Demonstration of Two Molecular Variants of Carcinoembryonic Antigen by Concanavalin A Sepharose Affinity Chromatography

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SUMMARY

The carcinoembryonic antigen (CEA) active glycoproteins from perchloric acid extract of liver-metastasized primary colon tumor have been separated by concanavalin A Sepharose (Con A Sepharose) chromatography. The CEA activities separated by Con A Sepharose chromatography were designated as loosely bound and tightly bound which, respectively, eluted on the Con A Sepharose column between 0.12 and 0.15 M and 0.3 M α-methyImannose in a linear gradient of α-methyImannose. Further purification of these activities by Sephadex G-200, Bio-Gels A-1.5m and P-300 yielded two variants of glycoproteins (B1 and C2) with CEA activity. Both purified preparations of CEA had similar immunochemical properties. Their A_{280}/A_{208} ratios were 1.30 and 1.56, respectively. The purified loosely bound CEA (B1) had immunological, chromatographic, and electrophoretic properties similar to those of α2M-CEA, whereas the tightly bound CEA (C2) had a lower molecular weight (120,000 to 140,000). Further, specificity of these two CEA’s was established by their reactions in immunoelectrophoresis with preparations of specific goat anti-CEA antiserum obtained from other investigators. The results indicate the practical use of Con A Sepharose affinity chromatography for the separation and characterization of glycoprotein tumor antigens.

INTRODUCTION

Studies previously reported from this laboratory revealed the potential use of Con A in the characterization of CEA as well as its utilization for the separation of CEA from perchloric acid extracts of liver metastasis from primary colon tumors (1). It has also been shown that Con A agglutinates embryonic and neoplastic cells with a preferential binding to epithelial cells of intestinal origin (8, 17). Although CEA was originally thought to be specific for tumors and fetal tissues of entodermally derived digestive systems (5), several authors have reported the existence of CEA-like antigens in normal tissues of nonentodermal origin and in the plasma of patients with cancer other than that of digestive tract (4, 9, 16). These CEA-like antigens appear to share a common determinant with CEA (15). These findings raise the possibility that the positive plasma values of CEA in nondigestive cancers (2, 10) may be due to cross-reactivity of CEA-like antigens. Since Con A binds to a variety of glycoproteins (13) including CEA (1), we feel that the affinity chromatography of crude extracts of liver-metastasized colon tumors on Con A Sepharose may be a better method for separating CEA from other CEA-like macromolecules. Further antigens thus separated may provide serological reagents with a better specificity in the radioimmunoassay. We report here some of our findings on one of the liver-metastasized colon tumors studied by Con A Sepharose affinity chromatography.

MATERIALS AND METHODS

Extraction of Tissue with Perchloric Acid. Frozen liver metastases of primary colon tumor were allowed to thaw at 4°C and then minced. All steps in the extraction procedure were carried out at 4°C. The tissue mince was suspended in 3 volumes of distilled water and homogenized in a Sorvall Omni-Mixer (Ivan Sorvall, Inc., Norwalk, Conn.). The homogenate was treated with an equal volume of 2 M perchloric acid, and the extraction was continued for 1 hr under stirring. The extract was centrifuged at 4000 × g (5000 rpm, GSA rotor; Sorvall Inc.), and the supernatant was dialyzed against cold tap water for 18 hr and then redialyzed against 3 changes of deionized water. The dialyzed material was clarified by filtration through glass wool and centrifugation. The clarified extract was concentrated 10-fold by using either UM-10 or PM-30 Diaflo membrane (Amicon Corp., Lexington, Mass.). The concentrated material was recentrifuged at high speed (31,000 × g) to remove aggregated material and then filtered through a Millipore filter (0.25 or 0.45 μm). The clear filtrate was lyophilized and stored at −20°C until needed for experiments.

Preparation of Antisera. Rabbit anti-CEA serum was
prepared by injection subcutaneously and in the hind footpad of a 1:1 emulsified mixture of sterile tumor perchloric acid extract (10 mg/ml) in 0.05 M phosphate buffer, pH 7.2, and Freund’s complete adjuvant (Miles Laboratories, Elkhart, Ind.). Each rabbit received a total of 2.0 ml of the emulsified preparation (10 mg of crude antigen). Three weeks after the primary immunization, a booster shot was given as described above. The rabbits were bled once a week after 2 weeks of the booster dose, and the antibody titers were checked by studying the binding capacity of 125I-CEA (Hoffmann-La Roche, Inc., Nutley, N. J.). Antisera showing a binding capacity of greater than 45 to 50% were used for immunochemical studies (3). Absorbed anti-CEA for routine use was prepared by absorption with 1.5 mg of normal liver perchloric acid extract per ml. Absorption procedure with other normal tissue extracts is described under “Immunological Methods.”

Affinity Chromatography of Perchloric Acid Extract on Con A Sepharose. Optimal conditions for the binding of CEA to Con A Sepharose and its elution from it were established by using 125I-CEA. Con A Sepharose (8 to 10 mg Con A per ml gel suspension; Pharmacia Fine Chemicals Co., Piscataway, N. J.) was packed into a 0.9- × 60-cm column which was then equilibrated in 0.1 M acetate buffer, pH 5.0, containing 0.1 M NaCl and 1 mM Ca2+, Mg2+, and Mn2+ chlorides (starting buffer). Lyophilized perchloric acid extract of the colon-metastasized liver tumor in starting buffer (20 mg/ml) was applied to the column. The column was washed with 8 ml starting buffer and then incubated for 2 hr at 25°. After incubation, the column was eluted with a linear gradient of 0 to 0.5 M α-MEM. The eluting buffer contained 0.5 M α-MEM in starting buffer and 0.2 M NaCl instead of 0.1 M NaCl. The volume of each buffer in the gradient was 70 ml. The flow rate was 12 to 14 ml/hr and 4-ml fractions were collected.

Determination of α-MEM Concentration. The concentration of α-MEM in column fractions after Con A Sepharose chromatography was determined by measuring their osmolality (osmole) on an osmometer (Osmette; Precision Instruments, Inc., Palo Alto, Calif.). The osmole was then converted to molar concentration of α-MEM with the help of a standard curve constructed by using known concentrations of α-MEM in appropriate buffers.

Sephadex Gel Chromatography. Appropriate pooled peak fractions from Con A Sepharose chromatography of tumour extracts were dialyzed against water, lyophilized, redissolved in PBS, and chromatographed on a calibrated Sephadex G-200 column (1.5 × 47 cm) equilibrated with PBS. Then 2.0-ml fractions were collected at a flow rate of 12 ml/hr and a pressure head of 16 cm. The absorbances of the fractions at 280 nm were measured and then assayed for CEA activity by immunodiffusion, immunoelectrophoresis, and a radioimmunoassay. The active fractions were pooled, dialyzed, and lyophilized. The lyophilized materials were redissolved in appropriate buffers and used for studies on purity and antigen specificity.

Radioimmunoassay of CEA. CEA activity of column fractions was determined by Hansen’s Z-gel assay with a slight modification described by Chu et al. (3). The column fractions were assayed before and after dialysis in varying quantities (10, 20, or 50 μl) depending upon the column fractions being studied. Fractions around 280 nm peaks were generally chosen for CEA assay. Binding capacity of CEA was determined by calculating the percentage of inhibition of binding of 125I-CEA in presence of unlabeled CEA in the radioimmunoassay (3).

Immunological Methods. Ouchterlony double diffusion study was carried out in Petri dishes containing 1% agarose (BioWare, Wichita, Kans.) in borate-buffered saline, pH 8.2. The immunoelectrophoretic method of Scheidegger (12) was used in conjunction with radioimmunoassay for CEA to monitor CEA or CEA-like substances obtained in various steps of purification of CEA by above-mentioned chromatographic techniques. In order to determine antigenic specificity of the purified CEA’s and the specificity of rabbit anti-CEA, absorption studies were carried out by incubating rabbit anti-CEA with whole human serum and perchloric acid extracts of normal liver, lung, colon, and kidney at 4° for 18 hr. On the basis of trial absorptions with normal liver perchloric acid extracts added in increasing amounts and monitored by immunoelectrophoresis for nonspecific antibodies, the rabbit anti-CEA was finally absorbed with lyophilized whole normal serum (3.0 mg/ml), perchloric acid extracts of normal colonic mucosa (2.0 mg/ml), normal lung (2.0 mg/ml), normal liver (2.5 mg/ml), and normal kidney (1.5 mg/ml). This absorbed antiserum was used in tests to determine the specificity of the antiserum as well as the purified CEA.

Polyacrylamide Gel Electrophoresis. Crude and purified preparations were submitted to electrophoresis on 4% polyacrylamide gel in 6 μ urea at pH 8.5 in Tris-glycine buffer, for 90 min at 110 or 300 V. At the end of the run the gels were stained in Amido black or periodic acid-Schiff reagent, destained electrophoretically, and observed for the stained bands.

125I-Radiolabeling of Purified CEA and Chromatography of 125I-CEA with 125I-CEA on Sephadex G-200. Iodination of purified loosely bound CEA obtained by Con A Sepharose chromatography with 125I was carried out essentially according to the method of C. W. Todd (personal communication). 125I-Labeled CEA was separated from free 125I with the use of Bio-Rad ion-exchange resin AG1 X8 (Bio-Rad Laboratories, Richmond, Calif.) equilibrated with 0.05 M PBS, pH 7.2. The 125I-CEA that eluted was further dialyzed against PBS, pH 5.5, and then checked for immunoelectrophoresis properties using unabsorbed rabbit anti-CEA serum.

RESULTS

Chromatography of Perchloric Acid Extracts on Con A Sepharose. Chart 1 shows a typical elution profile of a tumor extract, which reveals at least 3 protein (A280-absorbing material) peaks (I, II, and III) from the start of α-MEM gradient to its end. Peak I did not bind to Con A Sepharose since it eluted in the breakthrough volume, nor did it react with rabbit anti-CEA on immunoelectrophoresis...
Molecular Variants of CEA Separated by Con A Sepharose

Chromatography of perchloric acid extract of liver metastasis of colon tumor on Con A Sepharose. Column size, 0.9 × 60 cm. Gradient buffers: Buffer A, 0.1 M acetate buffer, pH 5.0, containing 0.1 M NaCl as starting buffer; Buffer B, 0.1 M acetate buffer, pH 5.0, containing 0.2 M NaCl and 0.5 M α-MEM as eluting buffer. Both gradient buffers contained 1 mM each Ca²⁺, Mn²⁺ and Mg²⁺ chlorides. CEA was determined by radioimmunoassay as described in the text.

or immunodiffusion. Peak II eluted approximately around 0.06 M α-MEM and showed some CEA activity as determined by radioimmunoassay (Peak A), although the peaks of CEA activity and protein absorbance did not overlap. This activity did not react with rabbit anti-CEA on immunodiffusion, but on immunoelectrophoresis a diffuse, faint precipitin arc was seen (Fig. 1). Peak III eluted approximately around 0.12 M α-MEM and revealed a CEA activity peak (Peak B) around 0.15 M α-MEM. This peak gave a single precipitin line on immunoelectrophoresis (Fig. 1) when tested against absorbed anti-CEA. However, with unabsorbed anti-CEA it gave at least 3 precipitin lines on immunoelectrophoresis including the CEA precipitin arc in the β-region (Fig. 1). We refer to this CEA activity peak (Peak B) as the loosely bound CEA.

Sephadex G-200 Gel Filtration of Loosely Bound CEA. Chromatography of the loosely bound CEA fraction (Chart 1, Peak B) on Sephadex G-200 demonstrated the presence of 3 A₂₅₀ peaks (Chart 2). Peaks B₁, B₂, and B₃ showed CEA activity upon radioimmunoassay (Chart 2). However, after immunodiffusion and immunoelectrophoresis, Peak B₁ gave a single precipitin line with absorbed and unabsorbed anti-CEA, whereas Peak B₂ reacted only with unabsorbed anti-CEA showing precipitin lines in α₂-α₁ region on immunoelectrophoresis (Fig. 2). The Peak B₃ CEA activity obtained after Sephadex G-200 chromatography, iodination with ¹²⁵I, and rechromatography on Sephadex G-200 with ¹²⁵I-CEA revealed identical migration patterns of both the ¹²⁵I- and ¹²⁵I-labeled CEA’s with a minor component of breakdown products (Chart 3). Polyacrylamide gel electrophoresis of the loosely bound CEA (Chart 2, Peak B₁) revealed a single, very-slow-moving protein with a penetration of less than 4 mm which was identical to that of ¹²⁵I-CEA.

Sephadex G-200 Gel Filtration of Tightly Bound CEA. The tightly bound CEA activity was immunochemically pure since it showed 1 precipitin line in the β region with absorbed and unabsorbed anti-CEA on immunoelectrophoresis (Fig. 1). However, after gel filtration through Sephadex G-200, it was resolved into 2 major protein peaks and 3 CEA activity peaks (Chart 4, Peaks C₁, C₂, and C₃). The C₁ CEA activity had very weak immunochemical reactivity, whereas C₂ CEA activity showed a vigorous reaction with anti-CEA in immunoelectrophoresis. This peak had an approximate molecular weight of 120,000 to 140,000 daltons. The 3rd peak of CEA activity (C₃) did not react with either absorbed or unabsorbed anti-CEA. The tightly bound C₂ activity was further chromatographed on a column (0.9 × 30 cm) of Bio-Gel A-1.5m (Bio-Rad) to separate the possible contaminating C₁ activity using identical buffer conditions as described in Sephadex G-200 gel filtration. The active peak was finally chromatographed on a column (0.9 × 15 cm) of Bio-Gel P-300 and found a single homogeneous peak (Chart 5). The A₂₅₀/A₂₅₀ ratio was used as one of the criteria for the characterization of B₁ and C₂.

Chart 1. Chromatography of perchloric acid extract of liver metastasis of colon tumor on Con A Sepharose. Column size, 0.9 × 60 cm. Gradient buffers: Buffer A, 0.1 M acetate buffer, pH 5.0, containing 0.1 M NaCl as starting buffer; Buffer B, 0.1 M acetate buffer, pH 5.0, containing 0.2 M NaCl and 0.5 M α-MEM as eluting buffer. Both gradient buffers contained 1 mM each Ca²⁺, Mn²⁺ and Mg²⁺ chlorides. CEA was determined by radioimmunoassay as described in the text.

Chart 2. Sephadex G-200 column chromatography of the loosely bound CEA activity (Chart 1, Peak B, Fractions 14 to 22) obtained by Con A Sepharose chromatography. Column size, 15 × 47 cm. Elution buffer: 0.05 M PBS. LDH, lactic dehydrogenase.
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Chart 3. Cochromatography of $^{131}$I-iodinated, loosely bound CEA (Chart 2, Peak B,) with $^{131}$I-CEA in 1 mg bovine serum albumin per ml prepared in PBS. $^{131}$I-CEA in PBS was mixed with $^{131}$I-CEA (3/1) and then chromatographed on a Sephadex G-200 column (0.7 x 20 cm; bed volume, 8.0 ml). PBS was used for elution. A, immunoelectrophoretic pattern of $^{131}$I-CEA after staining; B, radioautograph of A.

CEA activity. The $A_{280}/A_{260}$ ratios of the $B_1$ and $C_2$ after final purification were 1.30 and 1.56, respectively.

Immunological Specificity of Rabbit Anti-CEA and the Purified CEA's. Immunoelectrophoretic results show that the reaction of our rabbit anti-CEA with a purified preparation of CEA (ca. G-200, 17-P2) from Dr. C. W. Todd and Dr. M. L. Egan was identical to that of goat anti-CEA obtained from 2 different sources [Dr. H. J. Hansen; Hoffmann-La Roche, Inc., Goat 176; and Dr. Todd and Dr. Egan, City of Hope Medical Center, Goat ACE 67-70 (Fig. 3)]. Both of the goat anti-CEA as well as our rabbit anti-CEA reacted identically with CEA preparation from Dr. Todd's group showing a precipitin arc in the $\beta$ region (Fig. 3). Fig. 4 shows that the precipitin arc obtained with rabbit anti-CEA and the purified $B_1$ and $C_2$ CEA's reported in this study was similar to that obtained with goat anti-CEA from Hoffmann-La Roche. Both $B_1$ and $C_2$ showed a single precipitin arc with unabsorbed rabbit anti-CEA. They still retained their immunological reactivity even after extensive absorption of rabbit anti-CEA with whole normal serum and perchloric acid extracts of normal liver, colonic mucosa, lung, and kidney (Fig. 5). The straight precipitin lines between the troughs are indicative of the reaction of excess normal components present in absorbed antiserum with the unabsorbed antiserum. This further
sugests that the antiserum has been sufficiently absorbed.

Immunodiffusion experiments showed that the rabbit anti-CEA had a complete line of identity with both goat anti-CEA's (Ace 67-70 and Goat 167) when tested against CEA obtained from Dr. Todd and Dr. Egan or against B1 CEA.

**Binding Inhibition Studies.** Comparative inhibition curves demonstrating the ability of the B1 and C2 CEA activities to inhibit the binding of 125I-CEA by the goat anti-CEA antiserum are shown in Chart 6, which apparently reveals parallel inhibition curves for both activities with different slopes as compared to that of Hoffmann-La Roche CEA. The loosely bound CEA appeared to be less inhibitory than the tightly bound CEA.

**DISCUSSION**

The interaction of Con A with CEA (1) prompted us to investigate the selectivity of the binding reaction of CEA and perhaps its molecular variants as well as CEA-like glycoproteins. Indeed our data demonstrate that perchloric acid extracts of one of the colon tumor metastasized to the liver reported here contain at least 2 types of molecular variants of CEA (2 glycoproteins with CEA activity) that preferentially bind to Con A Sepharose as revealed by radioimmunoassay and immunochemical techniques. Since Con A has different properties under different conditions of pH, ionic strength, and requirement of divalent cations (7, 13), we chose such conditions for affinity chromatography of CEA on Con A Sepharose that would permit maximum stability of Con A and its binding capacity to CEA. We also investigated the sugars β-mannose and α-methyl-d-glucopyranoside for eluting CEA (based on batchwise technique) but found that α-MEM led to greater recovery of 125I-CEA. Therefore, α-MEM was routinely chosen in our system for separating molecular variants of CEA. Our studies with 125I-CEA also indicated an optimum ionic strength of 0.1 to 0.12 at a pH of 5.0 was needed for binding of CEA. Elution of CEA was best carried out by slightly increasing the ionic strength to 0.2 to 0.25 in the presence of α-MEM. Absence of α-MEM in the eluting buffer resulted in lesser recovery of the 2nd CEA activity which was tightly bound to Con A Sepharose. On the other hand, elution without increasing ionic strength of elution buffer in the gradient resulted in a poor separation of the A280-absorbing peaks. Recently, we discovered that the separation of protein (A280) Peak II from Peak III (Chart 1) could be widened by simply increasing the gradient volume of the starting and eluting buffer. We also checked the possibility of any residual CEA very tightly bound to Con A Sepharose after chromatography by washing the column with 0.6 to 0.8 M α-MEM in 1 M KCl in starting buffer. The wash, after dialysis and concentration by lyophilization, reacted with neither anti-CEA, nor Con A, nor anti-Con A. This suggested that all the CEA was removed from the Con A Sepharose column and that there was no leaching of Con A from the Con A Sepharose column by the buffer conditions used for elution of CEA. We also found it necessary to incubate the crude perchloric acid extracts at 25° for at least 2 hr, since elution without incubation resulted in poor binding of CEA to Con A Sepharose thus affecting resolution. The loosely bound CEA activity with β-electrophoretic mobility was the major activity (Chart 1) recovered from Con A Sepharose which was immunochemically and electrophoretically heterogeneous. Therefore, this peak was further chromatographed on Sephadex G-200, which separated the CEA activity from CEA-like substances (Chart 2). The 2nd peak of Sephadex G-200 gel filtration (Chart 2 B2) with a molecular weight of approximately 60,000 to 70,000 daltons mimicked CEA activity on radioimmunoassay, although immunoelectrophoresis with unabsorbed antiserum revealed precipitin lines in α2-γ1 region whereas with absorbed anti-CEA no precipitin lines were obtained. Whether this substance is the normal glycoprotein reported by von Kleist et al. (16) is yet to be determined.

Specificity of the loosely bound CEA (Chart 2, B1) was determined by comparing immunochromatographic, chromatographic, and polyacrylamide gel electrophoresis behavior with 125I-CEA and goat anti-CEA (Hoffmann-La Roche). These studies revealed that the loosely bound CEA migrated along with 125I-CEA on Sephadex G-200 (Chart 3) as well as on polyacrylamide gel and had identical immunological property on immunodiffusion and immunoelectrophoresis. The tightly bound CEA (Chart 1, Peak C) appeared to be immunochemically homogeneous and revealed immuno-
groups unique to each of these molecules in addition to CEA. We have immunized rabbits with the 2 variants on these molecules. We also feel that our loosely common determinant detected by rabbit and goat antisera prepared separately against each of these components reacted differently, suggesting additional determinant activity (C3) and the 2nd protein peak (Chart 4) did not react with either absorbed or unabsorbed anti-CEA serum on immunodiffusion, suggesting this activity to be nonspecific. Whether these observations are due to different sensitivities of the radioimmunoassay, immunolectrophoresis, and immunodiffusion still remains to be determined. The C2 activity after gel filtration on Bio-Gel A-1.5m to remove any overlapping C1 activity (Chart 4) revealed a homogeneous peak on Bio-Gel P-300 gel filtration indicating C2 activity to be chromatographically pure.

The tightly bound CEA is a minor component in the perchorlic acid extract of liver metastasis from primary colon tumor. Comparative inhibition of binding studies in the radioimmunoassay revealed that on a Lowry protein basis the tightly bound CEA had better binding capacity than that of loosely bound CEA, suggesting that the tightly bound CEA has better specificity towards the goat anti-CEA. Although the Hoffmann-La Roche CEA showed a greater binding capacity in the radioimmunoassay than did our B1 or C2 CEA, we feel that this may be due to differences in protein concentration of the CEA’s. We have determined the protein concentration by the method of Lowry et al. (6) using α1-acid glycoprotein as standard rather than albumin or human serum. Further, the solution of CEA supplied by Hoffman-La Roche appears to have been made up by initially weighing the purified CEA and then diluting it with a 10% normal plasma to a final concentration supplied to us. As a result a solution of CEA prepared by weighing may not necessarily correspond to the concentration of CEA determined by standard methods of protein determination.

While this manuscript was in preparation, Rogers et al. (11) reported that CEA can be separated into Con A binding and nonbinding components by affinity chromatography on Con A Sepharose. This component of CEA had identical immunological properties when tested against antisera raised to CEA by several investigators although antisera prepared separately against each of these components reacted differently, suggesting additional determinant groups on these molecules. We also feel that our loosely bound and tightly bound CEA’s may contain determinant groups unique to each of these molecules in addition to a common determinant detected by rabbit and goat anti-CEA. We have immunized rabbits with the 2 variants reported here and are following the course of antibody production to these.

The use of Con A Sepharose in the purification of glycoprotein antigens, such as CEA, has certain advantages since one might miss the molecular variants of CEA during a purification procedure with the conventional use of Sepharose 4B and Sephadex G-200 chromatography. Presence of a molecular variant of CEA is supported by the fact that the elution behavior on Con A Sepharose column and differences in molecular weight and in the binding capacity in the radioimmunoassay and the $A_{280}/A_{260}$ ratios of the 2 types of CEA studied by us are quite different. Since both types of CEA showed reaction in the radioimmunoassay and have same β-electrophoretic mobility, we suspect that the differences between the 2 CEA’s may be due to differences in the glycoprotein content. The specificity of the rabbit anti-CEA and the B1 and C2 activities sharing immunological identity with anti-CEA’s and CEA’s of other established investigators have been clearly demonstrated by the reaction of rabbit anti-CEA with CEA from another source (Dr. Todd’s CEA) and the reaction of our CEA activities with goat anti-CEA’s obtained from 2 different sources. Further, extensive absorption of the rabbit anti-CEA with whole serum and perchorlic acid extracts of normal liver, colon, lung, and kidney did not abolish immunochemical reactivity with the purified CEA’s. These observations do suggest that the 2 molecular variants reported in this study are indeed CEA’s according to the criteria defined by Terry et al. (14). The possibility that these 2 variants of CEA might show a common determinant to a variety of anti-CEA’s raised in different animals and with their own unique determinant groups has not been excluded. It is of interest to study which of these 2 variants has greater specificity in the radioassay of plasma samples from colon cancer and other nonentodermally derived cancers.

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Fig. 1. Immunoelectrophoretic pattern of peaks (Chart 1) obtained after Con A Sepharose chromatography of perchloric acid extract of liver metastasis of colon tumor. Antigen wells: 1, crude extract of liver metastasis, 20 mg/ml; 2, Con A Sepharose Peak I, 0.7 mg/ml; 3, Con A Sepharose Peak II, 1.6 mg/ml; 4, ascending arm of Con A Sepharose Peak III, 0.87 mg/ml; 5, descending arm of Con A Sepharose Peak IV, 0.73 mg/ml; 6, Con A Sepharose Peak C, 0.65 mg/ml. The troughs from the top contain alternatively absorbed and unabsorbed rabbit anti-CEA.

Fig. 2. Immunoelectrophoresis patterns of crude extracts of liver-metastasized tumor, normal liver, and purified fractions obtained by Sephadex G-200 column chromatography (Chart 2). Antigen wells: 1, crude tumor extract, 20 mg/ml; 2, normal liver extract, 20 mg/ml; 3, Peak B1 from Sephadex G-200 column (Chart 2), 0.88 mg/ml; 4, Peak B2 from Sephadex G-200 column (Chart 2), 0.61 mg/ml. The antiserum troughs contained unabsorbed rabbit anti-CEA.

Fig. 3. Antigen Wells 1, 2, and 3 contained purified CEA (0.51 mg/ml) supplied by Dr. C. W. Todd and Dr. M. L. Egan.

Fig. 4. Antigen wells from top to bottom contained C1 CEA (0.5 mg/ml), B1 CEA (0.86 mg/ml) and C2 CEA (0.82 mg/ml).

Fig. 5. Antigen wells from top to bottom contained B1 CEA (0.86 mg/ml) and C2 CEA (0.82 mg/ml).
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