clinical study of diazan for the treatment of this tumor. The deep and prolonged suppression of cell proliferation produced by NMU necessitates a revision of the view that NMU is ineffective against BC, which incidentally is based on only a small number of observations [6]. The study of the efficacy of NMU in the combined chemotherapy of BC will evidently also be fruitful.

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CHANGES IN ACTIVITY OF OXIDATIVE ENZYMES IN CBA MOUSE THYMUS CELLS DURING CARCINOGENESIS INDUCED BY SIMIAN ADENOVIRUS SA7(C8)

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UDC 616-006.6-02:578.827.22/-092.9-07:616.438-008.931:577.152.1

KEY WORDS: thymus; induced carcinogenesis; microspectrophotometry; activity of oxidative enzymes.

The thymus plays the leading role in the formation of the functional properties of the T lymphocytes and their immunocompetence. The least studied aspect of this problem at present is metabolism in the immunocompetent cells at the time of immunologic transformations [8]. The results of biochemical and histochemical studies of the thymus under normal conditions in animals of different species and in man have proved contradictory. The results of histochemical investigations of the thymus published in the literature are mostly based on visual estimate of activity of hydrolytic and oxidative enzymes [5, 7, 12]. There have been only isolated reports of changes in metabolic activity of thymocytes in different functional zones of the thymus during carcinogenesis [4].

The object of the present investigation was a microspectrophotometric study of changes in oxidative enzyme activity in mouse thymus cells in the postnatal period and during carcinogenesis induced by simian adenovirus SA7(C8), from the time of introduction of the virus until the period of progressive growth of the tumor.

EXPERIMENTAL METHOD

CBA mice were used. Simian adenovirus SA7(C8), in a titer of $10^{-4.5}$ CPD_{50}/ml, was used to induce the tumor and was injected subcutaneously into newborn animals during the first 24 h after birth in a dose of 0.1 ml. A tumor appeared in 95% of animals after 60 ± 4.5 days

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(Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Smol'yannikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 7, pp. 80-82, July, 1981. Original article submitted August 4, 1980.

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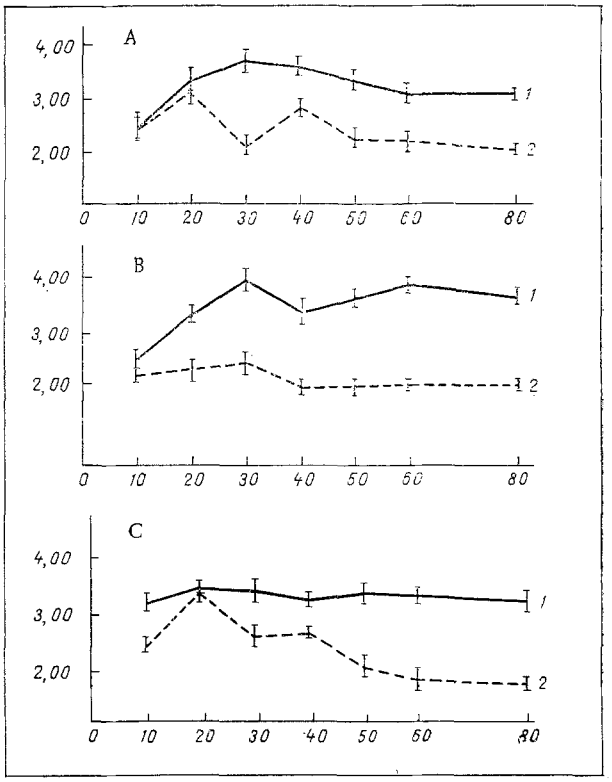

Fig. 1

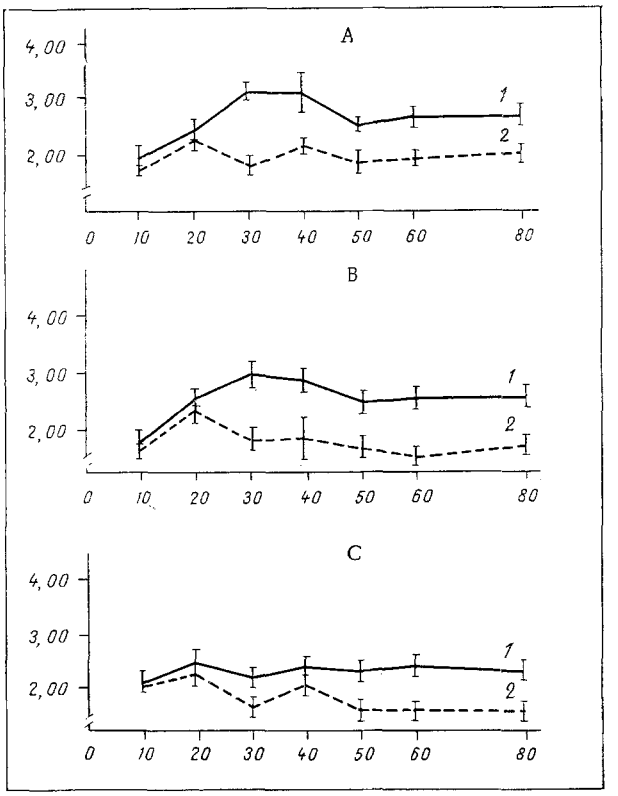

Fig. 2

Fig. 1. Dynamics of changes in oxidative enzyme activity in cortical zone of thymus depending on time after injection of virus. Here and in Figs. 2 and 3: A) NAD-diaphorase, B) NADP-diaphorase, C) βHBDH activity; 1) control, 2) experiment. Abscissa, time of experiment (days); ordinate, enzyme activity (in relative units).

Fig. 2. Dynamics of changes in oxidative enzyme activity in medullary zone of thymus depending on time after injection of virus.

of the experiment, and morphologically it was a hemangiopericytoma. The thymus was studied on the 10th, 20th, 30th, 40th, and 50th days of the latent period, and also on the 60th day (the stage of the primary tumor nodule) and the 80th day (the stage of intensive tumor growth). Thymus glands from healthy animals of the same age served as the control. The morphological investigation of the thymus was based on examination of the sections stained with hematoxylin and eosin, the histochemical investigations on cryostat sections. Activity of the following oxidative enzymes was determined: lactate, malate, isocitrate, succinate, and α-glucose-1-phosphate dehydrogenases (mitochondrial and cytoplasmic), and glucose-6-phosphate dehydrogenase (abbreviated to LDH, MDH, IDH, SDH, GPDH, and G6PDH respectively), NAD- and NADP-diaphorases, and β-hydroxybutyrate dehydrogenase (βHBDH). Activity of the enzymes coupled with NAD was determined by the method in [11], activity of those not coupled with NAD by the method in [9], and diaphorase activity by the method in [3]. For each of the above enzymes the microspectrophotometric relationship was established between optical density of the section, its thickness, and the incubation time. Analysis of the results showed a linear relationship between optical density and thickness of the sections in preparations 7-10 μ thick. A direct relationship was found between optical density and incubation time for NAD-diaphorase during incubation for 40 min, and for NADP-diaphorase and βHBDH during incubation for 60 min. Activity of the remaining oxidative enzymes in all zones of the thymus was very low at the times studied, so that they could not be analyzed quantitatively.

Microspectrophotometry of the preparations was conducted on a modified scanning integrating microspectrophotometer [1], using a 10 × 10 μ frame for determining optical density of dehydrogenases in thymocytes and a 5 × 5 μ frame for the corresponding determination in cells of the epithelial stroma, in a monochromatic beam of light with a wavelength of 560 nm. The results were subjected to statistical analysis with a 95% level of significance.

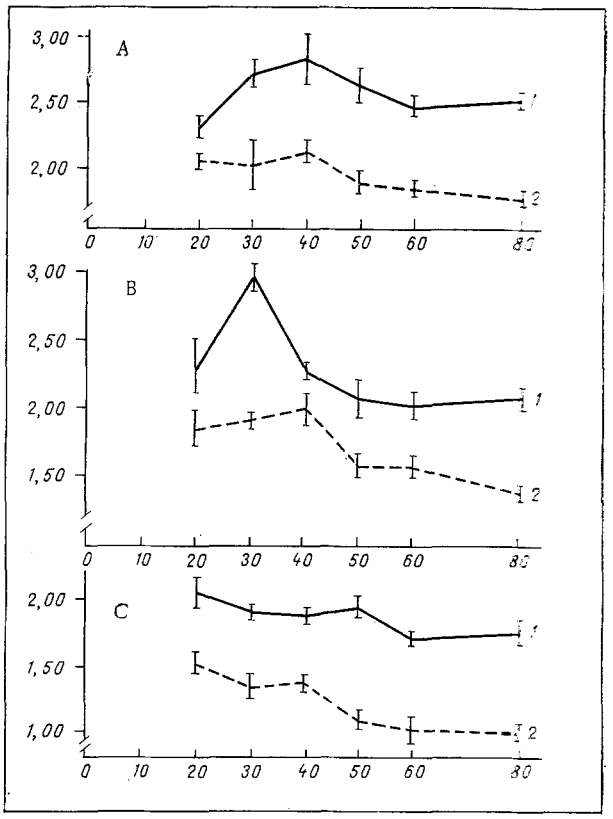

Fig. 3. Dynamics of changes in oxidative enzyme activity in cells of epithelial stroma of thymus, depending on time after injection of virus.

EXPERIMENTAL RESULTS

The comparative microspectrophotometric investigation of activity of oxidative enzymes was conducted on the following functional zones of the thymus: in the cortical and medullary zones, in cells of the epithelial stroma, under normal conditions and during tumor development.

In the control animals the character of distribution of diaphorase activity in the cortical and medullary zones of the thymus and in cells of the epithelial stroma was similar: The maximal level of enzyme activity was observed on the 30th day of postnatal development, when activity of NAD- and NAD-diaphorases was 1.6 times higher than on the 10th day (Figs. 1A, B and 2A, B), in the cells of the epithelial stroma NAD-diaphorase activity was 1.2 times, and NADP-diaphorase activity 1.3 times higher than the corresponding indices on the 20th day of development (Fig. 3A, B). Later, activity of the above-mentioned enzymes fell somewhat. Activity of βHBDH showed no significant change at all times of postnatal development (Figs. 1C, 2C, 3C).

In the animals receiving an injection of virus, the values of these indices fell even during the latent period of development of the tumor: NAD-diaphorase and βHBDH activity in the cortical thymocytes was significantly reduced by the 30th day of the experiment, by 1.8 and 1.3 times respectively compared with the control (Fig. 1A, C). In animals with a well-developed tumor (80th day of the experiment) the various indices were 1.5 and 1.9 times higher respectively than in the control. NADP-diaphorase activity in the cortical thymocytes of the experimental animals differed significantly from the control, starting with the 20th day of the experiment, and was 1.5 times lower (Fig. 1B). During the development of the tumor process this difference increased, to reach 1.7 times in animals with a well-developed tumor.

Activity of NAD- and NADP-diaphorases fell significantly in the medullary thymocytes compared with the control by the 30th day of the experiment. This difference was 1.8 times for NAD-diaphorase and 1.6 times for NADP-diaphorase (Fig. 2A, B). In the course of development of the tumor the difference increased, to 1.3 and 1.5 times respectively on the 80th

day of the experiment. Activity of βHBDH had two significant troughs: On the 30th and 50th days of the experiment, and in animals with a well-developed tumor it was 1.5 times below the control value (Fig. 2C).

The fall in oxidative enzyme activity in cells of the epithelial stroma of animals infected with virus began as early as the 20th day of the latent period. In mice with a well-developed tumor, NAD-diaphorase activity was 1.4 times lower, NADP-diaphorase activity 1.4 times, and βHBDH activity 1.7 times below the corresponding values in control animals of the same age (Fig. 3, A-C).

These results are evidence that in the postnatal period of development of the thymus, activity of oxidative enzymes reaches a maximum by the 30th day, indicating that the functional potential of the gland is at its highest before the onset of physiological involution [6, 13]. The low level of SHD, MDH, IDH, GPDH, and G6PDH activity in the thymus of the control animals is in agreement with data in the literature [5, 7, 12]. The present experiments showed that immunodepression induced by oncogenic adenovirus SA7(C8) [2] is accompanied by depression of metabolic activity of the maturing thymocytes and of the epithelial stroma, even during the latent period of carcinogenesis. This is in agreement with results obtained by other workers [4]: The most marked deviations in thymocyte metabolism are observed in a pretumor situation. Since the hormone thymosin synthesized by cells of the epithelial stroma is known to participate in the differentiation of maturing thymocytes [10], it can be tentatively suggested that the lowering of the level of cell metabolism of the epithelial stroma in adenovirus-induced carcinogenesis, in the early stages of infection, is one of the first links in the chain of disturbances leading to functional deficiency of the T stage of the immune response.

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