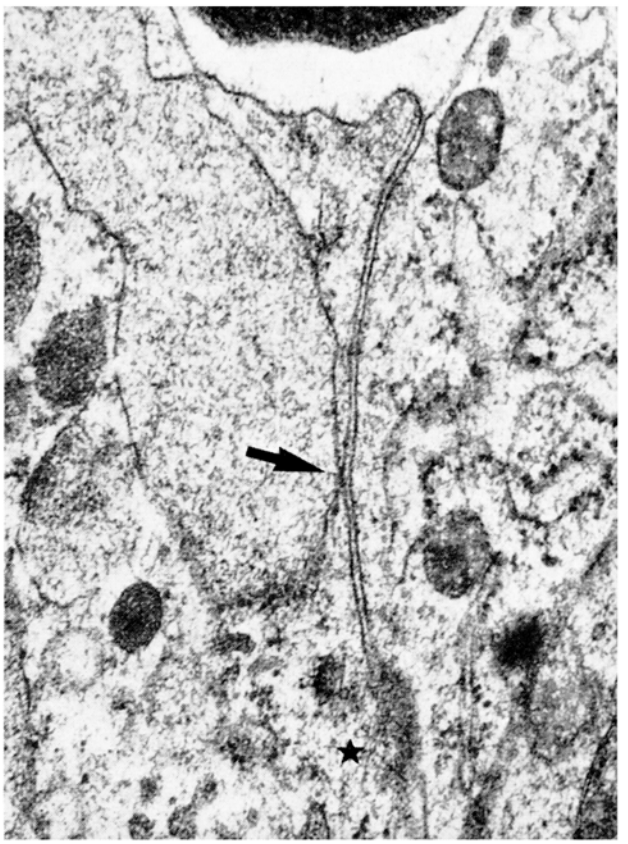

Fig. 1. A zone of contact between a dilated cisternae of the endoplasmic reticulum of a hepatectomy STH cell and the plasmalemma is indicated by the arrow. The star indicates a microvesicles aggregate in relation to a zone of increased density in the plasmalemma. ×35,000.

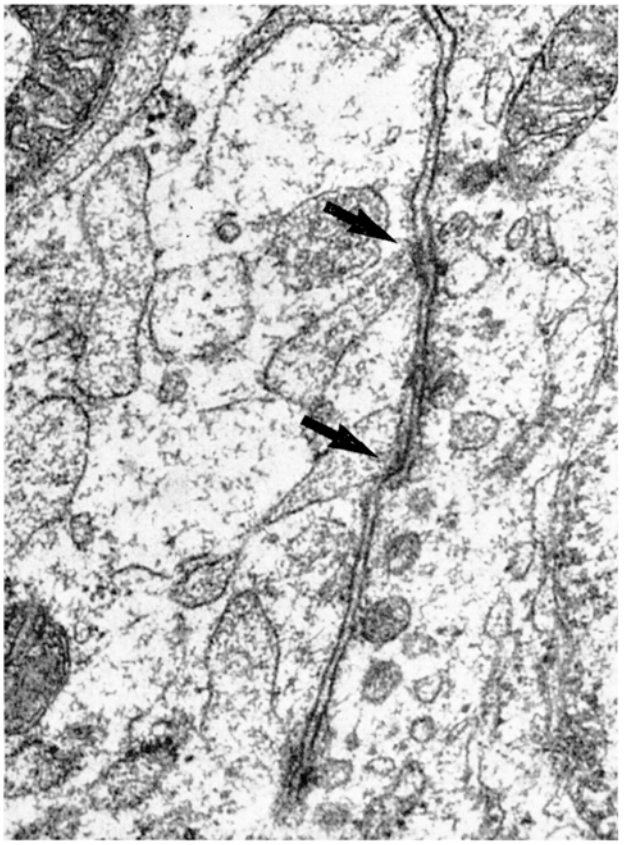

Fig. 2. The 2 arrows indicate zones of contact between the plasmalemma and 2 cisternae of the dilated endoplasmic reticulum. Increased density of the plasmalemma and dense material in the intersticial space are seen in these zones. ×35,000.

Golgi complex which, otherwise, is not inactive but greatly hypertrophied in hepatectomy STH cells[2], and contains many immature granules within the Golgi zone.

It is suggested that the deep metabolic changes appearing after hepatectomy, trigger, in some way yet unknown, this mechanism of short circuit direct release.

Resumen. El reticulo endoplasmico de las celulas somatotropas del raton hepatectomizado se encuentra muy dilatado y contiene material denso en el interior de sus cisternas. Estas se observan frecuentemente en contacto con la membrana celular en zonas en que ésta y el intersticio presentan un aumento de densidad. Se sugiere un mecanismo directo de secreción de la hormona sintetizada en exceso en el reticulo endoplasmico, sin pasar previamente por el complejo de Golgi.

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On the Mechanism of Modification of Radiation Effect by Dimethyl Sulfoxide

The effect of dimethyl sulfoxide (DMSO) on radiation response involves a dichotomy-protection in some instances and sensitization in others. It has been shown that DMSO is an effective radioprotective substance at the molecular[1], cellular[2] and animal[3] levels. DMSO is the initial topically effective protector against radiation cataract[4,5]. Moreover, concomitant sensitization of the irradiated cornea was observed with high concentrations

of DMSO. The drug has also been found to sensitize skin to ionizing radiation[6].

It has been ascertained that DMSO reversibly inhibits a number of biological processes[7–11]. These effects display a marked concentration dependence, and since radiosensitization seems to occur in tissues of highest DMSO concentration (cornea and skin), we have investigated the relationship between DMSO concentration and radiosensitivity in an in vitro cell system.

P815X2 mastocytoma cells were utilized in this investigation. The methods of culture, cell line characteristics, and survival assay have been described[12,13].

In each experiment a cell suspension containing a 10^5 log phase cells/ml was prepared in complete growth medium. All treatments were carried out on 2 ml aliquots of the cell suspension kept in an ice bath. Various amounts of iced DMSO (50% in distilled water) were added to obtain the desired final concentration of the drug, as indicated. Medium was then added so that the tubes contained equal volumes at the time of irradiation. In experiments involving the addition of DMSO following irradiation, the time between exposures and drug was less than 30 sec. Drug removal was accomplished by washing the cells 3 times with iced medium. All radiation exposures were administered while the cell suspensions were at 0 °C. Physical factors of irradiation were: 250 kVcp; 0.5 mm Cu + 1.0 mm Al added filtration; HVL 1.8 mm Cu; TTd 72 cm; exposure rate, as measured in air at the level of the cell suspension with a Siemen's condenser R-meter, was 60 R/min. Post-irradiation incubations were done in a water bath at 37 °C.

Fractional cell survival after various treatments is illustrated in Figure 1. In experimental design A, cells were irradiated with 350 R or 0 R at 0 °C, and DMSO was added immediately after exposure to a final concentration of 0, 3 or 15%. The cells were maintained for 3.5 h at 37 °C, then washed 3 times with iced medium, irradiated with a second 350 R, and assayed for survival. For the cell line employed, 350 R is on the exponential portion of the single dose survival curve. In B, the cells were irradiated with 700 R in the presence of 0, 3 or 15% DMSO. Immediately after exposure, they were washed and assayed for survival. In C, the cells were irradiated with 700 R in the presence of 0, 3 or 15% DMSO, and the drug was left in the cell suspension for a 3.5 h incubation at 37 °C. The cells were washed and assayed for survival. The data is expressed as fraction of corresponding unirradiated control.

As may be seen in Figure 1, 3% DMSO present between exposures of 350 R given 3.5 h apart did not influence survival; conversely, 15% DMSO greatly reduced cell survival. Marked radioprotection occurred at concentrations of both 3% and 15% when the drug was removed immediately after exposure (Figure 1 B). The results of adding the drug prior to exposure and allowing it to remain during the 3.5 h repair interval are shown in Figure 1 C. As anticipated, protection resulted from a 3% concentration. The degree of protection was quantitatively similar to that observed when the 3% DMSO was removed immediately after exposure (Figure 1 B).

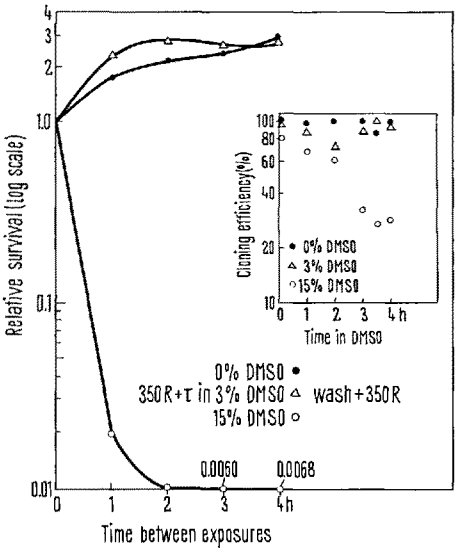

Fig. 2. Cell survival, relative to that following a single exposure of 700 R, obtained with various concentrations of DMSO present during the interval between successive 350 R exposures. Insert: cloning efficiency of unirradiated cells incubated in DMSO for various times at 37 °C.

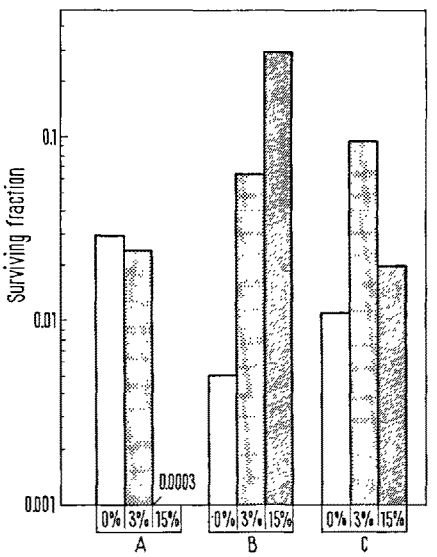

Fig. 1. Fractional cell survival following various radiation and drug treatments (see text).

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The data for 15% DMSO indicates a level of survival in between that observed when the drug was applied between exposures (1 A) and that which occurs when the drug is applied prior to irradiation but removed immediately thereafter (1 b). Under these conditions, survival reflects the net result of 2 opposing processes and is intermediate from the 2 extremes.

To elucidate the effect of DMSO on post-irradiation repair processes, the drug was added immediately after a conditioning exposure of 350 R, allowed to remain for various durations prior to removal, irradiation with a second 350 R, and survival assay. The data are shown in Figure 2. Split-dose survival is plotted relative to that following a single exposure of 700 R (note logarithmic scale on ordinate). The repair kinetics at a drug concentration of 3% do not differ appreciably from control (cf. Figure 1 A). If 15% DMSO only blocked repair of sublethal damage, a curve with 0 slope and an intercept of 1 should have been obtained. The observed curve for 15% indicates a definite additional radiosensitization. This, along with the previous data, suggests that DMSO affects repair of potentially lethal radiation damage. The degree of sensitization by 15% DMSO is seen to be greatest in the first hour following irradiation and reaches a plateau at about 3–4 h. The data in Figure 2 (insert) illustrate that 15% DMSO is progressively toxic to the cells. However, this is not nearly of sufficient magnitude to explain the decrease in survival observed, and all survival data are expressed in terms of respective unirradiated control values. Also, a striking decrease in radiation survival is seen at 1 h, when the cloning efficiency for cells treated with 15% DMSO is 70%. Therefore, the effect of 15% DMSO on cell survival is not simply an additive one of radiation damage and drug toxicity; rather, there appears to be a strongly synergistic relationship between irradiation and this concentration of DMSO.

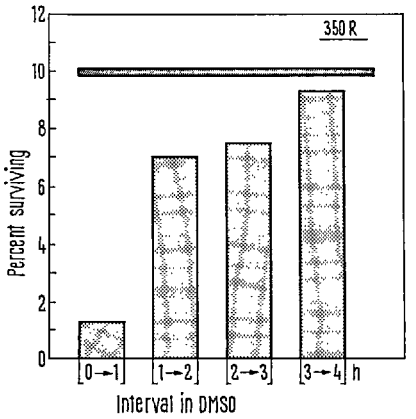

Fig. 3. Cell survival following a single exposure of 350 R. A 1h incubation in 15% DMSO at 37 °C was instituted commencing at various times after irradiation. Horizontal bar represents 350 R survival of cells not exposed to the drug.

Cells were irradiated with a single exposure of 350 R to examine the temporal course of DMSO action. A 1 h incubation in 15% DMSO at 37 °C was administered commencing at various times after exposure (Figure 3). The horizontal bar indicates the level of survival following 350 R for untreated cells. It is apparent that the sensitizing efficacy of DMSO decreases rapidly as the time between irradiation and onset of DMSO treatment increases. When the drug is applied in the 3–4 h interval following irradiation, survival approaches that of irradiated cells without subsequent exposure to DMSO. Conversely, it follows that repair of radiation damage (of the type inhibited by DMSO) proceeds rapidly following exposure and is essentially complete in 3–4 h. The temporal kinetics then are not appreciably dissimilar to those of split-dose type repair (Figure 2).

The data presented herein provide clarifying information on the dichotomous interaction between DMSO and X-irradiation.

Under conditions wherein the drug is applied prior to exposure in low concentrations, or is removed immediately following irradiation at 0 °C, i.e. wherein metabolic alteration caused by the drug is avoided, strictly concentration dependent radioprotection is observed. When 15% DMSO is allowed to remain for even a short time post-irradiation under conditions conducive to cellular metabolism, survival is drastically reduced from the maximum level[14, 15].

Zusammenfassung. Es wurde die früher in vivo beobachtete dichotome Wechselwirkung zwischen Dimethylsulfoxid (DMSO) und Röntgenstrahlen in vitro an einem System von Säugetierzellen untersucht. Wird 3% DMSO vor der Bestrahlung zugefügt, so wirkt es als Radioprotektor, gleichgültig, ob unmittelbar nach der Bestrahlung entfernt oder während der Reparationszeit belassen. Das Überleben wird auffallend erhöht, wenn eine Konzentration von 15% DMSO während der Bestrahlung vorhanden ist oder aber unmittelbar danach entfernt wird. Verbleibt der Wirkstoff während der Reparationszeit, so kommt es zu einer drastischen Herabsetzung der Überlebensquote.

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Variability of the Bohr Effect in Man

In a previous paper[1], we have shown that strong variations can be detected in the value of the Bohr effect, measured in crude haemolysates, in individuals with normal haemoglobin.

The highest Bohr effects were observed in Peruvian Indians living at 4000 m above sea level, but a considerable variability was also present in the European population. The conclusion was reached that the variation in the intensity of the Bohr effect may be due to some unknown factor other than the haemoglobin itself. In a paper by Dill et al.[2] some indication can be found that in patients with diabetic coma both the position of the oxygen dissociation curve and the intensity of the Bohr effect are not simply a function of pH.