RAPID DETERMINATION OF CARCINOGENIC ACTIVITY OF PREPARATIONS

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Correlation was found between erythropoietic activity and resistance of mice both to induced carcinogenesis and to growth of a transplanted tumor. By choosing animals with weakened erythropoiesis for the tests the time taken for investigation of carcinogenic activity of preparations can evidently be considerably reduced.

KEY WORDS: carcinogenesis; erythropoiesis; antitumor resistance.

There is at present an urgent need for the discovery of possible carcinogenic activity in chemical compounds within shorter times than can be done by modern methods of investigation. The most accurate method of determination of carcinogenicity is direct observation of the appearance of tumors after contact between the test factor and the tissues of experimental animals *in vivo*. However, this method requires a long period of observation, virtually until the end of the animals' life.

The leading role of resistance of the host in development of tumors was established long ago by A. A. Bogomolets and his school [1-8], K. P. Ulezko-Stroganova [9], and others, and it is now generally accepted. In animals with weakened reactivity of the physiological connective-tissue system tumors appear faster and they become malignant more frequently. If, therefore, the degree of reactivity of the host can be determined before the investigation of carcinogenicity begins, by choosing animals with weakened reactivity the time taken for testing the substances can evidently be shortened.

In 1941, Shreder [12] observed that erythropoietin, a specific stimulator of erythropoiesis, stimulates more rapid wound healing in rabbits. The present writer found [10, 11] that erythropoietin, when increasing erythropoietic activity, also stimulates the adsorptive power of the connective-tissue system. A true parallel evidently exists between erythropoietic activity and general reactivity of the organism.

With these observations in mind, it was decided to study to what extent the erythropoietic activity of mice reflects their general reactivity during induced carcinogenesis and after transplantation of tumors.

EXPERIMENTAL METHOD

Female F_1(CBA × C57BL/6) mice weighing 20-22 g at the beginning of the experiment were used. In series I (72 mice), to carry out the classical experiment of carcinogenesis, 3-methylcholanthrene in 0.5% solution in benzene was used as the carcinogen and applied once in a dose of 0.04 ml to the previously shaved skin of the interscapular region. Daily applications of 1% croton oil solution in benzene in a dose of 0.02 ml to the same area of skin began after 2 weeks and continued until the 22nd week of the experiment.

Erythropoietic activity was determined by the reticulocyte test after stimulation of erythropoiesis by hypoxic hypoxia [the mice were exposed to a rarefied atmosphere (0.5 atm) repeatedly in a pressure chamber for 6 h]. Reticulocytes were counted in the blood of the

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TABLE 1. Erythropoietic Activity and Development of Induced Papillomas in Mice

Index studied	Group of mice		
	1	2	3
Percentage distribution of mice	29,1	47,3	23,6
Mean erythropoietic activity (No. of reticulocytes in ‰)	54±2,02	70±2,14	80±3,5
Mean latent period of papilloma development (in weeks)	8±0,52	16±0,43	—

TABLE 2. Erythropoietic Activity and Development of Transplanted Sarcoma 37 in Mice

Index studied	Group of mice		
	1	2	3
Percentage distribution of mice	19,8	68,7	11,5
Erythropoietic activity (No. of reticulocytes in ‰)	20—30	32—50	52—62
Mean weight of tumor on ninth day (in g)	1,6±0,22	1,2±0,11	1,0±0,17

mice (stained with brilliant blue, 1000 erythrocytes counted) 72 h after removal from the pressure chamber. The carcinogen was applied, followed by the croton oil, 3 weeks later. The mice were observed until the 40th week from the beginning of the experiment. The time taken for the first papillomas to appear and for their malignant degeneration was determined. At the end of the experiment the mice were divided into three groups: Group 1 contained mice in which papillomas appeared after 4-11 weeks of the experiment, group 2 contained mice with papillomas developing after 12-22 weeks, and group 3 contained mice with no papillomas. The mean latent period of appearance of papillomas and the mean erythropoietic activity were determined for the mice of each group

In series II 96 mice were used. To stimulate erythropoiesis in this series, a single subcutaneous injection of 100 μg cobalt ($COCl_2 \cdot 6H_2O$) was given to stimulate endogenous erythropoietin production, although its effect is very much weaker than that of hypoxic hypoxia. After 72 h the reticulocytes in the blood were counted and 3 weeks later sarcoma 37 was transplanted into the mice. On the ninth day the mice were killed and the grafts were weighed. Mice in which erythropoietic activity was between 20 and 30% were included in group 1, those in which it was between 32 and 50% in group 2, and between 52 and 62% in group 3. The mean weight of the tumor also was determined for each group.

EXPERIMENTAL RESULTS

The results of the experiments of series I are given in Table 1. They show that in the mice of group 1 the functional erythropoietic activity was lowest and the mean latent period of appearance of papillomas was very short. In group 2 the mean erythropoietic activity was higher and the mean latent period was 8 weeks longer than in the group 1 ($P < 0.001$). Mice in which no papillomas whatever appeared (group 3), on the other hand, had the highest erythropoietic activity. It is interesting to note that of 72 mice at the 40th week of the experiment 16 (22%) had developed a tumor, and that the mean erythropoietic activity of these animals was at the lowest level (52 ± 2.5%).

Papillomas thus developed and became malignant sooner in mice with weakened erythropoietic activity. In this experiment these mice amounted to 29.1% of the total number, and mice with increased resistance, which developed no papillomas whatsoever, accounted for 23.6% of the total.

In series II (Table 2) in mice whose erythropoietic activity was lowest, the grafted tumors grew faster and had the greatest weight. In mice with highest erythropoietic activity the grafts grew much more slowly (difference in weight 0.6 g; $P < 0.05$).

In the writer's opinion these results suggest that erythropoietic activity reflects the general reactivity of the body and its immunological status. By investigating the carcinogenic activity of compounds on animals with weakened erythropoiesis, the duration of the tests can therefore be reduced by more than half. In addition, if the immunological status of the experimental animal is known, some conclusions can be drawn regarding the mechanism of action of the tested compound should it prove to be carcinogenic.

LITERATURE CITED

1. A. A. Bogomolets and I. M. Neiman, Vestn. Mikrobiol., 6, No. 1, 33 (1927).
2. G. F. Dyadyusha, "Protective functions of the connective-tissue system, their regulation, and their role in malignant tumors," Author's Abstract of Doctoral Dissertation, Kiev (1962).
3. R. E. Kavetskii, The Role of Active Mesenchyme in Disposition of the Organism to Malignant Tumors [in Russian], Kiev (1938).
4. R. E. Kavetskii, in: The Physiological Connective-Tissue System [in Russian], Kiev (1941), p. 342.
5. I. M. Neiman, Med. Biol. Zh., No. 3, 71 (1927).
6. I. M. Neiman, in: General and Special Oncology [in Russian], Vol. 1, Moscow—Leningrad (1940), p. 402.
7. I. M. Neiman, in: The Physiological Connective-Tissue System [in Russian], Kiev (1941), p. 539.
8. I. M. Neiman and A. Ya. Sinai, Byull. Éksp. Biol. Med., 19, No. 6, 56 (1945).
9. K. P. Ulezko-Stroganova, The Problem of Cancer and Active Mesenchyme [in Russian], Leningrad (1940).
10. M. A. Finogenova, Byull. Éksp. Biol. Med., No. 9, 90 (1972).
11. M. A. Finogenova, Pat. Fiziol., No. 5, 79 (1973).
12. V. N. Shreder, in: The Treatment of Wounds and Physiotherapy of Farm Animals [in Russian], Moscow (1941), pp. 14-18.