EXPERIMENTAL BIOLOGY

THE EFFECT OF ADRENALIN ON THE DIVISION OF ASCITIC CANCER CELLS AND OF NORMAL EPITHELIAL CELLS

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(Received July 24, 1958. Presented by Active Member AMN SSSR V. N. Chernigovskii)

In our previous research we showed that painful stimulation of an animal leads to a lowering of the mitotic activity of a subcutaneously transplanted Ehrlich's carcinoma [8] but causes no perceptible changes in the intensity of division of cells of an ascitic form of carcinoma [9].

A reactive decrease in the mitotic activity of a solid tumor has also been observed by other workers: Yu. N. Mol'kov — by excitation of mice with a sound stimulus [14], S. V. Sukhorukikh — during "motor excitation" of animals [16] and S. N. Aleksandrov — during mechanical stimulation alone by binding rats and mice to the bench [2]. It is interesting that, under the same conditions, Holmes [20] observed delay in the uptake of radioactive phosphorus by the nucleic acids of the tumor cells. S. N. Aleksandrov [2] points out, in addition, the significant fall in the number of mitoses in the cancer cells of an ascitic variety of carcinoma during painful stimulation of mice by the infliction of a burn on the skin of the thigh. He also observed inhibition of cell division in an ascitic tumor transplanted intraperitoneally during irradiation of the brain of animals. The lowering of the intensity of cell division in a malignant tumor is explained, as the author postulates [2], by the increased secretion of adrenalin as the result of excitation of the higher divisions of the sympathetic nervous system and of neurogenic stimulation of the medullary layer of the adrenals.

We know that during excitation of an animal, an increased concentration of adrenalin or of an adrenalin-like substance develops in the blood [12, 17]. The inhibitory action of this hormone on the processes of cell division has been demonstrated by a number of investigations in many normal tissues and organs, among these our own observations [4, 5, 10, 18, 19]. There is every reason to believe that adrenalin is one of the humoral links in the mechanism of regulation of cell division in the body.

It was interesting, in this connection, to find out whether adrenalin had any effect on the division of cells of pathologically abnormal tissues undergoing malignant change. This is all the more essential because many research workers now regard tumor growth as the result of the escape of normal tissue, of its metabolic processes and its cell division from the neurohumoral control, or of a considerable weakening of this control.

Investigations carried out with the aim of examining the effect of adrenalin on the growth of malignant neoplasms have given divergent results and the authors' conclusions are at times contradictory. The majority of specialists working in this field conclude, however, that adrenalin has a marked effect on tumor growth [1, 6, 11, 13], but under these circumstances mitotic activity was not investigated.

EXPERIMENTAL METHOD

We studied the effect of adrenalin on ascitic carcinoma in mice by the direct determination of changes in the division of the cancer cells. The tumor was transplanted intraperitoneally by injection of 0.4 ml of the ascitic form of Ehrlich's carcinoma. In view of the fact that adrenalin is unstable and is rapidly inactivated in the body, several injections were given to the animals. The hormone was injected subcutaneously or

TABLE 1

Mitotic Activity of the Cells of an Ascitic Carcinoma after the Subcutaneous Injection of Adrenalin, in a dose of 0.2 ml 3 times in the Course of 1 Hour

Time of taking material	Phases of mitosis				Atypical forms	Total number of mitoses
	P	M	A	T		
Animal No. 1						
Before injection of (control)	11	20	6	8	0	45
15 minutes after 3rd injection	14	29	6	9	4	62
Animal No. 2						
Before injection of adrenalin	9	21	10	12	1	53
15 minutes after 3rd injection	18	52	6	10	2	88
Animal No. 3						
Before injection of adrenalin	24	48	8	7	0	87
15 minutes after 3rd injection	26	68	14	15	2	125

Note. In this and in the subsequent tables, P, M, A and T designate pro-, meta-, ana- and telophase respectively.

intraperitoneally in a dose of 0.2 ml of a 1:20,000 solution (0.01 mg) for various periods of time. Ascitic fluid was taken from each animal before the injection of adrenalin (control) and several times afterwards. In fixed and stained films we counted 5000 cells and estimated the number of mitoses in each film. We tested altogether 42 samples of ascitic carcinoma. Furthermore, for purposes of comparison, we also determined the mitotic activity of the normal epithelium of the same animals.

EXPERIMENTAL RESULTS

In response to the subcutaneous injection of the hormone in a dose of 0.2 ml 3 times at intervals of 30 minutes, no decrease was found in the total number of mitoses in the carcinoma. On the contrary, as will be seen from Table 1, there was even a slight apparent increase in the number of dividing cells. In this experiment the material was taken 15 minutes after the third injection.

In the corneal epithelium of these mice (Table 2) a sharp suppression of mitotic activity could be observed. In some films not a single cell in a state of karyokinesis could be found in 100 fields of vision. (In the corneas of control animals an average of 150-200 mitoses was usually counted in this area of the film.)

In another group of animals adrenalin was injected 4 times in the course of 4 hours at equal intervals of time. The first sample of ascitic fluid was collected 1 hour after the first injection, the second 15 minutes after the third injection of the hormone, the third 15 minutes after the fourth injection, i.e., 4 hours 15 minutes after the first. The results obtained were the same, but were still more clearly expressed (Table 3). In this case too, a tendency was observed for the total number of mitoses to increase, especially on account of the metaphases.

On the following day the animals of the last group were again given adrenalin: 4 injections subcutaneously in the course of 2 hours (as always, ascitic fluid was taken first as a control). Material was taken 5 hours after the first injection. There was no doubt in the results obtained even at this stage.

TABLE 2

Sharp Fall in the Mitotic Activity of the Corneal Epithelium after Subcutaneous Injection of Adrenalin to Mice with Ascitic Carcinoma (corneas of control animals showed 150-200 mitoses per 100 fields of vision)

Animal No.	P	M	A	T	Total number of mitoses
1	0	0	0	0	0
	0	0	0	0	0
2	1	0	0	1	2
	0	1	1	0	2
3	0	0	0	1	1
	0	1	0	0	1

It can be seen from Table 4 that injection of the hormone under these conditions of the experiment leads, in fact, to an increase in the number of mitoses in the ascitic carcinoma, this taking place mainly as the result of an increase in the number of early stages of division, and especially of metaphases. In the corneal epithelium of these animals, on the other hand, complete suppression of cell division was observed; hardly any mitoses were found.

This experiment was performed 24 hours later on the same mice as the previous one, and it could therefore be observed that in the ascitic carcinoma films, after the increase in the number of mitoses resulting from adrenalin (Table 3), their number on the following day fell sharply (see control in Table 4).

We obtained similar results after intraperitoneal injection of adrenalin (Table 5). The hormone was injected twice into the mice of this group (the second injection was given $1\frac{1}{2}$ hours after the first). Samples were taken $1\frac{1}{2}$ and 3 hours from the moment of the first injection and in addition, on the following day; after this, the animal received a further injection of hormone (3 injections in the course of 1 hour at intervals of 30 minutes), and material was taken $1\frac{1}{2}$ hours after the first of the 3 injections. The trend of the changes in the processes of division is illustrated in Table 5. After injection of adrenalin directly into the ascitic fluid, i.e., intraperitoneally, the number of mitoses among the

TABLE 3

The Effect of Adrenalin on Division of Ascitic Carcinoma Cells after Subcutaneous Injection of a Dose of 0.2 ml 4 Times in the Course of 4 Hours

Time of taking material	P	M	A	T	Atypical forms	Total number of mitoses
Animal No. 1						
Before injection of adrenalin	20	30	8	19	2	79
15 minutes after: 1st injection	22	41	4	15	1	83
3rd injection	23	50	4	9	2	88
4th injection	43	136	6	12	3	200
Animal No. 2						
Before injection of adrenalin	25	21	11	22	2	81
15 minutes after:						
1st injection	36	28	11	20	1	96
3rd injection	25	36	8	12	1	82
4th injection	27	70	4	8	1	110
Animal No. 3						
Before injection of adrenalin	12	9	4	4	0	29
15 minutes after: 1st injection	8	16	1	6	0	31
3rd injection	10	22	2	6	3	43
4th injection	12	32	2	4	2	52
Animal No. 4						
Before injection of adrenalin	14	15	12	24	2	67
15 minutes after:						
1st injection	—	—	—	—	—	—
3rd injection	21	48	10	22	2	103
4th injection	—	—	—	—	—	—

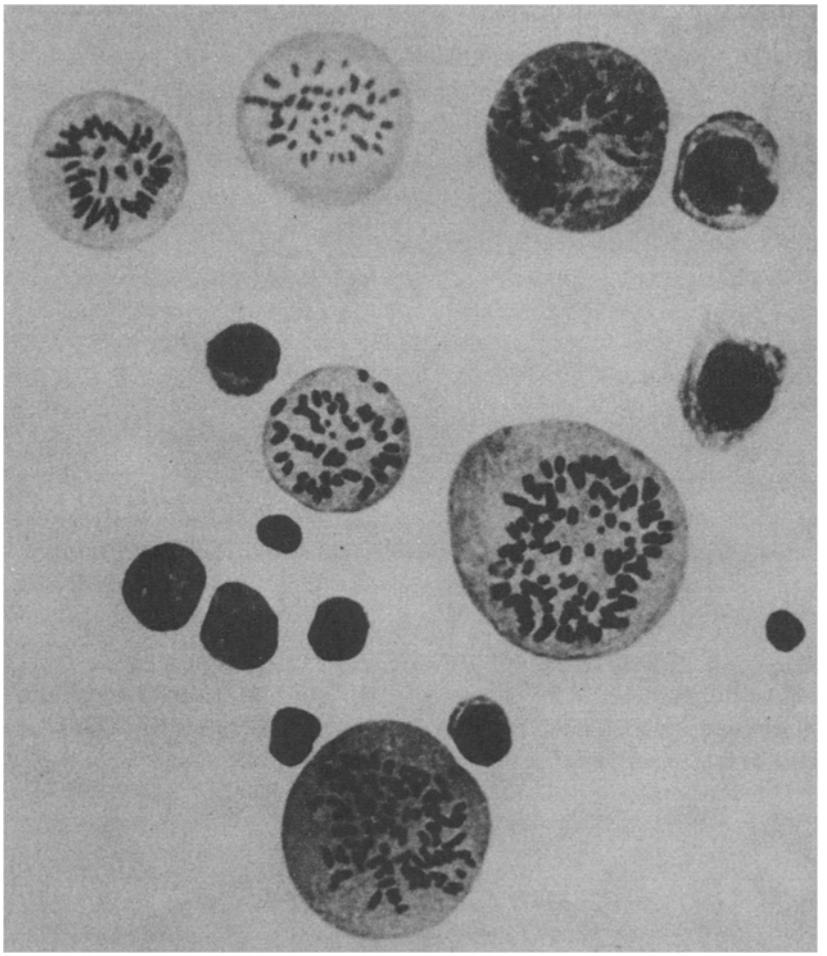
TABLE 4

Changes in the Number of Mitoses in an Ascitic Carcinoma after Subcutaneous Injection of Adrenalin in a Dose of 0.2 ml 4 Times in the Course of 2 Hours

Time of taking sample from the moment of the 1st injection	P	M	A	T	Atypical forms	Total number of mitoses
Animal No. 1						
Before injection of hormone	12	9	0	7	0	28
5 hours afterwards	26	92	14	18	5	155
Animal No. 2						
Before injection of hormone	10	2	2	2	0	16
5 hours afterwards	23	24	4	6	6	63
Animal No. 3						
Before injection of hormone	9	3	2	1	0	15
5 hours afterwards	27	32	3	7	4	73

Changes in the Mitotic Activity of Ascitic Carcinoma Cells after Intraperitoneal Injection of Adrenalin

Time of taking material	P	M	A	T	Atypical forms	Total number of mitoses
Animal No. 1						
Before injection of adrenalin	3	9	4	10	1	27
After 1st injection	5	15	3	5	1	29
After 2nd injection	12	40	6	10	3	71
On the following day	15	82	16	28	3	144
Animal No. 2						
Before injection of adrenalin	10	47	9	18	2	86
After 1st injection	18	60	18	26	1	123
After 2nd injection	22	47	12	26	5	112
On the following day	13	57	22	31	3	126
After 3 injections, each of 0.2 ml	27	110	28	25	5	195
Animal No. 3						
Before injection of adrenalin	7	14	6	10	0	37
After 1st injection	—	—	—	—	—	—
After 2nd injection	21	55	18	33	5	132
Animal No. 4						
Before injection of adrenalin	10	21	5	10	1	47
After 1st injection	10	17	8	9	2	46
After 2nd injection	23	43	10	19	2	97
On the following day	21	56	7	8	3	105
After 3 injection, each of 0.2 ml	30	123	17	41	4	219



A collection of mitoses at early stages of division, pro- and metaphases, in ascitic carcinoma cells after administration of adrenalin to a mouse.

carcinoma cells increased in all the animals, as may be seen in Table 5; this was also perceptible after the first two injections.

On the following day the number of dividing cells did not fall, as was observed when the hormone was injected subcutaneously, but continued at the raised level; this was probably due to the stronger influence of the adrenalin on the ascitic cancer cells when it was injected directly into the peritoneal cavity.

After a further injection of the hormone was given on the following day, the number of mitoses increased to an even greater degree. Mitoses were completely absent from the corneal epithelium of these animals, as in the previous experiments. Thus the adrenal medullary hormone, in the doses which we used, caused obvious changes in the mitotic proliferation of the tumor cells.

In all cases of use of adrenalin, the ascitic carcinoma films showed an increase in the number of cells in a state of karyokinesis which could be seen. A noteworthy feature was that after subcutaneous injection of this hormone, this increase always took place on account of the prophases and especially the metaphases; under these circumstances the number of late stages of karyokinesis usually showed no perceptible change. When adrenalin was injected directly into the ascitic carcinoma, a rise was observed in the numbers of all the phases of division. The changes in division in this case were more profound; this was also confirmed by the fact that the increased number of mitoses was also maintained on the following day. In our experiments we believe that adrenalin changed the processes of division in the normal tissue and the malignant tumor in the same direction but to a different degree. The tumor tissue was less sensitive to adrenalin. In the corneal epithelium of the experimental

animals the cells completely lost their ability to divide, but the mitoses which were taking place succeeded in completing themselves, whereas in the tumor the process of proliferation continued, although at a reduced speed. The course of mitoses itself was especially retarded, since the changes which took place in the cancer cells in the period of interkinesis, under the action of adrenalin, mainly affected mitosis, as the more vulnerable stage in the ontogenesis of the cell. Division, it must be thought, was even arrested, especially at the metaphase stage. This led to an increase in the number of visible mitoses in the ascitic carcinoma films.

Atypical mitoses with short, scattered chromosomes and very large, swollen dividing cells with vacuolated protoplasm were seen apparently slightly more often in the ascitic carcinoma cells after the action of adrenalin (see figure).

After giving single injections of smaller doses of adrenalin, I. A. Alov [3] observed no perceptible changes in division of carcinoma cells, whereas in the corneal epithelium he found severe suppression of division. In our investigations the tumor tissue showed lower reaction than did normal tissue to various stimuli [8, 10]. This all undoubtedly suggests that tumor cells have escaped to some degree from the neurohumoral control of the cell division of the body; they have becone less sensitive to the factors and mechanisms responsible for this control, although they have not ceased altogether to react to them. It is this latter circumstance which must explain the numerous reports in the literature of the influence of the nervous system and of the endocrine organs on tumor growth.

Our investigations show that one of the main humoral regulators of cell division in the body is adrenalin, and that after its repeated administration, it quite obviously has an effect on proliferation of cancer cells. This is also suggested by the observations of other authors [1, 2].

This peculiarity of the reactivity of tumor cells which we have described cannot be reduced to a simple quantitative phenomenon, i.e., to their lower degreee of sensitivity. Disturbance of the course of mitosis itself and the accumulation of metaphases (which was never observed in the corneal epithelium) show that there is also a qualitative aspect of the reactivity of carcinoma cells which distinguishes them from normal tissue.

SUMMARY

Experiments were performed on white mice with Ehrlich's ascitic carcinoma. 1: 20,000 adrenalin solution in a dose of 0.2 ml was repeatedly injected into the animals at different time intervals.

Under the effect of adrenalin a sharp depression of mitotic activity occurred in the corneal epithelium, and mitoses were absent almost completely. Conversely, a considerable increase in the number of cells, especially at the metaphase stage was noted in the carcinoma in these animals. The latter may evidently be explained by the diminished intensity of cellular reproduction as the result of a more prolonged period required for mitosis.

Obviously the cells of ascitic cancer were less sensitive to adrenalin than those of the corneal epithelium. While the latter completely lost their ability to multiply, cancer cells continued to divide — although at a slower rate—with the course of mitosis particularly delayed.

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