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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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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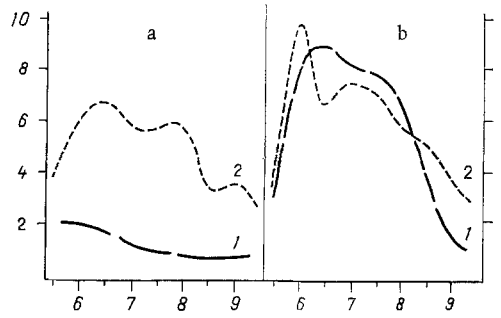

Fig. 1

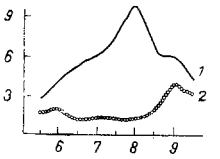

Fig. 2

Fig. 1. Dependence of protein kinase activity of MMC on pH of medium. Abscissa, pH values; ordinate, radioactivity (in cpm · 10^{-3}). a) Normal tissue, b) tumor tissue; 1) histone kinase activity, 2) casein kinase activity.

Fig. 2. Dependence of ratios between histone kinase and casein kinase activity on pH of medium. Abscissa, pH values; ordinate, histone kinase/casein kinase ratio. 1) Normal, 2) tumor.

TABLE 1. Activity of Protein Kinases (in units) in MMC of Healthy Subjects of Control Group and Patients with Carcinoma of the Colon (M ± m)

Test object	Histone kinase	Casein kinase	Casein kinase/ histone kinase
Control	763±84	118±25	9,95±1,38
Tumor tissue	1386±68*	819±77*	1,98±0,19*
MMC adjoining tumor (1 cm)	1448±75*	513±42*	3,15±0,23*
MMC at a distance from tumor (25-30 cm)	1342±51*	495±33*	2,99±0,21*

Legend. Mean values of ratios in protein kinase activity calculated from individual values of these parameters. *P < 0.01 compared with control.

EXPERIMENTAL METHOD

Altogether 65 persons, 24 of whom were clinically healthy and 41 had carcinoma of the colon, were studied. The diagnosis was based on clinical examination, with endoscopic investigation of the colon and histological study of biopsy specimens obtained under direct vision in every case. All the tumors studied were adenocarcinomas with different degrees of differentiation. None of the patients had been treated by radiotherapy or chemotherapy. The control group contained healthy volunteers with no pathological changes in the mucous membrane of the colon (MMC). Material for investigation from patients with carcinoma of the colon was taken from tumor tissue and from areas of MMC located 1 and 25–30 cm away from the tumor. The material was subjected to histological investigation and also frozen at the temperature of liquid nitrogen and kept until required for biochemical analysis.

Before determination of protein kinase activity, tissue samples (4–6 mg) were minced in a microhomogenizer in 5 volumes of 5 mM Tris-HCl buffer, pH 7.4, containing 2 mM EDTA, 0.5 mM dithiothreitol, 3 mM NaF, 0.5 mM phenylmethylsulfonyl fluoride, and 150 mM NaCl. The homogenate was centrifuged at 20,000g for 10 min and the supernatant was used for analysis. The protein kinase reaction was carried out in medium containing 50 mM Tris-HCl, pH 8.0, 10 mM $MgCl_2$, 1 mM EDTA, 2 mM NaF, 0.5 mM dithiothreitol, 50 μM [γ-^{33}P]-ATP (0.2–0.3 Ci/mmole), and 5 μg of tissue extract [3]. To determine cAMP-dependent histone kinase activity, histone H1 (2 mg/ml) and cAMP (5 μM) were added to the sample, and to determine casein kinase activity, dephosphorylated casein (3 mg/ml) was used as the phosphorylation substrate. The final volume of the

sample was 0.1 ml and the duration of incubation at 30°C was 10 min. The reaction was stopped by cooling the samples rapidly to 2–4°C, an aliquot of the incubation medium was transferred to a Whatman 3MM paper filter and fixed in 10% TCA solution, and then the filter was washed at 5% TCA, in a mixture of ethanol and acetone (1:1), and in acetone. Radioactivity of the filters was measured in standard toluene scintillator on a liquid scintillation counter. Enzyme activity was expressed in picomoles of orthophosphate transferred from ATP to 1 mg protein during incubation for 1 min at 30°C.

EXPERIMENTAL RESULTS

Dependence of activity of the protein kinases on pH of the incubation medium is shown in Fig. 1. Activity of cAMP-dependent histone kinase and, in particular, of casein kinase was significantly higher in MMC of the tumor than in normal tissue. The difference between protein kinase activities of tumor and normal tissue was particularly marked when ratios between histone kinase and casein kinase activity was compared (Fig. 2). Low values of this ratio, characteristic of tumor tissue, were due to a high level of casein kinase activity, which was most conspicuous in the weakly alkaline pH zone. The possibility cannot be ruled out that an abnormal form of casein kinase, with maximal activity at pH 7.0–9.0, may have appeared in the tumor tissue. However, a detailed study of the many different forms of this enzyme would be necessary to verify this hypothesis.

To characterize the clinical material the following parameters of protein kinase activity were used: activity of histone kinase and casein kinase, and also the ratio between activities of these enzymes, which evidently reflects a definite rule for the intensity of phosphorylation of proteins in normal and cancerous tissues. Being relative, this parameter depends only a little on some of the concrete conditions of determination of enzyme activity. The data given in Table 1 confirm a regular increase in casein kinase activity in tumor tissue, accompanied by a sharp decrease in the ratio between activities of these two protein kinases.

Levels of protein kinase activity characterizing MMC taken at distances of 1 and 20–30 cm from the tumor focus are very interesting. The high level of casein kinase activity and low values of the ratios between protein kinase activities indicate a close resemblance from the biochemical point of view between tumor tissue and MMC at a distance from the tumor. If certain levels of protein kinase activity are characteristic of tumor tissue itself, easily detectable by pathomorphologic methods, and if closely similar values are characteristic of MMC taken at a considerable distance from the tumor, and histologically indistinguishable from normal, it is evident that the levels of these parameters will be of definite diagnostic value for the detection of early stages of cancer. MMC located at a distance from the tumor, and outwardly intact, is perhaps transformed, although it does not exhibit morphological features of malignant growth. However, the results of the enzymic investigation indicate the presence of significant biochemical changes in protein phosphorylation systems of this histologically unchanged MMC, located at a considerable distance from the focus of malignant growth. A number of general hypotheses can be put forward on the basis of this observation. In the case of adenocarcinoma of the human MMC the process of malignant degeneration of the organ, it can be tentatively suggested, is more extensive (possibly affecting the whole organ) than the pathomorphologically demonstrable zone of tumor growth. Consequently, the tumor can be regarded as the local expression of a widespread but latent process of neoplastic transformation of the organ. From the diagnostic point of view the use of levels of tissue protein kinase activity may be useful for the discovery of risk groups during examinations of the population, and it may enable the results of radiotherapy, chemotherapy, and surgery and also the likelihood of recurrence of the disease, and so on, to be predicted.

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