with or separately from females, and in females which became pregnant.

Consequently, immunization with embryonic antigens during pregnancy neither inhibits nor stimulates growth of tumors induced by SV_{40} virus, as shown by comparison of the incidence of tumors in females becoming pregnant and in males (control). In a study by Parmiani and Lembo [3] it was shown that the incidence of primary methylcholanthrene tumors in female mice of various strains which became pregnant was lower than in females not becoming pregnant, although no male control group was present in this investigation. There are two possible explanations of these results. First, cells transformed by SV_{40} virus in the early stages of carcinogenesis may not contain antigens of embryonic type and, consequently, pregnancy during the latent period has no immunological effect on carcinogenesis. Such antigens perhaps appear in tumor cells in the later stages of tumor development and, in particular, in the cells of transplantable tumors. Second, it may be that embryonic antigens do not in general (because of tolerance or other reasons) play the role of transplantation antigens and cannot produce antitumor immunity. Both these possibilities are being studied experimentally.

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EFFECT OF SARCOLYSIN ON GLUTATHIONE PEROXIDASE AND GLUTATHIONE REDUCTASE ACTIVITY IN SARCOMA C-45

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A decrease in glutathione peroxidase and glutathione reductase activity in sarcoma C-45 was discovered during the period of its most rapid growth. Repeated injections of sarcolysin (1.2 mg/kg, intraperitoneally) caused a sharp decrease in the activity of both enzymes and a simultaneous decrease in the ratio between glutathione reductase and glutathione peroxidase activities. It is suggested that the glutathione redox enzyme system plays an important role in the mechanisms of the antitumor action of chemotherapeutic agents.

KEY WORDS: glutathione peroxidase; glutathione reductase; tumor growth; sarcolysin.

Investigation of glutathione peroxidase (GP) and glutathione reductase (GR) in tumor tissue is interesting from at least two aspects. First, by forming the glutathione redox system these enzymes, by their function, maintain a definite relationship between the oxidized and reduced forms of this tripeptide, which is of great importance in the regulation of cell division [5]. Second, it has now been shown conclusively that there is definite correlation between the level of lipid antioxidants and the rate of cell proliferation [1]. The ability of GP to perform its antioxidant function [3] on account of the safe utilization of hydrogen peroxide and of engodenous organic peroxide suggests that the state of the glutathione redox enzyme system can make a significant contribution to the free-radical mechanism of regulation of cell proliferation.

The object of this investigation was to study the dynamics of GP and GR activity in sarcoma C-45 during its growth and after administration of sarcolysin.

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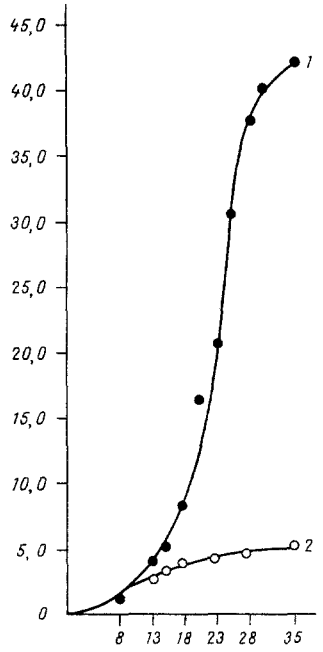

Fig. 1

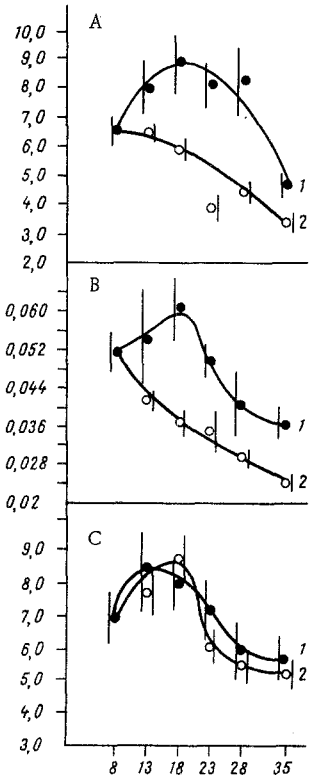

Fig. 2

Fig. 1. Effect of sarcolysin on growth of sarcoma C-45: 1) without sarcolysin; 2) with sarcolysin. Abscissa, days after transplantation of tumor; ordinate, volume of tumor (in cm^3).

Fig. 2. Changes in GP activity during tumor development and sarcolysin treatment. A) Activity calculated per gram wet weight of tissue, B) per milligram protein, C) per milligram DNA. Abscissa, days after transplantation of tumor; ordinate, units of enzyme activity. Remainder of legend as in Fig. 1.

EXPERIMENTAL METHOD

Noninbred male albino rats inoculated subcutaneously in the dorsal region with the tumor (sarcoma C-45) were used. Tissues of the tumor at different stages of its development and in the course of treatment with sarcolysin were investigated. The compound (1.2 mg/kg) was injected intraperitoneally on alternate days starting on the eighth day of the experiment. The course consisted of 10 injections. The animals were decapitated at various times, the tumor freed from its capsule and areas of necrosis, forced through a press with holes 0.5 mm in diameter, and homogenized in the cold in a homogenizer of the Potter type (Teflon–glass) in medium containing 0.25 M sucrose, 10^{-3} M EDTA, 0.1% Triton X-100, and 50 mM K-phosphate buffer, pH 7.4. The homogenate was centrifuged for 10 min at 800g and the supernatant used for the investigations.

The GP (EC 1.11.1.9) activity was determined by the use of tert-butyl hydroperoxide as the substrate [6]. The incubation mixture contained K-phosphate buffer, pH 7.0, 0.12 mM NADPH, 0.85 mM reduced glutathione, 1 unit yeast GR, 1 mM EDTA, and 0.4 mM tert-butyl hydroperoxide, GR (EC 1.11.1.6) activity was investigated by the method described by Gerasimov et al. [12]. GP activity was expressed in μmoles reduced glutathione hydrolyzed per minute, and GR activity in μmoles of oxidized glutathione reduced per minute. DNA was determined by Burton's method [4] and soluble protein by the biuret method.

EXPERIMENTAL RESULTS

Measurement of the tumors showed that the scheme of injection of sarcolysin used under these experimental conditions caused marked inhibition of their growth (Fig. 1), but did not however lead to their complete absorption. As a result, the dynamics of the indices can conveniently be regarded as characteristic for cells of the same time but with different rates of division. Curves of the dynamics of GP activity in the tumor tissue

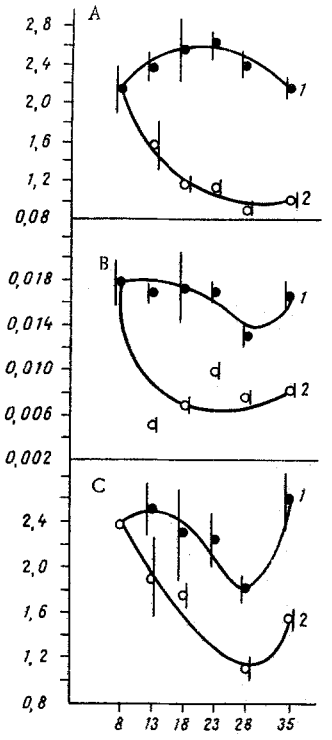

Fig. 3. Changes in GR activity during tumor development and sarcolysin treatment. Legend as in Fig. 2.

rose to a maximum corresponding to the 18th day of tumor growth (Fig. 2). The stage of exponential growth of the tumor (8th-25th days) thus proceeds against the background of increased GP activity, but in the period of most rapid growth of the sarcoma (18th-20th days) GP activity fell. Injection of sarcolysin caused a decrease in GP activity, calculated per milligram protein and per gram tissue. The change in GP activity calculated per milligram protein indicates that the action of sarcolysin has greater specificity toward this enzyme than to the main mass of proteins. Calculation per milligram DNA showed no significant changes in the enzyme activity under the influence of sarcolysin. The apparent contradiction between the results obtained by calculating activity relative to protein and to DNA disappears if it is recalled that the primary site of action of sarcolysin is DNA and that its concentration in the tumor falls more strongly than that of protein.

It is impossible to conclude from these results, primarily those characterizing GP activity calculated relative to DNA, that the rapidly proliferating cells are better provided with glutathione peroxidase. A more likely explanation is that increased GP activity precedes cell division, and during the period of more rapid proliferation the enzyme activity falls. Considering the functional role of GP, it must be emphasized that this decrease may be one of the essential causes of the increased consumption of antioxidants discovered during the period of active cell division and after administration of sarcolysin [1].

Changes in GR activity during tumor development were less marked than those of GP (Fig. 3). Meanwhile, sarcolysin sharply inhibited GR activity. The level of reduced glutathione is an essential factor in the regulation of cell division [5]. Since the ratio between reduced and oxidized glutathione depends on the ratio between the activities of GP and GR, the decrease in GR activity in the period of rapid growth of the sarcoma (18th-25 th days) and after injection of sarcolysin could be an important mechanism of spontaneous and sarcolysin-induced inhibition of tumor growth. Comparison of the curves showing the dynamics of GP and GR activity clearly shows a sharper decrease in GR than in GP activity after injection of sarcolysin. As a result of this, the ratio between GR and GP activities falls considerably, thus providing the basis for a shift of the thiol–disulfide equilibrium in the cell toward disulfide formation. It is worth noting that in some model systems the oxidation of glutathione inhibited protein synthesis [9].

Many carcinogens increased GR activity [5] and the ratio between GR and GP activities [7, 8]. As the results described above show, sarcolysin causes the opposite changes. Although the results do not answer the question whether a decrease in GR and GP activities is a condition or the result of the antitumor action of chemotherapeutic agents, the study of correlation between the therapeutic efficacy of antitumor compounds and their effect on enzymes of the glutathione redox system may prove promising for the rational search for such agents.

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