INVESTIGATION OF β-FETOPROTEIN BY CHROMATOGRAPHY ON SEPHADEX G-200 DEXTRAN GEL

S. S. Vasilelskii

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It was found by immunoelectrophoresis that β-fetoprotein is eluted from a chromatographic column with Sephadex G-200 in the intermediate zone between the 18S and 7S peaks, and in its molecular weight it thus occupies an intermediate position between these classes of proteins. These findings, together with the localization of β-fetoproteins in the β_2-globulin zone suggests that β-fetoprotein is related to two classes of newly discovered immunoglobulins: the 11S immunoglobulin of the colustrum and IgE.

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In previous investigations I discovered a new β_2-globulin component in the blood serum of newborn infants and attempted to define it physicochemically [1-4].

With the appearance of a new method of chromatography of proteins on columns packed with gel granules on the principle of the "molecular sieve", during recent years it has become possible to separate proteins with very similar properties and incapable of separation electrophoretically [5, 7, 8, 12-15, 17] and to obtain a definite idea of the molecular weight of a protein [16] even before it has been purified from accompanying components.

Having regarded these facts, in the present investigation I studied the behavior of β-fetoprotein during chromatography on a column with Sephadex G-200.

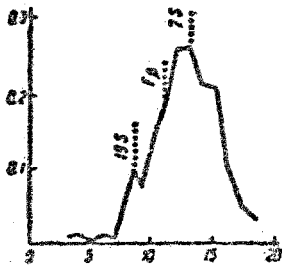

Fig. 1. Outflow chromatographic curve of experiment to fractionate neonatal serum on Sephadex G-200 column. The 19S peak is eluted with samples Nos. 7-9, the 7S peak with sample No. 10 and subsequently with a maximum in sample No. 13. β-Fetoprotein (Fβ) is found in sample No. 11. Ordinate, protein content, in mg; abscissa, serial No. of samples.

EXPERIMENTAL METHOD

Neonatal blood flowing freely from the placental end of the umbilical cord was collected. A mixture of sera from several infants was dialyzed against a buffer, in which the whole chromatographic procedure was subsequently carried out, and applied in a volume of 0.5 ml to a column with Sephadex G-200. The dimensions of the column were 20 × 300 mm. The column of buffer above the top level of Sephadex granules measured 140 mm. Both for suspension of the Sephadex and for elution (i.e., as chromatographic developer) 0.05 M veronal buffer, pH 8.5, with addition of NaCl up to 0.5 M was used. Chromatography continued for 3.5 h. During this period 18 samples (each of 6 ml) were collected.

A variant of chromatography with reflux of the buffer (from the bottom upward) as suggested by Porath [15] was also tested. This variant demands the use of a pneumatic "gate" in the upper part of the column, consisting of an inflated stopper through which the outflow tube passes, and this complicates the experimental procedure. We found only some speeding up of the chromatographic process without any increase in the resolving power of the method.

In each sample the protein concentration was determined by the method of Lowry and co-workers [11], after which the remainder of the

Laboratory of Biochemistry, All-Union Research Institute of Obstetrics and Gynecology, Ministry of Health of the USSR, Moscow (Presented by Active Member of the Academy of Medical Sciences of the USSR M. S. Malinovskii). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 66, No. 6, pp. 57-59, June 1968. Original article submitted August 13, 1968.

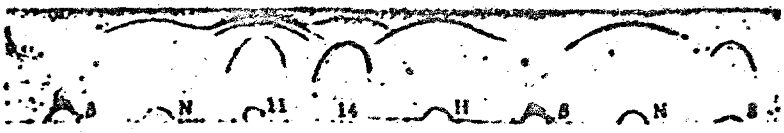

Fig. 2. Electrophoresis of samples obtained from column (Fig. 1). Electrophoresis of sera of newborn infants (N) and adults (A). Large wells were then cut out and the test samples from the column poured into them. The numbers denote the serial numbers of the samples. β indicates the line of β-fetoprotein.

fraction was dialyzed, concentrated by lyophilization, dissolved in the minimal volume (usually 0.3 ml) of 0.05 M veronal buffer, pH 8.5, and tested for the presence of β-fetoprotein as described previously [4]. The antiserum against β-fetoprotein was similar to that used in our previous investigations [1, 3, 4].

To detect hemoglobin and hematinalbumin after electrophoresis, a saturated solution of benzidine at 37° in 20% acetic acid with the addition 2-4 drops H_2O_2 was used.

EXPERIMENTAL RESULTS

A typical outflow chromatographic curve is illustrated in Fig. 1. Zone 19S was eluted from the column in samples Nos. 7-9, and this was followed by a sharp rise with a maximum in sample No. 13, corresponding to the 7S peak. At the end of this zone, the zones of albumin and hemoglobin appeared. To detect the latter, and also to fix the point of elution of β-fetoprotein relative to some more definite chromatographic fraction for reference, in addition to the sample for chromatographic separation, hemoglobin was also applied to the column. Since the serum for fractionation was neonatal and was practically free from haptoglobin, capable of binding hemoglobin, a reaction of transhemation took place with the formation of hematinalbumin. Hence, besides the test fractions, two reference substances migrated along the column: hematinalbumin and an excess of unreacted hemoglobin. These are very convenient labels, first, because they are fairly specific, and second, because it is unnecessary to concentrate the dilute solutions, such as the samples eluted from the column, to detect them, and 0.02 ml of material is sufficient for electrophoresis in agar and for staining with the benzidine reagent.

The hemoglobin stains were most intense in samples Nos. 15 and 16. Hematinalbumin moved slightly toward the high-molecular-weight region, overlapping the hemoglobin zone (maximal density in samples Nos. 14 and 15).

During tests for the presence of β-fetoprotein using specific antiserum (Fig. 2), the β-fetoprotein fraction was most marked in sample No. 11, located at the very beginning of the 7S peak, and separated from the maximum of this peak by two "empty" (free from β-fetoprotein) samples. The 19S peak also was free from β-fetoprotein. Hence, during fractionation based on molecular weight, β-fetoprotein lay in an intermediate region (molecular weight higher than 160 000 but considerably lower than 900 000), in agreement with the results of our early investigations of the behavior of β-fetoprotein during electrophoresis on a molecular sieve by Smithies' method and in sedimentation experiments on an ultracentrifuge in a sucrose gradient. It may be concluded from the results of the experiments under discussion and the position of -fetoprotein in the β_2-globulin zone that β-fetoprotein belongs to the class of immunoglobulins. There is information that a whole series of new, hitherto unknown components has been found in the β_2-globulin zone [9]. An immunoglobulin IgE [10] has been found with the mobility of β_2-globulin and a sedimentation constant of about 8S, performing the function of reagents in allergic reactions. In addition, a distinctive immunoglobulin has been discovered in colustrum, also with an intermediate molecular weight (11S) and with the mobility of β_2-globulins [13].

More definite conclusions regarding the relationship between β-fetoprotein and these proteins mentioned above may be drawn after its more complete purification.

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