PROPERTIES OF THE SERUM FACTOR OF NORMAL ANIMALS AND ANIMALS WITH TUMORS IN THE GEL DIFFUSION REACTION

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UDC 612.118.22+616-006-092.9-07:
616.15-097.5-071

The sera of normal Wistar rats and C3H/A mice give from 1-3 precipitation bands in the gel diffusion reaction with extracts from autologous and homologous tissues. This property of the sera of normal rats is depressed or is not observed when the reaction is carried out with extracts from embryonic and malignant tissues. The precipitating activity of the sera relative to extracts from normal tissues is depressed in rats dying from malignant tumors.

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The serum of normal animals has the ability to react in immunologic tests with extracts from autologous and homologous tissues. Some workers consider that these reactions take place because of the presence of normal tissue antibodies in these sera[3,4,7,9,11]. Others, on the other hand, do not regard these reactions as immunologic in nature [6, 8, 10]. The serum of normal animals apparently contains a natural factor capable of reacting in immunologic tests with extracts from autologous and homologous tissues. The properties of this factor have received little study.

The object of the present investigation was to study some properties of the natural factor in the sera of normal animals and the character of its reaction in the gel diffusion reaction with extracts from normal, embryonic, and tumor tissues, and also the state of this factor during growth of malignant tumors.

EXPERIMENTAL METHOD

Wistar rats of both sexes aged 6-12 months, rat embryos aged 12-17 days, and C3H/A mice were used as test objects.

The gel diffusion reaction and immunoelectrophoresis, performed by the method described previously [2], were used in the investigation. Tests were carried out on the sera of normal rats and mice and of rats with developing primary induced tumors, and also on saline extracts of autologous and homologous rat tissues: liver (105 specimens), kidney (103), thigh muscles (65), mammary gland (9), primary induced tumors of muscle tissue (53), spontaneous lymphosarcoma, and sarcoma (1 specimen of each), the liver and kidney of rat embryos (mixture from 52 embryos), and the corresponding mouse tissues: liver (5 specimens), primary induced hepatomas (2), and transplanted hepatoma XXIIa. The procedure of the test has been described previously [4]. To study the absorbability of the natural factor in normal sera, fractions of nuclei and mitochondria isolated from rat liver, kidney, and spleen tissues were used as absorbent [1]. In some experiments the sera of rats with developing primary induced tumors and the sera of guinea pigs immunized with extracts from rat liver and kidney tissues were used.

EXPERIMENTAL RESULTS

The results of the gel diffusion reaction are given in Fig. 1. They show that the sera of normal rats can form precipitation bands with extracts from the normal tissues of adult animals. Precipitation bands were formed most strongly and in a higher percentage of cases with extracts from kidney and liver tissues, less strongly with extracts from muscle and mammary gland tissues. In some cases the sera of normal rats gave two precipitation bands with extracts from liver, kidney, and muscle tissue (Fig. 2a). No differ-

Laboratory of Immunology of Growth and Development, Institute of Experimental Biology, Academy of Medical Sciences of the USSR, Moscow (Presented by Academician, N. N. Zhukov-Verezhnikov, Academy of Medical Sciences of the USSR.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 68, No. 7, pp. 81-85, July, 1969. Original article submitted October 9, 1967.

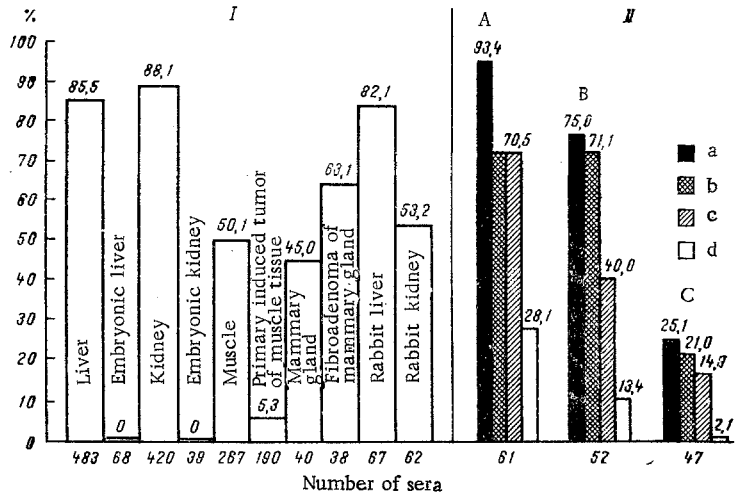

Fig. 1. Frequency of formation of precipitation bands (in %) in gel-diffusion reaction by sera of experimental rats with antigens from normal, embryonic, and tumor tissues of rats and rabbits. I) Sera of normal rats; II) sera of rats with developing induced tumors: A) 4-month tumors, B) 6-month, C) state preceding death; a) liver; b) kidney; c) muscle; d) autologous tumor.

ences were found between the reactions of these sera with extracts prepared from autologous and homologous tissues. The sera of normal rats did not form precipitation bands with extracts from rat embryonic liver and kidney tissues or from spontaneous lymphosarcoma and sarcoma (Fig. 2a, b). These sera gave a weak reaction with extracts from tissues of primary induced tumors. Of the 190 sera used in the experiments, for instance, only 5.3% gave precipitation bands. Meanwhile, precipitation bands were found when the sera of normal rats were tested with extracts from spontaneous fibroadenomas of the mammary gland (Fig. 2c). The sera of normal rats, absorbed by kidney nuclear fractions, gave no precipitation bands with kidney and liver extracts. However, when these sera were absorbed by liver nuclear fractions they formed precipitation bands with kidney tissue extract. A similar picture was observed when normal sera were absorbed by spleen nuclear fractions. No strict organ specificity was found when these sera were absorbed by the mitochondrial fractions of liver, kidney, and spleen cells. Immunoelectrophoresis showed that the active component of the normal sera of rats reacting with tissue extracts is located in the zone of α_2-globulins and also in the zone of β_1-globulins.

It has been reported in the literature that the sera of normal mice react in the gel-diffusion reaction with extracts from autologous or isologous liver, but do not form precipitation bands with extract from primary hepatoma tissue [6]. Our own investigations showed that the sera of normal C3H/A mice give from 1-3 precipitation bands with extracts from isologous liver (in 22 of 27 cases). One precipitation band was formed in each case in the reaction between normal sera and extract from primary induced hepatomas. In tests of extract from a 5-month hepatoma, precipitation bands appeared in 17 of 22 cases, and with extract from a 9-month hepatoma in 11 of 27 cases. However, in no case was the formation of precipitation bands observed by sera of normal mice with extract from primary hepatoma XXIIa tissue (Fig. 2d).

The sera of guinea pigs immunized with antigens from rat kidney and liver tissue gave from 2 to 4 precipitation bands in the gel diffusion reaction with these antigens, and one of them gave lines absolutely identical with those formed by the sera of normal rats when reacting with these antigens (Fig. 2e, f).

In the immunoelectrophoresis test, the sera of normal guinea pigs did not form precipitation bands with extracts from rat liver and kidney tissues. However, the sera of these animals, immunized with extracts from rat liver and kidney tissues, gave precipitation bands with extracts from these tissues in the zone of the α_2-, β_2-, and γ-globulins. In other words, the natural factor contained in the sera of normal rats behaves in the gel-diffusion reaction like certain immune antibodies present in the sera of immunized animals.

A study of the ability of the sera of rats with induced tumors at different times of development to react in the gel-diffusion reaction with extracts from homologous liver, muscle, and tumor tissues gave

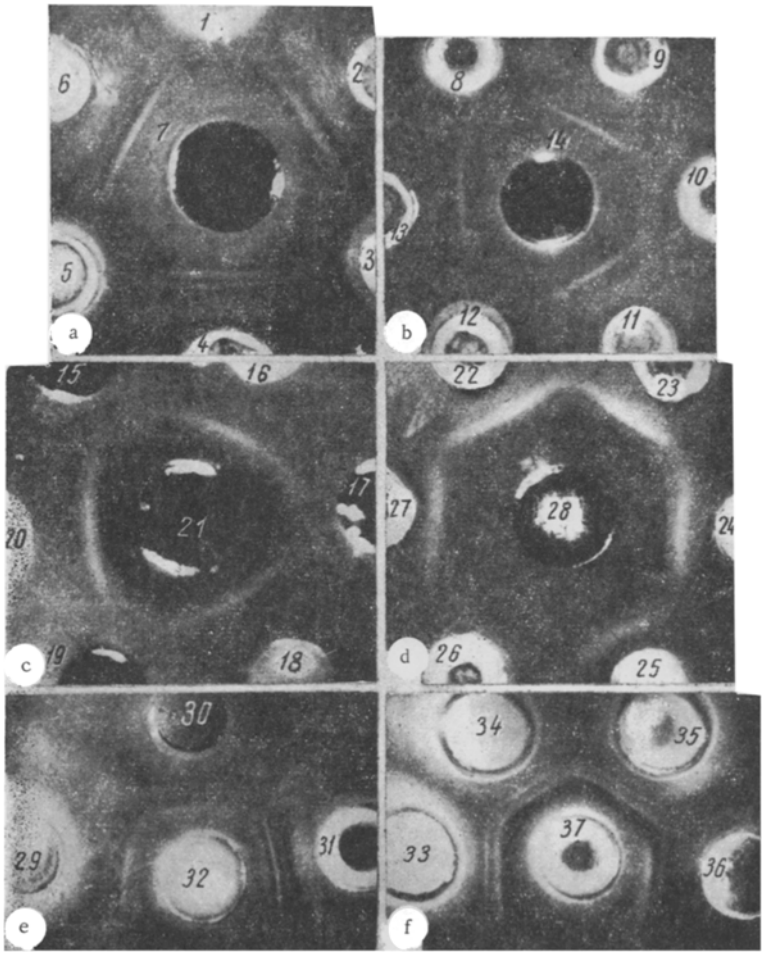

Fig. 2. Ouchterlony's gel-diffusion reaction. 7, 14, 21, 29, 30, 36) Sera of normal rats; 28) serum of normal C3H/A mouse; antigens from rat tissues: 2, 11, 32) liver; 4, 9, 37) kidney; 6, 13) muscle; 8) spontaneous lymphosarcoma; 10) spontaneous sarcoma; 12) primary induced tumor of muscle tissue; 16, 18, 20) spontaneous fibroadenoma of mammary gland; 1) embryonic layer; 3, 5) embryonic kidney antigens from mouse tissues; 23-25) liver; 22) primary induced hepatoma at 5 months of development; 27) primary induced hepatoma at 9 months of development; 15, 17, 19) physiological saline; 26) hepatoma XXIIa; 31) serum of guinea pigs immunized with antigens from rat liver tissue; 33, 34, 35) serum of guinea pig immunized with antigens from rat kidney tissue.

the following results. Sera of rats with 4-month induced tumors were indistinguishable in their precipitating activity from the sera of normal animals. Six months after the beginning of tumor development, slight weakening of the precipitating activity of the sera of these same rats was observed relative to extracts of liver, kidney, and muscle tissue. This weakening was more marked in animals on the brink of death. It is interesting to note that the sera of rats with induced tumors at 5-6 months of development as a rule formed precipitation bands with extracts from autologous liver, kidney, and muscle tissues, but did not react with extracts from the tissues of autologous tumors.

The results obtained are insufficient to determine the true nature of the factor investigated. A definite answer to this question can be obtained only by further research.

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