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INDIVIDUAL RESPONSIVENESS MANIFESTED AS CHANGES IN MITOCHONDRIAL CYTOCHROME CONCENTRATION IN CIRRHOSIS

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There is much information in the literature on changes in the content and stoichiometry of the mitochondrial cytochromes, which are responsible for the terminal stage of oxidative metabolism, coupled with energy accumulation, but the nature and role of these changes remain unexplained.

The aim of this investigation was to study the content of cytochromes in mitochondria (Mc) of hepatocytes during progressive cirrhosis of the liver in rabbits. With the more accurate determination of their concentrations, it was possible not only to track the dynamics of changes in the mean parameters, but also to analyze individual changes in the content of mitochondrial cytochromes in the animals.

EXPERIMENTAL METHOD

Three chronic experiments were conducted on mature male chinchilla rabbits. The model of cirrhosis was devised and built by V. N. Tugarinova [7]. Cirrhosis was induced by four cycles of combined treatment with the hepatotoxic agent CCl_4 and hepatogenic antigen [2]. Repeated biopsy operations for the morphological and metabolic investigations were performed simultaneously three times on the control and experimental rabbits: after 2-3 weeks, at the end of the 2nd and 4th cycles, and 2 months after the end of the injurious procedures. In all three years the experiments were done between February and June. The cytochrome concentrations were determined in biopsy material from 22 experimental and 22 control rabbits. Pieces of tissue were cooled in ice-cold 0.9% KCl solution and homogenized in medium of the following composition: sucrose 0.2 M, KCl 20 mM, KH_2PO_4 2 mM, $MgCl_2$ 0.5 mM, Tris-buffer 30 mM, versene 1 mM, pH 7.4, temperature 0°C. MC were isolated by differential centrifugation at −2°C: at 700g for 5 min and at 12,000g for 10 min. The residue of MC was suspended in the same medium. For spectrophotometry, 0.1 mM of 2,4-dinitrophenol and 2 mM amytal were added to the medium. To convert cytochromes a, b, c_1, and c into the reduced state 5 mM succinic acid (pH 7.4) and 3 mM KCN were added to one of the cuvettes; cytochrome b_5 in both cuvettes was reduced by the addition of 0.5 mM NADH. Difference spectra of the cytochromes were recorded on a multifunction differential spectrophotometer [3], constructed at the

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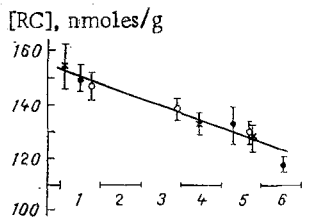

Fig. 1

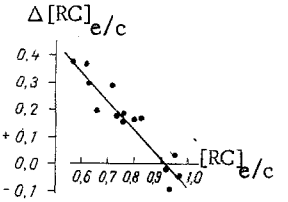

Fig. 2

Fig. 1. Seasonal course of number of respiratory chains in mitochondrial protein of hepatocytes in mature rabbits. Concentrations of respiratory chains averaged for groups in accordance with dates of their investigation for 3 different years.

Fig. 2. Dependence of change in concentration of respiratory chains by time of next investigation on its value at previous investigation for individual animals. e) Experimental; c) control.

Central Research Laboratory of the I. M. Sechenov First Moscow Medical Institute on the basis of a dual-beam monochromator [4]. The course of the beams through the cuvette was vertical. Spectrophotometric accuracy is determined by the shot noise of the photoelectronic multiplier, which in the absence of specimens is $1.5 \cdot 10^{-5}$ optical density unit with an optical slit width of 2 nm. Drift of the zero line is monotonic, stable in time, and does not exceed $1 \cdot 10^{-3}$ optical density unit in the working region of the spectrum from 750 to 380 nm. The densitograms were measured as in [1], at the maximum of the absorption bands of cytochromes a (605 nm), b (562 nm), c_1 (554 nm), and c (550 nm) relative to the base line, with reference wavelengths of 624 and 590 nm for a and 572 and 540 nm for b, c_1, and c. Concentrations of individual cytochromes were calculated by equations allowing for overlapping of absorption bands [2]:

$$\begin{aligned}
[a] &= 0{,}0521 \cdot A - 0{,}0023 \cdot B + 0{,}0021 \cdot C_1 - 0{,}0005 \cdot C,\\
[b] &= 0 + 0{,}0765 \cdot B - 0{,}0150 \cdot C_1 + 0{,}0104 \cdot C,\\
[c_1] &= 0 - 0{,}0074 \cdot B + 0{,}0656 \cdot C_1 - 0{,}0292 \cdot C,\\
[c] &= 0 + 0{,}0044 \cdot B - 0{,}0392 \cdot C_1 + 0{,}0689 \cdot C.
\end{aligned}$$

The concentrations of cytochromes [a], [b], [c_1], and [c] were calculated in nanomoles per milliliter of the suspension subjected to photometry; $A = \frac{\Delta I}{I} \cdot \frac{1}{d}$ for 605 nm, B for 562 nm, C_1 for 554 nm, and C for 550 nm; $\Delta I/I$ denotes the relative difference in intensities of light passing through the cuvettes; d the thickness of the layer of suspension subjected to photometry (in cm). The protein concentration in the MC suspension was determined by the biuret method, also on a differential spectrophotometer, as absorption at 540 nm relative to the base line with reference points at 480 and 630 nm. Statistical evaluation of the accuracy of protein determination gave a result of 2-3%; the error of determination of cytochrome concentrations in the MC suspension with two or three repetitions was reduced to 3-5% [2].

EXPERIMENTAL RESULTS

Morphological and function tests showed [5] that changes in the liver after the 2nd and 3rd cycles corresponded to the stage of precirrhosis, and after the 4th cycle to the beginning of monolobular cirrhosis. According to [8], these stages are the compensated phase of the process, and the activation of adaptive mechanisms is expressed by some normalization and stabilization of several parameters of function. The severity and rapidity of development of the process varied greatly in individual animals. Two months after exposure to the noxious agents signs of involution of the pathological process were observed only in individual animals, and in some rabbits the process continued to worsen.

In the experimental rabbits throughout the period of investigation the ratio between concentrations of cytochromes a, b, and c_1 was the same as in the control. Changes in their concentration can therefore be examined collectively as changes in the concentration of respiratory chains $[RC] = \frac{1}{3}([a] + [b] + [c_1])$.

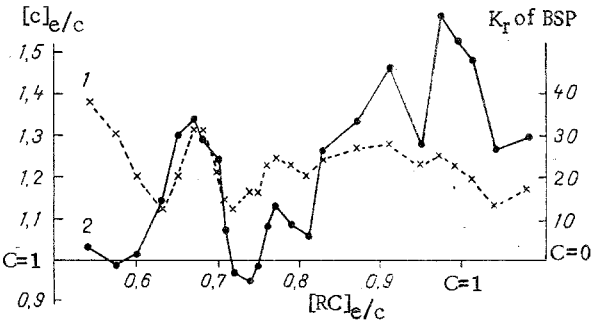

Fig. 3. Evolution of individual values of cytochrome c concentration and degree of disturbance of excretory function of the liver in relation to concentration of respiratory chains. Sliding means of three values of $[c]_{e/c}$ (1) and of K_r of BSP (2) following in succession in a series ranked according to value of $[RC]_{e/c}$.

According to the results of the experiments in three years a seasonal course of the number of respiratory chains in mitochondrial protein could be detected in the control rabbits (Fig. 1). The linear decrease in [RC] over 6 months (from February through July) amounted to 33 nanomoles/g protein, or 20% of the mean value. Changes in many physiological characteristics, including the decrease in basal metabolism in the adult animals, lay within the same limits. The decrease in [RC] is described by the regression equation: $[RC] = \alpha - \beta(t - t_m)$, where $\alpha = 138 \pm 1.5$, $\beta = 0.183 \pm 0.024$, t_m falls on April 24, and $\sigma = \pm 9.5$ nanomoles/g. The very small individual differences in [RC] will be noted in the animals, their upper estimate being the dispersion σ. Ratios of concentrations of cytochromes a, b, c_1, and c to the concentration of respiratory chains and their dispersion are given in parentheses: 0.85 (0.10), 1.23 (0.08), 0.90 (0.05), and 0.91 (0.09).

In the precirrhosis stage the number of respiratory chains in mitochondrial protein was 28-35% lower on average for the experimental group than for the control, indicating a considerable change in the mitochondrial enzyme constitution. Toward the end of the compensated phase, both 2 weeks and 2 months after the 4th cycle, average values of [RC] were close to the control, namely 97-102%. In some rabbits, data for which were not included in calculation of the means, marked cirrhosis was present. In these rabbits [RC] was again considerably reduced, by 30-50%. The phenomenon of a change, mainly an increase, in the [c]:[a] ratio, found by many investigators, when the body is subjected to various processes and influences, was confirmed. The [c]:[RC] ratio at the stages of cirrhosis studied was on average 1.5 times higher than the control, but varied widely. Analysis of the experimental data as a whole showed that the fall in [c] below the control level and values of [c]:[RC] above 2.4-2.5 discovered in individual rabbits were a poor prognostic sign.

Individual reactivity at the level of changes in the cytochrome enzyme system of Mc was manifested as wide variation of concentrations of the cytochromes in rabbits studied virtually simultaneously. Intervals of values for concentration of respiratory chains, expressed relative to the control (control values were calculated by a regression equation), namely $[RC]_{e/c}$, for two consecutive stages of the process were: 0.53-0.83 and 0.86-1.13. These consecutive values are ambiguous but do not overlap. Analysis of the time course of changes in [RC] in individual animals, which was made possible by the accuracy of the method used, gave the following results. The lower the value of $[RC]_{e/c}$ for a given rabbit during the previous investigation, the greater the magnitude of its change by the time of the next investigation (Fig. 2). Dependence of the difference $\Delta[RC]_{e/c}$ between preceding and succeeding values on the preceding value of $[RC]_{e/c}$ is described by the following regression equation:

$$\Delta[RC]_{e/c} = \alpha - \beta[RC]_{e/c},$$

where $\alpha = 1.00 \pm 0.02$ and $\beta = 1.09 \pm 0.12$. The closeness of correlation (dispersion $\sigma = \pm 0.06$) and the fact that $\Delta[RC]_{e/c}$ becomes negative in the region corresponding to the beginning of monolobular cirrhosis will be noted. At this stage $[RC]_{e/c}$ fell again during progression of the disease, as is confirmed by data for rabbits with definitively formed cirrhosis.

The great variation in individual values of [RC], [c], and the degree of disturbance of excretory function of the liver could be interpreted by the method of differential analy-

sis [10] of the results of three chronic experiments. Excretory function was characterized by the coefficient of retention of bromsulphthalein (K_r of BSP) [9]. Evolution of individual values of $[c]_{e/c}$ and K_r of BSP with an increase in $[RC]_{e/c}$ is given in Fig. 3, which shows sliding means of three values lying next in order in a series ranked according to the value of $[RC]_{e/c}$. For the region of lowest values of $[RC]_{e/c}$, the lower the concentration of respiratory chains in the hepatocyte mitochondrial protein of the rabbit, the more severely disturbed its excretory function, and the value of [c] falls (in some animals, actually below the control level). The subsequent narrow region of values of $[RC]_{e/c}$ — from 0.63 to 0.73 — is distinguished by an abrupt and significant (according to Student's test) peak of K_r of BSP and $[c]_{e/c}$. In the region corresponding to the beginning of cirrhosis excretory function stabilizes at the level of K_r of BSP = 15-25, but the cytochrome c concentration rises with an increase in $[RC]_{e/c}$ and again varies sharply. In the light of existing views on polyfunctionality of cytochrome c and the hypothesis that two pools of it exist, the following argument is interesting. At this stage of deviation of individual values of $[c]_{e/c}$ and K_r of BSP from their averaged course are as a rule in the same direction and correlate in magnitude. Moreover, among rabbits with established cirrhosis, in some [c] was twice the control value, whereas in others it exceeded the control only very slightly. This subdivision, it can be tentatively suggested, is connected with the great variability of values of [c] in the previous stage of the process. The very small individual differences in cytochrome concentrations can thus be regarded as pathogenetically important, and it can be postulated that the relationship shown in Fig. 3 between [RC], [c], and K_r of BSP and its phasic character reflect real correlations between concentrations of respiratory chains and cytochrome c and the excretory function of the liver while the process runs through the compensated phase.

The existence of individual reactivity at the mitochondrial enzyme constitution level was thus demonstrated in the case of progressive cirrhosis, in the form of shifts in the relative concentration of respiratory chains in the protein of these organelles. When the nature and role of changes in energy metabolism in pathological processes are investigated it is desirable to use analysis of individual differences as well as mean statistical values.

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