heating eggs of temperature-sensitive mutant flies (*12*). While these experiments are of considerable value to embryology and developmental biology, the techniques employed do not represent realistic means for inducing developmental abnormalities in natural insect populations. In my studies, however, morphological abnormalities resulted after cricket eggs were oviposited in sand contaminated with trace amounts of a teratogen. This finding indicates that a potential hazard exists for inducing morphological deviates in insects as well as other terrestrial invertebrates that undergo embryonic development in chemically contaminated substrates (*13*).

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References and Notes

1. Acridine (2,3,5,6-dibenzopyridine) was purchased from Aldrich Chemical Company, Inc., Lot Nos. 12267 PB and 082987 TC.
2. Orthoptera: Gryllidae.
3. In brief, female crickets oviposit in contaminated sand, and eggs develop in the sand for 5 days. The eggs are then separated by flotation in a 0.5*M* sucrose solution, collected, rinsed, and transferred to moist filter paper for incubation in a covered petri dish. Typically, nymphs begin to emerge on day 12 when incubated at 31°C [B. T. Walton, *Environ. Entomol.* **9**, 18 (1980)].
4. Acridine was purified by first dissolving it in hexane and washing several times with an aqueous solution of NaOH at *p*H 12.2 followed by two washings with distilled water. The hexane solution was then extracted with distilled water adjusted to *p*H 2.2 with H_3PO_4, which brought the acridine into the water phase and left the teratogenic fraction in the hexane. The *p*H of the water was returned to 12.2 with NaOH, and the acridine was back-extracted into clean hexane. The hexane was evaporated at room temperature to obtain acridine crystals.
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13. Multiple-eyed and two-headed crickets were produced in this assay after sand was contaminated with synthetic fuels obtained from coal liquefaction processes (B. T. Walton, unpublished).
14. ———, *Environ. Entomol.* **9**, 18 (1980).
15. I thank E. G. O'Neill for technical assistance and G. R. Southworth for helpful suggestions. Sponsored by the Office of Health and Environmental Research, U.S. Department of Energy, under contract W-7405-eng-26 with Union Carbide Corporation. Publication No. 1558, Environmental Sciences Division, Oak Ridge National Laboratory.

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Specific Antigen in Serum of Patients with Colon Carcinoma

Abstract. *The binding of monoclonal antibody specific for colon carcinoma was inhibited by serum from patients with adenocarcinoma of the colon but not by serum from patients with other bowel diseases or from healthy volunteers. Of other malignancies studied, serum from two patients with gastric carcinoma and two patients with pancreatic carcinoma also inhibited the specific binding of monoclonal antibody. The levels of carcinoembryonic antigen in these serum samples were not correlated with their levels of binding inhibition. Such monoclonal antibodies may prove useful for the detection of colorectal carcinoma.*

We have developed a binding inhibition assay for the detection of antigens unrelated to carcinoembryonic antigen (CEA) and present in the serum of patients with colon (colorectal) carcinoma (CRC). For this assay we use a monoclonal antibody that binds to CRC intact cells, cell membrane extracts, and antigen present in serum-free tissue culture media (SFTCM) from CRC cells.

Examination of the possibility of using CEA as a diagnostic tool for CRC revealed that, although the serum of most CRC patients contains CEA, the serum of many patients with ulcerative colitis, alcoholic cirrhosis, pulmonary emphysema, and other primary site carcinomas also binds antibody to CEA in radioimmunoassays (RIA) (*1*). Furthermore, the serum of approximately one-fifth of all healthy subjects who smoke cigarettes contains CEA (*2*).

These results led us to search for an assay that would distinguish between antigens that are specific for CRC and those that are shared with other tumors. The availability of monoclonal antibodies that recognize antigenic determinants associated only with CRC (*3, 4*) made possible a reinvestigation of the specificity of antigens circulating in the blood of patients with CRC.

Serum samples were obtained from 33 patients who had advanced adenocarcinoma of the colon and rectum with documented metastases in their liver or lungs; six patients with other bowel diseases, including two with multiple polyps; and 38 patients with forms of cancer other than CRC. All of the patients were hospitalized at the Oncologic Hospital, Fox Chase Medical Center, Philadelphia. Each serum sample was divided into small portions, coded, and frozen at −70°C. Preoperative patients and those with suspected but unproved recurrent cancer were not included in this study group. Serum samples were also obtained from 39 healthy volunteers: 15 females, 20 to 40 years of age; 10 females, 41 to 65 years of age; 9 males, 20 to 40 years of age; and 5 males, 41 to 65 years of age. Fifteen of these 39 healthy subjects were smokers.

Cells of CRC line SW 1116 (*5*) were used as a source of target antigen in the binding assay. This tumor is maintained in tissue culture and secretes its antigenic component (or components) into the tissue culture medium. Extracts (3*M* KCl) of cell membranes or cell-free SFTCM were used either as the target in RIA or as the inhibitor in preliminary inhibition assays. Hybridomas secreting antibody to CRC into the medium were grown according to a method described previously (*4*). The hybridoma medium contained approximately 10 μg of antibody per milliliter. The indirect RIA for the binding of antibody to its target has been described previously (*4*).

In the inhibition assay, dilutions of hybridoma antibodies were used that exhibited 40 to 50 percent maximum reactivity against a given concentration of target antigen in the form of either membrane extracts or SFTCM of cell line SW 1116. The percentage of specific inhibition of binding of hybridoma antibodies was calculated from the mean of triplicate wells according to the formula: percentage inhibition = $[100 - (R_T - R_C/R_{max} - R_C)] \times 100$, when R_T and R_C are, respectively, the radioactivity (measured in counts per minute) of the test and control cultures, and R_{max} is the maximum binding determined by incubating hybridoma antibodies with buffer solution instead of serum samples. The background radioactivity of the controls was determined by incubating test samples with nonspecific mouse immunoglobulin, a supernatant from myeloma P3 × 63Ag8 (*3, 4*). Statistically significant differences in inhibition of binding between test and control samples were calculated for each experiment by Student's *t*-test.

Of the large number of monoclonal antibodies that bind to CRC cells (*4*), an immunoglobulin G1 (IgG1) antibody was selected for the inhibition assay because of its wide range of reactivity with CRC cells of different origins. In preliminary assays we were able to inhibit effectively the binding of this antibody by the use of either cell membrane extracts of CRC, at concentrations of 5 to 20 μg of protein per unit assay, or SFTCM of CRC cultures at concentrations of 10 to 20 μg of

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Table 1. Inhibition of binding in RIA of CRC antibody to SW 1116 CRC antigen preparations by human serum samples of different origins. A preselected dilution of antibody was mixed in gelatin-coated polyvinylchloride plates (Cooke Engineering Co., Alexandria, Virginia) with an equal volume of dilutions of human serum. The mixtures were incubated overnight in a humidified chamber at 4°C and then transferred to wells of plates coated with either membrane extracts or serum-free supernatant of SW 1116 cells. After overnight incubation, the plates were washed with a cold Veronal-buffered NaCl solution and again incubated overnight at 4°C, this time with ^{125}I-labeled rabbit IgG antibody to mouse $F(ab)_2$. The plates were then washed, the wells were separated and counted in a Packard gamma counter, and the percentage of specific inhibition was calculated. Numbers in parentheses show percentages of the total number.

Percentage inhibition by human serum	Number of patients clinically diagnosed as			
	Advanced CRC	Other bowel diseases	Other malignancies	Healthy volunteers
< 10	3 (10)	5 (98)	34 (90)	38 (100)
10 to 19	6 (18)	1 (2)	2* (5)	0
> 19	24 (72)	0	2† (5)	0
Total	33	6	38	38

*Gastric carcinomas. †Pancreatic carcinomas.

protein per unit assay; binding was not inhibited when preparations of human melanoma cells or other tumors were used.

In the binding inhibition assay with patients' serum, a 1:500 dilution of monoclonal antibody to CRC was mixed with either a 1:3 or 1:9 dilution of patients' serum and, after incubation, transferred to wells of plates coated with 3*M* KCl membrane extracts of SW 1116 tumor cells containing 7 μg of protein per well. Inhibition of binding was then determined by RIA. The results are shown in Table 1.

Of the serum samples from 33 patients with advanced CRC, 24 (72 percent) inhibited the binding of hybridoma antibody to CRC by more than 19 percent ($P < .025$). Six samples inhibited binding by 10 to 19 percent, and three samples inhibited binding by less than 10 percent. Whether 10 to 19 percent inhibition of binding is significant must be determined by the results of more extensive studies. In an additional study of those patients whose serum inhibited less than 10 percent of the binding reaction, ascitic fluid obtained from one inhibited binding by 30 percent.

The average inhibition of binding by serum samples from 38 healthy volunteers, including heavy smokers, was 2.6 percent (Table 1). Of the samples obtained from 38 patients with other malignancies, significant inhibition was detected with the serum from two patients with carcinoma of the pancreas (35 percent and 60 percent) and from two patients with gastric carcinomas (10 to 19 percent range). None of the samples from the remaining 34 patients in this group, including those from six patients with breast carcinomas and 17 with melanomas, inhibited binding significantly. Serum from a patient with colitis inhibited binding by more than 10 percent in three different assays; however, none of the other samples from five patients with inflammatory bowel diseases such as colitis, proctitis, and diverticulitis, or from two patients with multiple colonic polyps, inhibited binding. Each serum listed in Table 1 was tested as a coded sample at least three times in the inhibition assay with reproducible results. Serum was used either freshly drawn or frozen and thawed once; serum that had been frozen and thawed several times gave erratic results.

Most of the tests developed for the immunodiagnosis of digestive system tumors by the detection of circulating tumor-associated antigens such as CEA, α-fetoprotein, and others lack the specificity necessary for an absolute distinction to be made between patients with and without cancer (*1*, *2*). Many healthy persons or patients with nonmalignant disease possess one or another antigen purported to be of value for diagnosing cancer. The antigen that we detected in patients with CRC could not be detected in healthy subjects or in patients with chronic inflammatory disease of the bowel or most other cancers. Its presence in tumors of endodermal origin (two patients with pancreatic and two with gastric carcinomas) may prove to be advantageous, given the usual difficulty in diagnosing these types of cancer during the early growth stages.

Table 2. Lack of correlation between the levels of CEA in serum and the percentage of inhibition of the binding of monoclonal antibody by serum of patients with CRC. The CEA was assayed by the Hoffmann–La Roche kit. The assay for inhibition of binding was as described in the legend to Table 1.

Serum sample number	CEA (ng/ml)	Percentage inhibition (mean of three consecutive assays)
67	2120	64
101	5.1	37
103	250	14
104	9.6	20
112	1200	78
120	6.8	26
121	16.4	27
136	1330	22
156	4.6	63
170	102	22
212	1610	19
214	40	50

The successful outcome of the binding inhibition assay may be attributable to the monoclonal antibody binding specifically to cultured CRC cells and not to cells of other cancers except those of the stomach and pancreas. The nature of the antigen recognized by the monoclonal antibody may account for the high specificity of the reaction.

There was no correlation between the levels of CEA in serum samples from colorectal carcinoma patients and the values obtained for the inhibitory capacity of these samples. For instance, as shown in Table 2, sample 67 contained high levels of CEA and showed high values in the inhibition assay, whereas samples 156 and 214 showed low levels of CEA and high values of binding inhibition. Furthermore, the binding of the CRC antibody to CRC cells was not inhibited by purified CEA obtained from the diagnostic kit of either Hoffmann–La Roche or Abbot Diagnostics (*6*). The antigen detected by the CRC antibody appears not be be related to other tumor-associated antigens such as β_2-microglobulin, because it is stable after incubation at 37°C for 2 weeks and after drying and heating to 85°C and thus is not a protein. Further studies have indicated that the antigen is a monosialoganglioside not found in normal adult tissues (*7*). Possibly the same ganglioside that is present in the serum of patients with CRC is also responsible for inhibition of the binding of monoclonal antibody to target cells. A chemically identified antigen may thus replace CRC cell material as the target for the binding of that monoclonal antibody which facilitates the inhibition assay.

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A Monosialoganglioside Is a Monoclonal Antibody-Defined Antigen of Colon Carcinoma

Abstract. *The antigen of a monoclonal antibody that is specific for cells of human carcinoma of the colon is a monosialoganglioside as determined by the direct binding of antibody to thin-layer chromatograms of total lipid extracts of tissues. Binding of antibody to chromatograms is detected by autoradiography after the application of iodine-125–labeled F(ab')$_2$ of rabbit immunoglobulin G antibodies to mouse immunoglobulins.*

In (*1*) a monoclonal antibody was described that is specific for colon carcinoma cells (*2*). The binding of this antibody to antigen is inhibited by the serum of most patients with advanced colorectal carcinoma but not by the serum of normal individuals, patients with inflammatory bowel diseases, or most patients with other malignancies. The data that we present here indicate that the antigen for this antibody is a monosialoganglioside.

The binding of the hybridoma antibody to colorectal carcinoma cell line SW 1116 was unaffected by treatment of the cells with ficin, but was abolished by treatment of the cells with neuraminidase. Since this behavior suggests that the antigen may be ganglioside (*3*), total lipid extracts of cells, which contain gangliosides, were chromatographed and then tested for antigen by autoradiography.

For the autoradiography we used a modification of a method developed for the detection of gangliosides that bind to cholera toxin (*4*). The total lipid extracts of colon carcinoma cells and melanoma cells (*1*) were obtained according to a method devised for the quantitative extraction of gangliosides from brain tissue (*5*). The tissue culture cells (1 g, wet weight) were homogenized in 3 ml of H_2O at 4°C with a Potter-Elvehjem homogenizer. The homogenate was added to 10.8 ml of methanol, to which 5.4 ml of chloroform was then added with constant stirring. The extract was stirred at room temperature for 30 minutes and centrifuged at 15,000*g* for 10 minutes. The pellet was rehomogenized in 2 ml of H_2O and extracted as above with 8 ml of a mixture of chloroform and methanol (1:2 by volume).

The supernatant solutions from both extractions were combined and evaporated under a stream of dry nitrogen, and the residue was dissolved in chloroform and methanol (2:1 by volume) for application to a thin-layer chromatography sheet (*6*). Samples of 1 μl of total lipid extract suitably diluted were spotted 1.5 cm from the bottom of the sheet which was then clamped in a sandwich chamber and developed in a chromatography tank containing chloroform, methanol, and 0.25 percent KCl (60:35:8 by volume). The chromatogram was air-dried, and then soaked for 10 minutes at 4°C in 0.01*M* sodium phosphate buffer, *p*H 7.2, containing 0.15*M* NaCl, 1 percent polyvinylpyrrolidone (molecular weight 40,000, pharmaceutical grade; Sigma) and 0.1 percent sodium azide (buffer A). The wet chromatogram was laid horizontally on a slightly smaller, parafilm-covered glass plate. Serum-free hybridoma culture medium containing about 10 μg of antibody per milliliter (*1*) was diluted 1:4 with buffer A and gently pipetted onto the chromatogram (about 50 μl per square centimeter of chromatogram). After incubation in a humid atmosphere for 6 hours at 4°C, the chromatogram was washed by dipping in six successive changes of 0.01*M* sodium phosphate buffer, *p*H 7.2, containing 0.15*M* NaCl (buffer B). The chromatogram was then laid horizontally as before and immediately layered with ^{125}I-labeled F(ab')$_2$ of rabbit immunoglobulin G antibodies to mouse immunoglobulins in buffer A (10^6 counts per minute per milliliter; about 50 μl/cm^2 of chromatogram). After incubation in a humid atmosphere for 12 hours at 4°C, the chromatogram was washed six times in buffer B, air-dried, and exposed to XR-2 x-ray film (Eastman Kodak) for 50 hours.

As shown in Fig. 1, antigen is detected in extracts of colorectal carcinoma cells (lanes 4 to 6) but not in extracts of human melanoma cells (lanes 1 to 3), which do not bind antibody (*1*). The antigen migrates between the standard gangliosides G_{M1} and G_{D1a} under the conditions described. Binding of antibody to the carcinoma cell extract is abolished by treatment of the extract with neuraminidase.

When the total lipid extract was chro-

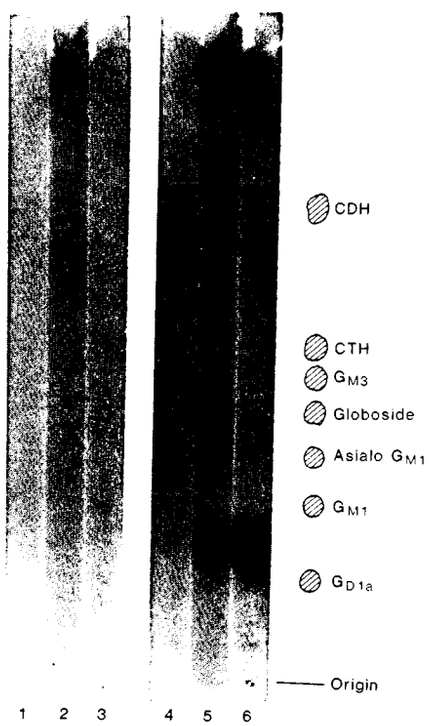

Fig. 1. Autoradiography of the antigen detected by a monoclonal antibody to colorectal carcinoma as described in the text. The amounts of extract chromatographed, expressed as the volume of packed cells from which the extract was obtained, are as follows: lanes 1, 2, and 3 contain lipids from 0.02, 0.1, and 0.5 μl of packed melanoma cells, respectively; lanes 4, 5, and 6 contain lipids from 0.02, 0.1, and 0.5 μl of packed colorectal carcinoma cells, respectively. The positions of some standard gangliosides and neutral glycolipids after chromatography are shown on the right. Abbreviations are: *CDH*, Galβ1-4Glcβ1-1Ceramide; *CTH*, Galβ1-4Galβ1-4Glcβ1-1Ceramide; G_{M3}, NeuNAcαa2-3Galβ1-4Glcβ1-1Ceramide; *Globoside*, GalNAcβ1-3Galα1 - 4Galβ1 - 4Glcβ1 - 1Ceramide; *Asialo* G_{M1}, Galβ1-3GalNAcβ1-4Galβ1-4Glcβ1-1Ceramide; G_{M1}, Galβ1-3GalNAcβ1-4[NeuNAcα2-3]Galβ1-4Glcβ1-1Ceramide; and G_{D1a}, NeuNAcα2 - 3Galβ1-3GalNAcβ1 - 4[NeuNAcα2 - 3]Galβ1-4Glcβ1-1Ceramide.

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