LITERATURE CITED

1. O. Gaal, G. A. Medgyesi, and L. Vereczkey, Electrophoresis in the Separation of Biological Macromolecules, Wiley, Chichester (1980).
2. M. F. Glazunov, Tumors of the Ovaries: Morphology, Histogenesis, Problems in Pathogenesis [in Russian], Moscow (1961).
3. H. Determann, Gel-Chromatography, Berlin–New York (1968).
4. H. Maurer, Disc Electrophoresis and Related Techniques of PAG Electrophoresis, De Gruyter, Berlin (1971).
5. J. Turkova, Affinity Chromatography [Russian translation], Moscow (1980).
6. N. I. Khramkina and G. I. Abelev, Byull. Éksp. Biol. Med., No. 12, 107 (1961).
7. B. C. Bast, M. Feeney, H. Lazarus, et al., J. Clin. Invest., 68, 1331 (1981).
8. M. Bhattacharya and I. I. Barlow, Cancer (Philadelphia), 48, 1616 (1978).
9. H. Bohn, Protein of the Placenta, Basel (1985), pp. 1-25.
10. P. A. Canney, P. M. Wilkinson, R. D. James, and M. Moore, Br. J. Cancer, 52, 131 (1985).
11. S. Knauf and G. Urbach, Am. J. Obstet. Gynecol., 131, 780 (1978).

IMMUNOHISTOCHEMICAL STUDY OF CARCINOEMBRYONIC ANTIGEN (CEA) IN NORMAL AND EMBRYONIC HUMAN TISSUES USING THE CEA-SPECIFIC ONCOPRECIPITIN "CRUSTACIN"

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Carcinoembryonic antigen (CEA) [3] is a complex glycoprotein and, as was originally considered, it is produced only by the entoderm of carcinomas of the digestive system and of the embryonic gut. Determination of CEA levels by immunochemical methods has been used successfully to monitor tumor growth after radical treatment, and also as an adjunct to methods of determining the pathology and clinical state of cancer patients [2, 5]. However, this test is ineffective for the early diagnosis of malignant tumors, due to the considerable immunochemical heterogeneity of CEA [8]. Definite progress in increasing the effectiveness of the test for CEA was made by the use of monoclonal antibodies against this antigen [7]. Recently a new class of substances — oncoprecipitins, which interact highly specifically with CEA in the manner of antibodies, has been discovered in marine invertebrates [1]. Oncoprecipitins, as has been shown by immunodiffusion and immunoenzyme methods, do not interact with antigens in extracts of normal human tissues or with other antigens of glycoprotein nature, including with normally cross-reacting antigen (NCA-1) [1].

The aim of the present investigation was to study the possibility of detection of CEA with the aid of an oncoprecipitin, namely crustacin (CR), in sections of human tissues and to compare the results with those of testing with CEA-specific antibodies.

EXPERIMENTAL METHOD

Normal tissues taken at autopsy on persons dying accidentally were studied. Embryonic tissues were obtained from Moscow gynecologic hospitals. Histological material was obtained by acetic–alcohol fixation of tissue fragments followed by embedding in paraffin wax [9]. Some histological material was obtained from the collection of the Department of Pathomorphology of the P. A. Gertsen Moscow Research Institute of Oncology, and was prepared by fixation of tissue fragments in buffered formalin, followed by embedding in paraffix wax. The thickness

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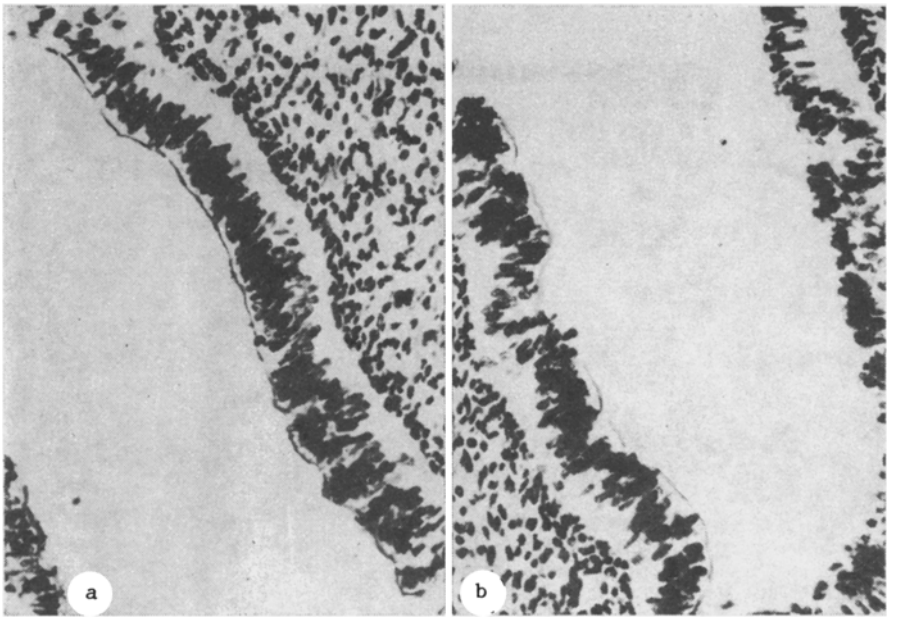

Fig. 1. Embryonic gut at 6-8 weeks of development. a) Immunoperoxidase reaction with CR reveals CEA along apical border of epithelial cells of gut; b) immunoperoxidase reaction using antibodies reveals CEA along apical border of epithelial cells of gut (very weak reaction) (arrow). Counterstained with hematoxylin. 360 ×.

of the sections used for treatment with antibodies and CR was 5-6 μ. The sections were dewaxed before immunochemical treatment.

The indirect histochemical method of enzyme immunoassay [6] was used. Sections were treated with first-order antibodies or CR in concentrations of 4-6 and 1 μg/ml respectively. Later the antibodies were revealed by a conjugate of donkey antibodies against rabbit γ-globulins with peroxidase in a dilution of 1:80. To detect tissue-bound CR, rabbit antibodies against CR, labeled with mark A peroxidase (Biolar, Olaine) were used by the method in [4] in a concentration of 20 μg antibodies/ml. The reagents were diluted and the sections rinsed with buffered physiological saline (pH 7.6). In the version with CR, 5 mM $CaCl_2$ and $MgCl_2$ was added to the buffer. Nonspecific binding was suppressed by the addition of bovine serum albumin (Sigma, USA) up to 1% to the solutions of antibodies and CR and to the second-order antibodies. Endogenous peroxidase was blocked by application of 3% H_2O_2 solution to the sections. A 0.05% solution of 3,3'-diaminobenzidine tetrachloride was used as chromagen for the peroxidase reaction. The results of the reaction were assessed by light microscopy after counterstaining of the sections with hematoxylin. The following controls were used: 1) incubation of tissue sections with antibodies and CR exhausted with a pure preparation of CEA 1 h before application; 2) treatment of sections with substrate and second-order antibodies by the traditional method; 3) treatment of sections of carcinoma of the colon, strongly positive for CEA, with antibodies and CR; 4) treatment of sections of normal adult human spleen and liver, negative for CEA, with antibodies and CR.

EXPERIMENTAL RESULTS

The results show that CR reacts with the embryonic gastrointestinal tract (6-8 weeks of development). The reaction with antibodies was much weaker (Fig. 1). In both cases the localization of the antigen was identical: along the apical border of the epithelial cells of the embryonic gut. Neither antibodies nor CR reacted with other tissues from embryos of this age.

In older fetuses (12-14 and 16-28 weeks of development) reactions with antibodies and CR were discovered in the intestine (Table 1). Their localization was identical — along the apical border and in the cytoplasm of the epithelial cells and also in the gut contents (meconium). The reaction with CR in the fetal stomach was stronger than that with antibodies. Meanwhile intensive reactions were found with antibodies in the esophageal epithelium, whereas

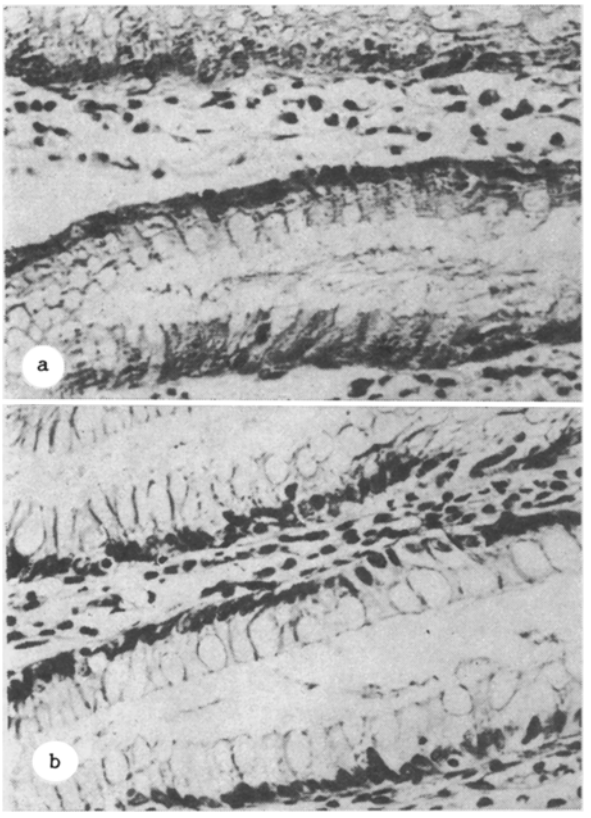

Fig. 2. Mucous membrane of proximal part of large intestine, removed during operation for carcinomas of the sigmoid colon. a) Immunoperoxidase reaction with CR reveals CEA in cytoplasm of intestinal epithelial cells (very weak reaction); b) immunoperoxidase reaction using CR neutralized beforehand with CEA preparation. Reaction negative. Counterstained with hematoxylin. 280 ×.

CR reacted strongly in only one case. Immune antibody—CEA and CR—CEA complexes were found in the mesothelium of the fetal ovary. In addition, both types of reaction were found in the glands and ducts of the prostate gland. One CR reacted with tissues of the parotid salivary gland and with the bronchial glands and endometrium. Antibodies and CR did not react with the remaining tissues studied.

In normal adult human tissues CEA was discovered by antibodies and CR in the epithelium of the large intestine. The reaction with antibodies was weak and revealed CEA along the apical border of cells of the upper third of the intestinal crypts. CR reacted more weakly still, revealing CEA in some cases predominantly within the cytoplasm of the goblet cells located in the upper third of the crypts of the large intestine. In other organs and tissues no reaction with antibodies and CR was found (Table 1).

In cases of intestinal mucosa removed during operations for tumors of this part of the gastrointestinal tract, the reaction was carried out on sections from regions adjacent to the neoplasms, and also on sections of parts of the intestine proximally and distally to the portion removed, with morphologically normal epithelium. Antibodies and CR reacted in all cases with the mucous membrane in the immediate vicinity of the neoplasms ("transitional mucous membrane"). In regions of the mucosa remote from the tumor, a reaction with antibodies and CR was observed in only some cases (Fig. 2), and CR, moreover, gave the weaker reaction. In two cases we observed a marked decrease in the reaction with antibodies and CR (virtually to negative) in the mucous membrane in one preparation in the direction away from the tumor.

Thus the similarity of the immunohistochemical reactions with antibodies and CR in normal human tissues is evidence of the close relationship between the determinants of CEA, revealed by these reagents. Despite the closeness of specificity of the two immunohistochemical tests studied, more conspicuous fetal features can be noted in CR—CEA, where they were

TABLE 1. Results of Immunohistologic Study of Adult Human Tissues and Tissues at Various Stages of Intrauterine Development

Test object	Antibodies against CEA	CR
Embryo 6-8 weeks	1/1*	1/1*
Fetus 12-14 weeks:		
Gastrointestinal tract	1/1	1/1
Lung	0/2	1/2
Other organs and tissues †	0/6	0/6
Fetus 16-28 weeks:		
Intestine	4/4	4/4
Stomach	2/7	5/7
Salivary gland	0/2	2/2
Bronchus	0/3	2/3
Endometrium	0/2	2/2
Ovary	1/2	1/2
Prostate	1/1	1/1
Other organs and tissues‡	0/9	0/9
Definitive tissues:		
Esophagus	0/1	0/1
Stomach	0/4	0/4
Small intestine	0/6	0/6
Appendix	0/1	0/1
Large intestine (autopsy material)	3/3	2/3
Large intestine (operation material)	12/15 **	10/15 **
Other organs and tissues ††	0/18	0/18

Legend. *) Reaction only in gastrointestinal tract; †) liver, muscle, rib, heart, upper limb, umbilical cord; ‡) brain, thyroid gland, lung tissue, liver, spleen, adrenal, kidney, gallbladder, placenta; **) reactions positive in all cases in areas of mucosa adjacent to tumors ("transitional mucous membrane"); ††) brain, cerebellum, muscle, skin, larynx, lung, heart, spleen, kidney, adrenal, urinary bladder, uterus (two cases), cervix uteri, ovary, uterine tubes, prostate gland, testes. Numerator indicates number of positive cases, denominator — total number of cases.

manifested as a fairly wide distribution of CEA in the immature epithelium. CR reacts with CEA located mainly within the cytoplasm of the goblet cells in the epithelium of the human colon, and the reaction was significantly weaker than the antibodies revealed. This is also confirmed by the low intensity of the reactions and the smaller number of positive observations.

The results may be evidence of structural differences in the antigenic determinants of CEA discovered by CR and by antibodies to CEA on the molecule of this antigen or of substances immunochemically similar to it, found in the cells of normal and embryonic tissues.

LITERATURE CITED

1. Yu. S. Ovodov, A. F. Pavlenko, A. V. Kurika, et al., Dokl. Akad. Nauk SSSR, 293, No. 4, 1009 (1987).
2. G. A. Tkacheva, Med. Radiol., No. 8, 69 (1983).
3. P. Gold and S. O. Freedman, J. Exp. Med., 122, 439 and 467 (1965).
4. A. G. Farr and P. V. Nakane, J. Immunol. Meth., 47, 129 (1981).
5. R. H. Fletcher, Ann. Intern. Med., 104, 66 (1986).
6. E. Heyderman, J. Clin. Path., 32, 971 (1979).
7. S. Jothy, S. A. Brazinsky, M. Chin-A-Loy, et al., Lab. Invest., 54, 108 (1986).
8. G. T. Rogers, Biochim. Biophys. Acta, 695, 227 (1983).
9. G.-J. Sainte-Marie, J. Histochem. Cytochem., 10, 250 (1962).