CORRELATION BETWEEN INTENSITY OF HEPATIC MICROSOMAL OXIDATION AND INDIVIDUAL LIFE SPAN

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The microsomal oxidative enzyme system, which is responsible for detoxication processes, plays an essential role in adaptation of the organism to external environmental conditions. During aging the adaptive powers of the system [2] decline and changes take place in the substrate specificity of cytochrome P-450 during exposure to foreign chemicals [6], which may have a significant effect on the individual life span (LS) of the animals. The duration of hypnosis induced by barbiturates is a physiological indicator of the functional state of the hepatic microsomal oxidative enzyme system. The duration of hexobarbital hypnosis, determined periodically in individuals throughout life, exhibits considerable variability [5], evidence of the heterogeneity of the population as regards activity of detoxication processes.

The aim of this investigation was to study correlation between activity of the hepatic microsomal oxidative enzymes and the individual LS of animals.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats aged 608 days (20 months). Pentobarbital sodium was injected intraperitoneally in a single dose of 2.5 mg/100 g body weight. The duration of drug-induced sleep was estimated by noting assumption of the side position before the appearance of locomotor responses. The animals were subsequently returned to the animal house (standard conditions) for observation of their survival rate and recording of the date of death between limits of 24 h. Dependence of mortality on the animals' age was assessed by the use of survival curves, and also by plotting between coordinates of Gompertz' equation: $R_t = R_0 \cdot \ell\alpha^t$, where R_t denotes mortality at time t, R_0 the hypothetical mortality at t = 0, and α is the coefficient of the increase in mortality with age [4]. In a separate series of experiments the animals were decapitated to study activity of the hepatic microsomal oxidation enzymes. The concentration of cytochromes P-450 and b_5 [7] and aminopyrine demethylase and aniline hydroxylase activity [1] were determined in liver homogenates (1:7, w/v, 1.15% KCl solution). The experimental results were subjected to statistical analysis and the significance of differences was assessed by Student's t test.

EXPERIMENTAL RESULTS

The study of the duration of drug-induced sleep in rats aged 20 months showed that this indicator is highly variable in the different individuals of the population (from 20 to 180 min). Depending on the duration of drug-induced sleep the animals were divided into three groups: group 1) 20-60 min (33 rats), group 2) 61-100 min (47 rats), and group 3) 101-180 min (32 rats). Animals of group 1 (short sleepers; SS group) and group 3 (prolonged sleepers; PS group) were used for the subsequent investigation.

Observations on the survival of the animals (Fig. 1) showed that the average specific LS in the PS group was 815.3 ± 24.9 days, and in the SS group 914.0 ± 26.4 days, or 12.2% higher ($p < 0.01$). The most substantial differences between the SS and PS groups of animals were revealed in the residual LS. In the PS group, for instance, the residual LS at 50% mortality was 196.5 days and at 80% mortality 329 days, whereas in the SS group the corresponding values were 277.0 and 434.0 days, or 51.1 and 31.9% higher respectively ($p < 0.05$).

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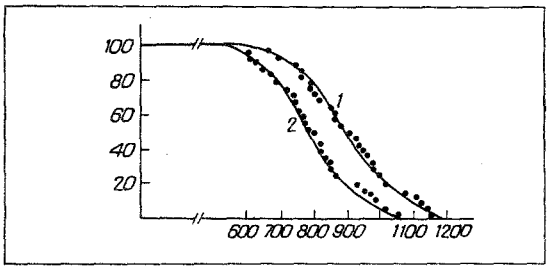

Fig. 1. Survival of rats of SS (1) and PS (2) groups. Abscissa, individual LS (in days); ordinate, percentage of animals.

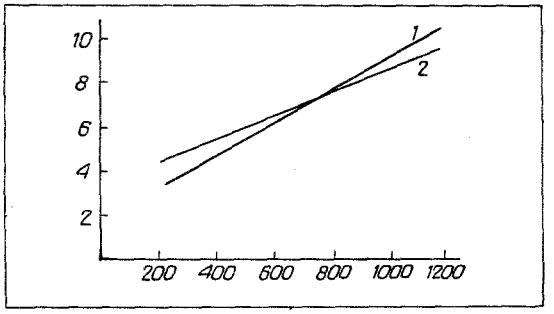

Fig. 2. Time course of mortality with age in coordinates of Gompertz' equation in rats of groups SS (1) and PS (2). Abscissa, life span (in days); ordinate, $\ln R_t$.

The maximal duration of residual life of rats of the SS group was 531.7 days, or 24.5% higher than in the PS group (427 days, $p < 0.05$).

Correlation analysis revealed negative correlation between the duration of drug-induced sleep and LS of the animals: in the SS group the coefficient of correlation $r = -0.99$ ($p < 0.05$). whereas in the PS group $r = -0.89$ ($p < 0.05$). Analysis of the survival curves (Fig. 1) between coordinates of Gompertz' equation enabled the time course of mortality of rats of the SS and PS groups with age to be evaluated. The great advantage of Gompertz' equation is linearization of the curves simply by taking logarithms. In this way estimates can be made of the constants R_0 (characterizing mortality in the early stages of the time range investigated) and α (the rate of rise of mortality in the later stages) to be estimated.

The regression equation $\ln R_t = \ln R_0 + \alpha t$ for animals of the SS group can be written as in $\ln R_t = 2.013 + 0.0072\ t$, and for the PS group: $\ln R_t = 3.370 + 0.0053\ t$ (Fig. 2). It will be clear from Fig. 2 and the regression equations that animals of the experimental groups differed not only with respect to LS, but also to the age dynamics of their mortality: rats of the SS group had a lower death rate at the age of 20 months ($\ln R_0 = 2.013 \pm 0.11$) than those of the PS group ($\ln R_0 = 3.37 \pm 0.07$; $p < 0.001$) and their mortality rate increased in the later stage of development ($\alpha_{SS} = 0.0072 \pm 0.0003$; $\alpha_{PS} = 0.0053 \pm 0.0004$, $p < 0.001$). It can be concluded from calculation of the constants of Gomertz' equation that animals of the SS group constitute a special subpopulation of long-living individuals.

As already stated, the duration of pentobarbital-induced sleep depends on activity of enzymes of the hepatic microsomal oxidation system. In a special series of experiments six animals were taken from each of the two (SS and PS) groups for a study of activity of their hepatic microsomal monooxygenases. The data given in Table 1 show that the content and activity of hepatic microsomal oxidation enzymes in rats of the SS group were much higher than in those of the PS group. For instance, the cytochrome P-450 concentration in rats of the SS group was 153.2%, of cytochrome b_5 216.9%, and their aminopyrine demethylase activity was 229.2% of the corresponding values for the PS group. Aniline hydroxylase activity was the same in both groups. The results of these investigations are evidence that animals of the SS and PS groups differed not only in the duration of their drug-induced sleep, but also in activity of their detoxication processes.

TABLE 1. Parameters of Hepatic Microsomal Oxidation Enzyme System in Animals of SS and PS Groups (M ± m; n = 6)

Parameter	PS group	SS group
Cytochrome P-450	5,45±0,89	8,35±0,49*
Cytochrome b_5	1,95±0,18	4,23±0,88*
Aminopyrine demethylase	26,30±3,54	60,27±12,10*
Aniline hydroxylase	2,52±0,70	2,54±0,57

Legend. Concentrations of cytochromes P-450 and b_5 given in nanomoles/g tissue; activity of aminopyrine demethylase in nanomoles formaldehyde/g tissue/min, and of aniline hydroxylase in nanomoles p-aminophenol/g tissue/min; *$p < 0.05$.

It can be concluded from the facts described above that positive correlation exists between activity of the hepatic microsomal oxidation enzymes and LS of the animals. A high level of detoxication processes, facilitating adaptation of the animal to the action of chemical external environmental factors may perhaps be one of the mechanisms of longevity ("vitauct" [3]), aimed at maintaining vital activity and making possible a long individual life span.

The use of this simple test based on the duration of drug-induced sleep, which characterizes the level of microsomal monooxygenases, enables groups of animals with high and low life expectation to be isolated from the general population, and this procedure may find an application in gerontological and pharmacological investigations of the late effects of gerontological and other drugs.

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