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STRESS AND RADIORESISTANCE (GENETIC ASPECTS)

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Abstract — Inbred mice and randomly bred stock were exposed to long-term adrenaline and hypothermia stress effects for unification of their functional status, followed by acute total gamma irradiation by equal-effect doses ($LD_{50/30}$). The significant reduction of lethality distribution in inbred mice after combined stress and radiation effects was observed. The spread of lethality in random bred stock was increased as compared to the control group. The post-irradiation mortality in experimental animals differed only slightly from the values in the control groups. Conclusions are drawn about the principal meaning of genotype in radiosensitivity, about the role of an organism's functional status in determination of death date and about the possible epigenetic structuralisation of linear mice by radioresistance.

INTRODUCTION

Large interactions of genotype with different environments are often seen as an indication of low stability of that genotype[1]. Genotype input into radioresistance is revealed with the interspecies and, especially, differences between strains in reactions to the same doses[2]. Differences between strains are considered to be determined mainly by genotypic factors, and those within a strain by environmental ones[3]. The problem of the relative roles of genetic characteristics and the functional status of animals in the radiosensitivity of the organism is not sufficiently studied nowadays. It is assumed that methods based on radiosensitivity comparisons of animals with different and similar genotypes can be used[4].

The aim of the present study was to reveal the relative roles of genetic and environmental factors in the formation of radiosensitivity characteristics of mammals using inbred mice and randomly bred stock.

The initial assumptions used were: (1) Animal strains are considered to possess the same level of genetotypic homogeneity as that in enzygotic twins[5]. (2) The functional status of mammals is regarded as the most significant component of radiosensitivity, conditioned by both genetotypic and environmental peculiarities[6]. (3) The idea that stress is a non-specific process of extraordinary mobilisation of all resources of the organism to respond to long-term effects of external origin[7].

MATERIAL AND METHODS

The study was carried out in two inbred strains of laboratory 4–5 month males BALB/c, CBA and randomly bred stock of the same sex and age. The BALB/c line was the most radiosensitive and very reactive to stress[8]. CBA was the most radioresistant[4,8,9]. The first stress factor was a sub-lethal dose of adrenaline for 10 days followed by acute total gamma irradiation, leading in each line to equal effects of $LD_{50/30}$ (line CBA, group 1; line BALB/c, group 2; random bred stock, group 3). In each case inbred and random bred mice were injected with physiological solution and irradiated simultaneously, thus making control groups. The second stress series was long-term hypothermia (14 h daily for 30 days, temperature 2–4°C) preceding the acute gamma irradiation (line BALB/c, group 4). The $LD_{50/30}$ values for inbred and randomly bred animals were estimated in our preliminary study[4]. An observation period of 30 days was used to determine lethality, lifespan and death spread. Ten animals exposed daily to adrenaline injections were killed over 10 days and the suprarenal index was estimated. A total of 627 animals were used in all experiments: 266 mice of CBA strain, 195 mice of BALB/c strain and 166 randomly bred ones. The results were examined using the Statgraph program added by routine statistical methods.

RESULTS AND DISCUSSION

Mortality

Semi-lethal doses between BALB/c and CBA strains differed by a factor of 1.55. Animal mortality in all experimental groups was higher, but only slightly so, from the values in the control groups, except for the hypothermia variant (Table 1). The total difference of mortality between experimental and control groups was 5.7%.

Average lifespan

The control and the experimental groups of the dead inbred and random bred animals differed significantly in average lifespan (Table 1). Average lifespan and mortality at equal-effects doses were fairly comparable in the groups of linear mice.

Lethality distribution

Change of mortality structure in the experimental groups of inbred mice — synchronising of death dates —

was observed. Comparative analysis of death dates variability showed that medically treated BALB/c strain animals died within the period of 4–16 days (Figure 1), but subjected to hypothermia within 4–12 days (Figure 2); however, in the control groups the period was 3–23 days. The effect of adrenaline in the dynamics of lethality was also evident in CBA strain mice (Figure 3); experimental animals died in the interval 6–16 days, controls between the 5th and 29th day. Comparison of dispersions using Fisher's criterion showed that variability of the distribution of post-irradiation deaths in the inbred BALB/c and CBA mice after the selected stresses was significantly ($P < 0.01$) lower than in the controls. The variability of lethality distribution in the random bred stock was quite different. Deaths in both groups were recorded during the period of 2–30 days. The analysis of death dates dispersion showed no significant differences in the variability of lethality distribution between the control and experimental groups. In the random bred stock values of variation coefficients differed by a factor of 1.2, while in the linear mice they were 1.6–1.9 times lower in the experiment than in the control.

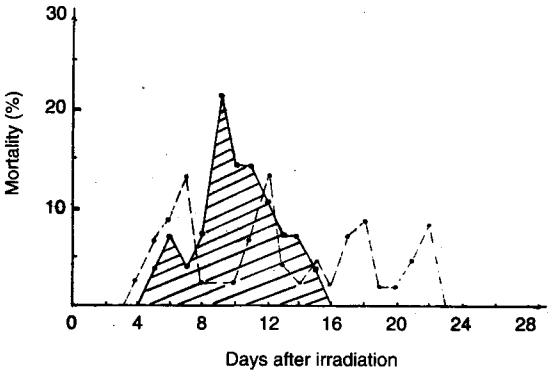

Figure 1. Lethality distribution of strain BALB/c mice (adrenaline ± $LD_{50/30}$ irradiation). Key: continuous curve and shading, experimental group; broken curve, control group.

Comparison of lethality distribution in BALB/c and CBA strains shows that they are similar and are not shifted on the axis relative to each other. Coincidence of changes in time and their similar direction suggests that the synchronisation of death dates in irradiated inbred mice may be connected with a certain unification of the functional status of their organisms, previously subjected to a long-term stress. Incomplete unification (surviving animals) may result from epigenetic differences between the surviving and the dead animals. The random bred stressed mice did not differ significantly from the controls in lethality distribution. However, the variance in the experimental group was 12% higher than in the control, which is evidence of a higher variability of this index. Hypothermia and adrenaline effects caused increased aggressiveness among animals. Morphologically chronic stress is displayed in suprarenal hypertrophy and thymus lysis[7]. Comparison of suprarenal indices of the experimental and control mice revealed a significant ($P < 0.05$) increase in their mass.

It is known that dying from irradiation in various strains is a long and irregular process with characteristic peaks[4,10]. Synchronous post-irradiation death dates may be explained by stress-caused unification of neuroendocrine and other system's functional interaction in

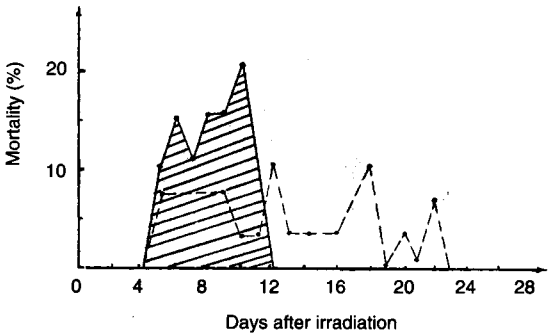

Figure 2. Lethality distribution of strain BALB/c mice (hypothermia ± $LD_{50/30}$ irradiation). Key as Figure 1.

Table 1. Mortality, average lifespan, coefficients of variation for inbred mice and random bred stock after combined stress and radiation ($LD_{50/30}$) effects.

Group	Group number	Mortality (%)	Average lifespan (days)	Variation coefficient
CBA adrenaline	1	57	12 ± 0.3	22
CBA control		51	14 ± 0.6	35
BALB/c adrenaline	2	58	10.1 ± 0.5	25
BALB/c control		54	12.8 ± 0.9	48
Random, adrenaline	3	52	12.7 ± 1.0	51
Random, control		51	9.3 ± 0.8	58
BALB/c Hypoth.	4	59	8.2 ± 0.44	24
BALB/c control		46	12.4 ± 1.0	42

the organisms of inbred mice, which originally possessed different functional status due to hierarchy in an artificial terrarium community. It is suggested that the absence of synchronisation in death dates in the stressed random bred stock might result from their genetic heterogeneity. It is difficult to explain the quantitative stability of the percentage (50%) of dead inbred animals despite the preliminary stress. Two explanations are possible:

(1) Differences in the phase conditions in the specimen homeostasis[11], including a concentration of endogenous radioprotectors. It is suggested, however, that the stress unified the animals with different physiological status. That was perhaps the reason for the synchronous death of inbred mice.
(2) Presence of hidden genetic structuring of linear mice by radiosensitivity.

Presence of within-strain genetic heterogeneity might be caused, firstly, by the fact that even close inbreeding does not result in a complete homozygosis for the most important genes which determine viability[12]: this caused genotypic variability of inbred mice. Other reasons are the appearance of new mutations, which are displayed by the altered frequency of alternative non-metric skeletal traits or fluctuating asymmetry (epigenetic shifts)[4,13]. Perhaps these epigenetic factors could explain the known observation that animals which have initial morphophysiological and metabolism indices close to average, prove to be the most resistant; individuals having extreme values of the studied parameters die first[6].

CONCLUSIONS

(1) Long-term stress before irradiation in $LD_{50/30}$ dose resulted in an insignificantly (less than 10%) increased mortality in inbred and random bred mice.
(2) Unification of post-irradiation reaction (synchronisation of death dates) after combined stress and radiation action was observed in linear mice. Lethality distribution in random bred stock was increased.
(3) The fate of an irradiated mammal organism depended basically on the genotype.
(4) The death date depended on the organism's functional status at the moment of irradiation.
(5) Stress before irradiation allowed an analysis of the correlation between the genotype and environments in radioresistance.

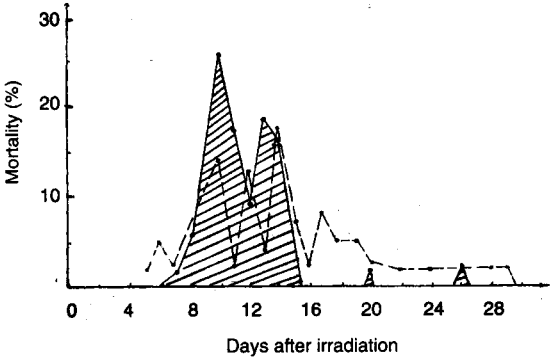

Figure 3. Lethality distribution of strain CBA mice (adrenaline ± $LD_{50/30}$ irradiation). Key as Figure 1.

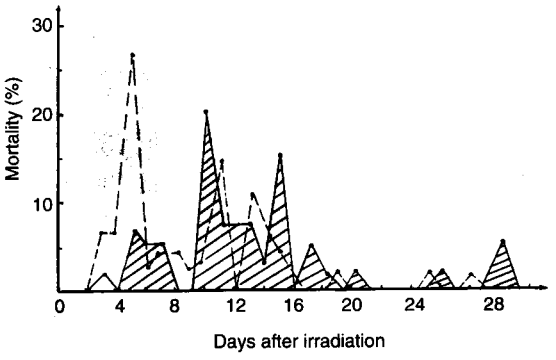

Figure 4. Lethality distribution of random bred stock (adrenaline ± $LD_{50/30}$ irradiation). Key as Figure 1.

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