on induction of tumors of the mammary gland or uterus. Second, the estrogen in this case stimulated growth of an induced connective-tissue malignant tumor, whereas previously the objects on which estrogens acted were as a rule tumors of epithelial origin.

As regards the mechanisms of the stimulating action of the estrogen on sarcoma development in the uterus, the possibility that the estrogen may affect binding of metabolites of DMH with cell macromolecules or stimulation of proliferation of the mesenchymal elements of the uterus must be borne in mind. The latter suggestion seems more important, considering the great increase in the frequency of tumors in the animals of groups 3 and 4 compared with group 2, in which EDP was given simultaneously with DMH and, consequently, it could affect interaction between carcinogenic metabolites and the cells. In groups 3 and 4, in which the highest frequency of development of uterine sarcomas was observed, EDP was given during the last 10 weeks (group 3) or 15 weeks (group 4), after exposure to DMH had ceased. Allowing for the very rapid excretion of DMH from the body (under 24 h) and the fact that its carcinogenic metabolites are short-lived compounds, the effect of EDP on interaction between metabolites and target cells under these conditions can be completely ruled out. Consequently, the difference in the frequency of appearance of tumors in the animals of group 2, on the one hand, and in the mice of groups 3 and 4, on the other hand, can be ascribed on reasonably solid grounds to the intensified proliferation of cells transformed by DMH, induced by EDP.

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EFFECT OF *Brucella abortus* ON GROWTH OF GRANULOSA-CELL CARCINOMA OF THE OVARY

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KEY WORDS: *Brucella abortus*; adoptive transfer; carcinoma of the ovary.

In recent years, besides systematic adjuvants such as *C. parvum* and BCG, the antitumor action of *Brucella abortus* has been intensively studied. An inhibitory action of *B. abortus* has been demonstrated on the development of Rauscher and Graffi virus leukemias and on the growth of ascites and solid tumors [1-5, 12].

The object of this investigation was to study the effect of *B. abortus* (strain 19-BA) on growth of granulosa-cell carcinoma OC-1-72 of the ovary. Optimal doses, ways, and methods of administration of *B. abortus* and the possibility of adoptive transfer of inhibition of tumor growth by peritoneal exudate (PEC) and spleen cells were studied.

EXPERIMENTAL METHOD

Living brucellosis vaccine from Omsk Research Institute of Natural Food Infections, prepared from vaccine strain 19-BA of batches 19-72 and 13-2, was used in the experiments. The vaccine was diluted with physiological saline and injected intraperitoneally, intravenously subcutaneously, or into the tumor nodule. The vaccine was killed by heating to 60°C for 1 h.

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TABLE 1. Dependence of Antitumor Effect of *B. abortus* on Time of Injection

Time of injection of B. abortus before transplantation of tumor, days	Number of experiments	Inhibition of tumor growth	Stimulation of tumor growth	No effect
43	3	1	1	1
34	1	1	0	0
28	3	0	1	2
14	3	0	0	3
0	8	3	2	3

Granulosa-cell carcinoma of the ovary OC-1-72 was obtained in the writers' laboratory in female (CMA × C57BL/61)F_1 mice [10] 10 months after an operation by Biskind's method. The first three passages were carried out on castrated females. The tumor was then subjected to passage through intact males and females, and it has so far gone through about 100 passages.

The source of Rauscher virus leukemia was blood plasma from BALB/c mice affected with Rauscher leukemia, with a titer of $5 \cdot 10^4$ ID_{50}/ml based on splenomegaly. The mice were infected intravenously in a dose of $1 \cdot 10^3$ ID_{50}. Splenomegaly, determined on the 18th-22nd days after injection of the virus, served as indicator of leukemia development.

To obtain serum and splenic extract for adoptive transfer, $2 \cdot 10^9$ bacteria were injected intraperitoneally into BALB/c and CDF mice, and blood was taken 14 days later from the retroorbital sinus. The serum was centrifuged for 15 min at 1200 rpm and for 1 h at 6000 rpm. The spleens were disintegrated in a Potter's homogenizer, 10 volumes of phosphate buffer, pH 7.4, were added, and the material was extracted overnight at 4°C and then centrifuged in the same way as the serum.

For adoptive transfer (Winn's test [11]) the PEC were removed from the peritoneal cavity without preliminary injection of an irritant, and the spleens were thoroughly homogenized in a glass homogenizer in medium No. 199, and then filtered and washed. The PEC or spleen cells were mixed with tumor cells in the ratio of 10:1, 50:1, 100:1, and 500:1 in 0.2 ml of medium and injected subcutaneously into the mice. The results were subjected to statistical analysis by Student's t-test.

EXPERIMENTAL RESULTS

Maximal inhibition of tumor growth was observed after intraperitoneal injection of $8 \cdot 10^9$ bacteria, but 90% of the experimental animals died 6-7 days after injection of the vaccine. Doses of $4 \cdot 10^9$-$2 \cdot 10^8$ had a significant therapeutic effect without causing death of the animals. Intravenous injection of $8 \cdot 10^9$ bacteria had a marked antitumor action. In that case 10% of the animals died. Tumor growth also was inhibited after intravenous injection of smaller doses of *B. abortus* ($4 \cdot 10^9$-$2 \cdot 10^7$ cells). Tumor growth was inhibited after subcutaneous injection of the vaccine but only in doses of $8 \cdot 10^9$-$2 \cdot 10^9$ cells.

The study of the times of injection of *B. abortus* relative to transplantation of the tumor showed that intraperitoneal injection of bacteria at the same time as or 14, 28, 34, and 43 days before injection of the tumor cells gave different effects: inhibition or stimulation of tumor growth or no effect (Table 1). The effect of injection of *B. abortus* into mice with a growing tumor depended on the size of the tumor. If the diameter of the tumor was 5-6 mm, *B. abortus* inhibited its development in all four experiments. If the tumor was larger (8-10 mm), injection of *B. abortus* led to significant inhibition of its growth in three of six experiments. Living vaccine, injected into the tumor nodule in a dose of $2 \cdot 10^9$ cells, delayed development of the tumors in two of three experiments. Injection of killed vaccine into the mice stimulated tumor growth. However, injection of killed *B. abortus* mixed with tumor cells did not inhibit growth of the tumor. In these experiments living or killed bacteria were incubated for 1 h at 37°C with tumor cells ($5 \cdot 10^3$ to $2 \cdot 10^9$ bacteria). When killed vaccine was used tumors appeared in 27.8% of cases compared with 81% in the control, but when living vaccine was used they appeared in only 15.6% compared with 92.7% in the control.

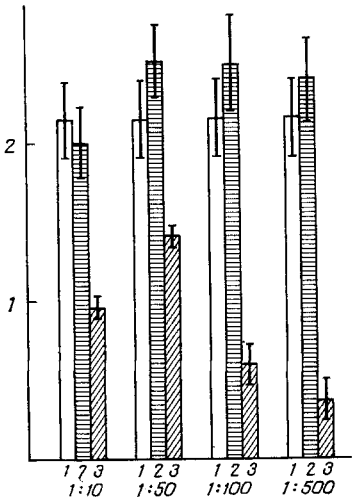

Fig. 1

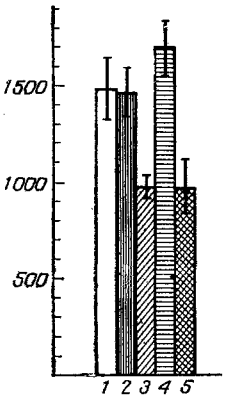

Fig. 2

Fig. 1. Adoptive transfer of inhibition of growth of OC-1-72 tumor by peritoneal exudate cells (PEC). 1) Control of tumor growth; 2) PEC of intact mice; 3) PEC of mice receiving *B. abortus*.

Fig. 2. Effect of serum and splenic extracts of mice inoculated 14 days previously with *B. abortus* on development of Rauscher virus leukemia (RVL). 1) RVL; 2) RVL + serum of intact mice; 3) RVL + serum of mice receiving *B. abortus*; 4) RVL + splenic extract of mice receiving *B. abortus;* 5) RVL + splenic extract of intact mice.

In Winn's test spleen cells and PEC from tumor-bearing mice, obtained 4 days after injection of *B. abortus*, were used. PEC and spleen cells of such mice inhibited tumor growth in vivo (Fig. 1).

The inhibitory action of *B. abortus* on development of Rauscher virus leukemia has been demonstrated in our laboratory by Veskova et al. [2]. We therefore studied the possibility that the effect of inhibition of leukemia development could be transferred with the aid of sera and extracts from the spleens of CDF and BALB/c mice, receiving *B. abortus* beforehand. The sera and extracts began to be injected 24 h after intravenous injection of the virus. Serum and extracts of CDF mouse spleen were injected into BALB/c mice intravenously four times, in a dose of 0.5 ml each time, with intervals of 72 h, and statistically significant inhibition of growth of the leukemia was observed (Fig. 2). However, after 10 intravenous injections at intervals of 24 h, this serum inhibited the development of leukemia.

Intraperitoneal and intravenous injection of *B. abortus* thus had a stronger antitumor action than subcutaneous injection. Injection of killed vaccine into the tumor, and also preliminary or simultaneous intraperitoneal injection of living *B. abortus* cells led in some experiments to stimulation of tumor growth. It can be tentatively suggested that *B. abortus*, like other systemic adjuvants, causes a disturbance of immunologic homeostasis, and in some cases it activates the cytotoxic effector cells whereas in others it activates suppressors [8, 9].

The antitumor effect of *B. abortus* can be transferred by spleen cells and serum. One question that still remains unanswered is: which cells and humoral factors are activated in the donor. All that can be said at present is that the humoral factor is not interferon, for *B. abortus* induces interferon production only during the first few hours after injection [6, 7].

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EFFECT OF FLUCTUATING ELECTROMAGNETIC FIELDS ON GROWTH AND CARCINOGENESIS

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Electrical fluctuations are an interesting variety of spectra of electromagnetic fields (EMF), whose effect on growth and development of living organisms had virtually not been studied. The use of such spectra in clinical and laboratory practice is limited at present to their application in physiotherapy for acute and chronic inflammatory conditions [1] and for electroanesthesia [7]. A special feature of fluctuating fields, which are usually distributed within a certain frequency range, is the presence of a "spectral distribution of energy" — a characteristic not typical of monochromatic frequencies.

The object of this investigation was to study the effect of electrical fluctuations with a gaussian spectral distribution on the kinetics of normal and malignant growth. The EMF chosen possessed minimal information content and maximal entropy.

EXPERIMENTAL METHOD

The kinetics of normal growth was studied on 120 young Wistar rats of the same age, divided into four groups. The animals of the first three groups were exposed daily to the action of a field, which began at different times — on the 28th, 45th, and 62nd days after birth, corresponding to the equivalent human age of 4.1, 7.24, and 10.37 years [2]. The duration of exposure was 15-20 days and the field intensity was 5 V/cm. The duration of each session was 1 h. The animals of group 4 were not exposed to any external influence and served as the control. Generators of types 12-1 and 12-12, by means of which the width of the spectrum* could be varied from 12 kHz to 6 MHz, served as the source of electrical fluctuations. Quantitative parameters of volume and linear growth of the rats were measured and calculated by Shmal'gauzen's method [4]. The experiments were carried out with three repetitions for each variant.

The action of fluctuating fields on malignant growth was studied on three strains of transplantable tumors: sarcoma 45, reticulosarcoma, and Walker's carcinosarcoma 256. Each series involved 140 adult rats (15-25 animals in each group). The tumors were inoculated subcutaneously into the rat's thigh as a 30% suspension. The field was applied by the con-

*The width of the spectrum of gaussian fluctuating fields usually corresponds to the frequency interval from zero to the upper limit of the spectral interval.

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