Table 3. Activity of α-glycerophosphate dehydrogenase in tissues of seal, rat, and mouse, expressed as micrograms of iodoformazan per microgram of tissue nitrogen. Numbers in parentheses denote number of samples.

Tissue	Weddell seal	Rat	Mouse
Liver	1.47 ± .17 (6)	5.55 ± .52 (12)	4.07 ± .43 (16)
Muscle	0.73 ± .11 (6)	4.00 ± .08 (11)	4.42 ± .58 (13)
Kidney	3.36 ± .59 (6)	2.26 ± .22 (12)	5.02 ± .52 (13)
Brain	0.83 ± .03 (6)	1.20 ± .04 (12)	2.85 ± .33 (14)
Adipose	6.94 ± .17 (6)		3.33 ± .44 (14)
Heart	0.29 ± .06 (5)	0.79 ± .05 (11)	Negligible

mammals (*5*) is not found in the Weddell seal, which indicates either that alternative pathways for the generation of reduced NADP exist in seal adipose tissue or that this tissue is not a major site of fat synthesis, because fatty acid production cannot occur without adequate amounts of the reduced coenzyme. Synthesis of fatty acids may occur elsewhere, and these acids may then be carried by the blood to adipose depots for assembly. However, the low activity of glucose-6-phosphate dehydrogenase in all the tissues assayed is not in accord with this hypothesis. Possibly, fat synthesis is not a major function of the adipose mass, but rather the high α-glycerophosphate dehydrogenase is an adaptation to oxygen deprivation associated with arterial constriction during diving (*6*). This enzyme functions similarly to lactate dehydrogenase in providing for a reoxidation of the reduced NAD arising during glycolysis, with a concomitant production of reduced metabolite. This may also apply in the kidney, where activity of α-glycerophosphate dehydrogenase is twice that in rat (Table 3).

The absence of intra-abdominal fat stores and the relatively coarse reticular structure of the insulating subcutaneous adipose tissue in seals (*7*) support the concept that adipose physiology is quite unique in these aquatic mammals. The absence of a hexose shunt pathway, coupled with high α-glycerophosphate dehydrogenase activity, provides an enzymatic reflection of this metabolic uniqueness.

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Sulfur Mustard and X-Rays: Differences in Expression of Lethal Damage

Abstract. *Before or after treatment with sulfur mustard, simple changes in the incubation conditions of cultured Chinese hamster cells lead to changes as great as a factor of 6 in survival-curve slopes. With x-ray treatment, changes are similar but much smaller in magnitude. These results suggest that the modes of action of these agents are not entirely the same.*

Bifunctional alkylating agents, which are considered to be radiomimetic (*1*, *2*), and x-rays kill cells, yield survival curves of similar shapes, produce chromosome breaks, lead to similar patterns of hematological changes, and are thought to produce similar results with respect to other end points. Lethal-repair processes relative to both agents are considered similar. Recovery from lethal damage by drugs is reported in microbes (*3*, *4*), and differences in the survival responses of different lines of mammalian cells are thought to reflect corresponding differences in their repair capacities after treatment with either type of agent (*2*). Because of these similarities and the use of both types of agent (singly and possibly together) for treating cancer, we undertook comparative study of their toxic properties. Sulfur mustard (*5*) was chosen because its reactivity in aqueous media permits closer approximation to acute irradiation than do other readily available alkylating agents. For Chinese hamster cells growing attached to a surface, we now report the influence on survival of some simple procedures entailing changes in medium and temperature before and after treatment. Our results show that these procedures have a strong influence relative to sulfur mustard treatment and only a weak influence relative to x-ray treatment.

Typical curves for the survival of the Chinese hamster subline *V79-661*, after exposure to different doses of sulfur mustard, are shown in Fig. 1. Cells were grown overnight attached to glass or plastic, and, after treatments to be described, were assayed for colony-forming ability by use of techniques similar to those reported (*6*). The medium used for surface-attached growth is a modification of Eagle's (*7*, *8*) and contains 15 percent fetal calf serum. The doubling time for these cells is 8 to 9 hours (*9*). As a result, after the overnight incubation of initially single cells, the population appears to be in asynchronous log-phase growth (*9*), with colony-forming units comprising an average of about three cells. Since Chinese hamster cells survive damage by either x-rays (*10*) or sulfur mustard (*10a*) independently, multiplicities greater than one introduce no serious complications with regard to analysis of survival curves.

Sulfur mustard (di-2-chloroethylsulfide), dissolved in absolute ethyl alcohol and stored in a deep freeze, was diluted before each experiment in the same solvent and then maintained at ice temperature. Immediately before each exposure, the solution was further diluted in buffer at *p*H 7.4 (Dulbecco's saline supplemented with 1 percent medium), also at ice temperature. Drug concentrations were adjusted so that 1.0-ml portions could be added to the medium or buffer in petri dishes to obtain the final concentrations desired. Accordingly, the final concentrations of alcohol (less than 1 percent) were always too low to affect survival. Treatment solutions were removed after 8 minutes and replaced by fresh medium or buffer as

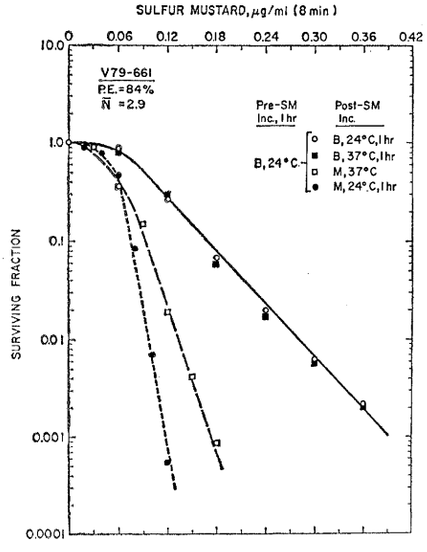

Fig. 1. Survival of surface-attached Chinese hamster cells exposed to sulfur mustard. Abbreviations for conditions of incubation before or after treatment: *M*, medium; *B*, buffer; *P.E.*, plating efficiency; $\bar{N}$, average multiplicity at the start of the treatments.

required. For x-irradiation, 55-kv x-rays (about 722 rad/min) were used as reported (*8*). All results shown in each figure were obtained with the same starting suspension of cells; standard errors in survival were smaller in size than the points plotted.

The data in Fig. 1 were obtained with cells incubated for 1 hour in buffer at 24°C before exposure to sulfur mustard. After treatment, incubation in buffer at 24° or 37°C, prior to incubation for colony formation, yielded the top curve. The result was virtually the same with buffer post-treatments if the pretreatment consisted of optimal growth conditions (medium, 37°C); in this instance, even medium at 37°C, after sulfur mustard, yielded survivals along the top curve (data not presented). In contrast, Fig. 1 shows that medium at 37°C, after sulfur mustard, resulted in a more rapid fall in survival (middle curve), and that medium at 24°C further increased the lethal effectiveness of this drug (bottom curve). As well as the same starting cell suspension, the same drug stocks were used for all results in Fig. 1. The data cannot reflect, therefore, the differential decay of drug stocks prepared at different times before the experiment. Furthermore, from survival results obtained over at least a 6-month period, no loss of potency was evident for our ethanol-stored material.

The survival-curve changes in Fig. 1 may be quantitated in terms of changes in the single-cell survival parameters $\tilde{D}_o$, and $\tilde{n}$ or $\tilde{D}_q$. $\tilde{D}_o$ is the concentration of sulfur mustard that reduces survival by a factor of $1/e$ in the exponential region; $\tilde{n}$, the extrapolation number, is the intersection with the ordinate, of a back extrapolate of the exponential portion of a survival curve, divided by the average cell multiplicity $\bar{N}$ (*8*, *10*). $\tilde{D}_q$ is a measure of shoulder width; for results obtained with single cells, it may be obtained from the intersection, of a back extrapolate, with the abscissa at surviving fraction 1.0. For results obtained with microcolonies as in Fig. 1, one obtains $\tilde{D}_q$ by decreasing the observed shoulder width by $\tilde{D}_o \ln \bar{N}$. Thus, in terms of these parameters and if one accounts for $\bar{N} = 2.9$ in Fig. 1, post-treatments in buffer result in $\tilde{D}_o \simeq 0.05$ μg/ml, $\tilde{n} \simeq 1.0$, and $\tilde{D}_q \simeq 0$. In contrast, post-treatments in medium lead to $\tilde{D}_o$ values about three- and sixfold smaller (medium at 37°C, $\tilde{D}_o = 0.018$ μg/ml; medium at 24°C, $\tilde{D}_o = 0.009$ μg/ml) and large increases in shoulder width (medium at 37°C, $\tilde{D}_q = 0.034$ μg/ml; medium at 24°C, $\tilde{D}_q = 0.043$ μg/ml).

To facilitate comparison of sulfur mustard with x-rays in connection with the treatment conditions described, we consider next the kinetics of the changes in Fig. 1. As we noted, optimal growth conditions before and after treatment with sulfur mustard lead to a survival curve virtually the same as the top one in Fig. 1. One survival point for optimal conditions throughout (medium, 37°C; sulfur mustard, 0.12 μg/ml for 8 minutes; medium, 37°C) is shown by the solid circle on the left ordinate in Fig. 2. A 1-hour pretreatment in buffer effects a tenfold drop in survival (open circle, left ordinate) provided that medium at 37°C follows exposure to sulfur mustard. The top and bottom curves show the effect of increase in periods in buffer at 26°C or in medium at 26°C, respectively. In both instances, the changes are completed in about 30 minutes, as is the case for buffer at 37°C, which yields a curve quite similar to that for buffer at room temperature (data not presented).

Precedence for the dependence, of the x-ray survival of mammalian cells, on post-irradiation changes of medium or temperature, exists in the results obtained with several lines of cells (*11*). In the main, however, the conditions used in these latter studies were quite different from those for Figs. 1 and 2. For this reason and because close correspondence has been proposed for the

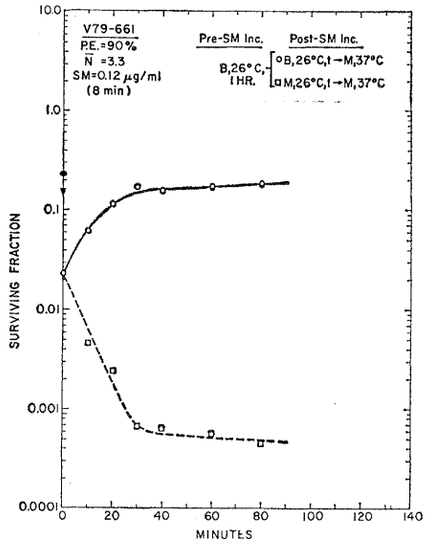

Fig. 2. Time courses of alterations of survival after treatment, for cells treated for 8 minutes with sulfur mustard at 0.12 μg/ml. The effect of 1-hour pretreatment in buffer (*B*) at 26°C is indicated by the drop in survival on the left ordinate. Otherwise as in Fig. 1.

actions of x-rays and an alkylating agent like sulfur mustard (*12*), we performed experiments similar to those described, except that x-irradiation was substituted for sulfur mustard treatment.

In Fig. 3, zero hour is the time of x-ray exposure for the top sets of data. (The times of irradiation or of changes in medium, or of both, for the bottom sets of data will be specified presently.)

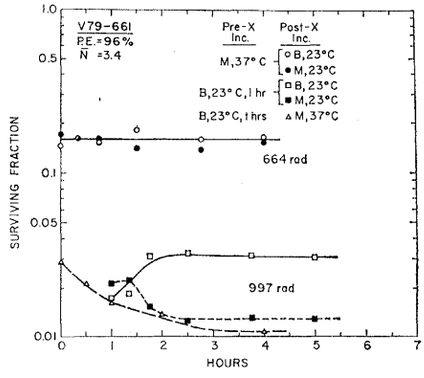

Fig. 3. Time courses of pre- and post-irradiation changes in incubation on the survival of Chinese hamster cells. For the top points, cells were irradiated at zero hour. For the triangles, cells were irradiated after the indicated periods in buffer (*B*) at 23°C. For the squares, cells were irradiated after 1 hour in buffer at 23°C; they were incubated thereafter for the indicated periods in either buffer at 23°C (open squares) or medium (*M*) at 23°C (closed squares). Abbreviations as in Fig. 1.

The results with buffer resembled those to which we have already referred. For sulfur mustard, medium at 37°C as a condition of pretreatment led to the same survival for subsequent treatment in room-temperature buffer or 37°C medium; similarly for x-rays, as indicated by the open circles. However, when the incubation after treatment was in room-temperature medium (that is, medium, 37°C; sulfur mustard; medium, ~24°C), a survival curve results (data not presented) having a slope equal to the steepest in Fig. 1. Thus, in contrast with the lack of effect after x-rays (closed circles), after a dose of sulfur mustard that would produce the same survival (about 17 percent) for optimal conditions throughout, medium at room temperature effected a 100-fold decrease.

The curve traced by the triangles in Fig. 3 shows that progressive fall in survival results from incubation, before irradiation, in buffer at 23°C. For sulfur mustard, the same pretreatment results in tenfold drop in survival in 1 hour (left ordinate in Fig. 2). A twofold drop results in the same period for x-rays. The dose used for the latter results was chosen to yield survival, after pretreatment with buffer for 1 hour, about equal to that at zero hour in Fig. 2 (open circle). The open squares in Fig. 3, obtained with the same conditions of post-treatment as for the upper curve in Fig. 2, show that survival after x-irradiation can be increased by room-temperature buffer but only about twofold. The closed squares refer to incubation after treatment in room-temperature medium. (This set of data was obtained about 1 hour after the open squares. The small difference between the first points on each curve probably reflects small differences in the compositions of the populations.) Compared to the situation after treatment with sulfur mustard, here too a decrease in survival results, but at most amounting to a factor of 2. $\bar{D}_0$ shifts of less than 25 percent can account for the survival changes in Fig. 3.

In summary: Simple alterations in pre- or post-treatment medium or temperature, or in both, can effect large changes in the survivability of surface-attached Chinese hamster cells after their exposure to sulfur mustard. Survival after x-ray exposure may be similarly affected, but the changes are much smaller. If this rough parallelism is evidence of the production of identical primary injuries by both agents, there must be significant dissimilarities in their secondary effects. The number and types of potentially lethal lesions may be the same, but a cell's ability to cope with them may reflect the agent as well as the conditions used. Alternatively, the quantitative differences in expression of lethal damage may indicate only a small degree of overlap of primary lesions in respect to number or type, or both.

In addition to radiomimetic properties, our results with sulfur mustard prompt us to consider the question of damage expression after treatment with such an agent. Generally speaking, we would expect optimal growth conditions throughout a treatment course to promote a considerable amount of repair of lethal damage. That incubation in buffer after treatment gives the same dose-effect curve as in medium at 37°C, when the pretreatment also is in medium at 37°C, is not inconsistent with this expectation. We need only assume either that active metabolism is unnecessary for the repair process or that repair is initiated, and is equally effective, after the buffer is removed. To explain the results in Fig. 1, however, we must also assume that: (i) pretreatment with buffer reduces the effectiveness of the repair system, and (ii) ability to repair can be reinstated by subsequent treatment with buffer (even at room temperature) but not with medium at room temperature. Thus our results with mammalian cells may reflect the same rescue processes that are thought to be active in bacteria (*4*) in which incubation in buffer, after exposure to a drug, also causes increases in survival, although over longer periods (2 to 6 hours versus 30 minutes).

While the foregoing may be essentially correct, on purely logical grounds the large fluctuations involved prompt us to mention another view. The degree of damage expressed after treatment with a given agent may involve *degradative* as well as *reconstructive* processes. Cell survival may reflect not only the number of initial lesions and the proportion of these that are repaired. The fate of a cell may also depend on whether or not the initial damage that can be expressed (amount or type) is changed by some treatment so that survivability is altered, even though ability to repair may not be. For example, in Fig. 1 post-treatment with medium leads to more fixation of damage, perhaps not because repair is inhibited but rather because medium increases the amount of damage requiring repair.

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Competent Chick Ectoderm: Nonspecific Response to RNA

Abstract. *Presumptive chick neuroectoderm responds to RNA from brain and heart by forming neural tubes, but it does not respond to liver RNA. This differential response can be correlated with the presence of Folin-positive material in those RNA preparations which elicit the formation of neural structures.*

There are divergent claims concerning the effects of RNA (extracted with phenol) on intact embryos and competent tissues. It has been stated that RNA acts as an "inducing" agent resulting in the formation of structures specific to the organ source of the RNA (*1–3*). Other workers, utilizing similar test systems, find no specificity related to tissue source (*4, 5*).