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# EFFECT OF ADRENALIN ON INHIBITION OF MITOSIS BY A CHALONE IN EHRLICH'S ASCITES CARCINOMA

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In 1964 Bullough and Laurence [7] postulated that cell proliferation is under the control of a chalone-adrenalin complex. This suggestion was confirmed by data obtained during the study of the action of a chalone-containing preparation from vertebrate skin on cell division in the epidermis [8, 9]. It was also shown that hepatic chalone is activated by adrenalin in vitro [13]. At the same time, granulocytic, erythrocytic [12], lymphocytic [10], and even epidermal [11] chalones are effective even in the absence of adrenalin.

The study of the kinetic parameters of cell proliferation under the combined influence of adrenalin and chalone showed that interaction between these substances is expressed not only as weakening or strengthening of chalone effects, but also as a change in the duration of action of chalones [3, 4].

In the investigation described below the effect of adrenalin on inhibition of mitosis by a chalone-containing preparation (CCP) isolated from Ehrlich's ascites carcinoma (EAC) was studied with respect to the time interval between administration of these substances.

## EXPERIMENTAL METHOD

Experiments were carried out on 350 noninbred male albino mice weighing 18-20 g. The animals were kept on a daily schedule of 12 h daylight (6 a.m. to 6 p.m.) and 12 h of darkness, at a temperature of 18°C, and received food and libitum. All the animals were inoculated intraperitoneally with a diploid strain of EAC (the tumor was obtained from the Institute of Experimental and Clinical Oncology, Academy of Medical Sciences of the USSR). The tumor was used on the 5th day of its development (in the exponential phase of growth). The CCP was obtained from a 13-day tumor by alcoholic fractionation, which was first used for purification of chalones by Bullough et al. [6], in the modification of Savchenko et al. [5]. The lyophilized CCP was dissolved in physiological saline and injected in a dose of 15 mg per animal in a volume of 0.5 ml. The 0.1% adrenalin solution was diluted with physiological

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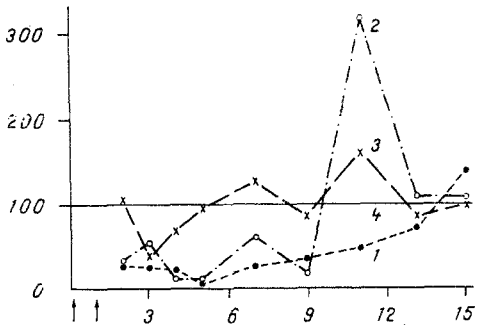


Fig. 1. Kinetics of changes in MI under the influence of adrenalin and chalone, with an interval of 1 h between their injection. Abscissa, time (in h) from beginning of experiment; ordinate, MI (in percent of control). 1-4) Groups of animals. Arrows indicate times of injection of adrenalin or physiological saline and CCP or physiological saline.

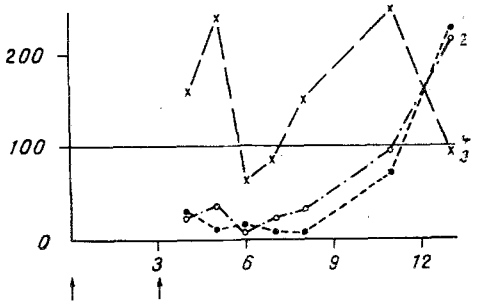


Fig. 2. Kinetics of changes in MI under the influence of adrenalin and chalone with interval of 3 h between their injection. Legend as to Fig. 1.

saline and injected in a dose of 1.5 μg/g body weight in a volume of 0.5 ml. Control animals received the same volume of physiological saline.

There were two series of experiments, starting at 9 a.m. In each series the animals were divided into four groups. Animals of group 1 received adrenalin and CCP, group 2 received physiological saline and CCP, group 3 received adrenalin and physiological saline, and mice of group 4 (control) received two injections of physiological saline. Injection of adrenalin or physiological saline always preceded injection of CCP.

In the experiments of series I CCP was injected 1 h after adrenalin and the animals were killed 2, 3, 4, 5, 7, 9, 11, 13, and 15 h after the beginning of the experiment. In series II, CCP was injected 3 h after adrenalin and the animals were killed 4, 5, 6, 7, 8, 11, and 13 h after the beginning of the experiment. At each point of the investigation 5-7 animals were used. Ascites fluid was taken for investigation and films prepared. Methyl alcohol was used as fixative. The films were stained with methylene blue, after preliminary hydrolysis in 1N HCl. From 3000 to 5000 cells were examined in films from each animal and the number of dividing cells was counted and the mitotic index (MI) calculated in promille. The results were subjected to statistical analysis by the Fisher–Student test. Differences were considered significant at the $p < 0.05$ level.

## EXPERIMENTAL RESULTS

As Fig. 1 shows, injection of CCP caused a significant decrease in MI during the first 8 h. This decrease in MI was maximal (by 92.6% compared with the control, $p < 0.001$) 4 h after injection of CCP. MI rose sharply (by 217% compared with the control, $p < 0.01$) 10 h after injection of the chalone, after which it again fell, to reach the control level after 11-14 h of action of CCP. Injection of adrenalin also was accompanied by an inhibitory

action of mitosis, but it began later than under the influence of CCP. Maximal inhibition occurred 3 h after the beginning of the experiment (MI was reduced by 62%, $p < 0.001$), but inhibition was weaker and shorter in duration than after CCP, and after 5 h the values of MI were virtually indistinguishable from the control. An increase in MI by 26% was observed 7 h after the beginning of the experiment, but this was not significant. After a second small decrease in MI after 9 h (by 17%, $p < 0.05$) there followed a much greater increase in MI (by 60%, $p < 0.01$) 11 h after injection of the hormone. After 13-15 h the values of MI were close to the control. If CCP was injected into the animals 1 h after adrenalin, the time course of cell division in EAC differed in character. A significant decrease in MI was observed during 13 h after the beginning of the experiment. The maximal inhibitory effect on mitosis (by 96.6%, $p < 0.001$) was observed 5 h after the experiment began, i.e., 4 h after injection of the chalone when CCP alone was given. Not until 15 h after the beginning of the experiment was an increase in MI by 39.5% compared with the control ($p < 0.01$) observed.

The results of the experiments of series I are evidence that the inhibitory effect of the partially purified chalone from EAC on mitosis was observed even in the absence of exogenous adrenalin. This confirms previous data obtained in vivo and in vitro for an aqueous tissue-specific extract isolated from EAC [2-4]. A marked synchronizing effect was observed 10 h after injection of CCP, evidence that cells held up in the $G_2$ phase of the mitotic cycle commence mitosis synchronously after removal of the chalone block. This also is in agreement with data for an aqueous extract isolated from EAC [1, 4]. Injection of adrenalin also caused a decrease in MI, but in this case the inhibitory effect did not begin until 3 h after injection of the hormone. Compared with the action of CCP it was weaker and shorter in duration, and synchronization of the entry of the cells into mitosis after their blockade in the $G_2$ phase was less well synchronized. The combined action of adrenalin and CCP prolonged the inhibitory effect on mitosis but just as in the case of CCP alone, the maximal decrease in MI took place 4 h after injection of the chalone. It must also be pointed out that in the case of the combined action of the hormone and chalone, their synchronizing effect on cell division was weak in the course of the experiment.

It follows from Fig. 2 that the kinetic parameters of the combined action of adrenalin and CCP, when injected with an interval of 3 h between them, were virtually indistinguishable from those resulting from the action of CCP alone. A significant decrease in MI by 27-29% was observed in both cases 1 h after injection of CCP. The maximal inhibitory effect on mitosis (by 94%, $p < 0.005$) following the combined action of adrenalin and CCP was found 4 h after injection of CCP. If CCP was injected without adrenalin, the maximal decrease in MI (by 90%, $p < 0.01$) was observed rather earlier — 3 h after injection of the CCP. A marked increase in MI (by 114-123%, $p < 0.05$) was found 10 h after injection of CCP in groups I and II. The kinetics of the action of adrenalin without CCP differed in character. MI 5 h after injection of the hormone was increased by 137% ($p < 0.05$). After 6 h MI was reduced by 40% ($p < 0.05$) and this was followed by a gradual increase in MI during the next 5 h after the beginning of the experiment; maximal values of MI were observed 11 h after injection of adrenalin. At this point of the investigation MI was 148% higher than in the control ($p < 0.05$). Values of MI after 13 h were virtually indistinguishable from the control. On the whole the character of the action of adrenalin in the experiments of series II did not differ from the results of series I. However, in the experiments of series II no significant inhibitory effect of adrenalin on mitosis could be detected, for judging by the results of series I, the effect was most marked 3 h after injection of the hormone, and the first observations were not made until after 4 h. The marked increase in MI after 5 and 11 h was most probably connected with the synchronizing action of the hormone after brief inhibition of cell division.

The results are evidence that the combined action of adrenalin and CCP in EAC depends on the time between injection of these substances. If CCP is injected 1 h after adrenalin, prolongation of the inhibitory action on mitosis by 4 h is observed, with a weak synchronizing effect on cell division in EAC during the experiments. If CCP is injected 3 h after adrenalin, the combined action of these substances is the same as the action of CCP alone.

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# PREPARATION OF A STABLE RAT EMBRYONIC FIBROBLAST LINE BY TRANSFECTION WITH DNA FROM A PLASMID CONTAINING POLYOMA VIRUS LARGE T ANTIGEN

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Transfection of plasmids containing early genes of DNA-containing viruses (the large T antigen of polyoma and SV40 viruses, the EIA region of adenoviruses), and also genes of RNA-containing viruses (myc of virus MC29) into a primary culture of rat embryonic fibroblasts leads to changes in the properties of these cells, the most important of which is that the cells acquire capacity for unlimited division. In some cases dependence of the cells on serum factors is reduced in these lines and sensitivity to the transforming action of viral and activated cellular oncogenes is considerably increased [4, 7, 10, 11]. This last factor may be of great importance for the identification of transforming genes in human tumors by transfection of genetic material of tumor cells into recipient cells. Usually NIH3T3 cells, a culture of mouse embryonic fibroblasts, immortalized spontaneously, are used as recipients. By means of this system, activated oncogenes belonging chiefly to the ras family have been identified in many tumor cells [2]. These cells are perhaps permissive for manifestation of the transforming activity of this family of oncogenes. There is therefore an urgent need for the creation of new models appropriate for revealing the action of other oncogenes, in order to detect them in human and animal tumor cells. In particular, the REF-1 cell line has now been obtained after transfection of primary rat embryonic fibroblasts with DNA from the early region of simian adenovirus SA7 [1], which is used as the recipient system for transfection with oncogene-containing plasmid.

The aim of this investigation was to obtain an immortalized line of normal rat fibroblasts after transfection of a plasmid containing sequences coding the large T antigen of polyoma virus.

## EXPERIMENTAL METHOD

Plasmid pPyLT1 was generously provided by Dr. Cuzin (France). It was constructed on the basis of plasmid pBR322 and contained an insert of sequences of the polyoma virus genome coding the large T antigen at BamH1 restriction sites [8].

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