THE ACTION OF TUMOR ANTISERUM ON THE MITOTIC ACTIVITY OF AN EHRLICH'S ADENOCARCINOMA DURING PHYSIOLOGICAL EFFORT BY THE ANIMAL

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It has previously been shown [1, 4-6] that tumor antiserum depresses mitotic activity of the tumor cells of Ehrlich's adenocarcinoma, but has no essential effect on the intensity of division of the cells of the corneal epithelium [6] nor of the intestinal crypts [1]. It must be mentioned that in these experiments the injections of serum were given when the animals were in a normal condition and were being kept in the usual way.

It is also known that the mitotic activity of a tumor is altered when various factors act on the body [2, 3]. Since work by Bullough [12] has shown that the mitotic activity of the epidermis of normal mice is diminished by an obligatory increase in their motor activity, we thought it of interest to ascertain whether such a relationship between the physiological state of the animal and the mitotic activity was also typical of a tumor, and also to determine the effect of tumor antiserum on the intensity of division of tumor and normal tissue cells, when administered during motor activity by the animals.

In the present work we accordingly studied the relationship between the mitotic activity of a subcutaneous Ehrlich's adenocarcinoma and the enforced performance of increased motor activity by the animals, and the effect of tumor antiserum on the mitotic activity of this tumor when given under identical experimental conditions. In the second part of the investigation we studied the intensity of division of the corneal epithelium and the intestinal crypts, as well as the tumor cells in the same experimental animals.

EXPERIMENTAL METHOD

In the first experiment we used 28 male white mice. The experiment took place during the morning (from 10:45 A.M) of the 9th day after subcutaneous transplantation of the tumor. Twenty mice were placed in a smooth-bottomed box, fixed to an apparatus which produced a uniform to-and-fro movement, to which the mice were subjected for 30 minutes; as a result they were kept in a state of increased motor activity during this time. Two groups of control animals (with 4 mice in each) were kept under ordinary conditions.

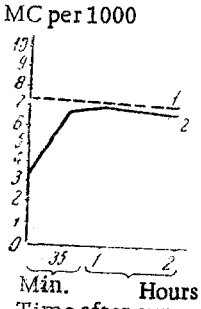
In order to determine the trend of the changes in mitotic activity of the tumor after cessation of the increased motor activity, the 20 experimental mice were divided into 4 groups with 5 mice in each. Each group of animals was killed at different times: group I – as soon as the experiment ended, group II – after 35 minutes, group III – after 1 hour and group IV – after 2 hours. The first control group of mice was killed at the same time as the first experimental group, and the second control group at the same time as the fourth experimental group. The tumors were fixed in Zenker's fluid, stained by Feulgen's method, and counterstained with light green.

The second experiment was carried out on 40 male white mice, which were divided into 4 groups, each of 10 animals. An Ehrlich's adenocarcinoma was implanted subcutaneously in the dorsal region of all the ani-

Mitotic Coefficient in a Subcutaneous Ehrlich's Adenocarcinoma after Injection of Tumor Antiserum

Mouse no.	Serum + motor activity (group I)	Serum under ordinary conditions (group II)	Motor activity alone (group III)	No additional agent (group IV)
1	2.26	3.82	6.15	6.74
2	5.52	4.40	6.33	7.26
3	5.94	4.57	6.45	7.32
4	6.18	4.70	7.39	7.40
5	6.24	5.01	7.49	8.00
6	6.30	5.37	9.24	8.57
7	7.84	5.52	9.77	8.60
8	7.98	5.78	10.16	8.98
9		6.20	12.69	10.62
10	Mouse died	6.39		17.24
Mean MC	6.03	5.18	8.14	9.07

mals. The mice of group I and III were kept in a state of increased motor activity for 30 minutes in the morning and evening for 4 days, starting on the 7th day after implantation of the tumor. Just before the beginning of the experiment in the evening (at 4 P.M.) on the 8th, 9th and 10th day of development of the tumor, the group I mice received an injection of 0.5 ml of serum beneath the skin of the dorsal region at a short distance from the tumor. The group II mice, kept under the usual conditions, received injections of serum by the same method and at the same times on the same days, but the group IV animals received no extra treatment in any form.



Mitotic coeficient in Ehrlich's adenocarcinoma after the cessation of increased motor activity by the animals. 1) control; 2) experiment.

The sera which we injected into the mice of groups I and II were obtained by intravenous immunization of chinchilla rabbits and tested by the complement fixation reaction at 37°. The sera reacted with tumor antigen in a dilution of 1:400 +++, and with antigens from liver and spleen in lower dilutions (1:200 ++ or 1:100 +++).

The experimental animals of all groups in the second experiment were sacrificed at 11 hours on the morning of the day following the last injection of serum. It was useless to sacrifice them sooner after the injection of serum since, according to the findings of P. I. Tseitlin and M. M. Kapichnikov [10], tumor antiserum increases the intensity of renewal of the protein tumors for roughly 20 hours after injection.

The tumors and intestine were fixed in Carnoy's fluid, and the cornea in a 5% solution of glacial acetic acid in 70° alcohol. All the material was stained by Carazzi's hematoxylin.

The mitoses in the mouse tumors in the first experiment were in each case counted in 500 fields of vision (about 100 000 cells from each tumor were examined). In the second experiment in the number of mitoses in the tumor was counted in 300 fields of vision (roughly 70 000 cells were examined), and the corneas – on both sides of two mutually perpendicular lines drawn in ink on the cover glass of the preparation (approximately 35 000 cells), and in the intestine – in 50 crypts, cut longitudinally (about 3500 cells). After counting the mitoses, we calculated the mitotic coefficient MC (the ratio between the dividing cells and the total number of cells in this preparation examined, per 1000) and the coefficient K (the ratio between the sums of the early and late phases of division). The experimental results were treated statistically by the Fisher-Student method.

EXPERIMENTAL RESULTS

First experiment. Counting the mitoses in the tumors in the mice of the first experiment showed that the mean percentage of the various phases of division in the experimental and control groups varied between narrow limits (P ≈ 4-7%; M ≈ 77-83%; A ≈ 5-8 % and T ≈ 7-11%), just as did the coefficient K (from 5 to 7).

The changes in the value of MC at different times after the end of the experiment are shown in the form of curves on the figure.

We see that the value of MC in the Ehrlich's adenocarcinoma was least (3.22 per 1000) in the experimental mice sacrificed immediately after cessation of increased motor activity; 35 minutes later the value of MC increased sharply to 6.92 per 1000 (the difference between the MC of this group and of the preceding group is significant $p = 0.004$). At later periods of investigation (after one and two hours) the value of MC remained at roughly the same level (7.03 per 1000 and 6.95 per 1000).

In the first control group of mice MC was equal to 7.31 per 1000, and in the second, 7.20 per 1000. The difference between the MC of mice sacrificed immediately after the end of the experiment (3.22 per 1000) and the MC of the mice of the first control group (7.31 per 1000), sacrificed at the same time as this experimental group, was statistically ($p = 0.015$). The mitotic activity in the subcutaneous Ehrlich's adenocarcinoma thus fell sharply after the end of the increased motor activity by the animals and was restored to the control level 35 minutes later.

The experimental results were in agreement with the findings of Bullough [12], obtained for normal tissue cells of animals exposed to similar conditions, and they suggest that the mitotic activity of tumor cells is to some extent dependent on the physiological state of the animal, which is also characteristic of the cells of normally derived tissues [7-9, 11].

Second experiment. During the period of the experiment one mouse from group I died from other causes; in a second animal the mitotic activity of the tumor cells could not be examined on account of its small dimensions (that of a grain of wheat). In one mouse of group III the mitotic activity of the tumor cells could not be investigated because of its extensive necrosis.

Counting the mitoses in the tumors of the remaining mice showed that the mean percentage of the various phases of division varied in all the groups studied between narrow limits ($P \approx 3$-6%; $M \approx 75$-80%; $A \approx 3$-4%; $T \approx 12$-16%), just as did the coefficient K (from 4 to 5%). The values of the mitotic coefficient for the tumors of the experimental animals of all the groups are given in the table.

It can be seen from the figures given in the table that the smallest, and roughly equal, mean values of MC were observed in the tumors of the group I animals, in which serum was injected in conjunction with motor activity (MC = 6.03 per 1000), and in those of the second group, in which serum was given under ordinary conditions (MC = 5.18 per 1000). In the mouse tumors of groups III and IV (no serum injected), the MC was considerably higher and also roughly equal (group III – 8.41 per 1000, group IV – 9.07 per 1000). The difference between MC for groups I and III and for groups I and IV was significant ($p = 0.024$), as also was that between groups II and III and between groups II and IV (in the first case $p = 0.000$ and in the second, $p = 0.002$).

As a result of the counting of the mitoses in the corneal epithelium of the experimental mice it was found that no essential differences were present in the proportions of the phases of division between the groups ($P \approx 15$-28%; $M \approx 33$-46%; $A \approx 6$-8%; $T \approx 22$-37%), just as in the case of the value of the coefficient K (≈ 2). The mean values of the MC in the corneal epithelium of the mice of all four groups varied within narrow limits (group I-4.02 per 1000, II-3.57 per 1000, III-3.19 per 1000 and IV – 3.16 per 1000); the difference between them was not significant.

Counting the mitoses in the crypts of the small intestine of the mice showed that the mean percentage of the different phases of division in all four groups differed only slightly (P 6-9%; M 53-60%; A 4-6%; T 26-35%), just as did the ratio between the early and late phases of division ($K \approx 2$). The mean values of the MC differed slightly in the various groups (group I – 28.82 per 1000, II – 34.20 per 1000; III – 32.85 per 1000 and IV – 31.67 per 1000), but the difference between the MC of all the groups was not significant.

The results of the second experiment thus showed that the mitotic activity in the tumors of mice sacrificed on the day following increased motor activity was unchanged from that in control animals kept under ordinary conditions, as might have been expected from the results of the first experiment.

From an analysis of the results of the second experiment it may also be concluded that tumor antiserum, when injected for a period of 3 days into animals kept under unaccustomed conditions (periodic enforced increased motor activity), possesses the same depressing action on the intensity of division of the cells of an Ehrlich's adenocarcinoma as when injected in ordinary conditions. At the same time, when tumor antiserum is injected

into mice in which an Ehrlich's adenocarcinoma is developing subcutaneously, kept under ordinary conditions or on repeated physiological effort (increased motor activity), it caused no essential changes in the corneal epithelium nor the crypts of the intestine of the experimental animals.

SUMMARY

As demonstrated on 68 white mice the mitotic activity of Ehrlich's tumor becomes temporarily reduced directly after intense motor activity by the animals. Anti-tumor serum administered to mice kept in the ordinary way or engaged in intensive motor activity depressed the mitotic activity of the tumor cells but did not exercise any significant effect on the intensity of cellular division of the corneal epithelium nor on that of the intestinal crypts in the same experimental animals.

LITERATURE CITED

[1] V. N. Dobrokhotov, Byull. Eksptl. Biol. i Med., No. 3, 59-62 (1955).

[2] V. V. Kozlov, Proceedings of the Fourth Conference of Senior Students and Interns of the First Leningrad Medical Institute, p.12, 1953 [In Russian].

[3] Yu. N. Mol'kov, Certain Relationships between the Cell Division, Growth and Metastasization of Tumors and Trauma. Candidate's dissertation, Moscow, 1952 [In Russian].

[4] S. V. Sukhorukikh, Problems of the Immunology of Normal and Malignant Tissues, pp.85-93, Moscow, 1956 [In Russian].

[5] S. V. Sukhorukikh, Proceedings of a Scientific Conference on Problems of Methods in the Immunology of Malignant Naoplasms, p.26, Moscow, 1956 [In Russian].

[6] S. V. Sukhorkikh, Byull. Eksptl. Biol. i Med., No. 7, 83-86 (1959).*

[7] L. A. Utkin, Relationship between the Form of Cell Division and the Physiological State of the Animal. Candidate's dissertation, Moscow, 1951.

[8] L. A. Utkin and L. P. Kosichenko, Byull. Eksptl. Biol. i Med., 41, 4, 62-66 (1956).*

[9] L. A. Utkin and L. P. Kosichenko, Byull. Eksptl. Biol. i Med., 41, 6, 65-69 (1956).*

[10] P. I. Tseitlin and M. M. Kapichnikov, Byull. Eksptl. Biol. i Med., No. 11, 60-62 (1955).

[11] L. I. Chekulaeva, Doklady Akad. Nauk SSSR, 106, 2, 371-373 (1956).

[12] W. Bullough, Proc. Roy. Soc. London. 1958, ser. B., v.135, p.233-242.

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