STUDY OF N-ACETYLTRANSFERASE ACTIVITY DURING GROWTH OF TRANSPLANTABLE PLISS LYMPHOSARCOMA AND DURING PREGNANCY IN RATS

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Direct correlation was demonstrated previously between the frequency of appearance of spontaneous tumors in mice and the level of acetylation due to activity of the enzyme N-acetyltransferase [1]. An increase in acetylation activity also has been observed during induction of tumors in various situations by means of 7,12-dimethylbenz(a)anthracene [2] and during growth of transplantable tumors in mice and rats [9]. It has been suggested that regular and consistent changes in N-acetyltransferase activity in animals with various types of experimental tumors and in man with tumors in various situations that the changes observed are a characteristic property of carcinogenesis [8]. Meanwhile the behavior of this enzyme system in other pathological or normal states (pregnancy) has been inadequately studied.

To continue the study of this phenomenon, in the investigation described below, activity of N-acetyltransferase was studied for the first time during growth of a transplantable tumor (Pliss lymphosarcoma). To determine the specificity of correlation between the rate of acetylation and tumor growth, changes in N-acetyltransferase activity during tumor growth were compared with those observed at different times of pregnancy in rats.

EXPERIMENTAL METHOD

Experiments were carried out on 89 noninbred male albino rats weighing 160-180 g. In the experiments of series I (10 rats) a Pliss lymphosarcoma was transplanted into the animals by the usual method and the time course of growth of the tumor was judged from changes in its volume [5]. In series II, 10 female rats with accurately dated onset of pregnancy were used. The day on the morning of which spermatozoa were found in vaginal smears of females kept in the same cage as males was taken as the first day of pregnancy.

Blood levels of N-acetyltransferase activity of the animals were determined before and in the course of the experiment: in animals with tumors on the 8th, 10th, 12th, 15th, 19th, 21st, and 24th days after transplantation, in pregnant rats on the 6th, 15th, and 21st days of pregnancy. At these times the rats were given an intraperitoneal injection of sulfadimidine in a dose of 50 mg/kg, and 5 h after the injection blood in a volume of 0.8-1.2 ml was taken from the orbital sinus under ether anesthesia. Since the intensity of acetylation of sulfadimidine reflects the level of N-acetyltransferase activity, the latter was assessed as the ratio of acetylated sulfadimidine to the total sulfadimidine concentration in the blood, expressed in percent [7].

The results were subjected to statistical analysis by the Wilcoxon–Mann–Whitney test [4] and rank correlation was evaluated as described previously [6].

EXPERIMENTAL RESULTS

Investigation of 89 intact females showed that the animals as a whole could be divided conventionally into three groups on the basis of N-acetyltransferase activity (Fig. 1): those with low values (4-10%) — 23 rats, those with intermediate values (11-18%) — 46 rats, and those with high values (19-24%) — 20 animals. Differences in the type of acetylation in the rats

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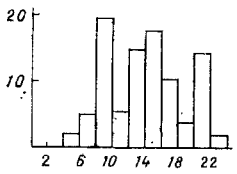

Fig. 1

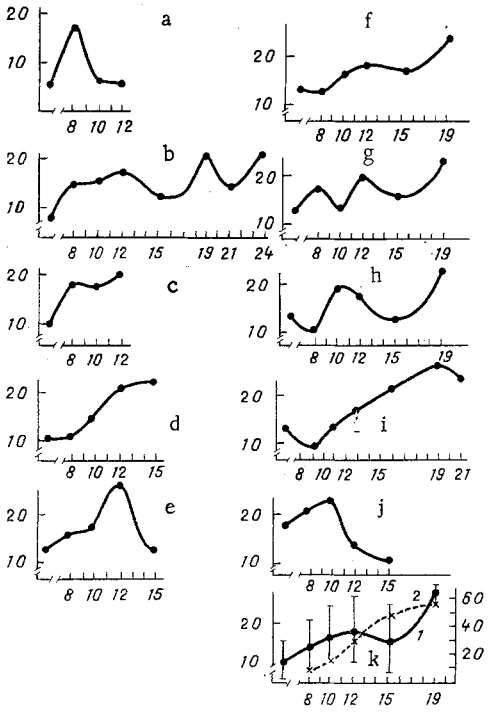

Fig. 2

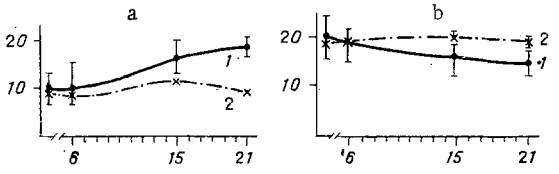

Fig. 3

Fig. 1. Distribution of female noninbred albino rats by acetyltransferase activity. Abscissa, N-acetyltransferase activity (in %); ordinate, number of animals (in %).

Fig. 2. Changes in N-acetyltransferase activity during growth of Pliss lymphosarcoma: a-j) individual animals in order of increasing initial acetylation phenotype; a-c) "slow," d-i) "intermediate," j) "fast" acetylation phenotypes (each point is mean value of three measurements); k) averaged data: $P < 0.05$ for 12th and 19th days relative to initial level. Observations made before death of animals. Abscissa, time of observations (in days, counting from 1st day after transplantation); ordinate (left) — N-acetyltransferase activity (in %); ordinate (right), weight of tumor (in g). 1) N-acetyltransferase activity, 2) weight of tumor.

Fig. 3. Changes in N-acetyltransferase activity during pregnancy in noninbred albino rats. a) "Slow" and "intermediate" acetylation phenotypes, $P < 0.05$ for 15th and 21st days relative to initial level; b) "fast" acetylation phenotype, $P < 0.05$ for 21st day relative to initial level. Abscissa, time of observations (in days, counting from 1st day of pregnancy); ordinate, N-acetyltransferase activity (in %). 1) Pregnant rats: a) three animals, b) four animals; 2) rats with interrupted pregnancy: a) one animal, b) two animals.

resembled the analogous distribution in mice [3], rabbits [10], and man [12]. The results suggest the presence of individuals among the noninbred rats studied with monozygotic "slow" (relatively low N-acetyltransferase activity) or "fast" (high N-acetyltransferase activity), and also heterozygotic individuals with an "intermediate" acetylation phenotype.

The study of the intensity of acetylation in rats during tumor growth revealed an increase in N-acetyltransferase activity on the 12th and 10th days after transplantation (by 60 and 130% of the initial level, respectively, $P < 0.05$; Fig. 2k). Individual differences also were observed in the time course of the change in enzyme activity (Fig. 2, a-j). On the whole no cor-

relation could be found between the initial acetylation phenotype, on the one hand, and individual differences in the time course of the change in activity of the enzyme or length of survival of the animals with tumors, on the other hand. Nevertheless, it must be pointed out that death of the animals as a rule (in seven of 10 cases) was preceded by an increase in the level of enzyme activity. However, no correlation could be found between changes in enzyme activity and the increase in weight of the tumor (Fig. 2k).

N-Acetyltransferase activity in rats on the 15th and 21st days of pregnancy differed, and the character of its change likewise differed toward the end of pregnancy (Fig. 3). For instance, in rats with "slow" and "intermediate" acetylation phenotypes the level of enzyme activity rose from the 15th to the 21st day of pregnancy, to reach 180% of its initial level ($P < 0.05$; Fig. 3a), whereas in rats with a "fast" acetylation phenotype it was 30% lower at these same times ($P < 0.05$, Fig. 3b). N-Acetyltransferase activity in the early stages of pregnancy (6th day) was virtually indistinguishable from its initial level. In rats with interrupted pregnancy, no change was observed in N-acetyltransferase activity at the above-mentioned times after fertilization (Fig. 3).

Both growth of a transplantable tumor (Pliss lymphosarcoma) and the development of pregnancy were thus accompanied by changes in N-acetyltransferase activity in rats. However, we know that activity of this enzyme in intact sexually mature rats is relatively stable [2]. The change in intensity of acetylation during tumor growth or pregnancy must evidently be regarded in conjunction with growth of the cell mass of the tumor or fetus.

Considering that the weight of the fetus on the last days of pregnancy is approximately 4-5 g, and the weight of the tumor on the 8th day after transplantation is similar (4-8 g), the vector character of the changes in N-acetyltransferase activity in each animal was compared at these particular periods, i.e., on the 21st and 8th days, respectively, during pregnancy and tumor growth. According to the results of histological investigations, growth of Pliss lymphosarcoma during the first 8-10 days was not accompanied by any appreciable death of the cells, and a tumor of this weight contained no necrotic areas. As will be clear from Fig. 2, in rats with the "slow" acetylation phenotype, N-acetyltransferase activity on the 8th day of tumor growth was substantially (by 100-200%) increased (Fig. 2, a-c), in rats with the "intermediate" acetylation phenotype it was increased (Fig. 2e, g), reduced (Fig. 2h, i), or remained at its original level (Fig. 2d, f), and in rats with the "fast" acetylation phenotype it remained at its initial level (Fig. 2j). Definite similarity could thus be observed in the vector character of the change in N-acetyltransferase activity. It must be pointed out, however, that whereas in pregnancy changes in N-acetyltransferase activity were characterized by a stereotype, determined unambiguously by the initial phenotype, during tumor growth considerable individual variability in the character of the changes was observed. These differences are evidently linked with the character of the processes studied: pathological in one case, normal and "programmed" in the other case.

Since the metabolic activity of the embryonic liver relative to many compounds, including xenobiotics, is low [11], it can be tentatively suggested that the enzyme located in the maternal liver is more likely to be responsible for the changes observed in the intensity of acetylation. Changes in the level of acetylation during tumor growth evidently reflect in a similar way the influence of tumor growth on the liver cells in which, as has been shown [13], most N-acetyltransferase activity is concentrated.

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MORPHOLOGICAL CYTOCHEMICAL CHARACTERISTICS OF SPONTANEOUS HEMOBLASTOSIS OF AKR MICE

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Spontaneous hemoblastoses, causing death of 70-90% of AKR mice in the first year of life, have been widely used in various branches of experimental oncology and hematology. However, available information on the morphological characteristics of hemoblastosis arising in mice of this strain is highly contradictory. For example, one investigator has described all the known morphological variants of hemoblastosis in mice of this strain [5], whereas others [1] have described AKR leukemia as hemocytoblastosis and myelosis, and a third group [2] have stated that the myeloid form of leukemia is observed most frequently in the animals which they studied. Finally, in more recent investigations the view has been expressed that hemoblastoses in AKR mice are lymphoid in genesis [4] and some workers consider them to be generalized lymphosarcomas, whereas others are inclined to regard them as lymphatic leukemias [3, 6]. The contradictory nature of these data to some degree reflects present difficulties in the differential diagnosis of tumor-like leukemias and generalized lymphosarcomas, for there are as yet no morphological criteria for differentiation of these two processes. In view of the facts described above it was decided to study the morphological characteristics of hemoblastoses in AKR mice, using modern methods of cytological and cytochemical analysis.

EXPERIMENTAL METHOD

Experiments were carried out on 500 AKR mice of both sexes obtained from the "Stolbovaya" nursery, Academy of Medical Sciences of the USSR, at the age of 2 months. The animals were kept on a standard diet. Of the total number of mice 400 were kept under observation until natural death; from the remaining group of 100 animals ten were killed for investigation every month. All animals which died or were killed were autopsied. Peripheral and abdominal lymph nodes, the thymus, spleen, liver, lungs, and kidneys were taken for histological analysis. The organs were fixed in 10% formalin and embedded in paraffin wax; sections were stained with hematoxylin and eosin. Squash preparations of internal organs and bone marrow films stained by Leishman's method were used for cytological analysis. Parallel with the cytological investigation, in 36 of 400 mice with hemoblastosis diagnoses on the basis of inspection and hematologic investigation a cytochemical study was made of squash preparations of lymph nodes, spleen, liver, lungs, and kidneys, including the PAS reaction and determination of activity of the following enzymes: α-naphthyl acetate esterase, peroxidase, acid and alkaline phosphatases. Parallel tests were carried out with sodium fluoride, an inhibitor of α-naphthyl acetate esterase, and with potassium-sodium tartrate, an inhibitor of acid phosphatase.

EXPERIMENTAL RESULTS

Among the 400 animals kept under observation until natural death, hemoblastoses were found in 77.5% of mice. Clinically these mice showed marked dyspnea and a barrel chest, due to the presence of an enormous tumor of the thymus. Analysis of the peripheral blood picture of most of these mice revealed wide variation in the numbers of leukocytes and blast cells. At autopsy

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