EXPERIMENTAL BIOLOGY

CELL DIVISION AND VITAMIN A

I. A. Alov

From the Dept. of Histology (Chief Assistant – Prof. I. A. Alov), Khabarovsk Medical Institute

(Received June 20, 1956. Presented by Prof. D. N. Nasonov, Active Member of the Academy of Medical Sciences, USSR)

Cell multiplication in the different tissues is controlled by neurohumoral regulation, which is accomplished both by regulating mechanisms (the nervous system, the hormones of the suprarenal, hypophisis, thyroid and sex glands), and by certain local factors (cell functional activity, the process of cell differentiation, tissue alteration, etc.). The mitotic rate of the tissues evidently also depends on the exchange of the vitamins which play an important part in the different physiological and biological processes. Certain observations [7, 9] permit the hypothesis that vitamin A is one of the essential links of the cell division regulation system.

The purpose of this work was to study the role played by vitamin A in regulating cell division.

EXPERIMENTAL METHODS AND RESULTS

We conducted three sets of experiments. In the first set, the influence of vitamin A injections on the intensity of cell division in different organs of white mice was studied.

A butyrous solution of vitamin A was injected subcutaneously in 500, 1000, or 3000 international units per 24 hours. The injection was repeated after a period of 5 days (the last injection being 7 hours before death). The control group of mice was injected with an equal quantity of butter. All the animals were kept in identical conditions and on a regular diet. Mitotic activity was studied in the epithelium of the cornea, intestine and tongue. The intensity of the mitoses was judged according to the number of cells dividing in a constant area (1.65 mm^2) and according to the coefficient of the phases (the relation of the first two phases of mitosis to the last two phases). Calculations were done on total preparations of the cornea and sections (10 microns) of the cornea and other organs.

The results of the experiments are given in Table 1.

TABLE 1

The Influence of Vitamin A on Cell Division

Mouse group	Number of animals	Intensity of mitoses (M ± m) and coef. of the phases (K)		
		Cornea	Intestine	Tongue
Control	7	105± 6.7; K=2.4	273±10; K=1.2	27±0.8; K=1.8
Vitamin A, 500 i.u.	8	101±12.7; K=2.2	368±10; K=1.5	29±0.7; K=1.7
Control	6	115±18; K=1.4	289± 2,5; K=1.3	22±0.6; K=2.0
Vitamin A, 1000 i.u.	6	172±12; K=1.2	640±15; K=1.3	36±3.0; K=2.2
Control	9	59± 8,8; K=1.9	391± 7,0; K=1.6	23±5.2; K=2.8
Vitamin A, 3000 i.u.	9	111±15; K=1.7	337±17; K=1.7	48±5.0, K=2.7

As is evident from Table 1, vitamin A stimulated cell division. When relatively small quantities of the vitamin were injected (500 i.u.), mitotic intensity increased in the intestinal epithelium only. Larger doses of the vitamin (1000 i.u.) caused mitotic stimulation in the spithelium of the cornea and of the tongue. In one of the experimental groups (data not included in Table 1), the number of dividing cells in the epithelium of the tongue did not increase, and growth was noted only in the early phases of mitosis (increase of the coefficient), which seemed to be associated with change in the multiplication rate.

That the degree of increase in mitotic activity varied in the different groups of animals was probably due to their individual singularities and to the pre-experimental diet regime. The most significant mitotic stimulation was found in the intestinal epithelium. The intestinal epithelium also reacted to hypervitaminosis earlier than the others by suppressing mitotic activity. The 3000 i.u. injection of vitamin A stimulated mitotic activity in the epithelium of the cornea and tongue, but, in the majority of mice, lessened it in the intestinal epithelium. Since increased mitoses were noted in the intestinal epithelium of some mice, the average indices of mitotic activity with the 3000 i.u. vitamin A injection, given in Table 1, were slightly lowered. The high sensitivity of the intestinal epithelium to vitamin A seems to be explained by the fact that this organ, as does the liver, forms vitamin A from carotene [8]. In the second series of experiments, changes in mitotic rate under conditions of vitamin A deficiency were studied.

White mice were put on a vitamin A deficient diet for 20 days: wheat flour, lard, yeast and a salt mixture [5]. The control animals were kept on their regular diet.

By about the 20th day, a typical picture of vitamin deficiency developed in the experimental mice (growth sharply arrested, loss of weight, dishevelled fur). Avitaminosis did not result in the development of xerophthalmia in any of the experiments.

The results of the experiments are given in Table 2.

TABLE 2

Mitotic Activity Change in A Avitaminosis

Mouse group	Number of animals	Intensity of mitoses (M ± m) and coefficient of the phases (K)		
		Cornea	Intestine	Tongue
Control	9	97±13.0; $K=1.7$	347±18; $K=1.6$	27±3.4; $K=2.0$
Avitaminosis	7	91±13.0; $K=1.6$	336±20; $K=1.6$	27±5.3; $K=2.5$
Avitaminosis + thyroidin	7	161±15.0; $K=1.8$	299±16; $K=1.5$	33±6.0; $K=3.6$

As Table 2 shows, vitamin A deficiency in the experimental animals did not cause any appreciable change in mitotic activity. Only when the preparations were stained on to ribonucleic acid according to Brash could one note certain histochemical changes in the mitotic process of the intestinal epithelium: the normal gradual decrease in the concentration of ribonucleic acid could not be observed in part of the dividing cells, and the high ribonucleic acid content was retained in the metaphase.

When we analyzed the results of both series of experiments, we noticed that the influence of vitamin A on the mitotic process of the tissues was very similar to that of the thyroid gland. Thyroidin stimulates mitotic activity just as vitamin A does. Thyroidin had a greater activating influence on cell division, but it, too, was most sharply expressed in the intestinal epithelium. Methylthiouracil blockade of the thyroid gland in adult animals, like A avitaminosis, did not cause any appreciable change in mitotic activity [1].

These comparisons led us to include a third group of animals in the experiments with avitaminosis, which were given thyroidin with their food (18 mg of thyroidin per mouse in a period of ten days) on a background of vitamin deficiency. In this group of mice (Table 2), as is usual in experiments with thyroidin, a rather considerable

crease in mitotic activity was observed in the corneal epithelium. Only a small increase was observed during the early mitotic phases in the epithelium of the tongue. In the epithelium of the intestine (the organ most sensitive to thyroidin and vitamin A), mitotic stimulation not only did not occur, but mitotic activity was even somewhat decreased.

Thus, although avitaminosis was not attended by noticeable change in the number of dividing cells, it did lead to profound disturbance in the regulation of cell division. In vitamin A deficiency conditions, a powerful mitosis stimulator – thyroidin – did not cause an increase in mitotic intensity in the epithelium of the intestine or the tongue. Since the thyroidin hormone is the activator of carotenase (the enzyme which transforms carotene into vitamin A), one can propose that the thyroid gland acts on cell division through the synthesis of vitamin A. In every case, the thyroid gland hormone could only regulate the mitoses if vitamin A was present in the tissues. There are evidently similar correlations between other hormones that regulate cell division and vitamins. Adrenalin, in particular, seems to belong in this group as its antimitotic action was not discernable in guinea pigs deprived of the ability to synthesize ascorbic acid [6].

We have demonstrated in previous papers [1, 2, 3, 4] that the different factors of neurohumoral regulation of cell division in normal tissues (adrenalin, cortisone, thyroidin) do not change mitotic activity in tumors.

In a third series of experiments, we studied the influence of vitamin A on cell division in mice with Kroker's sarcoma. Beginning the 9th day after the sarcoma graft, the mice were injected with 1000 i.u. per day of vitamin A for 5 days.

TABLE 3

Vitamin A Influence on Cell Division of Mice with Grafted Tumors

Mouse group	Number of animals	Intensity of mitoses (M ± m) and coefficient of phases (K)		
		Cornea	Intestine	Sarcoma
Control	8	323±16; $K=1.8$	331±16; $K=1.3$	179±8.3; $K=2.6$
Vitamin A, 1000 i.u.	9	338±16; $K=1.2$	443±18; $K=1.7$	178±6.3; $K=2.7$

The results of the experiments (Table 3) showed that the vitamin A injection stimulated mitotic activity in the intestinal epithelium of the mice with the sarcomas. Mitotic intensity did not change in the corneal epithelium of these animals. Evidently, the reaction of normal cells to the action of vitamin A is lessened under the influence of the growing tumor. However, it would be premature to draw final conclusions as the reaction of the tissues to the vitamin injection varied considerably as to intensity in the different groups of mice (Table 1).

In the actual tumor, mitotic activity was not affected by vitamin A. Mitotic intensity in the tumors of both the experimental and control mice remained the same. Thus, vitamin A, like adrenalin, cortisone and thyroidin, does not influence the division of tumor cells. Cell division in tumors is out of the control of the system regulating these processes in normal tissues.

At this time, it is still difficult to explain the relative areactivity of the tumor cells. If one agrees that cell division in normal tissues is connected with nucleic exchange, then the different mitotic regulators, all things considered, seem to effect mitotic activity through cell nucleic exchange. Perhaps other exchange processes cause division in tumor cells. It is also possible that the nucleic exchange of tumor cells is different, and that the mitotic regulators do not change in, or even that, in a tumor cell, those intermediate links through which the mitotic regulators change the nucleoprotein exchange drop out. The first proposal is less probable: the experiments conducted in our L. M. Ermolenko laboratory showed that blockading nucleic exchange by different inhibitors deters cell division in both normal and tumor tissues. Changes in the actual nucleic exchange of the tumor cells or those intermediate links through which the mitotic regulators influence nucleic exchange are more probable. The possibility of such changes is indicated by the second series of experiments, in which cell reaction to thyroidin was disturbed by the exclusion of vitamin A from the exchange. Nor must one exclude the possibility

that similar correlations are formed in the growing tumor. Further experiments are needed to prove the validity of this working hypothesis.

SUMMARY

Vitamin A injected into white mice stimulates cell division. The epithelium of the intestines is the most sensitive to the vitamin. Vitamin A deficiency does not lead to any considerable changes in the number of mitoses in the tissues but the regulation of cell division is strongly disturbed. In cases of avitaminosis even such a powerful stimulator of mitosis as thyroidin fails to increase mitotic activity. The presence of vitamin A in the tissues is indispensable for the stimulative effect of thyroidin on cell division. Experiments on mice with Kroker's sarkoma demonstrated that vitamin A does not change the intensity of tumor cell division, since in tumors that process is out of control of the neurohumoral system which regulates the mitotic activity in normal tissues.

LITERATURE CITED

[1] I. A. Alov, Byull. Eksptl. Biol. i Med. Vol. 40, No. 12, pp. 47-50.

[2] I. A. Alov, Voprosy Onkol. 1955, Vol. 1, No. 6, pp. 40-45.

[3] I. A. Alov, Doklady Akad. Nauk SSSR 1956, Vol. 107, No. 5, pp. 745-747.

[4] I. A. Alov, in the book: Works of the Khabarovsk Medical Institute,* 1956, Vol. 15, pp. 63-71.

[5] P. P. Smilyanskaya and K. Z. Tulchinskaya, in the book: Vitamins in Theory and Practice,* Moscow 1953, pp. 152-153.

[6] L. V. Suvorova, Reactive Inhibition of Cell Division in Animals and the Development of a Capacity for it in Ontogenesis,* Dissertation, Leningrad 1955.

[7] E. Bujard, W. Jadassohn and R. Brun, Compt. rend. soc. biol.* 1954, Vol. 148 No. 9-10, pp. 778-780.

[8] R. F. Krause and H. B. Pierce, Arch. Biochem. 1948, Vol. 19, pp. 145-148.

[9] A. Studer and I. A. Frey, Dermatologica, 1952, Bd. 104, N. 1, S. 1-18.

* In Russian.