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A COMPARATIVE STUDY OF PATHOLOGICAL CHANGES IN INFORMATIONAL PARAMETERS OF RAT LIVER DUE TO EXPERIMENTAL EXPOSURE DURING REPRODUCTIVE AND SENILE PERIODS

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ABSTRACT — The informational parameters characterizing the adaptive-compensatory resources of the liver of Wistar rats in the reproductive period and the period of pronounced senile changes in norm and pathological conditions were studied. The same informational parameters were also determined for some non-oncological pathologies, for hepatocellular adenoma and for hepatocellular carcinoma at both ages. It is established that in ontogenesis there is an increase in the level of real structural diversity (H) and coefficient of relative entropy of the system (h). At the same time a decrease of level of organization of system (S) and coefficient of relative organization of system (R) are noted. In the studied periods of ontogenesis, at similar pathologies and pathological processes in liver, in reproductive period of ontogenesis the organ is characterized by fewer devia-tions from the norm and higher level of adaptation and compensative abilities than in the period of pronounced senile changes. Changes in informational condition of liver at hepatitis have the same direction as ontogenetic changes. They lead to an increase in the disorder of the system and a decrease in the level of its integrity, which results in a decrease in the level of adaptive and compensatory resources. In tumors, a decrease in H and h was found with a simultaneous increase in S and R compared to age norms, which are more pronounced in malignant neoplasms. For all the studied pathologies, changes in the information state are more prominent in the liver of rats in the period of pronounced senile changes.

KEYWORDS — liver, entropy, hepatocyte, hepatitis, morphometric measurement, tumor.

INTRODUCTION

The morphofunctional changes in pre- and postnatal ontogenesis are increasingly considered as phenomena caused by the dynamics of adaptive and compensatory capabilities of living systems of various hierarchical levels [2, 18]. The adaptive-compensatory abilities of biological systems largely determine the success of maintaining homeostasis through ensuring the structural and functional unity of the system components, both in normal condition and in pathology [8, 13].

Various criteria are used to assess the adaptive-compensatory resources of these systems, but each of them is not completely objective and integrative and does not allow a comprehensive assessment of the potential capabilities of the studied system in the process of adaptation and/or compensation, reflecting mainly its functional and/or morphological features.

At the same time, the concept of *information* has become one of the key concepts for actively developing branches of biology. Information parameters can be considered as diagnostic criteria for pathological conditions. The use of information parameters as criteria for assessing the state of various systems and organs of a mammal is increasingly noted. Among the systems of information parameters used to characterize the adaptive and regenerative capabilities of systems, the concept of entropy is most often used [10–14].

It can be assumed that namely the entropy of biosystems can be the criterion that comprehensively characterizes the complex of adaptive-compensatory capabilities of bio-systems under various functional states. G.G. Avtandilov put into practice several information parameters that are derivatives of entropy that can be used for the informational characterization of systems. Our previous studies suggest that the above informational criteria are quite informative indicators characterizing the level of adaptive-compensatory resources of the organism [3–6].

Moreover, it has been reported that it advisable to calculate the information parameters of organs and tissues based on variations in the volume of hepato-

cyte nuclei. The cell nucleus is the most labile and significant indicator of the morphofunctional state of the cell. The shape and size of the nucleus are those indicators by which it is possible to determine the state of the cell itself, one or another stage of its life cycle. The change in the average size of the nuclei or the cells themselves (cross-sectional area or volume) is an integral derivative of two factors. First, it may be due to a true functional fluctuation in the size of the nuclei. Secondly, a change in the average size of cell nuclei occurs due to a change in the ratio of di- and polyploid cells (partial polyploidy). Both the first and second factors are very important for assessing the adaptive reserves of an organism [9, 15, 16, 17, 20, 21].

In this regard, we considered as the subject of current interest the study of the informational state of the liver of white Wistar rats, which characterizes the level of adaptive and compensatory resources of organ in norm, at chronic and leptospiral hepatitis, hepatocellular adenoma and hepatocellular carcinoma in the reproductive period and the period of pronounced senile changes.

MATERIALS AND METHODS

All the experimental protocols were performed in accordance with ethical guidelines approved by the Ethics Committee of the Research Center for Biology of Cells and Applied Biotechnology, Moscow Region State University, (Moscow, Russia) prior to conducting the experiments. Experiments were performed as per "Directive 2010/63/EU of the European Parliament for animal use for scientific purpose" and "NIH Guidelines for the Care and Use of Laboratory Animals". In the description of the neoplasms, the WHO International Histological Classification of Tumors of Domestic Animals (AFIP, Washington, DC, 1999.) was used. Qualitative and quantitative characteristics of the studied material are presented in Table 1.

pathologies in the reproductive period and the period of pronounced senile changes the pathologic materials from the archives of the cell biology laboratory at the Research Center for Biology of Cells and Applied Biotechnology, Moscow Regional State University; and the laboratory of experimental biology and biotechnology at the Research and Educational Center, Moscow Region State University were used.

The liver of animals with leptospiral hepatitis was obtained from animals infected in-traperitoneally with a LD50 of five-day culture of Leptospira of following serological groups — Canicola, Icterohaemorrhagiae, Grippotyphosa [19].

Chronic hepatitis was induced by subcutaneous administration of CCl4 (Adams et al., 2016). For euthanasia, a CO_2 camera equipped with an overhead gas supply was used. The chamber volume was filled with gas (100% CO_2) at a rate of 20% per minute to avoid dyspnea and pain in animals. To determine the informational status at focal lesions of the liver, pieces of tissue were taken from the least altered areas on the border of macroscopically visible lesions. In case of visual homogeneity of organ, the material for research was taken from random part of it. The criteria for the inclusion of archival material in the study were the verified diagnosis and accurate information about the age of the animal from which the material for study was obtained. For further research, the histological preparations stained with hematoxylin-eosin were prepared by standard methods.

The following informational parameters were determined: H_{max} — maximal structural entropy of the system (informational morphological capacity), H — informational morphological entropy (real structural diversity of the system), S — informational morphological organization (the indeterminacy, implemented in the system), h — the relative morphological entropy (coefficient of compression of information), R — coefficient of the relative organization of the system (or coefficient of redundancy), e — informational morphological equivocation (value of system reliabil-

Table 1. *Qualitative and quantitative characteristics of the material studied to determine the informational condition of the rat liver in the reproductive period and the period of pronounced senile changes in normal and pathological conditions*

Period of ontogenesis	The number of animals used in the study in the norm and in pathologies and pathological conditions.				
	Norm	Chronic hepatitis	Leptospiral hepatitis	Hepatocellular adenoma	Hepatocellular carcinoma
I (6–18 months)	20	10	5	8	5
II (24–40 months)	20	10	9	10	6

Hereinafter: *I — parameters of the organ in the reproductive period of ontogenesis, II — parameters of the organ in the period of pronounced senile changes*

Rat liver in normal condition was obtained from healthy animals of the corresponding age periods. In research of the informational state of rat liver at

ity). Since the principles and methods for calculating of entropy parameters were proposed and developed in the framework of classical thermodynamics, the traditional letter designations of the quantities were also used. As a unit of information, a bit was used, as suggested by G.G. Avtandilov [3–5].

For determining the above parameters, a karyometric analysis of histological preparations stained with hematoxylin-eosin was carried out with a further disaggregation of the obtained data into classes. For further analysis, the Hmax is calculated, i.e. the *maximum structural diversity*, according to the formula: $H_{max}=log_2 n$; where n — number of classes. This parameter, calculated with use of a real number of classes, reflects the structural diversity for the organ with an already embedded account of the individual characteristics of the organism.

Then the calculation of *real structural diversity of the system* (*informational morpho-logical entropy*) H is made, which is characterizing the degree of determinancy of a morphofunctional system in time and space. Calculation was made by formula: $H=-\sum P_i log_2 P_i$; where $\sum P_i$ — the sum of the probabilities of stay of the measured cell parameter in one of the presented classes; $log_2 P_i$ — the logarithm of the probability of presence in one of the possible classes. Moreover, P_i is defined as the classical probability. Knowing the maximum and actual structural diversity, we can calculate the organization of system (S), — the indeterminacy, implemented in the system: $S= Hmax–H$. Then the *coefficient of relative entropy of system* (*coefficient of compression of information*) h is defined by formula $h=H/H_{max}$. A high level of relative morphological entropy indicates a disordered in system and a significant decrease in the level of its structural integrity. *Coefficient of the relative organization of the system* (*coefficient of redundancy*) R is calculated by formula: $R=(S/H_{max}) \times 100\%=(1–h)/100\%$. Knowing these data, the researcher is given the opportunity to calculate equivocation of system (value of system reliability) e: $e=H_n–H_p$; where H_n — real structure diversity in norm, H_p — real structure diversity at pathology (Avtandilov, 2006).

Microscopy of histological preparations was carried out on a Nicon Eclipse 80I digital microscope using a Nicon DL-FI digital camera (Japan). From each studied preparation, 10 digital images of randomly selected fields of vision were made at a magnification of ×400, ×1000. For morphometric studies, the ImageJ program (USA) was used [7]. Statistical analysis of the data was performed using GraphPad Prism V6.01 for Windows. The data are presented in the form M±m where m is the standard deviation. To assess the significance of differences, the Student t-test was used. Changes were considered reliably significant at $p \leq 0.05$.

RESULTS

For liver of rats in the reproductive period the following indicators are characteristic — H_{max} — 3.32±0.00 bits, parameter H makes 2.552±0.014 bits, consequently, S is 0.768±0.014 bits, h — 0.7687±0.0042 bits, R is equal to 23.13±0.42%. In period of pro-nounced senile changes in liver at H_{max}, equal to 3.32±0.00 bits the H increases to 2.732±0.010 bits, S decreases to 0.588±0.01 bits, h raises to 0.8229±0.0022 bits, but at the same time the decrease in parameter R to 17.71±0.22% is noted (Fig. 1).

In case of the considered non-tumor pathologies and tumor pathological processes, we found similar changes in the studied parameters, relative to age norms (Fig. 2).

In particular, we found an increase in the levels of real structure diversity (H) and coefficient of relative entropy of system (h). At the same time a decrease in levels of organization of system (S) and coefficient of relative organization of system (R) are noted. The noted changes have different degrees of severity in the studied periods of ontogenesis. In the reproductive period, an increase of H and h is on average 5.71±1.01%, and in the period of pronounced senile changes, the increase is significantly higher — 9.01±1.03%. Decrease of S and R makes 18.98±4.78% in first and 41.72±6.98% in second studied period of ontogenesis. Value of equivocation of system (e) averages 0.163±0.02 bits and 0.275±0.020 bits respectively. Thus, with unidirectional changes in information parameters at pathologies, the level of adaptive resources of organs is higher in reproductive age (Fig. 3).

Analysis of the informational state of the liver in neoplasms made it possible to establish a decrease in values H and h, and, consequently, increase in values of S and R (Fig. 4.).

The degree of difference between the studied parameters and the age norm depends on the period of ontogenesis and the type of pathology. So, in liver with adenoma the values of H and h are less than the age norm by 6.3±0.80% and 15.93±1.21% in each of the studied periods of ontogenesis, but in case of cancer these differences are 15.71±2.21% and 33.93±4.85% respectively. Similarly, S and R are over the age norm by 20.96%±3.81 and 72.45%±8.22 at adenoma, and by 52.21%±5.22 and 157.65±9.84% at carcinoma. Equivocation makes -0.161±0.013 bits at adenoma in reproductive period of ontogenesis, and -0.432±0.01 bits in period of pronounced senile changed, and at cancer this value makes -0.401±0.013 bits and -0.927±0.054 bits respectively (Fig. 5).

DISCUSSION

Our study demonstrates that during ontogenesis at norm the liver as a biological system tends to

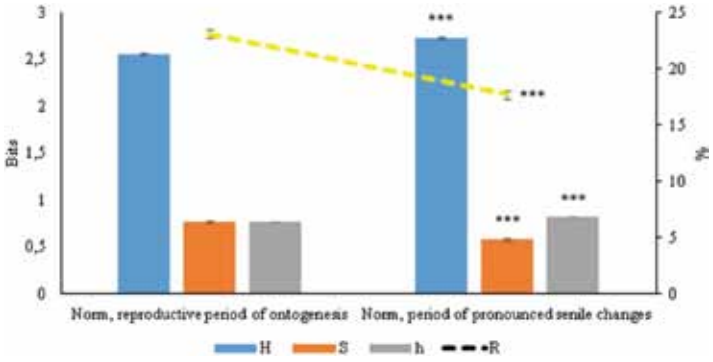

Fig. 1. *Informational parameters characterizing the liver of rats in norm in the reproductive period of ontogenesis and in the period of pronounced senile changes.*
Note: **(P≤0,05); **(P≤0,005); ***(P≤0,0005) — statistical significance of differences in comparison with parameters in the reproductive period of ontogenesis*

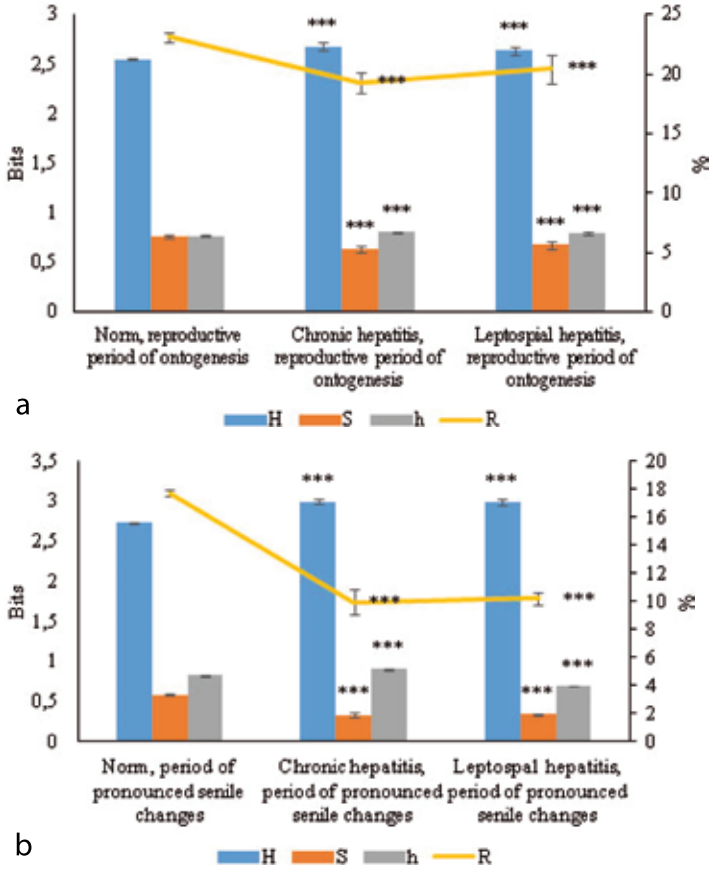

Fig. 2. *Changes in informational parameters at chronic and leptospiral hepatitis in reproductive period of ontogenesis (A) and in period of pronounced senile changes (B)*
Note: **(P≤0,05); **(P≤0,005); ***(P≤0,0005) — statistical significance of differences in comparison with parameters at norm*

destruction, loss of structural integrity and functional interconnection of elements, reduction of the reliability of the system, increased risk of disruption of compensatory processes, and a decrease in adaptive reserves and system organization degree.

In studied periods of ontogenesis, at similar pathologies and pathological processes in liver, in reproductive period of ontogenesis this organ is characterized by fewer deviations from the norm and higher level of adaptation and compensative abilities, then in period of pronounced senile changes.

Changes of informational condition, revealed in liver with hepatitis, have the same direction, as ontogenetic changes, and testify about increase in disorder in the system and a decrease in the level of its integrity, and generally about a decrease in the level of adaptive and compensatory resources.

In both considered types of neoplasms, the information parameters of the organ characterize the systems as aimed at a progressive increase in the number of structural elements of the system. Thus, with tumors, a simplification of the informational system of the liver occurs and, as a result, an increase in its reliability, ordering, and the system itself is aimed for growth. In all cases considered by us, in tumor pathologies there is a change in the existing structural diversity, i.e. there is a decrease in the number of system components, its simplification. Such a system is aimed at maintaining functional activity, but at the same time weakly responds to influences from the external and internal environment.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

COMPLIANCE WITH ETHICS GUIDELINES

All the experimental protocols were performed in accordance to ethical guide-

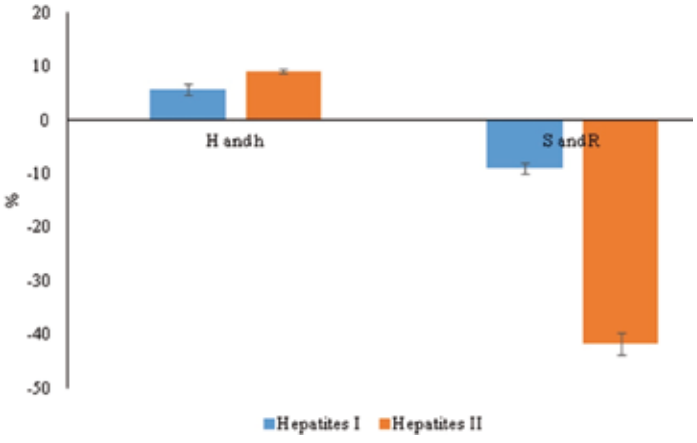

Fig. 3. Changes in some information parameters of rat liver in comparison with the age norm at non-tumor pathologies and pathological processes

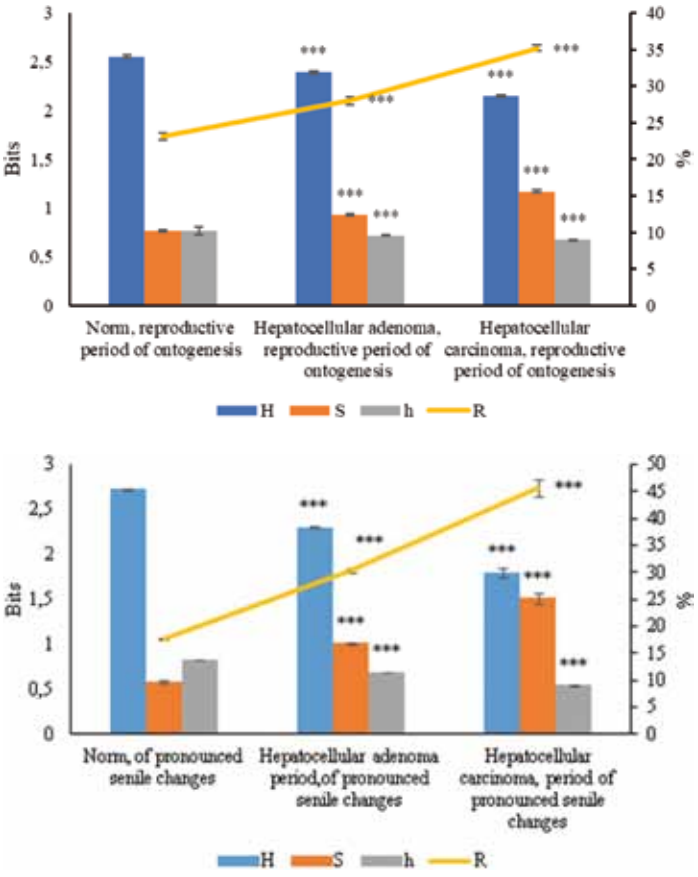

Fig. 4. Changes in informational parameters at hepatocellular adenoma and hepatocellular carcinoma in reproductive period of ontogenesis (A) and in period of pronounced senile changes (B)
Note: **(P≤0,05); **(P≤0,005); ***(P≤0,0005) — statistical significance of differences in comparison with parameters at norm*

lines approved by the Research and Ethics Committee of the Moscow State Regional University. Experiments were performed as per "Directive 2010/63/EU of the European Parliament for animal use for scientific purpose" and "NIH Guidelines for the Care and Use of Laboratory Animals". In the description of the neoplasms, the WHO International Histological Classification of Tumors of Domestic Animals (AFIP, Washington, DC, 1999.) was used.

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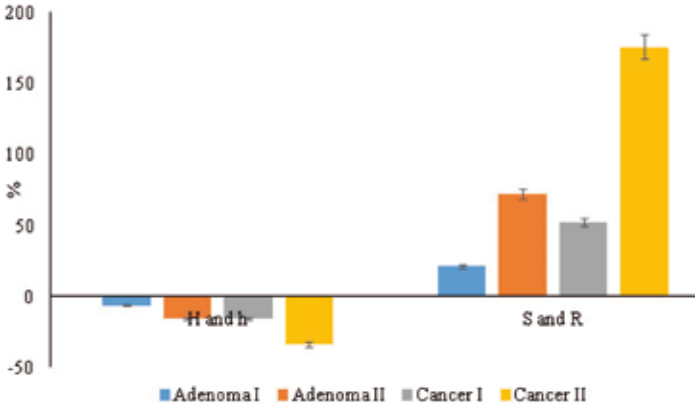

Fig. 5. Changes in some informational parameters of rat liver with tumor pathologies relative to the age norm

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