SERUM ALPHA-FETO-PROTEIN*

III. ELECTROPHORESIS OF SERA FROM CASES OF PRIMARY CANCER OF THE LIVER: AN ELECTROPHORETIC VARIANT

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Serum alpha-feto-protein has been found to be present in the sera of up to 78% of cases of primary cancer of the liver. The serum concentrations studied by quantitative immunodiffusion[1] ranged from 0·1 to 710 mg./100 ml.

Although the presence of alpha-feto-protein in human foetal serum was initially demonstrated by Bergstrand and Czar,[2] and there have been studies on the comparative aspects of electrophoresed foetal sera in a variety of species by Gitlin and Boesman,[3] there appear to have been no reports on the appearance of the electrophoretogram in cases of primary cancer of the liver.

In this study we report on the electrophoretic appearance of sera from cases of primary cancer of the liver using a variety of techniques.

METHODS AND MATERIALS

Antisera were prepared by the same method as in a previous report.[1] Sera from 150 cases of primary cancer of the liver were available for electrophoresis. All sera were stored frozen at −20°C when not in use. Every serum was electrophoresed, including those in which the alpha-feto-protein test was negative.

Cellulose acetate electrophoresis. A standard Beckman Microzone apparatus was used and the strips were scanned with an Analytrol scanner.

Starch-gel electrophoresis. A number of different methods were tried. Vertical starch gel according to the method of Smithies[4] did not give satisfactory runs, as the alpha-feto-protein band was diffuse and partly covered by the albumin.

Horizontal starch-gel electrophoresis, using a Shandon tank, proved to be the most successful with the following buffer system: gel buffer (pH 8·65) 0·076 M Tris and 0·005 M citric acid; and bridge buffer (pH 8·0) 0·3 M boric acid with sodium hydroxide to correct pH.

Various sizes of gels were used with appropriate current changes, e.g. a constant current of 20 mA for 3 - 4 hours was used on a gel 18 × 10 × 0·5 cm.

The gels were sliced and a middle slice was stained overnight with Amidoschwartz and eluted with a mixture of water : methanol : acetic acid in the ratio 5 : 5 : 1.

The method of Kunkel[5] was used for preparative starch-block electrophoresis, and the method of Scheidegger[6] was used for immuno-electrophoresis.

RESULTS

In high concentrations, alpha-feto-protein with the cellulose acetate electrophoresis technique can be seen as a separate band between albumin and alpha$_1$-globulin. The more usual result, if the separation is not perfect, is that the alpha$_1$-globulin band appears to be displaced slightly towards the anode when compared with normals (Figs. 1 and 2). This appearance has never been noted by our laboratory in any other condition.

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The alpha-feto-protein appears to run in a similar position in agar using the immuno-electrophoretic technique (Fig. 3) and in horizontal preparative starch blocks.

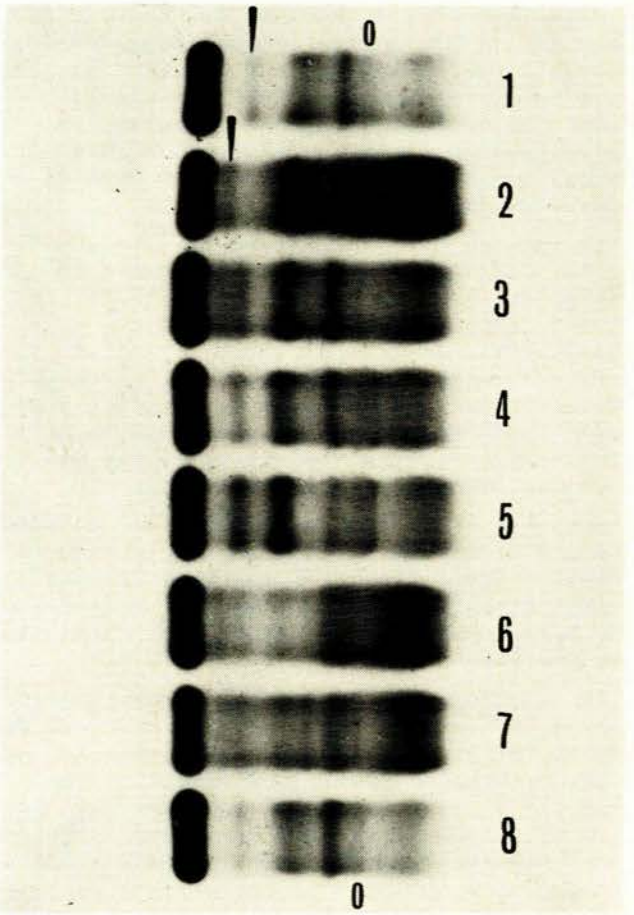

Fig. 1. Cellulose acetate electrophoresis of sera from cases of primary cancer of the liver. Samples 1 and 8 are the same normal case with a pointer in 1 indicating the position of alpha$_1$-globulin as it is always found in non-hepatoma cases. The origin is indicated by (o). Samples 2 - 7 are from cases of primary liver cancer and have alpha-feto-protein levels (by immuno-assay) of 174, 155, 40, 174, 175 and 125 mg./100 ml. The pointer in 2 indicates the alpha-feto-protein position.

Using the horizontal starch-gel technique, the alpha-feto-protein migrates as a single band in the postalbumin position superimposed on the faster migrating of the two thin bands (Gc-globulins) in the postalbumin position but having no immunological relationship to them. Occasionally, in cases of severe liver dysfunction or other pathology, one or both of these postalbumin bands becomes very intense.

Alpha-feto-protein partly purified by starch-block electrophoresis and incorporating all the fractions found to contain alpha-feto-protein by immunological testing, has

given only a single band with all the sera tested so far, with no suggestion of any heterogeneity of the protein.

A single case has shown a different electrophoretic mobility on horizontal starch-gel electrophoresis (Fig. 4). In this case the band has consistently shown a slightly slower mobility than the usual alpha-feto-protein band. This difference is present in all 4 samples available for testing, obtained over a 7-month period. As all the other samples had received similar handling in storage, we conclude that the difference is not a storage artefact but a true variant of the protein. There appears to be complete immunological identity of this protein with foetal and hepatoma alpha-feto-protein by immunodiffusion techniques, although lack of material has prevented our testing further for immunological equivalence.

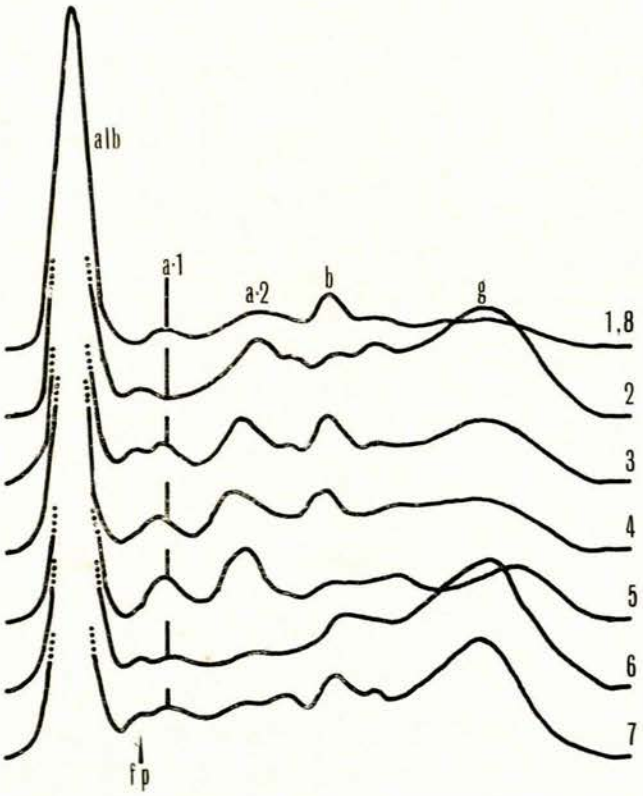

Fig. 2. Optical density scans of the cellulose strip shown in Fig. 1. The albumin peaks were all aligned on the diagram. The amplitude of the albumin peak was adjusted to approximately the same height when the Analytrol scanner was used, thus the peak area does not relate directly to the alpha-feto-protein content. The alpha$_1$- peak shift, which is present, for example, in 4 and 5, is more striking when a single hepatoma serum is run with 7 normals on the same strip. Also note the clean area between albumin and alpha$_1$- which is present in all normals and the blurring in the same position in samples 4 and 5 (see also Fig. 1).

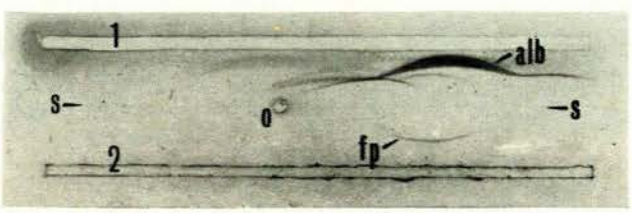

Fig. 3. Immuno-electrophoresis of serum containing alpha-feto-protein. Using the method of Scheidegger[6] serum was electrophoresed from the well (o), and after the run the agar was slit along (s-o-s) and the two halves were separated to prevent the contents of troughs 1 and 2 reacting with each other (this has not been totally avoided). Trough 1 was filled with antiserum against foetal blood and trough 2 contained the same antiserum absorbed with pooled normal human adult serum. The alpha-feto-protein precipitation line (fp) can be clearly seen on both sides and in relation to the albumin (alb). There are no other precipitation lines on the side of trough 2.

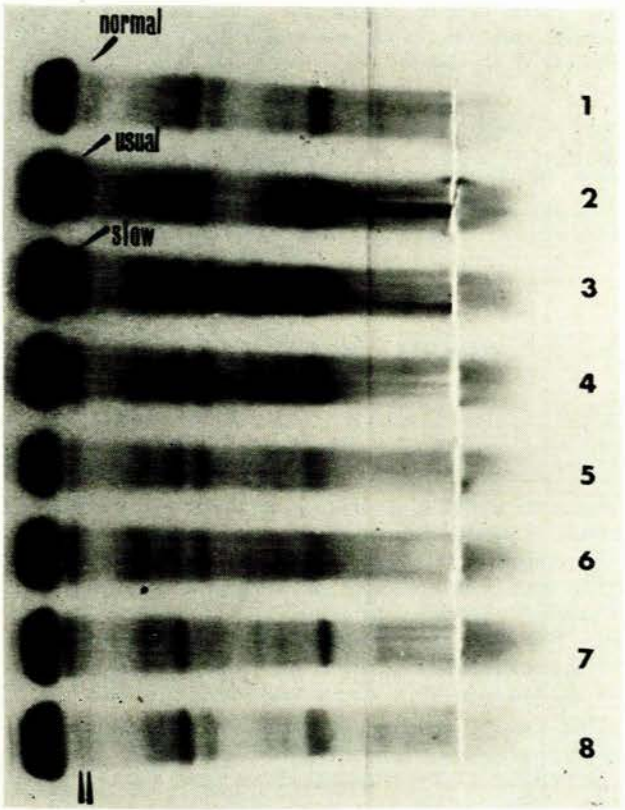

Fig. 4. Slow electrophoretic variant of alpha-feto-protein demonstrated by horizontal starch-gel electrophoresis. Samples 1 and 8 are both normal and the faint single or double postalbumin bands are marked with arrows. Samples 2 and 7 are from cases of primary cancer of the liver having alpha-feto-protein levels of 290 and 131 mg./100 ml. respectively and show the 'usual' alpha-feto-protein band. Samples 3, 4, 5 and 6 are from the same case over a 7-month period and the alpha-feto-protein values are 280, 128, 62 and 128 mg./100 ml. respectively.

The 'slow' mobility of the alpha-feto-protein is consistent. Mixtures of 'usual' and 'slow' alpha-feto-protein containing sera yield two distinct bands. An appearance which has been noted several times and is present here is that although samples 2 and 3 have almost identical alpha-feto-protein levels, the intensity of the band in sample 3 appears greater. This might reflect a lesser degree of immunological reactivity of this 'slow' variant.

All sera positive to the alpha-feto-protein test were examined visually to compare the density of the alpha-feto-protein band with the immuno-assay result. When the value was over about 30 mg./100 ml., the band could always be seen and there were no obvious discrepancies between the immuno-assay result and the density of the band. Comparable immuno-assay results in different cases also always produced comparable band densities.

DISCUSSION

Simple cellulose acetate electrophoresis and starch-gel electrophoresis have proved to be useful tools for confirming immunological alpha-feto-protein assay results. The shift towards the anode of the alpha$_1$-globulin band in cellulose acetate electrophoresis has the same specificity

as the alpha-feto-protein assay result itself, although very much less sensitive. No false positive electrophoretic result has been observed by us in our routine laboratory investigations.

The presence of an electrophoretic variant adds interest to the study of this protein and should stimulate an even more careful search for other forms of heterogeneity and for other proteins produced by primary cancers of the liver.

SUMMARY

Alpha-feto-protein is detectable on electrophoresis on cellulose acetate or in starch gel in the serum of cases with primary liver cancer. An electrophoretic variant has been found.

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