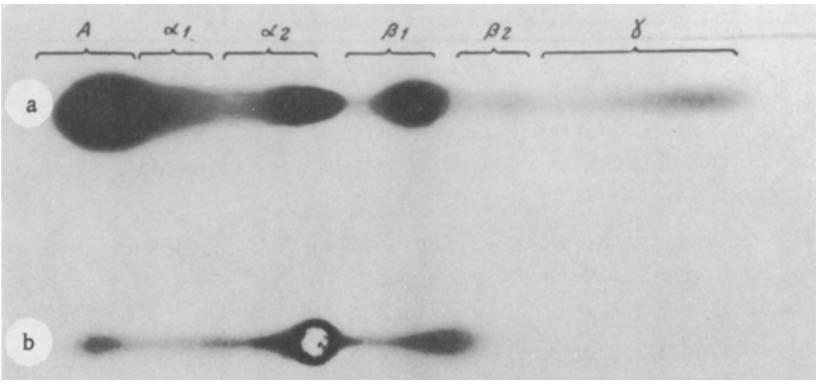

Fig. 1. Electrophoregrams of proteins from blood serum (a) and placental extract (b).

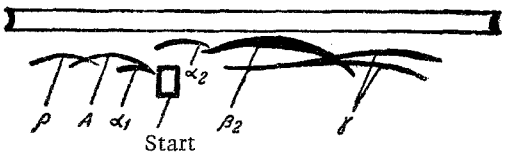

Fig. 2. Immunoelectrophoregram of placental extract (scheme). A) albumins, p) prealbumins.

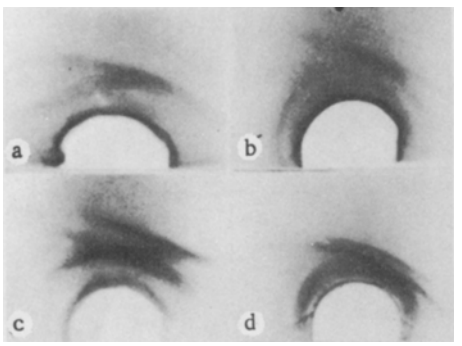

Fig. 3. Transverse sections of the stereoimmune electrophoregram of placental extract (right) and umbilical blood serum (left). Development by antiserum against placenta. a) Albumin region. The "spore phenomenon" can be seen; b) region of the α_2-globulins. A common line and a line only on the side of the placental extract can be seen; c) region of the β_2-globulins. One common line and one line only on the side of the placental extract can be seen; d) region of the γ-globulins. One common line and two lines only on the side of the placental extract can be seen.

in its mobility to the principal stain of the electrophoregram of the extract in the region of the β_2-globulins; weakly stained components: one in the prealbumin and one in the albumin region, one in the region of the α_1-globulins, one in the region of the α_2-globulins, and two in the region of the γ-globulins (Fig. 2). When antiserum against human blood serum was used as developer, revealing 8 components in the electrophoregram of the blood serum, in the electrophoregram of the placental homogenate some barely perceptible arcs were seen in the region of the β_2-globulins.

Meanwhile, antiplacental antiserum revealed 6 components in the electrophoregram of the fetal blood serum: one in the prealbumin and one in the albumin region, one in the region of the α_1-globulins, one in the region of the α_2-globulins, one in the region of the β_2-globulins, and one in the region of the γ-globulins.

After these results had been obtained, experiments were conducted to reveal the identity of the placental proteins and the serum proteins of the umbilical blood.

The experiment was conducted using the short trough method and development with placental antiserum; this showed that the principal component of the placenta (β_2-fraction) gave an arc running into that of the β_2-globulin of umbilical blood serum.

When the method of paired stereoimmunological development was used to investigate the electrophoregram of the placental extract (Fig. 3), identical components were revealed in the pair from the electrophoregram of umbilical blood (confluent lines): one in the albumin and one in the α_2-globulin regions, one (the most intensive line) in the β_2-globulin and one in the γ-globulin regions.

Surplus components were discovered: one surplus line on the placental extract side in the α_2-globulin and one in the β_2-globulin regions, and two surplus lines in the γ-globulin region. The last two lines were considerably brighter than the identical components in the γ-globulin region.

The "spore phenomenon" was noted in the albumin region (reaction of incomplete identity).

Hence, the placenta evidently is capable, independently, of synthesizing several protein components, and these may possibly be transferred to the fetus. By means of Grabar's immunoelectrophoretic method, it was established that some protein components of the fetal serum and the placenta are identical; on the other hand, by means of Vasileiskii's paired stereoimmunological development method, it was confirmed that some components were completely identical and others not identical.

The identity of the components discovered evidently reflects the immunological species specificity, and the nonidentity of the individual proteins reflects the organ specificity.

SUMMARY

By employing Levy-Polonovski and Wadsworth-Hanson immunoelectrophoretic methods with short trough, and also theVasileisky method of stereoimmunological development it was shown that electrophoregrams of placental homogenate and umbilical cord blood serum possess identical components: 1) one in albumin fraction, 2) one in β_2-globulin fraction, 3) one in α_2-globulin fraction, and 4) one in γ-globulin fraction; cross-reacting components (one in β_2-globulin fraction and two in γ-globulin fraction), as well as related but not identical components in albumin fraction (spore). The placenta used in the experiments was duly washed of blood, so that, following immunoelectrophoretic tests by Grabar-Williams and antiserum development as against adult human serum in optimal concentration the arcs were barely perceptible. However, the same antiserum, applied to a human serum electrophoregram, gave eight quite distinct bands.

LITERATURE CITED

1. S. S. Vasileiskii, Biokhimiya, 5, 855 (1959).
2. P. Grabar and C. A. Williams, Biochim. biophys. Acta, 1953, v. 10, p. 193.
3. T. Itasaka, Okayama Igakkai Zasshi, 1958, v. 70, p. 2707.
4. H. Kusumoto, Ibid., 1957, v. 69, p. 2931.
5. G. Lévy and J. Polonovski, Bull. Soc. Chim. Biol. (Paris), 1958, v. 40, p. 1293.
6. Y. Miyoshi, Okayama Igakkai Zasshi, 1959, v. 71, p. 555.
7. O. Ouchterlony, Lancet, 1949, v. 256, p. 346.
8. D.Subrahmanyam and P. H. Maurer, J. Immunol., 1959, v. 83, p. 327.
9. M. Torimaru, Okayama Igakkai Zasshi, 1957, v. 69, p. 3073.
10. C. Wadsworth and L. A. Hanson, Int. Arch. Allergy, 1960, v. 17, p. 165.
11. E. Zapp, Mschr. Kinderheilk., 1960, Bd. 108, S. 120.*
12. E. Zapp, Mschr. Kinderheilk., 1960, Bd. 108, S. 120.*

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.

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