the tumors in them. The essential features of this relationship between the species of animal and the tumor spectrum requires elucidation.

LITERATURE CITED

1. V. V. Khudolei, in: Carcinogenic N-Nitrocompounds: Action, Synthesis, Determination (Proceedings of the Second Symposium) [in Russian], Tallin (1975), pp. 109-111.
2. V. V. Khudolei, Usp. Sovr. Biol., 81, No. 1, 157 (1976).
3. M. Balls and R. H. Clothier, Oncology, 29, 501 (1974).
4. Editorial, Lancet, 1, 1414 (1975).
5. J. R. Halver, in: Trout Hepatoma Research Conference Papers. Research Report No. 70, Washington (1967), p. 78.
6. IARC Working Group of the Evaluation of Carcinogenic Risk of Chemicals to Human Sex Hormones, Lyon (1974), pp. 55-76.
7. A. Ingram, Brit. J. Cancer, 26, 206 (1972).

IMMUNOCHEMICAL IDENTIFICATION OF A NEW EMBRYONIC ANTIGEN IN OVARIAN TUMOR TISSUE

V. V. Kalashnikov, S. A. Borisenko, and Yu. S. Tatarinov

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A new embryonic antigen was found in ovarian tumors by methods of immunodiffusion analysis. This antigen consists of two components with different electrophoretic mobilities: The slow peak migrates in the α_0-globulin zone, the fast peak in the prealbumin zone. It is immunologically different from α-fetoprotein and from the carcinoembryonic antigen of the large intestine. The antigen was found in fetal and neonatal blood serum and also in tissue extracts of ovarian tumors. It was not found in normal adult human tissues or in the blood serum of healthy donors and cancer patients.

KEY WORDS: ovarian tumor; tumor antigens; embryonic antigens.

Embryonic reversion of tumor tissue has now been demonstrated in tumors of the liver [1], large intestine [8], and pancreas [6]. The discovery of embryonic antigens in patients' blood serum is the basis for the serological diagnosis of some tumors [3, 6, 10]. The writers previously found both heterologous organ antigens [2] and the carcinoembryonic antigen of the large intestine [4] in ovarian tumor tissue.

This investigation is a continuation of earlier work aimed at finding embryonic antigens in ovarian tumor tissue.

EXPERIMENTAL METHOD

Tumor tissue (adenocarcinoma of the ovaries) was obtained during operations and it was processed within 2-3 h. A weighed sample of tissue with the addition (1:1) of Tris–glycine buffer containing detergent (Triton X-100) was homogenized with powdered glass. The resulting homogenate was frozen and thawed twice and then centrifuged at 12,000 rpm. The supernatant was lyophilized. Extracts from normal organs were prepared in the same way from the cadavers of persons dying from injuries. Extracts containing 50,000 μg protein/ml were used.

Department of Biochemistry and Problem Laboratory for Immunochemistry of Malignant and Embryonic Tissues, N. I. Pirogov Second Moscow Medical Institute.

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TABLE 1. Immunochemical Determination of Embryonic Antigen in Various Tumors and Normal Human Tissues

Material studied	No. of indiv. tests	Result of determination of embryonic antigen		
		positive	negative	titer
Ovarian adenocarcinoma	21	9	12	1:1
Benign ovarian tumors	8	1	7	1:1
Adenocarcinoma of the uterus	3	0	3	
Adenocarcinoma of the large intestine	8	1	7	1:1
Hypernephroma of the kidney	9	0	9	-
Normal tissues: ovary, stomach, small intestine, large intestine, lung, spleen, liver, kidney, heart, brain	4	0	4	-
Blood serum:				
donor's	80	0	80	-
of pregnant woman	114	0	114	-
fetal (18-38 weeks)	27	27	0	1:2-1:32
neonatal	60	60	0	1:1-1:2
of patient with ovarian neoplasia	127	0	127	-
Total	497	98	399	

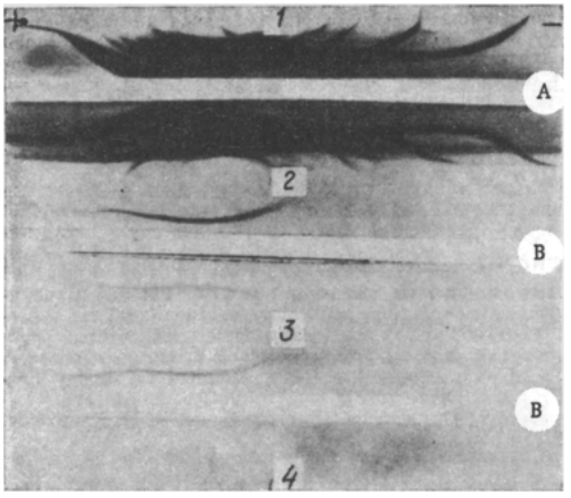

Fig. 1. Immunoelectrophoretic characteristics of embryonic antigen in fetal blood serum: 1,4) donor's blood serum; 2) fetal blood serum (28 weeks); 3) fetal blood serum (20 weeks); A) polyvalent antiserum against human blood serum proteins; B) antiserum against tissue proteins of ovarian adenocarcinoma exhausted by donor's blood serum proteins and by tissue antigens of healthy human organs.

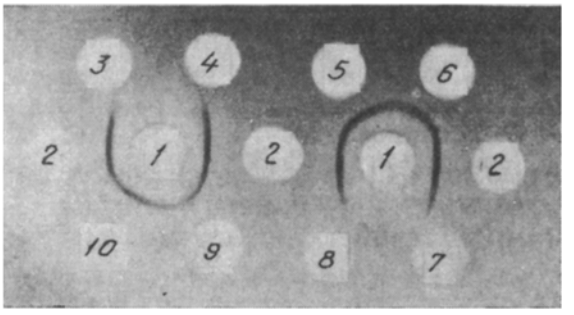

Fig. 2. Immunodiffusion analysis with standard test system for embryonic antigen: 1) monospecific antiserum against embryonic antigen; 2) purified preparation of embryonic antigen; 3,7) physiological saline; 4,8) donor's blood serum; 10) fetal blood serum (25 weeks); 9) tissue extract of adenocarcinoma of large intestine; 5) neonatal blood serum; 6) tissue extract of ovarian adenocarcinoma.

Animals (nine rabbits) were immunized with extracts of ovarian adenocarcinomas. One group of rabbits (five animals) was immunized with comparatively large doses of antigen for 1 month; each dose (100 mg protein), with the addition of Freund's complete adjuvant, was divided into eight to ten portions and injected subcutaneously and intramuscularly into different regions. Altogether five such fractional injections were given at intervals of 9 days. The total quantity of antigen given to each rabbit was 400 mg protein. The second group of

animals (four rabbits) was immunized with comparatively small doses for 1.5 months. The first injection (10 mg protein) was given with the addition of Freund's complete adjuvant. Three cycles of immunization were given at weekly intervals after 15 days; in each cycle 7 mg antigen in potassium alum was injected daily for 3 days, the sites of injection being alternated (subcutaneously, intramuscularly, intravenously). The total quantity of antigen injected into each rabbit was equivalent to about 70 mg protein. Blood was taken on the seventh, ninth, and 12th days after the last injection of antigen and antisera were obtained.

The resulting antisera were exhausted with dried human plasma (30-40 mg plasma was added to 1 ml) and with freeze-dried extracts of normal organs (40-60 mg of the tissue mixture was added to 1 ml). Exhaustion of the antisera was controlled by immunodiffusion analysis.

Immunoelectrophoresis was carried out by the method of Grabar and Williams [9] and immunodiffusion analysis by Ouchterlony's method in Khramkova and Abelev's modification [5].

EXPERIMENTAL RESULTS

Immunochemical analysis of the absorbed immune sera revealed specific antibodies (in one of the nine antisera) precipitating with an antigenic component whose migration zone stretched from the α_0-globulin to the prealbumin region. As Fig. 1 shows, this antigen exhibited well-marked heterogeneity: The slow peak migrated in the α_0-globulin zone, the fast peak in the prealbumin zone. It was also shown that the antigen is not identical with α-fetoprotein or with the carcinoembryonic antigen of the large intestine. A comparative immunochemical investigation of the antigen with the rho antigen discovered in human fetal serum was decided upon [7].

As Table 1 shows, the antigen discovered may belong to the group of embryonic proteins, for it was found in all the fetal and neonatal blood sera but was not present in the donor's blood serum. Neither was it found in the blood serum of pregnant women or in tissue extracts of healthy organs. The highest concentration of this antigen in fetal blood serum was found during the first half of pregnancy, when its titer was 1:32. However, later its synthesis decreased and in the neonatal sera it was found only at the tail end of the standard test system (about 1-3 μg/ml).

Immunochemical analysis of the embryonic antigen in tissue extracts of human tumors showed that it was present in nine of 21 ovarian adenocarcinomas, in one of eight benign ovarian tumors, and in one of eight adenocarcinomas of the large intestine (Fig. 2).

In extracts of adenocarcinoma of the uterus and hypernephroma of the kidney, and also in blood serum from cancer patients, this nitrogen could not be found. In the future it is intended to investigate the embryonic antigen in the blood serum of cancer patients by the use of highly sensitive methods of radioimmunological analysis.

LITERATURE CITED

1. G. I. Abelev, S. D. Perova, N. I. Khramkov, et al., Biokhimiya, No. 4, 625 (1963).
2. S. A. Borisenko, V. V. Kalashnikov, and Yu. S. Tatarinov, Akush. Gin., No. 4, 71 (1974).
3. Yu. S. Tatarinov, Vopr. Med. Khim., No. 1, 90 (1964).
4. Yu. S. Tatarinov, V. V. Kalashnikov, S. A. Borisenko, et al., Byull. Éksp. Biol. Med., No. 1, 50 (1975).
5. N. I. Khramkov and G. I. Abelev, Byull. Éksp. Biol. Med., No. 12, 107 (1961).
6. O. Banwo, J. Versey, and J. R. Hobbs, Lancet, 2, 643 (1974).
7. P. Burtin, S. Von Kleist, and D. Buffe, Bull. Soc. Chim. Biol., 49, 1389 (1967).
8. P. Gold and S. O. Freedman, J. Exp. Med., 122, 467 (1965).
9. P. Grabar and O. A. Williams, Biochim. Biophys. Acta, 10, 193 (1953).
10. D. Thomson, J. Krupey, and S. O. Freedman, Proc. Nat. Acad. Sci. USA, 64, 161 (1969).