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# Synergistic Interaction of Detrimental Factors Can Intensify Disaster Consequences

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## ABSTRACT

Experimental data obtained for simultaneous action of ionizing radiation with different physical or chemical agents on various cellular systems evidence that the lesser the intensity of physical factor or the concentration of chemical agents, the smaller the temperature that has to be used to provide the highest or a definite level of synergistic interaction. On this basis, it is inferred that the synergism may take place at small intensities of harmful environmental factors existing in the biosphere and can intensify disaster consequences. Hence, the assessment of health or environmental risks both in normal and disaster conditions should take into account the synergistic interaction between harmful agents.

## INTRODUCTION

Combined exposures are an essential feature of modern life. It is well known that almost all physical and a wide array of chemical harmful agents, both natural and

man made, are capable of interacting with each other in a synergistic manner when the final biological effect exceeds the sum of individual effects produced by interacting agents. Therefore any risk assessment must consider the question whether or not combined effects will influence the health outcome. Of all possible situations of the combined actions, long term exposure of living objects to low levels of the agents widely presented in the nature is especially important. However, the assessment of potential significance of synergistic interaction between adverse environmental factors acting together at the level of intensity and concentration found in biosphere is still an intriguing and unresolved problem. Real experiments with low intensities found in environmental and occupational settings are prone to large uncertainties. A feasible approach to this problem is to analyze the dependence of the efficiency of synergistic interaction on the intensity of agents used. Hypothetically, some possibilities could be realized. The case where synergism is decreasing with intensities of deleterious agents applied is unimportant. The same is true for the situation when a decrease in the intensity of one factor should be accompanied by an increase in the intensity of another to retain their synergistic interaction at the same level. The only possibility would be of great importance: if the lesser intensity of one of the agents is applied, then the smaller intensity of another agent should be employed for the display of the highest or a definite level of synergy. In such a case, it would be expected that even at low intensity environmental agents may, in principle, interact with each other in a synergistic manner and thereby to enhance their harmful action. The same is true for different kinds of local or national disaster resulting in an increase both in a number of harmful agents and their intensity. In this paper, we present conclusive evidence confirming these opportunities for various cellular systems and acting noxious agents.

## RESULTS AND DISDCUSSION

The material and methods have been described in detail elsewhere [1,2,3]. It is now generally accepted that the highest synergistic interaction is observed under the simultaneous action of harmful agents. The increasing interval between exposure results in a diminution of synergy. That is why in this paper we analyse only simultaneous application of agents. Cell killing is the main end-point envisaged. Fig. 1 provides an example of the basic experimental data used in this investigation. To estimate quantitatively the sensitization action of hyperthermia, one can apply the thermal enhancement ratio [4] defined as the ratio $D_3/D_1$ or $t_3/t_1$ (Fig. 1). This ratio indicates an increase of cell radiosensitivity by high temperature. However, it does not reflect the kind of interaction (whether it was independent or synergistic). To calculate the synergistic effect we used the synergistic enhancement ratio (k), defined as the ratio of the calculated radiation dose (assuming an additive effect of radiation and hyperthermia) to that observed from the experimental survival curve for the simultaneous action of radiation (or other agents employed) and hyperthermia at a fixed level of survival. For example for 1% survival, $k = D_2/D_1 = t_2/t_1$ (Fig. 1). For exponential survival curves, this parameter is independent of the survival level for which it is calculated. For sigmoidal survival curves, the synergistic enhancement ratio was calculated for 10% survival. Both these parameters are plotted in Fig. 2 against the irradiation temperature for XS800 diploid (Fig. 2a) and S288c yaploid (Fig. 2b) *Saccharomyces cerevisiae* cells simultaneously exposed to $^{60}$Co γ-rays (10 Gy/min) and high temperature. The noticeable feature of Fig. 2 is that the thermal enhancement ratio (curves 1) increases indefinitely with increasing exposure temperature, while the synergistic enhancement ratio (curves 2) at first increases, then

reaches a maximum, which is followed by a decrease. This implies that the synergistic interaction between hyperthermia and ionizing radiation is observed only within a certain temperature range. Noteworthy is the fact that such a dependence of synergistic effect on temperature under which the exposure was occurred was also obtained upon the simultaneous combination of hyperthermia with many other physical and chemical agents [1-3]. Hence, one can conclude that for a given intensity of physical factors or concentration of chemical agents there would be a specific temperature that maximizes the synergistic interaction. Any deviation of the acting temperature from optimal value results in a decrease of synergism.

To evidence the importance of synergistic effects at low intensity of inactivating agents, we analysed the dependence of synergistic interaction on the intensity of physical factors or on the concentration of chemical agents applied in combination with hyperthermia. Using survival curves data published for simultaneous action of hyperthermia and ionizing radiation on bacteriophage, bacterial spores, yeast and mammalian cultured cells, we were able to calculate the synergistic enhancement ratio for various cell systems and irradiation condition. It allowed us to establish the correlation between the dose rate and the exposure temperature, which both provide maximum or other arbitrary levels of synergistic interaction (Fig. 3). Open circles denote the results of our calculations based on the published experimental results. One can see that linear relationships are found between these values for various cellular objects. This means the general importance of the dose rate of ionizing radiation in the manifestation of synergistic interaction. It can be inferred that the temperature at which ionizing radiation is delivered should be diminished to obtain the maximum or a definite synergistic effect with dose rate decreasing and *vice versa*.

To check the universality of this regularity the data on the simultaneous effect of hyperthermia combined with UV light or ultrasound on yeast cells, as well as with tris(1-aziridinil)-phosphine sulfide (thio-TEPA) and cis-diamminedichloroplatinum (II) (cis-DDP) on cultured mammalian cells were involved. The last two sets of data include the relationship between exposure temperature, concentration, and rate of cell inactivation for chemical agents used in clinical chemotherapy. Hence, they have no direct attitude toward environmental harmful agents and they were used here to verify the universality of the rule manifested. Using the published experimental data, we could obtain the relationships between the intensity of physical factors or the concentration of chemical agents with the exposure temperature which both provide the greatest synergy (Fig. 4). Here again, open circles denote the results of our calculations based on the survival curve data published earlier. In all cases, at a smaller intensity of the physical factor or concentration of the chemical agents, it was required to reduce the acting temperature to preserve the highest synergistic effect.

Thus, we can conclude that for any constant intensity of physical agent or concentration of chemical compounds there is an optimal temperature at which their synergistic interaction shows the highest effectiveness. In other words, there exists a definite temperature range inside which the synergistic interaction takes place. For temperatures below this range, the synergistic interaction is not observed and cell killing is mainly induced by the physical or chemical factor applied. On the contrary, for temperatures above this range, the synergistic interaction is also lacking while cell killing is caused for the most part by heat. A similar rule reveals for other deleterious agents and not only on cellular but also on the whole organism level.

The second relevant feature, followed from the data presented in this paper is the evidence of the importance of synergistic effects both at low and high intensity of

acting agents. Experimental results obtained for the simultaneous treatment of hyperthermia with ionizing radiation, ultraviolet light, ultrasound and some chemical drugs on various cell systems clearly indicate that the intensity of the physical factor or the concentration of chemical agent predestines the effectiveness of their synergistic interaction with heat. One can conclude therefore that the time factor may be considered as a determinant of synergy. It was shown that the lesser intensity of one of the agent applied, the lesser temperature under which the treatment occurred should be used to provide the highest or a specified level of synergism and *vice versa*. These results seem to be related to the fact that, if the intensity decreases, then the effective lethal dose is delivered over a long time, so that the duration of heat incubation increases, which could explain the lower temperature that should be applied to the cells.

We tried to extrapolate to the region of low dose rate the data on the dependence of synergistic effect of simultaneous treatment of heat and ionizing radiation on cultured mammalian cells on dose rate. Although the results were widely scattered, it was suggested that the existence of a certain interval of dose rates ($10^{-5}$-$10^{-3}$ Gy/min) inside which the synergistic interaction of ionizing radiation and heat may be observed at physiological body temperatures. It is curious that such dose rates were measured within the 30-km zone around the Chernobyl nuclear reactor.

Dineva et al. (1993) [5] studied the genetic consequences of the combined action of chronic irradiation with ionizing radiation and lead nitrate on *Arabidopsis thaliana* seeds. Seed were collected from natural populations growing for five years in the 30-km zone around the Chernobyl nuclear reactor at areas with different levels of radioactive contaminant (1, 5, 25, and 300 μGy per hour), then treated with lead nitrate (3.39 g/l) and tested for the frequencies of mutant embryos and embryos with

lethal genotypes. The authors [5] showed the dependence of synergism on the dose rate of chronic irradiation of populations studied. The synergistic effect reached the greatest value (about of 2.5) at an optimal dose rate (5 μGy/h), being much lower at other rates.

Taking all these data as a whole, one can conclude that for a long duration of interaction, which are important for problems of health physics, small intensities of deleterious environmental factors may synergistically interact with each other either with environmental heat or physiological temperatures of homoiothermal animals and man. Hence, the assessment of health or environmental risks both in normal and disaster conditions should take into consideration the synergistic interaction between harmful agents.

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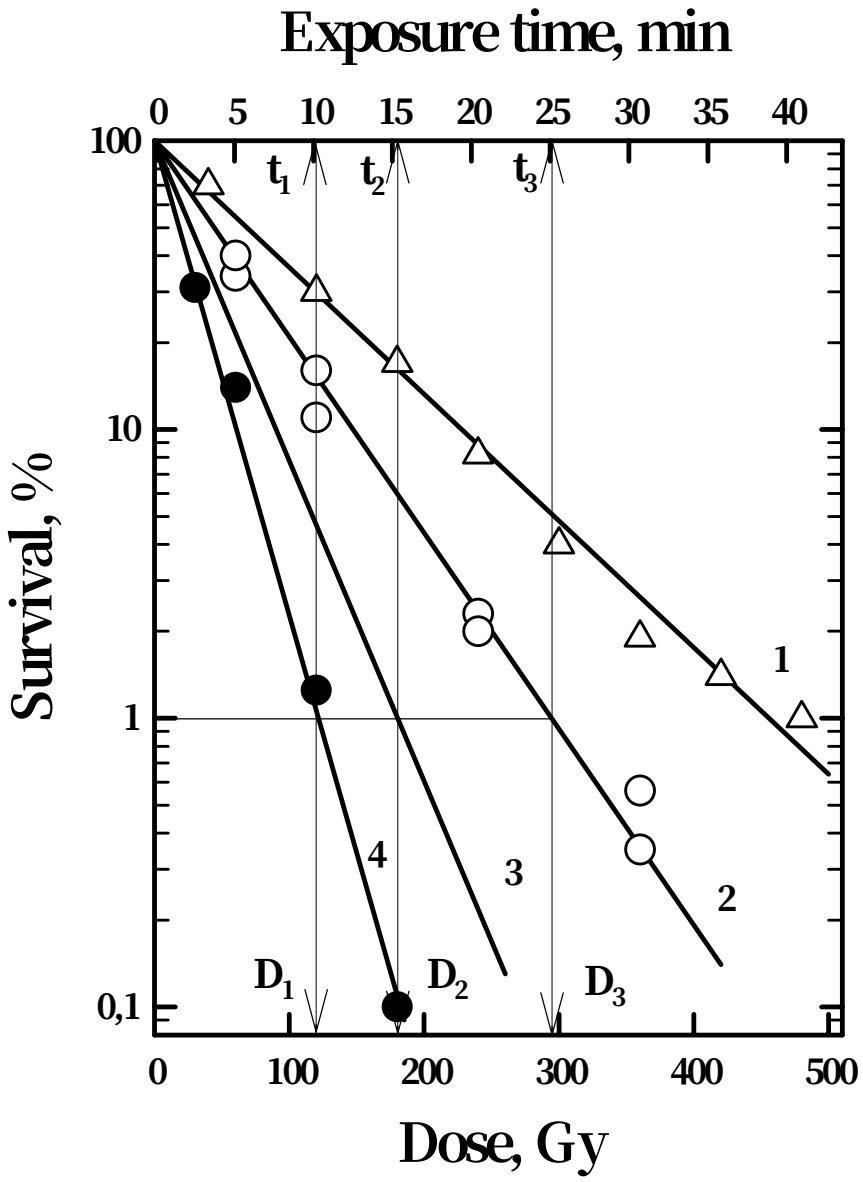


**Fig. 1.** Survival curves of Zygos*accharomyces bailii* haploid yeast cell: curve 1 - heat treatment (45 $^{o}$C) alone; curve 2 - ionizing radiation ($^{60}$Co) at about 10 Gy/min and room temperature; curve 3 - calculated curve for independent action of ionizing radiation and heat; curve 4 - experimental curve after simultaneous thermoradiation action.

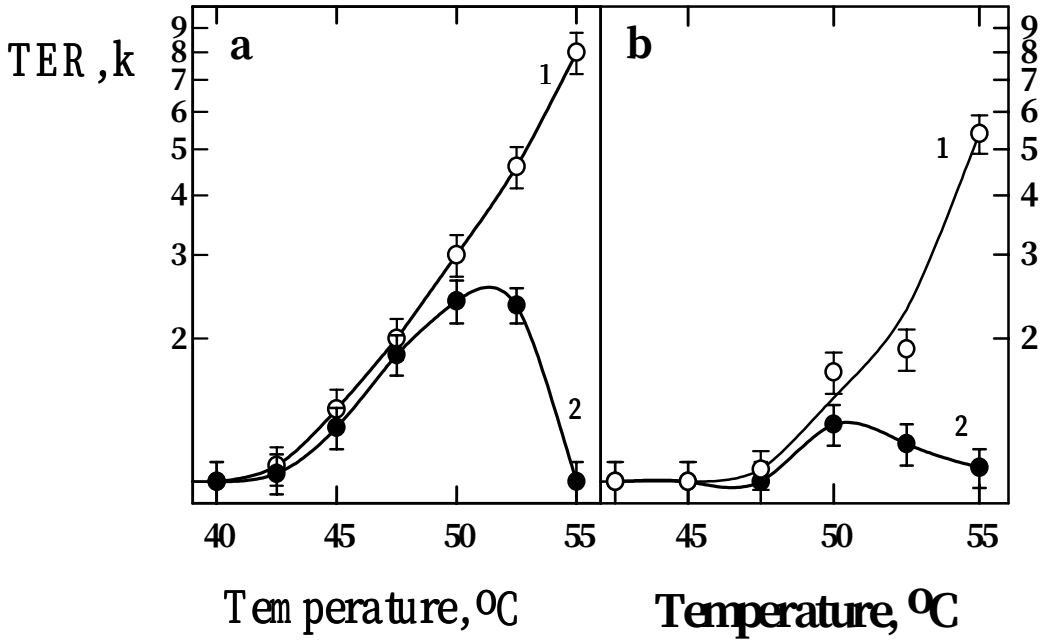


**Fig. 2.** Thermal enhancement ratio (curves 1) and synergistic enhancement ratio (curves 2) of *Saccharomyces cerevisiae* diploid yeast cell (**a**, strain XS800) and haploid (**b**, strain S288C) as a function of temperature exposed ($^{60}$Co) at 10 Gy/min.

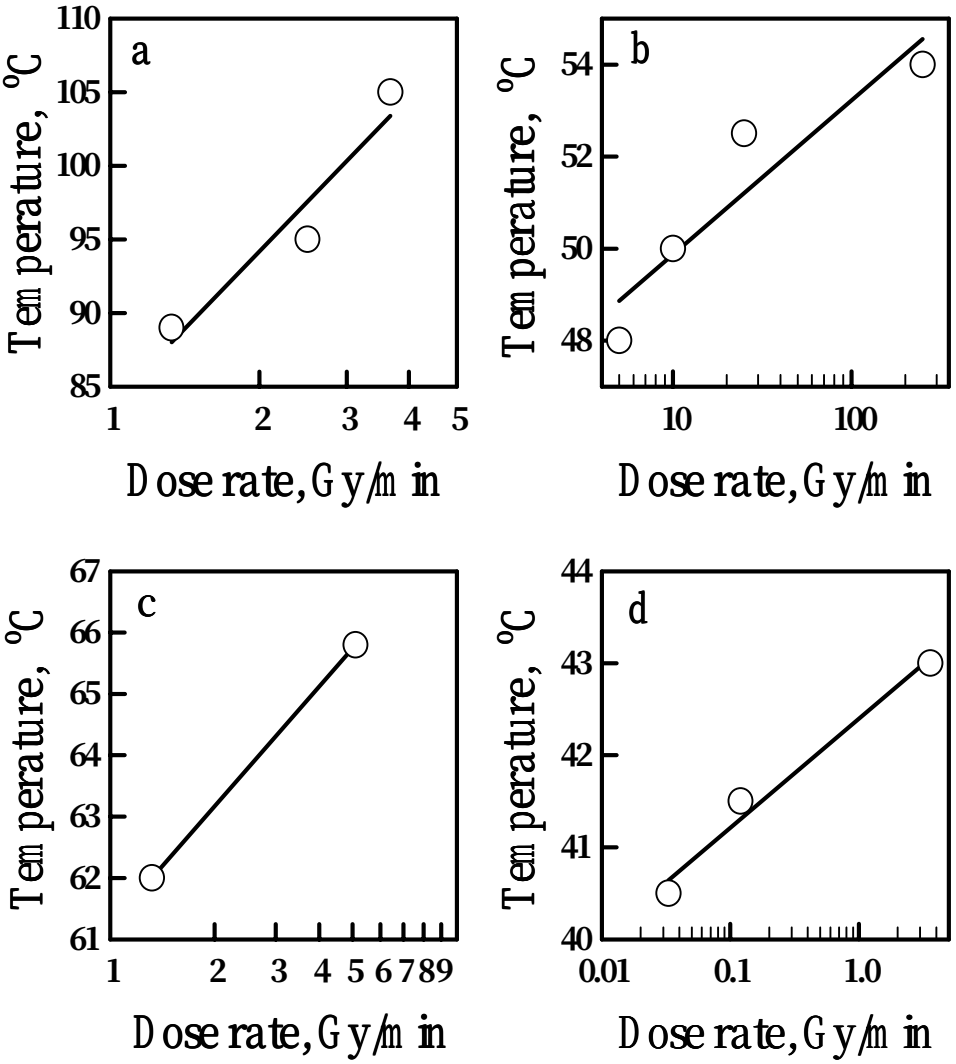


**Fig. 3.** Correlation of dose rate and exposure temperature providing the same synergistic interaction under simultaneous thermoradiation action: **a** - bacterial spores (*Bacillus subtilis*); **b** - diploid yeast cells (*Saccharomyces cerevisiae*, XS800); **c** - bacteriophage (T4); **d** - cultured mammalian cell (Chinese hamster cells).

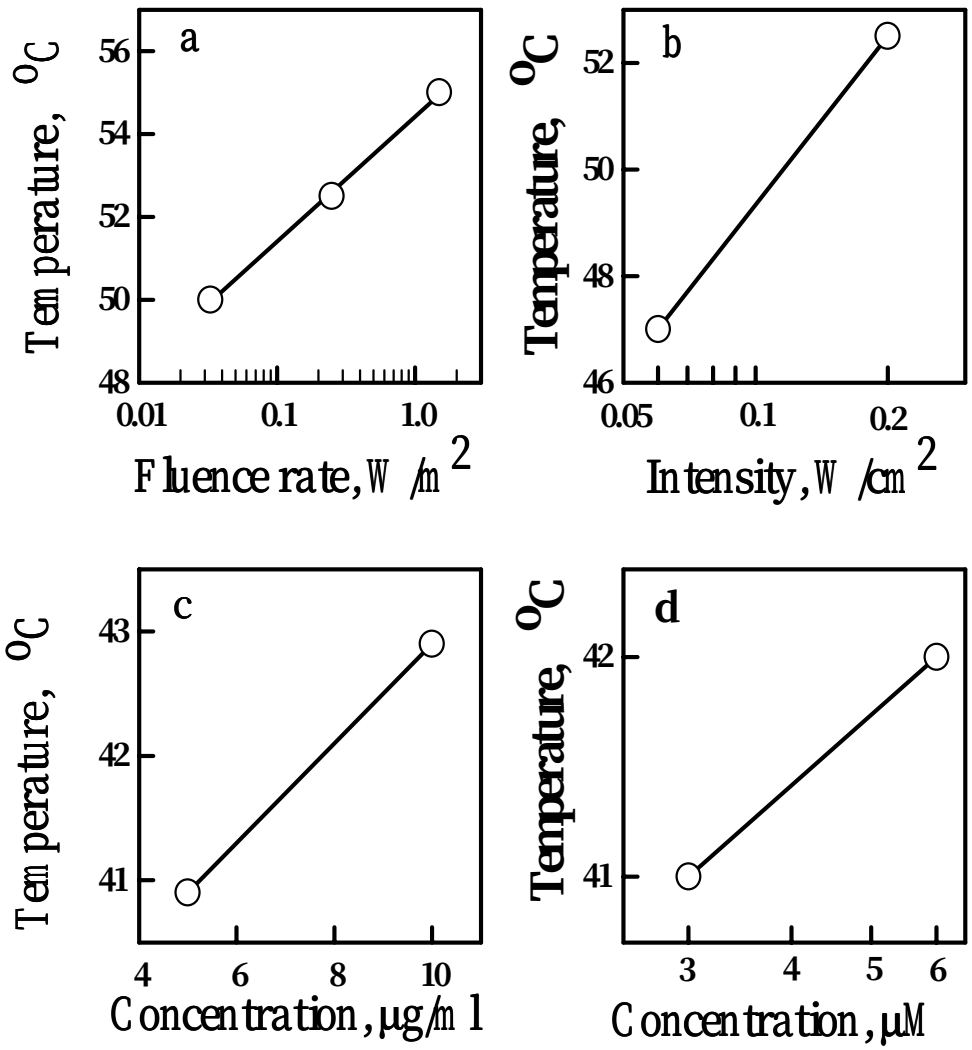


**Fig. 4.** Correlation of exposure temperature with UV light fluence rate (**a**), ultrasound intensity (**b**), and concentration of tris(1-aziridinil)-phosphine sulfide (thio-TEPA) (**c**) and cis-diamminedichloroplatinum (II) (cis-DDP) (**d**) providing the highest synergistic interaction under their simultaneous action on yeast cells (**a, b**) and cultured mammalian cells (**c, d**).