The results of this investigation thus show that sarcomas developing in radiation chimeras as a result of subcutaneous implantation of pieces of plastic film in the late periods after irradiation arise as a rule from cells of the irradiated animal and not from bone marrow cells transplanted into it. However, the presence of T6 markers in one of the tumors does not rule out the possibility that, in principle, sarcomas can develop through induction by plastic film from elements of bone-marrow origin.

If connective-tissue cells used up as material for the formation of these tumors in mice are in fact replaced by bone-marrow cells, the process takes place extremely slowly. Therefore, the formation of connective-tissue cells from bone-marrow precursors evidently does not play an essential role in the histogenesis of sarcomas induced by implantation of plastic film.

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ACTION OF SARCOLYSIN AND ASALINE ON INCORPORATION OF THYMIDINE-H^3 INTO DNA OF SARCOMA 45 AND TISSUES OF TUMOR-BEARING RATS*

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The action of sarcolysin and asaline on DNA synthesis in sarcoma 45 and in the spleen, thymus, bone marrow, and liver of tumor-bearing rats was studied in relation to the time of administration of the compounds. The selectivity of action of sarcolysin and asaline, which differ in the structure of the carrier, was found to be directly dependent on the ability of these compounds to depress DNA synthesis in the tumor and in normal tissues.

KEY WORDS: sarcolysin; asaline; DNA synthesis; sarcoma 45.

Larionov's view [5] that the effectiveness of antitumor alkylating compounds may depend substantially on the structure of the carrier has now obtained adequate experimental confirmation [3, 6]. Meanwhile the search for new chemotherapeutic agents with more selective action calls for the study of the molecular mechanisms of their cytostatic action [3]. One possible approach to the solution of this problem would be by studying DNA synthesis, which is easily disturbed by alkylating agents [7] and ionizing radiation [9].

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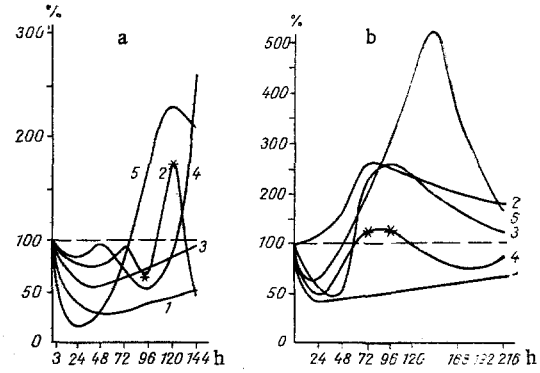

Fig. 1. Dynamics of incorporation of thymidine-H^3 into DNA of tissues of tumor-bearing rats depending on time of injection of the compounds: a) sarcolysin; b) asaline. 1) Sarcoma 45; 2) bone marrow; 3) spleen; 4) liver; 5) thymus. Broken line, control. Asterisks denote that differences are not significant. Mean results of three experiments shown. Abscissa, time after injection of compound (in h); ordinate, ratio of experimental to control value (in %).

The object of this investigation was to compare the action of sarcolysin and asaline in vivo on DNA synthesis in a tumor and in various tissues of tumor-bearing rats. Asaline is a dipeptide, the ethyl ester of N-acetyl-sarcolysyl-valine, i.e., it differs from sarcolysin in the more complex structure of the carrier. Possessing high antitumor activity, it causes less damage than sarcolysin to the hematopoietic organs of animals [2, 8]. It was accordingly decided to compare the results of chemotherapeutic [2, 8] and biochemical investigations of the effect of sarcolysin and asaline on DNA synthesis.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 100-200 g. Sarcoma 45 and the thymus, bone marrow, spleen, and liver of the tumor-bearing rats were investigated. Sarcoma 45 (sensitive to sarcolysin) was obtained from the strain laboratory of the Institute, transplanted, and maintained by the usual method. The alkylating agents were injected once only, intraperitoneally, in the maximal tolerated dose, usually on the fifth to seventh day after transplantation: sarcolysin (10 mg/kg) in 0.14 M NaCl solution, asaline (100 mg/kg) as a suspension in 2% starch.

The disturbance of DNA synthesis in the test tissues was assessed from the incorporation of thymidine-H^3 (13.5 Ci/mmole), which was injected intraperitoneally in 0.14 M NaCl solution (100 μCi/100 g body weight). The dynamics of incorporation of thymidine-H^3 into DNA was studied 3, 24, 48, 72, 96, 120, and 144 h after injection of sarcolysin and 24, 48, 72, 96, 120, 168, 192, and 216 h after injection of asaline into the animals. At each time point, two or three rats were used in the experiment. The animals were decapitated 2 h after receiving the injection of thymidine-H^3 and DNA was extracted. For this purpose the tissues were placed in glass vessels (50 cm^3), cooled to 0°C, minced with a hand press (except the bone marrow), and homogenized at 0°C (in 5 ml of 0.14 M NaCl solution with 0.01 M Na citrate) in a glass homogenizer with Teflon pestle. Usually 0.3 g spleen, 0.15 g thymus, 0.5 g of the tumor, 0.15 g of bone marrow, and 0.5 g of liver were used in the experiment. Along with RNA, DNA was extracted by the phenolic method [1] after a single deproteinization. The DNA content in the samples and the radioactivity of the specimens were determined by the method of Schmidt and Thannhauser [12], in conjunction with a millipore technique, which excluded contamination by RNA and radioactive low-molecular-weight impurities. The procedure of preparation of the samples for determination of the DNA content and radioactivity was as follows: 0.5 ml of the original DNA solution was added to 0.5 ml 1 N NaOH, the mixture was incubated at 37°C for 45 min, and after cooling to 0°C it was treated with 2 ml of 25% TCA. The mixture was quickly mixed with an ultrasonic vibrator, which also facilitated the formation of a finely dispersed residue of DNA, which was applied to a millipore filter and washed three times with 2 ml cold 5% TCA. The excess of TCA was removed by washing three times with 70% ethanol or ether. The filters were further dried by leaving them in the incubator at 37°C overnight. To determine the DNA content the filters were placed in tubes with ground glass stoppers, 4 ml of 5% perchloric acid was added, and the samples were hydrolyzed for 20 min at 100°C. The DNA content in the samples was determined spectrophotometrically [10].

The procedure of preparing samples for the study of radioactivity was the same except that the DNA content in the samples was 25 μg. Millipore filters (Synpore-6, Czechoslovakia) 25 mm in diameter, with a mean pore size of 0.4 μ, were used. The radioactivity of the DNA samples was measured on the Mark II scintillation counter (USA). The scintillator was 5 g PPO and 0.3 g POPOP to 1 liter toluene. The results were expressed in counts/min/mg DNA and as percentages of the control. The results were analyzed by Student's method.

EXPERIMENTAL RESULTS

The dynamics of incorporation of thymidine into DNA of sarcoma 45 and of the thymus, spleen, bone marrow, and liver of the rats in relation to the time of action of sarcolysin is shown in Fig. 1a. Clearly under

the influence of sarcolysin, given as a single injection of the maximal tolerated dose, incorporation of thymidine-H^3 into DNA was considerably reduced (by 70%) after 24 h, and it remained reduced by 50% in the final stages of the experiment. Compared with the tumor, in normal rat tissues (spleen, bone marrow, and thymus) DNA synthesis was inhibited for a shorter time. However, in the initial periods of the experiment, DNA synthesis in the bone marrow was found to be more resistant to the action of sarcolysin than in the spleen: After a very small decrease between 24 and 48 h, it returned close to normal after 72 h. However, later DNA synthesis became unstable, for a tendency was observed both for it to diminish and to be activated. Characteristically in the thymus a sharp decrease in DNA synthesis was replaced after 24-48 h by considerable stimulation (by 250%).

The results of these experiments indicate that sarcolysin inhibits DNA synthesis in the tumor more selectively and that it affects normal tissues to a lesser degree. Bone marrow hematopoiesis in tumor-bearing rats, moreover, is impaired less than lymphopoiesis. These observations are in good agreement with the results of the chemotherapeutic investigations, in which the action of various derivatives of dichloroethylamine on hematopoiesis in animals was studied [3, 8]. The results of the present experiments are basically analogous to those of biochemical investigations [7] of the action of sarcolysin on incorporation of P^{32} into DNA of sarcoma 45 (normal strain) and in the spleen of rats. However, 3 h after injection of sarcolysin into the animals, stimulation of DNA synthesis was not observed, as was reported by the author cited above [7] 6-12 h after injection of the compound.

The results of the present experiments with asaline are shown in Fig. 1b. Asaline, like sarcolysin (but by a somewhat lesser degree), persistently inhibited the incorporation of thymidine-H^3 into DNA of sarcoma 45 (by 50-40%) at all times of the experiment. However, it must be pointed out that the dynamics of DNA synthesis in normal rat tissues in the experiments with asaline differed significantly from its dynamics in the experiments with sarcolysin. This was reflected in the fact that the phase of inhibition of incorporation of thymidine-H^3 into DNA in the spleen and thymus (with a maximum at 24 h) was followed by a phase of marked stimulation (by 200 and 500% respectively, with maxima at 96 and 120-144 h). Moreover, by contrast with sarcolysin, asaline in general did not inhibit DNA synthesis in the bone marrow in the initial periods of the experiment, and later it stimulated it considerably (an increase of 250%).

Besides strongly inhibiting DNA synthesis in the tumor, unlike sarcolysin, asaline thus inhibited DNA synthesis to a lesser degree in the normal tissues of the tumor-bearing rats. However, in their action on the tissues of hematopoietic organs, asaline and sarcolysin have much in common. For example, both asaline and sarcolysin inhibited DNA synthesis more effectively in the spleen and less effectively in the bone marrow. Both these compounds in the initial stages of the experiments inhibited DNA strongly in the thymus, but later activated it considerably. The phenomenon observed in this investigation may have great importance for the understanding of the mechanism of the immunobiological response of the recipient to the action of alkylating agents. Stimulation of DNA synthesis in the cells of hematopoietic tissues, as was observed in the present experiments, is evidently connected with stimulation of regeneration in these tissues after initial damage.

The data on incorporation of thymidine-H^3 into DNA in the liver of tumor-bearing rats indicate that under the influence of alkylating agents DNA synthesis may be disturbed not only in actively proliferating tissues. Differences observed in the dynamics of incorporation of thymidine-H^3 into liver DNA in response to injection of asaline and sarcolysin suggest that disturbances of DNA synthesis may also play an important role in the chemotherapeutic effect, as was also concluded from the work of Wheeler and Alexander [11].

In conclusion, the results of these comparative investigations of the action of sarcolysin and asaline on DNA synthesis agree well with the results of chemotherapeutic, cytological, and cytogenetic observations [2, 4, 8] and they emphasize the great importance of the structure of the carrier in the antitumor effect of alkylating agents on the body. The cytostatic action of asaline, like that of sarcolysin [7], is evidently based on disturbance of DNA synthesis.

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ENZYMIC AND IMMUNOLOGICAL ACTIVITIES OF LYMPHOCYTES DURING CHEMICAL CARCINOGENESIS

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During chemical carcinogenesis in Wistar rats the activity of acid phosphatase and certain dehydrogenases in the blood lymphocytes was compared with the development of cellular immunity revealed by the macrophage migration inhibition test. In the early periods of carcinogenesis the changes in succinate dehydrogenase and acid phosphatase activity were shown to correspond to a high level of immunological reactivity of cellular type in 66% of the animals. During progressive growth of the tumors in the rats the enzyme balance in the lymphocytes was disturbed and, at the same time, the immunological response was depressed.

KEY WORDS: chemical carcinogenesis; lymphocytes; enzyme activity; cellular immunity.

Because of the inadequacy of immunological protection against malignant growth, the discovery of the mechanism of this phenomenon is extremely important. It follows from communications published previously [1, 2, 4, 7-10, 12] that during the development of the immune response of the organism to antigens of infectious and noninfectious nature definite changes arise in metabolism of the lymphocytes. Suggestions have been made for an approach to the elucidation of the degree of informativeness of the cytochemical changes in the cells of the immunocompetent system as a means of predicting the course of diseases and the effectiveness of treatment [3].

The object of this investigation was to study the enzyme activity of lymphocytes and to compare this activity with the manifestation of reactions of cellular immunity in the course of chemical carcinogenesis. No investigations of this type on this particular model have been described.

EXPERIMENTAL METHOD

The carcinogen DMBA was injected intramusuclarly in a dose of 3 mg per animal into Wistar rats. Before treatment with the carcinogen and for the 5 months after its injection, the dynamics of activity of three enzymes was studied in the blood lymphocytes: succinate dehydrogenase (SD; EC 1.3.99.1), α-glycerophosphate dehydrogenase (α-GPD; EC 1.1.2.1), and acid phosphatase (AP; EC 3.1.3.2). Activity of the dehydrogenases was determined by Nartsissov's method [6] and of AP by the method of Goldberg and Barka [11]. In order to characterize the activity of these enzymes after the number of stained granules had been counted in 50 lymphocytes, mean values per cell were obtained.

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