The results of these experiments (Figs. 2 and 3) agree well with each other and can be interpreted as evidence of partial synchronization of the cells under the influence of loading of the MPS with colloidal gold.

Since colloidal gold has no direct effect on malignant erythroblasts, but is phagocytosed by macrophages, modifying their activity, the results indicate that proliferative activity of both normal and malignant hematpoietic cells depends on the state of the macrophages in vivo and, consequently, on the possibility of oriented regulation of the process by means of agents affecting MPS.

The method of determining the strength of DNA–protein interactions, developed as described above, is therefore useful for examination of several problems in experimental oncology. It can also evidently be used in clinical oncology for the study of samples of bone marrow, lymph node, and spleen cells and also of blood cells of patients with leukemias, containing a high proportion of blast cells.

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EFFECT OF SYNTHETIC β-CAROTENE ON CYTOLYTIC T LYMPHOCYTE FORMATION

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The possibility of using vitamin A and its derivatives for the prevention and treatment of malignant neoplasms in man and animals has been reported several times in the recent literature [2, 3, 6]. The mechanism of the antitumor action of retinoids has not yet been studied. Meanwhile Lotan and Dennert [4] have shown that vitamin A, in doses giving a therapeutic effect, can stimulate the formation of cytolytic T lymphocytes (CTL) in vitro. These observations suggest that the antitumor action of vitamin A is connected with increased ability of the host to produce effector cells concerned with rejection of the tumor. The widespread use of vitamin A for the prevention and treatment of malignant tumors is restricted because this preparation, in therapeutic doses, has a toxic action associated with manifestations of hypervitaminosis. Accordingly the possibility of using β-carotene, a precursor of vitamin A which is nontoxic even in large doses, is particularly interesting.

The aim of this investigation was to study the effect of various doses of β-carotene on CTL formation in vitro, to determine the optimal dose and schedule of administration of this substance, and also to study its effect on growth of transplantable sarcoma 180 and the length of survival of the animals.

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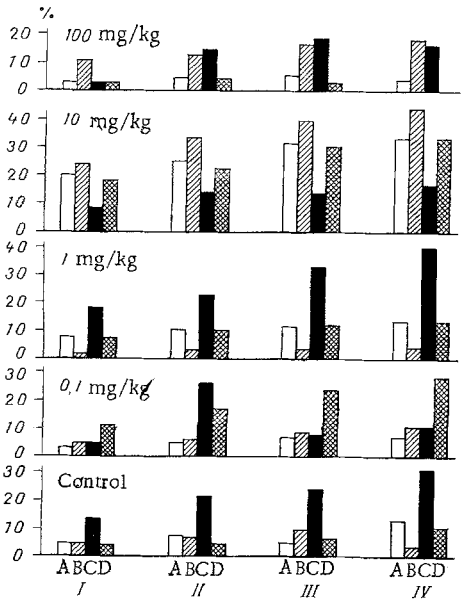

Fig. 1

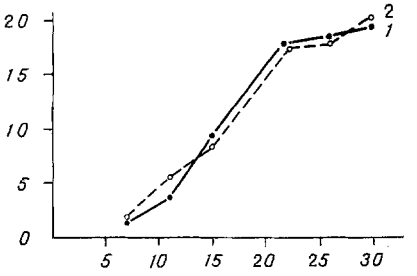

Fig. 2

Fig. 1. Effect of various doses and schedules of administration of β-carotene on cytolytic activity of lymphocytes. A, B, C, D) Groups of BALB/c mice receiving a single intraperitoneal injection of β-carotene in doses indicated on figure, 9, 6, 3, and 1 day respectively before experiment. I, II, III, IV) Ratio of lymphoctyes to target cells 12 : 1, 25 : 1, 50 : 1, and 100 : 1 respectively. Ordinate, percentage of specific lysis.

Fig. 2. Effect of β-carotene on duration of survival of mice with transplanted sarcoma 180 and on size of tumor: 1) mice receiving injections of olive oil (control); 2) mice receiving injections of 10 mg/kg β-carotene. Abscissa, duration of survival of animals (in days); ordinate, size of tumor (in cm^3).

EXPERIMENTAL METHOD

Experiments to determine the effect of β-carotene on CTL formation were performed on adult male BALB/c ($H\text{-}2^d$) mice weighing 18–20 g, obtained from the "Stolbovaya" Nursery, Academy of Medical Sciences of the USSR. β-Carotene was synthesized in the Laboratory of Chemistry of Polyene Compounds, "Vitaminy" Research-Production Combine. The compound was injected intraperitoneally into BALB/c mice in various single doses, in 100 μl of olive oil, 1, 3, 6, and 9 days before setting up the mixed lymphocyte culture (MLC). The spleens were removed under sterile conditions, suspensions of spleen cells were prepared, and $25 \cdot 10^6$ lymphocytes from each group of mice were mixed in the ratio of 5 : 1 with irradiated splenocytes of C3H ($H\text{-}2^k$) mice and incubated at 37°C in 6-well plates (Linbro 76-047-05) in 5 ml of DMEM medium (Flow Lab.), containing 10% embryonic calf serum (Gibco), $2 \cdot 10^{-3}$ M L-glutamine, and $5 \cdot 10^{-5}$ M 2-mercaptoethanol. The cytotoxic activity of the lymphocytes was determined on the 6th day of incubation as the quantity of $Na_2{}^{51}CrO_4$ isolated from lysed L-929 ($H\text{-}2^k$) target cells by the method described previously [1].

To determine the antitumor activity of β-carotene an oily solution of the compound was injected intraperitoneally into BALB/c mice 6 days before transplantation of sarcoma 180 cells into the animals. On the day of transplantation the injections were repeated, and the compound continued to be injected every 6 days until the animals died. The therapeutic effect was assessed by the effect of β-carotene on the size of the growing tumor and on the length of survival of the animals.

EXPERIMENTAL RESULTS

The results of determination of the cytotoxic activity of lymphocytes obtained from mice receiving different doses of β-carotene are given in Fig. 1. It can be concluded from comparative analysis of the data that the greatest cytotoxic effect was obtained after injection of the compound in a dose of 10 mg/kg, 6 days before setting up the MLC. To determine whether β-carotene, given in the optimal dose and schedule, has a therapeutic effect during development of a malignant neoplasm, its effect was studied on growth of transplantable sar-

coma 180 and on the length of survival of animals with the tumor. The results are given in Fig. 2. They show that β-carotene, injected in a dose of 10 mg/kg, had no significant effect either on the size of the growing tumor or on the duration of survival of the animals.

These experiments thus showed no direct relationship between the immunostimulating and antitumor activity of β-carotene. According to data in the literature, the antitumor action of β-carotene could be demonstrated only on a limited number of slowly growing strains of tumors, mainly induced by ultraviolet radiation [5]. The degree of stimulation of CTL by β-carotene is evidently insufficient to inhibit growth of the rapidly growing sarcoma 180. The possibility likewise cannot be ruled out that the antitumor activity of the compound may be linked with its ability to stimulate other types of effector cells of the immune system such as macrophages, normal killers, and K cells. This hypothesis may be tested in future experiments.

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PROTEIN KINASE ACTIVITY LEVELS AND BIOCHEMICAL DIAGNOSIS OF MALIGNANCY

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KEY WORDS: protein kinases, diagnosis, oncology, mucous membrane of the large intestine.

Many attempts to use biochemical and enzymic tests for the diagnosis of malignant tumors have not yet resulted in their widespread application in clinical practice. As before, the leading role in this field is still played by histologic and pathomorphologic methods of investigation of biopsy material, but this does not effectively ensure detection of early stages of cancer. Meanwhile it is evident that the initial stages of neoplastic transformation of cells and tissues, without any clearly distinguishable external manifestations, are realized initially at the molecular level, with involvement of the genetic material of the cell in the process [4]. As a result of expression of the modified genome, changes in biochemical processes in the cell, in the form of a change in its sensitivity to external regulatory factors, weakening of intercellular interactions, etc. [1, 6], become observable. Considering that it is disturbances at the level of regulation of biosynthesis and coordination of metabolic activity that are the features which distinguish the transformed from the normal cell, the writers have concentrated their attention on the study of protein phosphorylation systems. Changes in the degree of protein phosphorylation are known to be closely connected with modifications of the functional and metabolic states of cells. Changes in protein kinase activity are observed when various disturbances of the proliferative status of the cell are present and, in particular during tumor growth [2, 5].

The aim of this investigation was to study the possibility of using protein kinase activity levels for the biochemical diagnosis of malignancy, using carcinoma of the colon, a very common disease, as the example.

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