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 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, and there have been studies on the comparative aspects of electrophoresed foetal sera in a variety of species by Gitlin and Boesman, 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. 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 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 was used for preparative starch-block electrophoresis, and the method of Scheidegger 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-globulin. The more usual result, if the separation is not perfect, is that the alpha-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.

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.

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 (Ge-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 lower 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.

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-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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REFERENCES


PLASMA RENIN CONCENTRATIONS IN CASES WITH RENOVASCULAR HYPERTENSION*

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There has been a great deal of controversy over the role played by renin-angiotensin in causing and maintaining hypertension in patients with renovascular abnormalities. It has been suggested that narrowing of the renal artery stimulates the release of renin from the juxtaglomerular cells. The renin acts upon plasma substrate to produce the pressor peptide angiotensin, which then raises the arterial pressure by a direct vasoconstrictor effect.1-3

This theory has been criticized by various workers, mainly because concentrations of renin and angiotensin in experimental hypertension have been inadequate to cause significant pressor response.4-6 It is currently felt that the renin-angiotensin system regulates aldosterone secretion, and that the aldosterone plays an important role in maintaining the hypertension.7,8

The three cases of renovascular hypertension to be discussed in this paper proved very good experimental models for the study of renin excretion. Renin estimations were done before and during surgery on all cases.

CASE REPORTS

Case 1

Mr A. M., a 52-year-old White masseur and part-time karate instructor, incurred an injury to the left side of his abdomen while taking part in a karate exhibition during 1968. Four months after this injury he was seen in the outpatient department because of severe headache and loss of vision. He was found to be hypertensive and was placed on antihypertensive therapy (guanethidine, methyldopa and hydrochlorothiazide) for a period of 2 weeks. At reassessment he was still severely hypertensive, and it was decided to admit him for a full investigation.

In the ward he was agitated and was prone to attacks of violence. His blood pressure was 220/130 mm.Hg in both arms and 240/140 mm.Hg in the legs. No abdominal bruits were heard. His fundi showed grade IV KW changes. There were no other abnormalities found in any other system.

Laboratory and radiological tests. The full blood count was within normal limits. Urinalysis showed a SG of 1·010 with a 4+ proteinuria and 4-5 red blood cells per high-power field. Further pertinent laboratory data are summarized in Table I. An intravenous pyelogram showed normal excretion from the right kidney but non-visualization of the left (Fig. 1).

Fig. 1. See text.

A retrograde pyelogram showed a normal-sized right kidney, but a small left one. A right femoral aortogram showed normal vasculature on the right, but absent vasculature on the left.

A hippuran renogram done on this patient showed a flat curve over the left kidney, with a normal-appearing pattern over the right kidney. Systemic venous blood had

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