repair of DNA-protein cross-linkages is possible has assumed great biological importance at the present time [1]. Moreover, data have been published to show that in certain inherited pathological conditions and, in particular, in xeroderma pigmentosum, the fibroblasts of the skin evidently cannot repair DNA-protein cross-linkages induced by UV-radiation [9]. The role of repair systems in the elimination of cross-linkages must therefore await further investigation.

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LIMIT OF CERTAIN REACTIONS OF THE CELL NUCLEUS IN OLD AGE AND IN CHRONIC CCl_4 POISONING

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UDC 612.014.22:612.67+616-018.13

The number of nucleoli and the structure of chromatin bound with the nucleolus (as reflected in the number of chromocenters) were investigated in two groups of mice: a control group and an experimental group receiving weekly injections of carbon tetrachloride for one year. During aging a gradual increase in the number of nucleoli and in the number of chromocenters bound with the nucleolus was observed in the control animals. During CCl_4 administration both these indices rapidly reached their maxima, which were the same as or a little higher than the values for the old animals. During subsequent development of the pathological process no further change took place in these indices.

KEY WORDS: nucleolus; chromatin; old age; carbon tetrachloride.

The similarity between age changes in the structure and function of organs and changes arising as a result of stress reactions [3, 7] or prolonged hyperfunction of an organ [2] has been described. The essential nature of the processes causing more rapid aging of organs affected by a pathological process has not yet been explained. The explanation given by most investigators, in its general form, can be reduced to the statement that the body possesses a certain reserve of adaptive capacity which becomes exhausted with age. In disease intensive mobilization of this reserve takes place, so that it becomes exhausted more rapidly.

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(Presented by Academician of the Academy of Medical Sciences of the USSR M. I. Kuzin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 86, No, 9. pp. 367-370, September, 1978. Original article submitted July 25, 1977.

0007-4888/78/8609-1248$07.50

Fig. 1. Electron-microscopic autoradiograph of nucleolus in hepatocyte of mouse receiving CCl_4 injections for 6.5 months. Arrows point to two chromocenters connected with nucleolus. Distribution of grains of silver shows that RNA synthesis took place only in body of nucleolus. 20,000 ×.

The object of this investigation was to compare changes taking place in the nuclei of liver cells during aging and during chronic CCl_4 poisoning.

EXPERIMENTAL METHOD

Two groups of noninbred albino mice were studied. Once a week the animals of the first group received a subcutaneous injection of 0.2 ml of a 40% solution of CCl_4 in peach oil; the other group served as the control. The experiments were carried out over a period of one year. Every week the liver of two experimental and one control animals was fixed for histological and autoradiographic investigation. In 200 randomly chosen hepatocytes from each animal the number of nucleoli and the number of chromocenters associated with the nucleolus were counted. An electron-autoradiographic investigation of RNA synthesis was undertaken. For this purpose uridine-5-^{3}H was injected into the animals in a dose of 100 μCi/g. Pieces of liver 4 h after injection of uridine were fixed with glutaraldehyde and osmium tetroxide and embedded in Epon. A monolayer of M emulsion was applied to the sections and exposure lasted 30-120 days. The statistical significance of differences between the experimental and control series was determined by Wilcoxon's criterion.

EXPERIMENTAL RESULTS

The number of nucleoli in the hepatocytes of the control mice increased with age, in agreement with data showing an increase in the ploidy of liver cells during life [1]. The mean number of nucleoli in the sections through hepatocytes of the control mice under 7 months of age was 2.7, and in older animals (7-12 months) the number increased to 3.6. In the experimental animals hyperplasia of the nucleoli took place much faster, and even in the younger age group of mice (under 7 months), the mean number of nucleoli after a course of CCl_4 injections lasting 1.5-6 months was 3.6, i.e., the same as in the older control animals. This number remained constant in the future, despite the increase in the animals' age and in the number of CCl_4 injections they had received.

Hyperplasia of the nucleoli undoubtedly reflected a reparative response aimed at maintaining the necessary level of synthesis of ribosomal RNA under conditions when some of the liver cells had been killed by the action of CCl_4. The same clearly defined quantitative limit was found when the other reaction of structural rearrangement of chromatin bound with the nucleus was studied.

In sections through hepatocytes of the younger mice most frequently one chromocenter was associated with the nucleolus. Frequently, however, a nucleolus with two chromocenters could be found, and for that reason the mean number of chromocenters per nucleolus in the mice aged 4-7 months was 1.33. As the animals aged a tendency was observed for this index to increase to 1.41 in the mice aged 7-12 months, although the difference in the mean values in the younger and older groups of the control in these experiments was not statistically significant.

Fig. 2. Electron-microscopic autoradiograph of nucleolus of mouse muscle fiber. High concentration of label discovered above body of nucleolus; heterochromatin bound with nucleolus (arrow) not labeled. 20,000 ×.

The pathological process led to a more rapid reorganization than aging of the chromatin bound with the nucleolus. In the mice aged 4-7 months, after exposure to CCl_4 for 1.5-6 months the mean number of chromocenters bound with the nucleolus increased to 1.65 (statistically significantly compared with the control of the same age; $P < 0.01$). This figure represented the maximum in the present experiments, like the figure of 3.6 characterizing hyperplasia of the nucleoli. The subsequent course of the pathological process and the increase in the animals' age caused no further increase in the number of chromocenters associated with the nucleus; their mean number in the mice aged 7-14 months receiving CCl_4 for 6-12 months was 1.58.

How is the increased number of chromocenters associated with the nucleolus reflected in the function of the organelle? RNA synthesis in liver cells takes place only in the body of the nucleolus (nucleonema); the template for this synthesis is evidently the intranucleolar euchromatin (Fig. 1). In electronmicroscopic autoradiographs grains of silver are frequently found above the heterochromatin bound with the nucleolus, but the density of their distribution per unit area does not differ significantly from the background. Similar results were obtained by the writers during an electron-autoradiographic investigation of RNA synthesis in connective tissue, muscle, glandular, and nerve cells (Figs. 2 and 3). The passiveness of the nuclear heterochromatin in relation to RNA synthesis was described in the very first electron-autoradiographic studies of this problem [5, 6]. Modern views of the more condensed "packing" of DNA molecules not utilized in transcription are based to a large extent on these results. The increase in the number of nucleolar chromocenters was therefore regarded as a response connected with the decrease in the number and volume of templates for RNA synthesis, condensation of nonfunctioning templates, and their rearrangements on the surface of the nucleolus, where they form additional chromocenters.

In the writers' view, hyperplasia of the nucleoli and the increase in the number of nucleolar chromocenters are interconnected reactions proceeding in opposite directions: the first contributes to increased production of ribosomal RNA, the second to its reduced production. The unity of these reactions is emphasized by the similarity of their manifestations during normal development of the animal and in disease. Accordingly an attempt may be made to analyze the factors responsible for the parallel trend in the formation and precise demarcation of the range of hyperplasia of the nucleoli and the increased number of chromocenters. In a diploid cell there are two sets of genes which control nucleolar function – the nucleolar organizers [4]. Under normal conditions, however, there is only one nucleolus in the cell. The organizer which has not formed a nucleolus is a compensation reserve which can be mobilized in the presence of an appropriate change in the internal and external conditions for life of the cell. One such change may be an increase in the mass of heterochromatin (or in the number of chromocenters) in the working nucleolus on account of injury to the templates on account either of their own work or of the pathogenic factor. The reparative response in this particular situation is the formation of a second nucleus by the reserve nucleolar organizer. These arguments apply to polyploid cells also, in which the number of nucleolar organizers is greater, but is also strictly limited. The increase in the number of chromocenters with age and in chronic diseases can be attributed to inactivation of templates.

Fig. 3. Electron-microscopic autoradiograph of nucleolus of neuron. Selective concentration of grains of silver only above body of nucleolus and grains completely absent above heterochromatin connected with nucleolus (arrow). 20,000 ×.

The limit of this response is determined by the limited number of templates, which is determined by the constancy of the content of cell DNA in general and nucleolar DNA in particular. In turn, the appearance of a large number of less active nucleoli with an increase in the number of chromocenters and death of a certain number of liver cells as a result of CCl_4 poisoning stimulate hyperplasia of the nucleoli. The limit of this hyperplasia is determined by the limited number of nucleolar organizers in the cell.

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