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5'-Nucleotide Phosphodiesterase Isoenzyme Pattern in the Serum of Human Hepatoma[1]

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SUMMARY

The isoenzyme patterns for 5'-nucleotide phosphodiesterase in human sera have been studied by the use of a 5'-(5-iodoindoxyl) nucleoside phosphodiester as a histochemical substrate after electrophoresis on polyacrylamide gel support. Normal sera show two major, fast-moving bands and two weak, slow-moving bands. All six sera from patients having histologically identified primary hepatoma show an additional, fast-moving isoenzyme band compared to normal serum, and a benign liver adenoma serum shows no such additional isoenzyme band. Furthermore, this last, fast-moving isoenzyme is not seen in neonatal serum.

Since these six cases of hepatoma sera are all negative with anti-α-fetoprotein serum by the double immunodiffusion test, the presence of this isoenzyme in these sera could be an alternate diagnostic aid.

INTRODUCTION

Although the incidence of human hepatomas is low in the United States, the seriousness of this disease requires a sensitive test as a diagnostic aid. To date, the most useful test has been that for α-fetoprotein (1, 2). This protein is of hepatic origin and is present in fetal sera but reappears only in 40 to 50% of adult sera in Western countries when primary hepatoma is present (2, 3, 5). This large number of false negative cases has been attributed to the difference in carcinogen that induced such hepatoma (2, 12) as well as the low antigen concentration (7).

In our laboratories, a method originally developed for histochemical demonstration of 5'-nucleotide phosphodiesterase (14) has been adapted to a serum isoenzyme study. Because this enzyme is elevated in the regenerating liver of the rat (15) and is high in transplanted hepatoma of the rat (17), and in some human cancer sera (6), investigation of isoenzyme patterns of 5'-nucleotide phosphodiesterase has lately been of particular interest in human hepatomas. The present study reports the serum isoenzyme pattern of this enzyme from a preliminary study of 6 human primary hepatomas, one benign liver adenoma, a neonatal (cord) serum, and their comparison with normal and other disease sera.

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MATERIALS AND METHODS

Fresh serum was prepared from blood in the usual manner. Serum samples could be stored frozen in 2-ml vials for up to 12 months without causing any change in enzyme patterns. The hepatoma serum samples were obtained over the course of 18 months at the Hospital of the University of Pennsylvania. All hepatomas were diagnosed by biopsy specimen prior to the test, and 2 cases have been subsequently confirmed by autopsy. This limited number is unavoidable because few diagnosed cases are found in the United States.

Substrates: 5'-(5-Iodoindoxyl)-Phosphodiester of Nucleosides. The syntheses of the 5'-(5-iodoindoxyl)-phosphodiester of nucleosides substrates have been reported elsewhere (13, 16). The most useful ones are the thymidine and the 5-fluorodeoxyuridine derivatives. The substrate solutions were 0.2 mg of ammonium 5'-(5-iodoindol-3-yl) thymidylate per ml for phosphodiesterase and 0.1 mg of 5-nitroindoxyl phosphate (10) per ml for the alkaline phosphatase (18) in 0.05 M Tris-HCl buffer (pH 8.1).

Electrophoresis. A 7% polyacrylamide gel in running buffer was prepared in gel tubes in the usual manner (11). The running buffer was 0.09 M Tris-boric acid, pH 9.4, which was used in both electrophoresis chambers.

Each gel was loaded with 20 μl of serum, thoroughly mixed with 20 μl of saturated sucrose. One sample of each run was dyed with bromphenol blue and the electrophoresis was stopped when most of the dyed albumin had left the gel. The electrophoresis was run at a constant current of 2.0 ma/tube at 3-4° (7).

The gels were removed from the tubes and incubated overnight at 37° in the substrate solutions for 5'-nucleotide phosphodiesterase or alkaline phosphatase. After incubation, the gels were rinsed and then preserved in 8% acetic acid; the indigo color is stable.

Immunodiffusion (Micro-Ouchterlony) for α-Fetoprotein (8, 9). Double immunodiffusion was done in 1% agarose in Veronal buffer, pH 8.2, 0.04 M, using a standard 5-well pattern with antiserum to α-fetoprotein (Princeton Laboratories, Princeton, N. J.) in the center well. The sera for testing, including cord serum, a positive control and normal serum, were placed in the outer wells. Cord serum and the positive control gave precipitation lines within 5 to 6 hr, and all other sera were negative after 48 hr. Both concentration by freeze-drying and serial dilutions of the hepatoma sera also gave negative results. Since negative results

Table 1
Summary of 5'-nucleotide phosphodiesterase in human sera

Diagnosis	No. of sample	Band I	II	III	IV	V	Remarks
Primary hepatoma	6	N-VW[a]	N-W	M	W	M	1 case shows extra bands near origin
Cancer with liver involvement	5	N-VW	W	S	VW	W	1 case breast cancer with liver involvement; no autopsy granted. 3 cases of cancer of the colon and 1 pancreatic cancer with liver involvement.
Cancer other than hepatoma	26	N-VW	N-W	M	M	N	Stomach, rectum, lung, breast (unmetastasized)
Benign liver adenoma	1	VW	W	S	M	N	
Jaundice or liver diseases	6	N-VW	N-W	S	M	N	
Other diseases	11	N-VW	N-VW	M	M	N	Lupus, diabetes, sickle cell anemia, emphysema
Neonatal (cord)	1	M	VW	M	VS	N	
Normal	12	VVW	W-VW	M	M	N	Including 2 pregnancies: 1 2nd trimester and 1 3rd trimester

[a] VW, very weak; W, weak; M, medium; S, strong; VS, very strong; and N, absence of band.

were obtained with 3 different preparations of antiserum to human α-fetoprotein from 2 different suppliers for the hepatoma samples and other known positive samples did give positive results, the hepatoma sera were shown to contain either no or too little (<2 μg/ml) (3) α-fetoprotein to be detected by this method.

RESULTS AND DISCUSSION

Serum Isoenzyme of 5'-Nucleotide Phosphodiesterase. The normal serum pattern (Fig. 1*A*) of this enzyme shows 2 major bands (III, IV) in the α-globulin region and, in some persons, 2 weak bands (I, II) close to the β-globulin region. The intensity of the isoenzyme bands varies from person to person. It is likely that Band IV may be associated with liver as in practically all jaundice and nonmalignant liver diseases, Band III is stronger and Band IV is weaker than normal.

The serum of each of 6 patients with a positive biopsy diagnosis of primary hepatoma give a negative α-fetoprotein test, yet it showed a definite, additional, fast-moving isoenzyme band (V, Fig. 1*D*). This band is not present in 6 cases of other liver diseases, and is occasionally present in sera of patients with other forms of cancer (5 out of 31 cases, Table 1), but only when there is liver involvement. This result is especially significant considering that the serum of a patient with benign liver adenoma, surgically confirmed, has been found to be free of this isoenzyme prior to operation (Fig. 1*C*).

Neonatal serum was obtained from cord blood at birth and was compared in the same study. The isoenzyme associated with Band I is more prominent than the same isoenzyme found in adult serum from an age group of 20 to 50 (Fig. 1*B*).

Serum Isoenzyme of Alkaline Phosphatase. Normal serum shows a broad band of alkaline phosphatase occurring in the region between phosphodiesterase isoenzyme Bands III and IV, and including the region of Band III, (Fig. 1*E*). In some serum samples this band may be resolved into 2 components. Hepatoma serum shows the same alkaline phosphatase band but of greater intensity than in normal serum, and, in addition, some samples show a weaker, slower-moving band in the region of phosphodiesterase isoenzyme Band II, (Fig. 1*F*).

The hepatoma phosphodiesterase isoenzyme, Band V, and also phosphodiesterase isoenzyme, Band IV, are definitely not associated with alkaline phosphatase isoenzyme. Further work is in progress to demonstrate the independence of alkaline phosphatase and phosphodiesterase isoenzymes in the Band III region.

This investigation describes the use of 5'-nucleotide phosphodiesterase isoenzyme patterns in serum as a possible diagnostic aid for human hepatoma. An isoenzyme typical of human hepatoma has been found. Thus far, this isoenzyme is exclusive of other liver diseases. As cirrhosis is sometimes associated with primary hepatoma, a larger sampling will be necessary. The fact that a benign liver adenoma gives a negative test is significant in that such decisions are difficult to arrive at without a biopsy specimen.

We believe that further study of this new serum isoenzyme associated with human hepatomas will yield valuable information. At present, the diagnostic value of this new method for hepatomas is indicated, especially in α-fetoprotein-negative cases.

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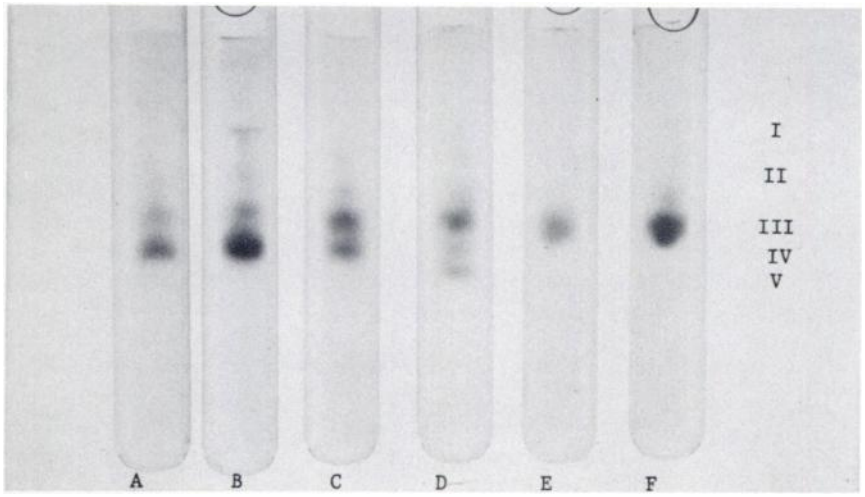

Fig. 1. Gel electrophoresis of human sera incubated with an indigogenic phosphodiester (*A* to *D*) and phosphomonoester (*E* and *F*), showing one picture of 6 different gels: *A*, normal; *B*, neonatal (cord); *C*, benign liver adenoma; *D*, primary hepatoma; *E*, normal; and *F*, primary hepatoma.