# CARCINOEMBRYONIC ANTIGEN IN THE BLOOD SERUM OF RATS WITH NONSPECIFIC INTESTINAL LESIONS

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The sera of rats in which an extensive lesion had been produced in the mucous membrane of the cecum were tested by the agar diffusion method. A carcinoembryonic antigen appeared in the sera of 84% of the animals 24 h after injury, and in most of them it persisted for 15-20 days, corresponding to the period of most intensive regeneration of the intestinal mucosa.

KEY WORDS: carcinoembryonic antigen; nonspecific lesions of the intestine; proliferation of the intestinal epithelium.

A previous investigation [1] showed that induced intestinal tumors in rats contain a specific glycoprotein which, in many of its physicochemical properties, resembles the carcinoembryonic antigen (CEA) found in neoplasms of the human large intestine. In 70% of cases it was detected by the double immunodiffusion in agar test in the blood serum of rats with tumors.

The object of this investigation was to detect CEA in the blood serum of rats with nonspecific lesions of the intestine accompanied by intensive proliferation of enterocytes. This investigation was motivated, first, by data in the literature on the appearance of CEA in the blood serum of patients with chronic nonspecific ulcerative colitis [6, 7] and, second, by personal observations showing an increased quantity of this antigen in the blood serum of rats with spontaneous ulcerative lesions of the large intestine [1].

## EXPERIMENTAL METHOD

To produce massive lesions of the intestinal mucosa, after laparotomy a purse-string suture was tied around the cecum, so that "diverticula" were formed when the threads were tied. In the course of a few days the sutures were cut out and the mucous membrane developed necrosis in areas corresponding to the "diverticula," surrounded by a zone of increased proliferation of the intestinal epithelial cells: The labeling index of the enterocytes 1 mm from the edge of the wound increased from $25.1 \pm 1.8\%$ under normal conditions to $36.1 \pm 2.1\%$. The presence of a ligature in the wall of the cecum was a factor causing continuous trauma to the mucous membrane in the late periods after the operation also, and this was accompanied by a local increase in the number of proliferating enterocytes. By changing the perimeter of the purse-string suture, the size of the intestinal lesions could be varied at will. Most frequently in these experiments the zone of injury occupied about one-third of the area of the cecum.

Operations were performed in this manner on 35 noninbred male rats (weight 200-250 g) from the "Rappolovo" nursery of the Academy of Medical Sciences of the USSR. A similar operation, but with a zone of injury eight to ten times smaller, was performed on ten similar rats. In addition, experiments were carried out on five rats with an artificial anus made at the level of the proximal part of the ascending colon, and with injury to the gastric mucosa, and also rats undergoing laparotomy but no manipulations of any sort on the gastrointestinal tract.

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Laboratory of Experimental Tumors, N. N. Petrov Research Institute of Oncology, Ministry of Health of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR, V.I. Ioffe.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 81, No. 4, pp. 467-470, April, 1976. Original article submitted June 10, 1975.

TABLE 1. CEA in Sera of Rats with Lesions of Intestinal Mucosa

| No. animals | Results of determination of CEA: before injury (twice) | days after injury: 1 | 3 | 5 | 7 | 10 | 12 | 15 | 20 | 25 | 30 | 35 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | — — | ++++ | +++ | +++ | ++++ | ++++ | ++++ | +++ | +++ | ++ | ++++ | ++ |
| 2 | — — | ++++ | ++++ | ++++ | ++++ | +++ | ++ | ++ | + | — | — | — |
| 3 | — — | +++ | ++++ | + | ++ | + | + | + | + | +++ | + | — |
| 4 | — — | + | ++ | +++ | ++++ | ++++ | ++ | + | ++++ | ++ | + | — |
| 5 | — — | +++ | +++ | ++ | ++ | ++ | + | — | — | — | NT | NT |
| 6 | — — | — | — | — | — | + | — | — | — | ++++ | ++ | ++++ |
| 7 | — — | ++++ | ++++ | ++++ | ++++ | NT | NT | NT | NT | NT | NT | NT |
| 8 | — — | ++++ | + | + | + | + | — | — | — | — | NT | NT |
| 9 | — — | ++ | +++ | + | + | + | + | + | — | — | NT | NT |
| 10 | — — | ++++ | ++ | ++++ | NT | NT | NT | NT | NT | NT | NT | NT |
| 11 | — — | ++++ | ++++ | ++++ | ++++ | NT | NT | NT | NT | NT | NT | NT |
| 12 | — — | ++++ | +++ | NT | NT | NT | NT | NT | NT | NT | NT | NT |
| 13 | — — | +++ | ++++ | NT | NT | NT | NT | NT | NT | NT | NT | NT |
| 14 | — — | ++++ | ++ | — | + | + | + | + | + | + | + | + |
| 15 | — — | +++ | — | — | + | — | + | + | — | + | — | — |
| 16 | — — | + | — | + | + | + | + | + | +++ | — | — | NT |
| 17 | — — | ++ | + | — | — | ++ | — | — | + | — | — | NT |
| 18 | — — | + | + | ++ | +++ | ++ | ++ | +++ | + | — | NT | NT |
| 19 | — — | +++ | + | — | NT | NT | NT | N T | NT | NT | NT | NT |
| 20 | — — | — | — | + | ++ | — | + | + | — | — | — | NT |
| 21 | — — | + | — | — | NT | NT | NT | NT | NT | NT | NT | NT |
| 22 | — — | + | — | + | — | — | — | — | — | — | NT | NT |
| 23 | — — | + | — | — | — | NT | NT | NT | NT | NT | NT | NT |
| 24 | — — | — | — | — | — | — | — | — | — | — | — | NT |
| 25 | — — | — | — | — | NT | NT | NT | NT | NT | NT | NT | NT |

Legend. NT) Not tested.

TABLE 2. Comparative Data showing Relationship between Quantity of CEA in Rats' Sera and Size of Lesion of Intestinal Mucosa

| Size of injury to mucosa | No. animals | Results of determination of CEA: before injury | days after injury: 1 | 3 | 5 |
|---|---|---|---|---|---|
| Extensive damage to one-third of area of intestine | 1 | — | ++++ | +++ | ++++ |
| | 2 | — | ++++ | ++++ | ++ |
| | 3 | — | ++++ | ++++ | + |
| | 4 | — | +++ | +++ | +++ |
| | 5 | — | +++ | ++ | ++ |
| | 6 | — | ++ | +++ | ++ |
| | 7 | — | ++ | + | + |
| | 8 | — | ++ | + | — |
| | 9 | — | — | — | — |
| | 10 | — | — | — | — |
| Small zone of injury ($^1/_{24}$ - $^1/_{30}$ of area of intestine) | 1 | — | ++++ | ++ | + |
| | 2 | — | +++ | ++ | — |
| | 3 | — | ++ | + | — |
| | 4 | — | ++ | + | — |
| | 5 | — | + | + | ++ |
| | 6 | — | — | — | — |
| | 7 | — | + | — | — |
| | 8 | — | — | — | — |
| | 9 | — | — | — | — |
| | 10 | — | — | — | — |

Both CEA and antigens of the normal mucosa of the large intestine of the rats were determined in the blood serum by the agar diffusion test twice before the operation and frequently between the first and 35th day after injury. Blood was taken from the caudal vein of the rats.

Isolation of CEA from tumors and preparation of the monospecific immune serum were described previously [1]. Serum against tissue-specific antigen of enterocytes was obtained in a similar way: Rabbits were immunized for a long time with a saline extract of the separated mucosa of the large intestine and the immune serum was exhausted with extracts of all the other organs and the blood serum of normal rats. Each of the immune sera formed a single line of precipitation in the agar diffusion test with the homologous antigen.

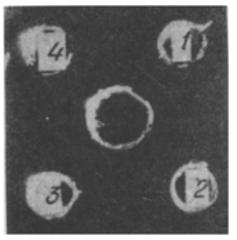


Fig. 1

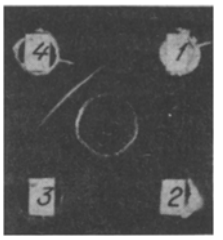


Fig. 2

Fig. 1. Agar diffusion test between immune serum against CEA (in center), homologous antigen (1), blood serum of a rat with regenerating mucous membrane of the cecum, taken 3 days after operation (2), blood serum of normal rat before operation (3), and saline extract of mucous membrane of large intestine of a rat (4). Reaction negative with last two samples.

Fig. 2. Agar diffusion test between immune serum against tissue-specific antigen of enterocytes (in center) and homologous antigen (4): 1) saline extract of organs of normal rats; 2-4) as in Fig. 1. Reaction negative with all samples except 4.

The intensity of the reaction was assessed visually by the character and position of the precipitation line: ++++ indicates a very strong reaction (very distinct line, displaced toward the well containing the immune serum, antigen in excess with respect to antibodies); +++ a strong reaction (distinct precipitation line half-way between the wells with the reagents); ++ a moderately strong reaction (weak precipitation line displaced toward the well with the antigen); + a weak reaction detectable only from deviation of precipitation line in test system [2]; and – a negative reaction.

## EXPERIMENTAL RESULTS

As Table 1 shows, before injury to the cecum CEA could not be found by the method used in the blood serum of the rats. No antigen characteristic of the normal intestinal mucosa also was found in the blood serum of the animals.

After the operation CEA appeared in large amounts in the serum of most animals within 24 h, and it persisted until the 15th-20th day (in some rats even until the 35th day) after the beginning of the experiment (Fig. 1). It is during this period that the most intensive regeneration takes place in the mucous membrane of the injured intestine.

Correlation was found between the size of the injury inflicted and the relative quantity of CEA detected, in particular 3-5 days after the operation (Table 2). In this connection it should be noted that CEA was discovered in the rats 24 h after the formation of an artificial anus, but this reaction was transient and 3 days later the antigen could be found in the blood serum only of a few animals in trace amounts. Injury to the gastric mucosa also was accompanied by the appearance of CEA in the serum of four of the five rats.

Altogether in these experiments CEA was found in 84% of rats with injury to the intestinal mucosa. However, it was never found after simple laparotomy. Tissue-specific antigen of the mucous membrane of the large intestine likewise was never found in the serum of rats with injury to the intestine (Fig. 2). This fact, in the writers' opinion, is evidence that the appearance of CEA in the serum under these conditions was not due to necrotic changes in the mucous membrane or to the absorption of breakdown products of the enterocytes.

Production of the CEA in the adult state is thus not specific for neoplasm alone, and it is very possibly synthesized by the normal epithelial components of the intestine, so that their intestine proliferation leads to the appearance of this antigen in considerable amounts in the blood serum. This interpretation is in harmony with data in the literature showing that CEA can be found, although in very small amounts (not more than 2.5 ng/ml), in healthy human blood serum [4, 5, 7]. The correlation observed in these experiments between the quantity of CEA entering the blood stream and the size of the regenerating zone of in-

testinal mucosa is indirect confirmation of this view. Considering that the increase in proliferative activity of the epithelium evidently takes place chiefly through precursors of the enterocytes starting the mitotic cycle, it can be postulated that CEA is in fact produced by these cells. The appearance of CEA in animals with nonspecific intestinal lesions and the appearance of $\alpha$-fetoprotein after partial hepatectomy [2] probably have a similar mechanism.

The results of this investigation provide an explanation of the false positive results of the diagnosis of carcinoma of the large intestine in man with the aid of CEA [3, 4, 7] and its discovery in an increased titer in the blood serum of patients with chronic ulcerative colitis of varied etiology [6-8].

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