# REACTION OF CELL DIVISION IN EHRLICH'S MOUSE ASCITES TUMOR TO ADRENALIN

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A characteristic feature of the action of adrenalin on several tissues is brief inhibition of cell division [2, 4, 5, 9]. This is due, it has been suggested, to the development of a $G_2 \rightarrow M$ block in the cell cycle [6, 8, 10, 11]. However, certain tissues do not respond to adrenalin by inhibition of cell division [1, 3, 7, 8]. The action of adrenalin on cell division also varies in tumors such as, for instance, in Ehrlich's ascites tumor. Not only inhibition, [12] and stimulation [4] of mitotic activity have been observed under the influence of adrenalin, but also no change [1]. The reasons for this action of adrenalin have not been adequately studied, and for that reason the role of adrenalin in the regulation of cell division in tumors requires further investigation.

The object of this investigation was to study cell division in Ehrlich's ascites tumor after injection of different doses of adrenalin into mice.

## EXPERIMENTAL METHOD

Experiments were carried out on 200 noninbred male albino mice weighing between 18 and 20 g and aged 1.5-2 months. The animals were kept under conditions of equal light and darkness (12 h: 12; light from 6 a.m. to 6 p.m.). An ascites tumor was transplanted by intraperitoneal injection of 0.2 ml of ascites fluid, containing 10 million tumor cells. The experiments were carried out on mice with ascites of 5 days' duration.

Adrenalin hydrochloride in doses of 1, 1.5, and 2 $\mu$g/g body weight in a volume of 0.2 ml was injected intraperitoneally into the animals, always at 12.30 p.m. Physiological saline was injected into the control animals. The mice were killed 0.5, 1, 2, 3, and 4 h after injection of the substances. At each stage of the experiment 8 to 10 animals were used.

Films of the tumor were fixed with methanol and stained with methylene blue, after preliminary hydrolysis in 0.1 N HCl at 56°C. To assess proliferative activity the mitotic index (MI) and the index of phases of mitosis (PrI, MetI, AnI, and TelI) were calculated and expressed in promille. In each case 5000 cells were counted. The significance of differences between the values obtained was assessed by the criteria of Student and Fisher. Differences were considered to be significant at the $P \leq 0.05$ level.

## EXPERIMENTAL RESULTS

Significant inhibition of cell division in Ehrlich's ascites tumor was observed 30 min after injection of all doses of adrenalin (Fig. 1). Maximal inhibition of mitosis (by 52.7%; $P < 0.001$) occurred after the action of a dose of 1 $\mu$g, and minimal inhibition (by 22%; $P < 0.05$) after injection of 2 $\mu$g adrenalin. A dose of 1.5 $\mu$g inhibited mitotic activity by 45.3% ($P < 0.001$). After 1 h of the experiment the number of dividing cells continued to be reduced by 36% ($P < 0.001$), 47.8% ($P < 0.001$), and 21.3% ($P < 0.02$) after injection of the hormone in doses of 1, 1.5, and 2 $\mu$g, respectively.

Significant inhibition of cell division continued 2 h after administration of adrenalin in doses of 1.5 $\mu$g (by 41.1%; $P < 0.001$) and 2 $\mu$g (by 22.5; $P = 0.05$). MI 3 h after the beginning of the experiment, with doses of 1.5 and 2 $\mu$g was lower by 51.5 and 63.1%, respectively ($P < 0.001$). Inhibition of MI amounted to 58 and 42%, respectively ($P < 0.01$ and less than 0.05) 4 h after administration of these doses.

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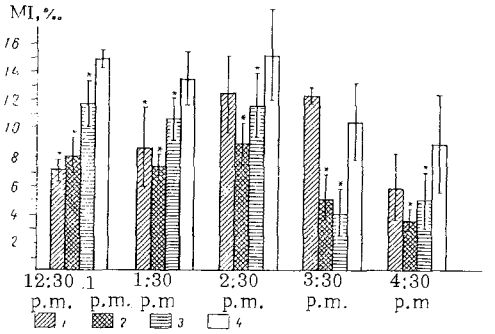


Fig. 1. MI in Ehrlich's ascites tumor cells after injection of different doses of adrenalin. Abscissa, time of day; ordinate, MI (in $^0/_{00}$. 1, 2, 3) Doses of adrenalin per gram body weight 1, 1.5, and 2 $\mu$g, respectively; 4) control.

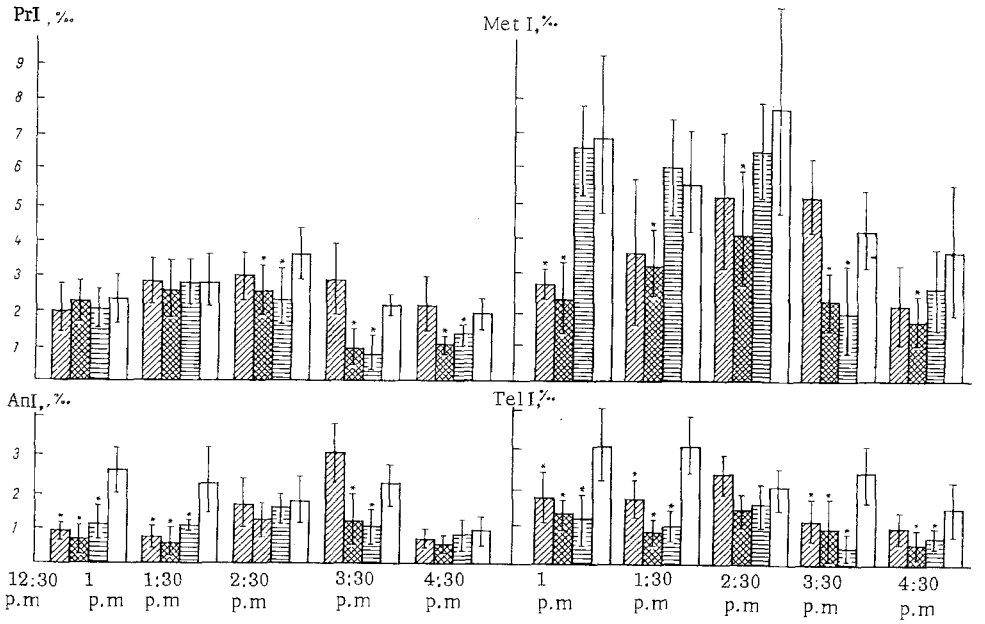


Fig. 2. Indices of phases of mitosis in Ehrlich's ascites tumor after injection of different doses of adrenalin into mice. Ordinate, indices of phases of mitosis (in $^0/_{00}$); remainder of legend as to Fig. 1.

All the doses of adrenalin used thus had an inhibitory effect on cell division in Ehrlich's tumor, but with a dose of 1 $\mu$g this effect lasted only 1 h and was followed by normalization of MI, whereas with doses of 1.5 and 2 $\mu$g it was observed throughout the experiment.

The study of phase indices (Fig. 2) showed that the prophase index (PrI) did not change significantly after all doses of the hormone during the first hour of the experiment. A significant decrease in PrI was observed, however, 2 h after injection of adrenalin in doses of 1.5 and 2 $\mu$g ($P < 0.02$ and $< 0.001$), and this continued until the end of the experiment ($P < 0.001$). A significant fall in the metaphase index (MetI) was found 30 min after administration of 1 and 1.5 $\mu$g adrenalin ($P < 0.01$); the decrease continued throughout the experiment after a dose of 1.5 $\mu$g adrenalin ($P < 0.001$). With a dose of 1 $\mu$g, MetI was restored to normal 1 h after the beginning of the experiment, but with a dose of 2 $\mu$g it again fell after 4 h of the experiment ($P < 0.01$). Values of the anaphase (AnI) and telophase (TelI) indices after 30 min and 1 h were significantly below the control ($P < 0.001$) when all doses were used and the normal level was restored by the end of the second hour of the experiment. After 3 h a marked wave of inhibition of AnI and TelI was again observed following administration of doses of 1.5 and 2 $\mu$g ($P < 0.01$ and less than 0.001). At this time a decrease in TelI also was found after administration of 1 $\mu$g of the hormone ($P < 0.01$). Values of AnI 4 h after the beginning of the experiment did not differ from the control, whereas TelI was reduced only as a result of the action of doses of 1.5 and 2 $\mu$g ($P < 0.01$ and $< 0.02$).

The results show that mitotic activity in cells of Ehrlich's ascites tumor in the early stages of the experiment (after 0.5-1 h) was reduced mainly on account of anaphases and telophases, after the action of all doses, and of metaphases after administration of 1 and 1.5 μg of adrenalin. In the later stages of the experiment inhibition of mitosis through the action of doses of 1.5 and 2 μg took place as a result of depression of all phases of mitosis.

The changes found in mitotic activity in Ehrlich's ascites tumor as a result of injection of different doses of adrenalin are evidence that the tumor responds by inhibition of mitosis to administration of the hormone, and so preserves the adrenoreceptor sensitivity characteristic of normal tissue.

However, the degree of inhibition was less marked although it lasted longer than, for example, in stratified squamous epithelium [1, 2, 4-6, 9]. The shorter duration of inhibition of mitosis as a result of exposure to adrenalin in a dose of 1 μg compared with the other doses used (1.5 and 2 μg) was evidently connected with differences in their action on the individual phases of mitosis. Whereas after administration of 1 μg adrenalin, a decrease in TrI was not observed at any period of the experiment, and a decrease in MetI likewise was not found 1 h after the beginning of the experiment, with other doses of the hormone both these indices were reduced in the late stages of the experiment. Changes in AnI and TelI indices in the course of the experiment were more similar after administration of all doses of adrenalin.

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