MICROSPECTROMETRIC POSTMORTEM STUDY OF CHANGES IN THE NUCLEAR DNA CONCENTRATION IN THE LIVER, SKELETAL MUSCLE, AND MYOCARDIUM OF RATS

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UDC 612.398.145.1.015.349

A comparative microspectrophotometric investigation was made of changes in the DNA content in cell nuclei of the liver, skeletal muscle, and myocardium of noninbred male albino rats dying from head injury, in the course of the 3 days after death. The decrease in the nuclear DNA content within the 36-72 h interval is described mathematically by regression curves of the type:

$$M_{(t)} = e^{-at+b}$$

On the basis of the results a mathematical description is given of the postmortem change in the DNA content in the test material.

KEY WORDS: microspectrophotometry; nuclear DNA; postmortem period.

Considerable material has been gathered in recent years during the study of the dynamics of the DNA content in cell nuclei of different organs during malignant growth and other pathological changes [1, 2, 4, 5]. However, the principles governing changes in the quantity of genetic material in cell nuclei during their necrobiosis and necrosis after death of the organs have hardly been studied at all, although the examination of these problems is linked with an evaluation of the role of microspectrophotometry for the investigation of autopsy and biopsy material.

Accordingly the main purpose of the present investigation was to study the principles governing changes in the nuclear DNA content in tissue cells at various times after death.

EXPERIMENTAL METHOD

Experiments were carried out on 90 noninbred male albino rats weighing 180-220 g. For a week the animals were kept on a standard diet in individual cages. All food was removed from the cages 16 h before sacrifice of the animals, but water continued to be provided ad lib. All the animals were killed (by head injury) at the same time of day. The time at which death took place was taken to be the end of agony, the duration of which was 4-5 min.

Material for microspectrophotometric investigation was taken immediately after the end of agony and after the lapse of 2, 4, 6, 12, 24, 36, 48, and 72 h. The cadavers were kept in the laboratory at 18-20°C in a relative humidity of 40-60%.

Pieces of the soleus muscle, myocardium, and liver (and lymph nodes also in the control) were fixed with Carnoy's mixture and all pieces from the same animal were embedded in one paraffin wax block. Ten sections were cut from each block for microspectrophotometry. Microspectrophotometry of the nuclei was performed on sections stained by the Feulgen reaction. The following conditions were observed during the reaction: all sections were divided into groups with nine sections in each group; each section in such a group represented one stage of the experiment. All subsequent processing of sections belonging to the same group was carried out simultaneously and under absolutely identical conditions. The sections were hydrolyzed with 1N HCl at 60°C for 8 min. The Feulgen reaction was then carried out on the sections. The sections were

Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow. Izhevsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 87, No. 6, pp. 620-622, June, 1979. Original article submitted July 31, 1978.

0007-4888/79/8706-0659$07.50

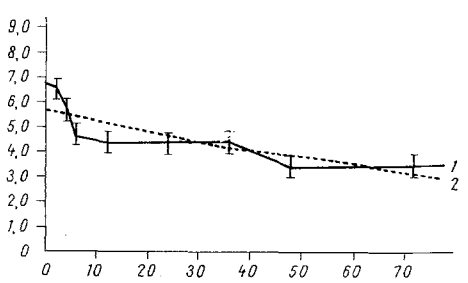

Fig. 1

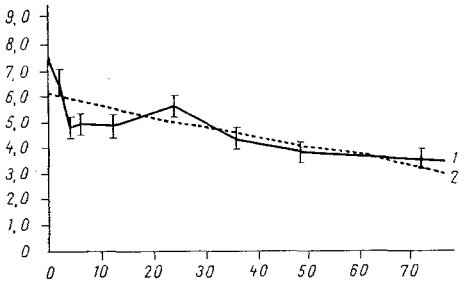

Fig. 2

Fig. 1. Dynamics of DNA content in rat liver cell nuclei (1) and regression curve of decrease in DNA content (2) in postmortem period. Here and in Figs. 2 and 3: abscissa, time (in h); ordinate, DNA content (in conventional units; M ± 3m).

Fig. 2. Dynamics of DNA content in rat skeletal muscle cell nuclei (1) and regression curve of decrease in DNA content (2) in postmortem period.

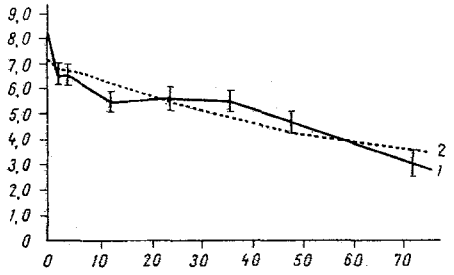

Fig. 3. Dynamics of DNA content in rat myocardial cell nuclei (1) and regression curve of decrease in DNA content (2) in postmortem period.

dehydrated, cleared, and mounted in Canada balsam. The DNA content in the nuclei was investigated on a frame-integrating microspectrophotometer [3] at a wavelength of 550 nm (the maximum of absorption of basic fuchsin in the visible part of the spectrum) and with a probe with an area of 1 μ^2. At each time of the experiment 125 arbitrarily chosen nuclei were subjected to microspectrophotometric investigation. The modal class of optical density of the nuclei of small lymphocytes in sections through lymph nodes of control rats was taken as the indicator of diploidy. Statistical analysis of the results was carried out by methods of variance analysis. The results were compared by Student's t test. Changes in the DNA content in the cell nuclei from individual structures in the course of the experiments were studied by methods of regression analysis. Regression curves showing DNA levels in cell nuclei of the test tissues as a function of the time elapsing after death were calculated by the equation:

$$M_t = e^{-at+b},$$

where e is the base of natural logarithms; t the time elapsing after death; a and b coefficients obtained by the method of least squares, using the mean index M for 125 cells of each tissue.

Mathematical analysis of the data was carried out on the MIR-1 computer.

EXPERIMENTAL RESULTS

Dynamics of the DNA Content in the Liver Cell Nuclei during the First 72 h after Death. Data showing changes in the DNA content in hepatocyte nuclei are given in Fig. 1. The regression equation for the DNA content of the hepatocyte nuclei is of the type:

$$M_t = e^{-0.013t+6.576}\cdot 10^{-2}.$$

The values of the DNA content in the hepatocyte nuclei of the control animals were significantly higher than those for nuclei of small lymphocytes. This indicated the presence of a certain number of polyploid nuclei and must affect the final result for the population as a whole. A sharp decrease in the DNA content

(below values corresponding to a diploid set of chromosomes) was observed in the hepatocyte nuclei 2 h after death. During the next 4 h of the postmortem period the DNA content in the hepatocytes did not change significantly. In the period from 2 to 6 h, the activity of DNA breakdown in the liver diminished compared with the previous period. During the next 6 h, processes of DNA catabolism in the organ were again intensified. Later (between 12 and 36 h) the process showed some degree of stabilization.

Dynamics of the DNA Content in Nuclei of Skeletal Muscle Fibers. Data showing the postmortem dynamics of the DNA content in nuclei of skeletal muscle fibers are illustrated in Fig. 2. The regression equation for the DNA content of the skeletal muscle nuclei is in the following form:

$$M_t = e^{-0.008t + 6.337} \cdot 10^{-2}.$$

The content of nuclear DNA in the muscle fell steadily until 12 h of the postmortem period. Between 12 and 36 h there was no evidence of any intensive decrease in the DNA content in the muscle. The second stage of decrease in the DNA content was observed 48 h after death.

Dynamics of the DNA Content in the Cardiomyocyte Nuclei. The dynamics is shown in Fig. 3. The regression equation for values of the DNA content is as follows:

$$M_t = e^{-0.009t + 6.416} \cdot 10^{-2}.$$

The DNA content fell rapidly during the first 4 h after death, but 12 h after death it showed no significant change. After 24 h the DNA content in the cardiomyocyte nuclei, judging from the mean values measured, increased. This fact can evidently be explained by differences in the initial functional state of the cells. After 24 h a gradually progressive process of DNA breakdown was observed in the myocardium. The increase in the intensity of the Feulgen reaction in the cell nuclei in the postmortem period could be connected with liberation of DNA from nucleoprotein complexes [6].

It can be concluded from this investigation that certain general rules can be discerned in the dynamics of the DNA content in the cell nuclei of the tissues studied in the postmortem period. The type of curve obtained, reflecting changes in M as a function of t, usually describes a decremental process and disintegration of structures, such as is observed during dying of liver, skeletal muscle, and myocardial cells. This is also demonstrated by the values of the coefficients: coefficient a for the liver -0.013, for skeletal muscle -0.008, and for myocardium -0.009; coefficient b for the liver $+6.575$, for skeletal muscle $+6.337$, and for the myocardium $+6.416$.

The experimental curves reflecting the DNA content in the nuclei of the test tissues correspond to some degree to the course of the regression curves (Figs. 1-3) for the period between 36 and 72 h after death. In the earlier stages of the investigation the experimental curves were periodic in character and the amplitude of the fluctuations of these curves differed for different organ structures. The largest number of fluctuations was a feature of the curve reflecting the dynamics of the DNA content in the cardiomyocytes, the smallest its dynamics in the soleus muscle, and the curve showing changes in the DNA content in the hepatocytes occupied an intermediate position.

The features of the curves reflecting the decrease in the DNA content in the cell nuclei of tissues investigated in the postmortem period, described above, can evidently be explained by differences in the initial functional state of the cells.

The regression equations showing the decrease in the DNA content in cell nuclei of the liver, skeletal muscle, and myocardium of the experimental animals can be used also to determine more precisely the time of death after an interval of between 36 and 72 h, by means of the equations:

$$\text{for the liver } t = \frac{6.576 - \ln M \cdot 10^2}{0.013};$$

$$\text{for skeletal muscle } t = \frac{6.337 - \ln M \cdot 10^2}{0.009};$$

$$\text{for the myocardium } t = \frac{6.416 - \ln M \cdot 10^2}{0.009};$$

furthermore, the values of the postmortem decrease in the DNA content in the cell nuclei must be taken into account when microspectrophotometric investigations are made of autopsy and biopsy material.

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COURSE OF DEVELOPMENT OF ACUTE TOXIC HEPATITIS IN RATS STIMULATED WITH PRODIGIOSAN

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UDC 616.36-002.1-099-092:615.331

Acute toxic hepatitis was induced in Wistar rats by a single injection of 40% CCl_4 in peach oil in a dose of 0.2 ml/100 g body weight. During stimulation with the bacterial polysaccharide prodigiosan the resistance of the hepatocytes to CCl_4 rose sharply and this was reflected in a decrease in the severity of destruction of the hepatic parenchyma.

KEY WORDS: toxic hepatitis; prodigiosan; resistance of hepatocytes.

Previous investigations showed that the course of reparative regeneration of hepatocytes after partial resection of the liver in rats depends essentially on the initial functional state of the Kupffer macrophages [3]. Blockade of the Kupffer cells with granules of colloidal iron carbonyl, if carried out before or during the first few hours after the operation, appreciably inhibited the rate of DNA synthesis and of mitotic division of the hepatocytes. Conversely, preliminary stimulation of the mononuclear phagocyte system (MPS) with prodigiosan provided the conditions for more rapid regeneration of the hepatocytes in partially hepatectomized rats. The problem thus arose of the course of regeneration of the pathologically charged liver during functional reorganization of its stroma.

The results described in this paper reveal certain special features of the structural recovery of the hepatocytes after CCl_4 poisoning in rats with an activated MPS. The MPS was stimulated by means of the bacterial polysaccharide prodigiosan, a substance which has proved itself to be a reliable inducer of tachyphylaxis in many clinical and experimental investigations [2].

EXPERIMENTAL METHOD

Wistar rats of both sexes weighing 200-250 g were used. Toxic hepatitis was induced by a single injection of a 40% solution of CCl_4 in peach oil in a dose of 0.2 ml/100 g body weight. An intraperitoneal injection of 50 μg prodigiosan (experiment) or of 0.85% NaCl (control) was given to the rats 24 h before poisoning. Changes in the structure of the liver were noted 16, 24, 48, and 72 h after poisoning. Zones of damage were measured under a magnification of 100 times by means of a grid mounted in the ocular, dividing the field of vision into 16 squares, in sections stained with hematoxylin-eosin and by the PAS method. The mean number of points of intersection of the lines of the grid falling on the area of the zone of damage was determined in 30 fields of vision. The numerical results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

In the rats of the control and experimental groups the formation of acute toxic hepatitis on the first day after poisoning was accompanied by the appearance of foci of necrosis in the central zones of the hepatic lobules, and by signs of "balloon" and eosinophilic degeneration spreading from the center of the lobule to its

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(Presented by Academician of the Academy of Medical Sciences of the USSR V. P. Kaznacheev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 87, No. 6, pp. 622-624, June, 1979. Original article submitted August 6, 1978.

0007-4888/79/8706-0662$07.50