COMBINED ACTION OF GROWTH-INHIBITING EXTRACT OF EHRLICH'S ASCITES CARCINOMA AND ADRENALIN ON CELL DIVISION OF THIS TUMOR *in vitro*

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The mechanism of action of tissue-specific extracts (chalones) on reproduction of cells and their interaction with stress hormones has not been adequately studied. It was first suggested that adrenalin forms an unstable complex with chalone and that, as a cofactor of chalone, it potentiates its mitosis-inhibiting effect nonadditively [2]. According to other observations, adrenalin and chalone interact at the cell membrane level through the adenylate cyclase—cyclic AMP system [8]. With the discovery of antichalone [7], the mechanism of their interaction has come to be explained by inactivation of this factor by adrenalin [6]. Meanwhile investigations both *in vivo* and *in vitro* have shown that many chalones are biologically active even without adrenalin [4, 5]. The question of the role of adrenalin in chalone regulation of cell division thus requires further investigation.

The object of this investigation was to study the combined action of a growth-inhibiting extract of Ehrlich's ascites carcinoma (EAC) and adrenalin on cell division in this tumor.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albine mice weighing 18-20 g aged 1.5-2 months. A diploid strain on EAC, adapted to conditions of culture *in vivo* and *in vitro*, was transplanted into animals by intraperitoneal injection of ascites fluid containing 3-4 million tumor cells every 7 days. Cells for culture were taken from the tumor on the 5th day of its development. An aqueous extract of tumor cells containing growth-inhibiting factor was isolated from the tumor on the 13th day of its development by the method described previously [1]. The cells were cultured in penicillin flasks containing 2-3 ml medium and a cell suspension in a concentration of $1 \cdot 10^6$-$2 \cdot 10^6$/ml. The culture fluid consisted of 70% medium No. 199, 30% Eagle's medium, and 20% calf serum relative to the total volume. Glutamine was added in a concentration of 1 ml to 100 ml medium. Flasks with the samples were closed with sterile gauze plugs and were adapted to the conditions of culture for 2-4 h before the beginning of the experiments until the appearance of new mitoses, reflected in an increase in the mitotic index (MI) in the tumor cells. Preliminary experiments revealed the doses of the extract (0.5 and 1.0 ml per sample) and of adrenalin (2.5 μg/ml) which inhibited mitosis, and these were subsequently used in the main experiment. Extract and adrenalin were added to the culture at the end of its period of adaptation. The experiments included six series: 1) addition of 0.5 ml extract to the culture; II) 1 ml extract; III) adrenalin; IV) 0.5 ml extract and adrenalin; V) 1 ml extract and adrenalin; VI) physiological saline. In all series the material was investigated 30 min and 1, 2, 3, and 4 h after addition of the substances. Films were prepared, fixed with methanol, and stained with methylene blue. To assess the mitosis-inhibiting effect of the substances used, MI was calculated for 5000 cells in each preparation, and expressed in promille. On average in each experiment three parallel tests were set up, and allowing for their repetition, there were from 3 to 12 tests at each point. The significance of differences in the values was estimated by the Student—Fisher criterion. Differences were considered to be significant for which $P \leqslant 0.05$.

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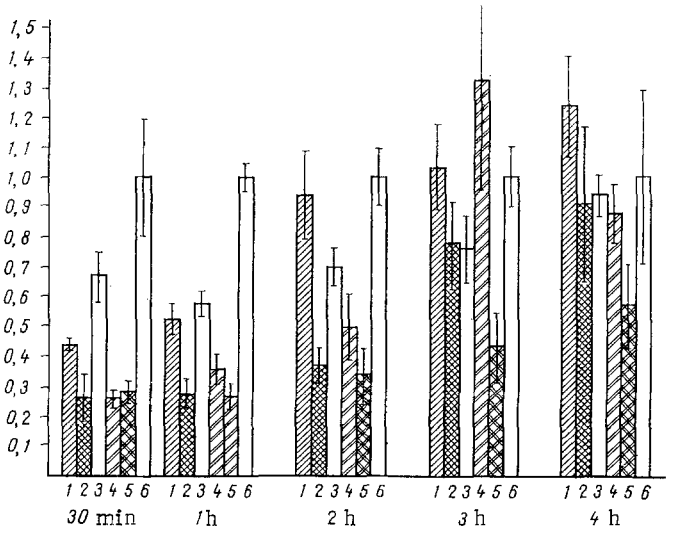

Fig. 1. Changes in cell division in mouse EAC *in vitro* at different times after separate and combined addition of extract from EAC and adrenalin. Abscissa, time of experiment (in h); ordinate, ratio of MI in experiment to MI in control. 1) 0.5 ml extract, 2) 1 ml extract, 3) 2.5 μg/ml adrenalin, 4) 0.5 ml extract + 2.5 μg/ml adrenalin, 5) 1 ml extract + 2.5 μg/ml adrenalin, 6) control.

EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that 30 min after addition of all substances significant inhibition of cell division was observed in the EAC culture. Under the influence of extract in a dose of 0.5 ml inhibition amounted to 56% ($P < 0.001$) and in a dose of 1 ml to 74% ($P < 0.001$); under the influence of adrenalin it amounted to 33% ($P < 0.02$). Addition of adrenalin to samples with extract led to a more marked mitosis-inhibiting effect compared with the action of adrenalin alone (inhibition was 74 and 72% respectively; $P < 0.001$). Addition of 0.5 ml of extract caused inhibition of cell division after 1 h by 47% ($P < 0.001$), 1 ml extract by 72% ($P < 0.001$), and adrenalin by 42% ($P < 0.001$). The use of 0.5 ml extract with adrenalin had a stronger inhibitory effect on cell division than the action of the same dose of extract and of adrenalin separately (inhibition 64%; $P < 0.001$). Extract in a dose of 1 ml, added together with adrenalin, inhibited cell division by 73% ($P < 0.001$), i.e., the same as the extract alone. Extract in a dose of 0.5 ml did not inhibit mitosis after 2 h, whereas in a dose of 1 ml it still continued to exert an inhibitory action (MI reduced by 63%; $P < 0.001$). At this time adrenalin reduced the number of mitoses by 30% ($P < 0.001$). When added together with 0.5 ml of extract it reduced MI by 50% ($P < 0.001$) and with 1 ml of extract by 66% ($P < 0.001$). After 3 h of the experiment significant inhibition of cell division was found only after the action of adrenalin (by 24%; $P < 0.05$) and the combined action of 1 ml of extract and adrenalin (by 57%; $P < 0.01$). After simultaneous addition of 0.5 ml of extract and adrenalin MI was increased by 33% compared with the control, but this change was not significant. By the 4th hour of the experiment MI was reduced only after the action of 1 ml extract together with adrenalin (inhibition by 43%; $P < 0.02$). In other series of experiments MI was indistinguishable from the control.

Both extract and adrenalin, when added separately, thus has an inhibitory effect on division of EAC cells *in vitro*. A stronger inhibitory action on cell division was observed by the action of extract in a dose of 1 ml than in a dose of 0.5 ml. It will also be noted that whereas in the first case inhibition continued for 2 h, in the second case it lasted only 1 h. Adrenalin inhibited cell division during 3 h of the experiment, but the degree of inhibition was weaker than when extract was added in a dose of 1 ml. Meanwhile addition of adrenalin to the samples with extracts prolonged the action of the latter: With extract in a dose of 1 ml inhibition continued for 4 h, but in a dose of 0.5 ml it continued for only 2 h of the experiment. As Fig. 1 shows, the action of extract with adrenalin is reversible in principle. Their combined action on cell division was expressed as the strengthening of inhibition and an increase in its duration.

The results are evidence that the mitosis-inhibiting activity of EAC extract can be manifested in the absence of adrenalin. This is in disagreement with the results of an investigation by Cooper and Smith [3], who found that the action of EAC chalone is dependent on adrenalin. In their investigation the effects of the combined action of chalone and adrenalin was determined only 4 h after their addition, and this may perhaps have led them to draw the wrong conclusion. Meanwhile, as the results of the present investigation show, the combined use of EAC extract, containing chalone, and adrenalin modifies the character of their effect on mitotic activity in a culture of EAC cells. The reasons for this effect are not clear and require further study.

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CHANGES IN AUTOIMMUNE ANTIBODY LEVELS DURING GROWTH OF TRANSPLANTABLE TUMORS IN MICE

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Much evidence has been obtained in clinical oncology to show that during tumor growth autoimmune antibodies, including antibodies reacting with blood cells, can be found in the patient. Antilymphocytic and antierythrocytic antibodies are often found in patients with hematogenous tumors. According to statistics given by various workers [13] autoimmune hemolytic anemias are observed in 14.3-82.6% of patients with lymphoid tumors, and their frequency is particularly high in chronic lymphatic leukemia. Antierythrocytic antibodies [7] were found in 23 of 120 patients with lymphogranulomatosis. In some cases autoimmune hemolytic anemias have been observed in patients with Kaposi's sarcoma [6]. Autoantibodies against blood cells are also found in a wide variety of nonhematogenous tumors: carcinoma of the ovary [1], adenocarcinoma of the cecum [3], carcinoma of the cervix uteri [10, 14], epithelioma of the stomach [9], and so on. In some cases autoimmune antibodies were found initially in these patients but later the tumor itself was found [1, 6, 8, 9]. After removal of the tumor the autoimmune symptoms as a rule cleared up. Several surveys dealing with relations between autoimmune syndromes and tumor growth have been published [4, 5].

The object of this investigation was to study the dynamics of appearance of autoimmune antibodies during growth of various syngeneic transplanted tumors in mice.

EXPERIMENTAL METHOD

Experiments were carried out on mice of the CBA and 129 lines aged 4-5 months. The following syngeneic tumors were used: carcinoma of the cervix uteri RShM-5 at the 8th passage (the recipients were CBA females) and strains of teratocarcinomas maintained by a group under

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