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MORPHOLOGICAL MANIFESTATIONS OF COMPENSATORY AND ADAPTIVE PROCESSES IN THE LIVER DURING AGING

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Aging is characterized by reduced reliability of the maintenance of homeostasis, the material basis of which is regeneration. Meanwhile, a combination of compensatory and adaptive mechanisms aimed at preserving viability under conditions of a changed external environment operates in the body [9]. An important feature distinguishing changes in the liver during aging is reduction of the number of hepatocytes due to their death from external and internal causes, with the result that the organ atrophies. The aim of this investigation was to study compensatory and adaptive processes and mechanisms of regeneration developing in this situation.

EXPERIMENTAL METHOD

Experiments were carried out on 15 male Wistar rats belonging to three age groups: 8, 24, and 30 months (five animals in each group). Pieces of liver for light microscopy were fixed in acetic-alcohol-formalin by Brodskii's method; paraffin sections 7 μ thick were stained with hematoxylin and eosin, with magnification of 90, 1.6, and 10 times, in fields of vision with an area of 0.78 mm^2, taken as the unit of measurement; the total number of hepatocytes, the number of binuclear hepatocytes and the number of sinusoidal cells were counted; the mean area of the mononuclear hepatocytes and of their nuclei was determined on the Leitz ASM instrument, using a KONFI program. Cytochrome oxidase was determined by Burstone's method. For electron microscopy (EM) material was fixed in 3% glutaraldehyde in phosphate buffer, pH 7.4, postfixed in 1% osmic acid solution, dehydrated, and embedded in Epon 812. Sections cut on the LKB-III Ultrotome were stained by Reynolds' method and studied in the JEM-100B electron microscope.

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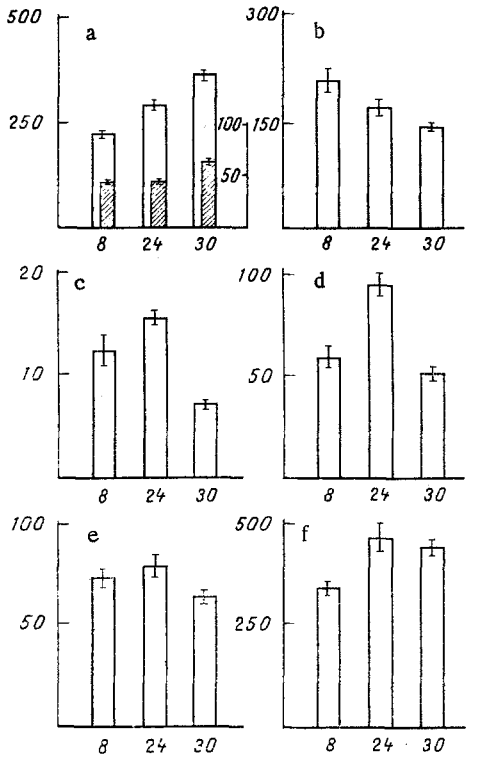

Fig. 1. Age changes in section through hepatocytes and their nuclei. Abscissa, age, in months. Ordinate: a) area of hepatocytes (left) and their nuclei (right), b) total number of hepatocytes, c, d) number of binuclear hepatocytes, e, f) number of sinusoidal cells.

EXPERIMENTAL RESULTS

The light-optical study of the liver revealed an exaggerated lobular structure due to connective-tissue fibers in the 24- and 30-month-old animals compared with those aged 8 months, although the regularity of the trabecular structure was basically preserved in all age groups. The area of the hepatocytes and their nuclei was increased in animals aged 24 and 30 months (Fig. 1a). The numerical density of the hepatocytes, calculated per unit area, gradually decreased regularly with age (Fig. 1b). The discovery of enlargement of the nuclei during aging is evidence of an increase in their ploidy which, in turn, is one cause of hypertrophy of hepatocytes. The degree of ploidy in the liver has been shown to be directly proportional to the size of the nuclei and age both in laboratory animals and in man [4, 12]. During EM, the normal arrangement of packing of the cytoplasmic and nuclear structures, as well as their normal structure, were observed in the hepatocytes of rats aged 24 and 30 months. Against this background, in some hepatocytes hyperplasia of the smooth endoplasmic reticulum was particularly noticeable (Fig. 2), and this can be interpreted as an adaptation to the increase in the load on the detoxicating system of the hepatocyte during aging, although activity of cytochrome oxidase, the key enzyme of this system, as our histochemical investigations showed, was considerably reduced in the animals aged 30 months. Incidentally, no partial necroses, vacuoles, infiltration with large lipid droplets, or edema, i.e., all those features which could simulate enlargement of the hepatocytes revealed at the light-optical level, and which are not manifestations of compensation but, rather, evidence of dystrophy, could as a rule be found.

Consequently, hypertrophy of the hepatocytes and their nuclei arising during aging is the result of hyperplasia and hypertrophy of cytoplasmic and nuclear ultrastructures. This manifestation of intracellular regeneration is the principal form of maintenance of structural homeostasis and one which lies at the basis of all compensatory and adaptive processes in the cell [5]. However, intracellular regeneration in some hepatocytes is delayed or is not observed at all, as shown by the standard deviation from the mean values of parameters of hepatocytes and their nuclei, which rises in old rats, showing that besides hypertrophied

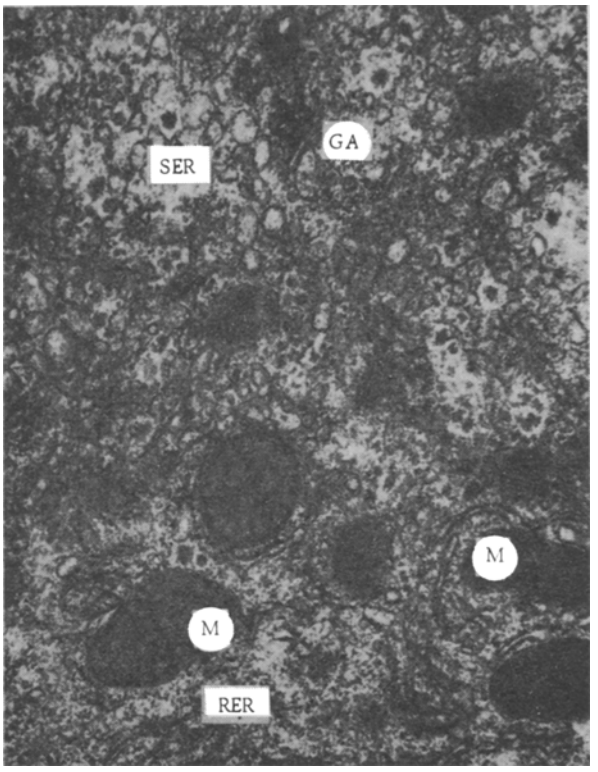

Fig. 2. Hyperplasia of smooth endoplasmic reticulum (SER) in hepatocyte of rat aged 30 months. RER) Rough endoplasmic reticulum, M) mitochondria, GA) Golgi apparatus. 20,000×.

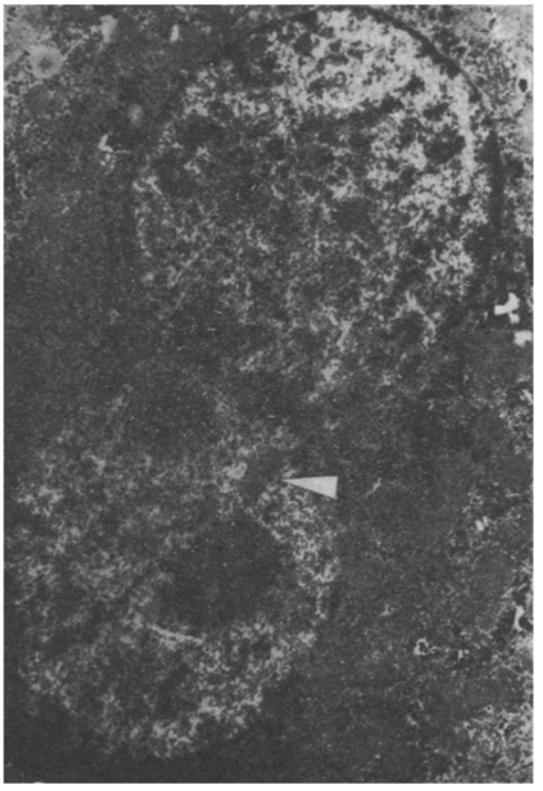

Fig. 3. Binuclear hepatocyte of rat aged 24 months. Intranuclear lipid inclusion visible in one of the nuclei (arrow). 6000×.

cells, there are others which are considerably reduced in size. It is this last fact which can explain the reduction of the detoxicifying and protein-synthesizing functions of the liver during aging, discovered by various investigators [2, 3]. In other words, the manifestations of hepatic failure which develop with age are due to reduction of the capacity of the hepatocytes for physiological regeneration.

The next morphometric parameter reflecting processes of compensation and adaptation is the number of binuclear hepatocytes. These are formed from mononuclear cells by acytokinetic mitosis and, in the opinion of Brodskii and Uryvaeva [1], they are precursors of polyploid cells, and thus represent a stage of regeneration (Fig. 3). Regeneration in the liver usually takes place in two ways: by hyperplasia and hypertrophy of the cells.

Counting the binuclear hepatocytes revealed an increase per unit area and also relative to the number of mononuclear cells in animals aged 24 months (Fig. 1c, d), evidence of switching of regeneration from physiological hyperplasia of the cells to their hypertrophy. A further decrease in the number of binuclear cells in animals aged 30 months (Fig. 1c, d) against the background of a considerable increase in size of the mononuclear hepatocytes (Fig. 1a), which are polypoid cells dividing with difficulty, suggests that hypertrophy of the cells is the predominant regenerative process in animals of this age group, unlike in those aged 8 months.

Compensatory processes similar to those in postmitotic nonrenewing cells (neurons, cardiomyocytes) in which intracellular regeneration, leading to cellular and nuclear hypertrophy, takes place throughout ontogeny [7, 8], develop during aging in hepatocytes, which constitute a self-renewing cell population.

The data given above play an important role because all the many different functions of hepatocytes, such as synthesis "for export," detoxication, and metabolism take place during aging in a number of hepatocytes in large cellular and nuclear volumes, with an increased quantity of genetic material so that special conditions are created for their course and regulation both intracellularly and from external sources.

The function of hepatocytes is directly linked with the sinusodial cells surrounding them. As regards the relative number of its stromal cells the liver occupies a special position because of their diversity and the importance of their function. They include endotheliocytes, which play a barrier and transporting role; stellate reticulo-endotheliocytes, representatives of the mononuclear phagocyte system participating in the immune response and correcting regenerative processes in the parenchyma; fat cells, analogs of fibroblasts which accumulate vitamin A and retinol; pit cells which, according to the latest data, are members of the APUD-system [6] and also play an active role in immunity [10].

An increase in the number of sinusoidal cells relative to hepatocytes was found morphometrically in groups of 24- and 30-month-old animals (Fig. 1f), corresponding to the larger size of the hepatocytes in these animals. Meanwhile the numerical density of the stromal cells in animals aged 24 and 30 months per unit area remained statistically the same relative to animals aged 8 months (Fig. 1e). Not until the age of 24 months was an increase observed in the mean values of this parameter, evidently in association with an increase in the number of pit cells, identified by EM, in this age group.

It follows from the facts described above that during aging sinusoidal cells are quantitatively more static than hepatocytes, in agreement with the results of other investigations, in which morphometric analysis of various sinusoidal cells revealed no age changes [11].

Compensatory and adaptive processes in the liver in response to reduction of the number of parenchymatous cells developing during aging are thus manifested by compensatory hypertrophy of hepatocytes, which is based on intracellular regeneration, leading to polypolidization, hyperplasia, and hypertrophy of protein-synthesizing and energy-forming structures. The transition by regenerative processes in the liver to a path of predominant hypertrophy of hepatocytes is biologically justified, for along this pathway of compensation cells are not excluded from their tissue-specific function, which is important when the number of functioning cells is reduced.

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