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.

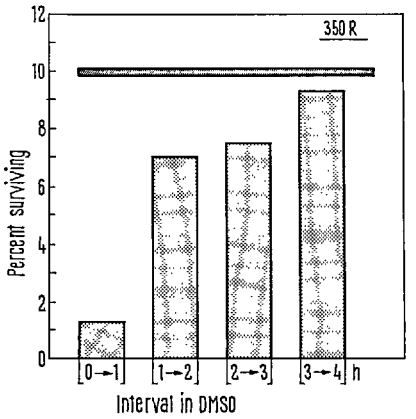

Fig. 3. Cell survival following a single exposure of 350 R. A 1h incubation in 15% DMSO at 37 °C was instituted commencing at various times after irradiation. Horizontal bar represents 350 R survival of cells not exposed to the drug.

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.

R. F. Hagemann and J. C. Schaer

Cell and Radiation, Biology Laboratories, Allegheny General Hospital, 320 East North Avenue, Pittsburgh (Pennsylvania 15212, USA), 31 August 1970.

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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.

In the present paper we examine the Bohr effect in the haemolysates of 15 diabetic individuals with high glycemia (from $2^0/_{00}$ to $5^0/_{00}$).

Hb oxygen dissociation curves were determined according to LEGGIO and MORPURGO[3] in 0.1 *M* phosphate buffer at pH's of 7.4 and 6.7. Results are reported in the Table and in Figure 1. It can be seen that diabetic patients have at P_{50} (the partial pressure O_2 at half saturation) a higher Bohr effect than the normal individuals. This is due essentially to a shift toward the right of the oxygen dissociation curve determined at pH 6.7. The same phenomenon has been observed in the haemolysate of the Peruvian Indians.

Comparison of partial oxygen pressures required for 50% (P_{50}) saturation of haemoglobin from normal individuals and diabetic patients (with standard deviations of the mean). The Bohr effect is calculated as the difference between the values obtained at pH 6.7 and 7.4; for remanent details see text

	No. of individuals	pH 7.4	pH 6.7	Bohr effect
Normal individuals	18	19.5 ± 1.5[a]	26.4 ± 1.48	6.9
Diabetic patients	15	20.6 ± 1.9	31.9 ± 1.6	11.3
Normal purified by sephadex	3	19.3 ± 0.5	26.3 ± 0.5	7.0
Diabetic purified by sephadex	4	19.0 ± 0.8	25.7 ± 1.2	6.7
Diabetic sephadex[a] Normal filtrate	1	19	26	7
Normal sephadex[a] Diabetic filtrate	1	20	30	10
Normal sephadex[a] Normal filtrate	1	20	26	6

[a] Standard deviation of the mean.

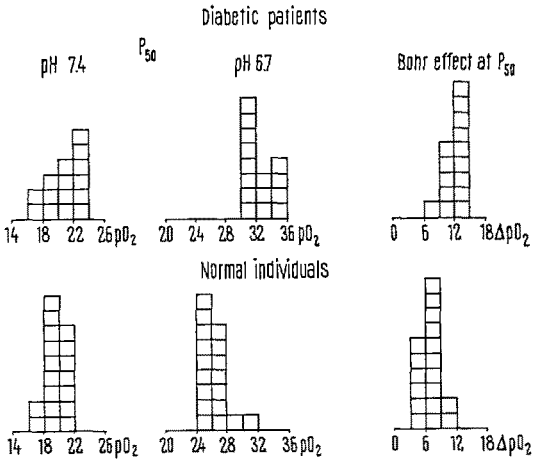

Fig. 1. Histograms showing the individual distributions of partial oxygen pressure required for 50% saturation (P_{50}) of haemoglobin in haemolysates diluted in 0.1 *M* phosphate buffer, at pH 7.4 and pH 6.7, from diabetic patients and normal individuals. Each square represents 1 person. The histogram on the right shows the Bohr effect at P_{50}.

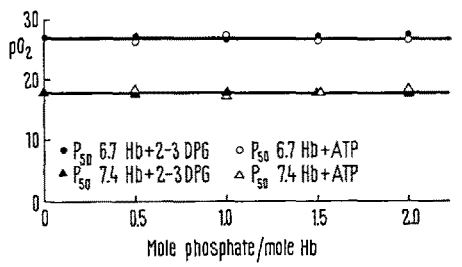

Fig. 2. PO_2 at half saturation of Hb purified by Sephadex at the pH's of 7.4 and 6.7 with increasing concentration of ATP and 2–3 DPG. Temperature 37 °C in 0.1 *M* phosphate buffer.

The haemolysates of 4 diabetic patients were examined by starch gel electrophoresis according to GOLDBERG[4] and only normal haemoglobins ($A + A_2$) were detected. The same haemolysates were purified with two columns of Sephadex G 25 equilibrated with the phosphate buffers at the pH's of 7.4 and 6.7. Purification by Sephadex resulted in a strong reduction of the Bohr effect which is almost identical with that of the control (Table). In one other case, the haemolysates were deprived of the haemoglobin by filtration through XM 50 Amicon (a membrane which retains proteins above 50,000 mol. wt.).

The haemoglobin of a normal individual purified by Sephadex was then diluted with the filtrate free of haemoglobin of the diabetic patient and the oxygen dissociation curve was determined at pH 6.7. In this condition a shift toward the right of the curve can be observed. On the contrary, the position of the curve resulting from stripped haemoglobin of a normal or diabetic individual diluted in the filtrate free of haemoglobin of a normal individual remains unchanged (Table).

It is well known[5,6] that in man the position of the oxygen dissociation curve is determined by the concentration of organic phosphate. It is however 'a priori' unlikely that in our case the factor responsible for the shift at pH 6.7 is 2–3 DPG or ATP. Organic phosphate, in the physiological range, has no effect on the affinity of Hb for the oxygen in phosphate buffers at the ionic strenghth we used[6].

At any rate we have added as a control increasing concentrations of ATP and 2–3 DPG to an haemolysate purified by Sephadex of a normal individual in 0.1 *M* phosphate buffer. 2–3 DPG sodium salt was converted into pure acid with Nalcite HCR and titrated prior to determination of the curve according BARTLETT[7].

No shift of the oxygen dissociation curve was observed (Figure 2).

This experiment shows that the Bohr effect in man is under the control of a low molecular weight factor specifically affecting the Hb-O_2 affinity at the acidic pH.

Riassunto. L'effetto Bohr è notevolmente e significativamente più elevato nei diabetici che negli individui normali. Si dimostra che tale differenza non è dovuta ad un cambiamento nella molecola dell'emoglobina, ma alla presenza di un cofattore diverso da quelli noti e cioé dai fosfati organici ATP e 2–3 DPG.

P. BATTAGLIA, G. MORPURGO and S. PASSI

Istituto Superiore di Sanità, Laboratori di Chimica Biologica, Roma (Italy), 26 August 1970.

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