BEHAVIOR OF β-FETOPROTEIN DURING ELECTROPHORESIS IN A VERTICAL STARCH GEL

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Previous investigations [1, 2, 4, 5, 6] showed that, besides fetoprotein migrating during electrophoresis between albumin and the α_1-globulin zone, the serum of the human fetus contains a second fetoprotein in the β_2-globulin zone. Whereas the α-fetoprotein disappears at the 20th-30th week of intrauterine life, the β-fetoprotein persists until the 5th week of postnatal development [1]. It has also been claimed that the β-fetoprotein discovered in the author's laboratory is identical with that found in the blood of patients with hepatocellular carcinoma [3]. It was later shown that β-fetoprotein is present in fetal monkeys but not in adult monkeys [2].

In the present investigation the behavior of β-fetoprotein was studied during starch-gel electrophoresis, using a method described in the literature [11].

This investigation is urgently required for the following reasons. In the method adopted, electrophoresis is combined with molecular filtration, and localization of the individual proteins and the starch gel differs in principle from the technique used in ordinary electrophoresis. Proteins with lower molecular weight overtake proteins with higher molecular weight. Hemoglobin, for example, overtakes the haptoglobins, whereas during electrophoresis on paper or in agar, the opposite relationship holds good. The picture obtained by electrophoresis using Smithies' technique gives valuable information about the properties of a protein, and its localization by starch-gel electrophoresis is an independent constant.

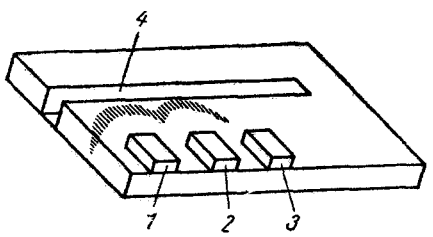

Fig. 1. Immunodiffusion using a piece of starch-gel cut from the block after electrophoresis: 1) piece of starch-gel; 2, 3) pieces cut from the β region of the gel after electrophoresis of sera of a newborn infant (the "positive" standard) and of an adult person (the "negative" standard) respectively; 4) gutter containing antiserum against β-fetoprotein AS(F_β).

On the other hand, because the separation of proteins taking place in starch gel is very clear cut, the method possesses high resolving power, and a protein of high purity can be obtained.

EXPERIMENTAL METHOD

For a long time there was no satisfactory method of extracting the protein from the zones of starch gel after electrophoresis by Smithies' method [11]. A technique of extraction has now been developed [12], using electrophoretic migration of the protein into special compartments of a divided dish, and the investigator now possesses a powerful instrument for the preparative separation of proteins.

Both these methods were used. Because of the advantages of starch-gel electrophoresis, as far as possible all the experimental conditions were adapted to the preparative variant, i.e., all the experiments were carried out in very thick starch gel – in dishes up to 10 mm thick. The start wells in this case contained 0.2 ml serum. These large dimensions demand (as an essential condition) a vertical position of the dish during electrophoresis [11] to prevent interference with convection.

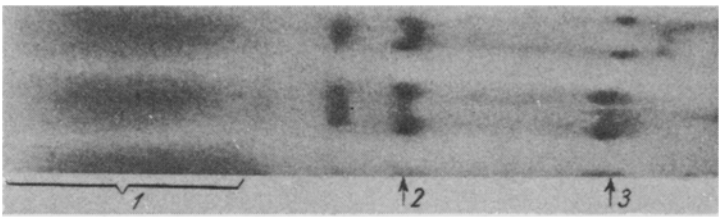

Fig. 2. Vertical starch-gel electrophoresis of the serum of a newborn infant: 1) albumin; 2) transferrin; 3) $S\alpha_2$ zone.

The buffer used was obtained by mixing a 0.2 M solution of H_3BO_3 and 0.05 M solution of $Na_2B_4O_7 \cdot 10H_2O$. To prepare the gel the solutions were taken in the ratio of 7.5 : 17, and for the electrode cells in the ratio of 3 : 7. With a 12% gel and a potential gradient of 2.5 V/cm the optimal time of electrophoresis was 18 h.

To detect β-fetoprotein in the zones of the starch-gel, at first the following technique was used for searching [7]. Separate pieces cut out of the starch-gel were placed with their cut surface on agar gel and were left for 24 h to allow diffusion; reference blocks of gel were then placed between these cut-out pieces, consisting of pieces cut from another agar gel used for electrophoresis (Fig. 1). This series of pieces of starch-gel, interspersed with reference agar blocks, were arranged along a gutter into which was poured antiserum against β-fetoprotein. After a further 24 h, when counter diffusion of antibodies and antigens had taken place, precipitates were formed as a wavy line (see Fig. 1).

In addition, ultracentrifugation was carried out on the Spinco-L preparative centrifuge in a sucrose gradient (CB-39 bucket rotor).

In the main series of experiments in which preparative partition [12] was used to test the fractions extracted from the starch gel, an immuno-diffusion technique was adopted, using a cross gutter. This has been described previously [2] and is a derivative of the classical methods [8, 9, 10].

RESULTS

In preliminary experiments an attempt was made to determine the localization of β-fetoprotein on the starch-gel after electrophoresis without using immunodevelopment. The method was based on the fact that β-fetoprotein is not present in the serum of certain fetuses (in approximately 20%). The sera of such fetuses were used as negative controls. As a first step the two sera for comparison were fractionated by electrophoresis in agar gel, the zone of the β-globulins was cut out, the proteins were extracted from it by freezing and thawing, and then they were dried. The preparations, when purified in this way, were compared by starch-gel electrophoresis.

Electrophoresis of both samples revealed a very bright band belonging to transferrin in its characteristic place. In addition, electrophoresis of the sample obtained from the serum containing β-fetoprotein gave a pale stain in the region of $S\alpha_2$-globulin, slightly displaced towards the cathode. No such stain was found after electrophoresis of the serum not containing β-fetoprotein.

The preliminary conclusion was thus drawn that β-fetoprotein migrates at the speed of $S\alpha_2$-globulin. However, because of the unsatisfactory quality of the results obtained by electrophoresis in this manner, the method was changed and experiments were carried out as described above [7]. In this series of experiments the preliminary conclusions were confirmed. In fact, β-fetoprotein was found in the region of $S\alpha_2$-globulin, and an immunoprecipitation line was seen above the piece cut out of the $S\alpha_2$ zone. This line was confluent with the corresponding lines above the reference block of β-fetoprotein and it did not extend across to the region lying above the "negative" standard, i.e., that corresponding to the β-fraction of the adult human serum after electrophoresis.

Attempts were then made to extract the proteins of the $S\alpha_2$ zone from the starch-gel (Fig. 2) and to discover whether the β-fetoprotein could be eluted. By means of the method described above [12] the β-fetoprotein could be extracted from the zone of starch-gel and obtained as a solution.

Above the well into which the fluid extracted from the corresponding compartment after electrophoretic elution was poured a precipitation line was formed which merged with the line of β-fetoprotein but did not extend across to the region corresponding to the adult human serum (Fig. 3). The other lines present on the preparation

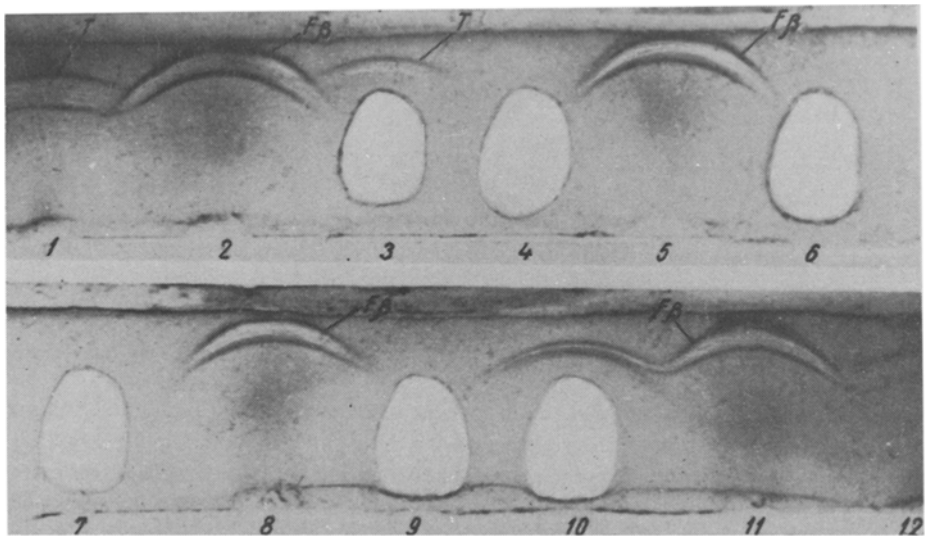

Fig. 3. Tests of fractions obtained by Tsuyuki's method [12]. Electrophoresis of the reference sera was first carried out: 2, 5, 8, 11) serum of newborn infant; 1, 12) serum of adult person. Large wells were then cut out, into which the preparations for testing were poured: 10) $S\alpha_2$; 3) zone of transferrin; the other wells contained borate buffer. Line T) transferrin; F_β) β-fetoprotein.

were precipitates of protein impurities; these also extended across to the region of migration of proteins of adult human serum. All the lines were clearer than on the preparations obtained by the technique described above [7].

The experiments thus showed conclusively that β-fetoprotein moves in a vertical starch-gel at the speed of the $S\alpha_2$-fraction. Transferrin, the chief protein impurity during electrophoresis in agar gel, lies far ahead in starch-gel. No β-fetoprotein is found in the transferrin zone (see Fig. 3).

It may be postulated that the molecular weight of β-fetoprotein is fairly high, higher than that of hemoglobin and transferrin. Ultracentrifugation confirmed this suggestion: the sedimentation constant was 10S.

The results of this investigation show that much higher purification of β-fetoprotein is possible than can be achieved by simple electrophoresis.

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SUMMARY

Using vertical starch-gel electrophoresis after Smithies' and subsequent isolation of protein fractions according to Tsuyuki it was shown that β-fetoprotein discovered earlier migrates in the $S\alpha_2$ band. Using this procedure, it can be easily separated from transferrin.

It is believed that β-fetoprotein has a larger molecular weight than transferrin, i.e., over 90,000.

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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 the first issue of this year.
