the immature spinal cord. The lack of chlorpromazine effect on the mature spinal cord further supports the existing disagreements concerning the effects of chlorpromazine upon adult spinal levels[6].

It can be speculated that the higher AChE activity in the chlorpromazine-treated 9-day-old spinal cord explants may reflect enzyme induction by this drug. Chlorpromazine, like other CNS drugs such as barbiturates, diphenylhydantoin and chlordiazepoxide, has been reported to induce enzymes[6]. Further studies, however, are required in order to propose such a selective action of chlorpromazine on AChE.

AChE activity has been used as evidence for the presence of acetylcholine[12], a proposed neurotransmitter in many CNS areas[13]. Thus, the higher AChE activity in the maturing spinal cord observed in this study and also in previous studies[1] suggests that chlorpromazine influences cholinergic systems of the developing spinal cord. Moreover, chlorpromazine has been found to increase the sensitivity of the developing CNS to seizures elicited by electroshock stimulation in chick embryos[1] and in maturing rats (unpublished observations). Spinal reflex systems play a substantial role in the integration of seizure activity. The influence, therefore, of chlorpromazine on cholinergic systems of the developing spinal cord may be an underlying factor in the sensitivity to electrical convulsions induced by chlorpromazine[14,15].

Résumé. L'activité acétylcholinesterasique de fragments de moelle d'embryon de poulet de 9 jours augmente lorsqu'ils sont maintenus en culture organotypique, après addition de Chlorpromazine au milieu standard de EAGLE[3].

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[15] Chlorpromazine HCl was made available through the generosity of Smith, Kline and French Laboratories. The able technical assistance of Mrs. JUDITH SHEARER is greatfully acknowledged.

Radioprotection and Recovery by Dithiothreitol

It is well known that many chemical compounds provide protection against the effects of ionizing radiations in mammals and living organisms. The most effective class of radioprotective molecules seems to be the aminothiols. The presence of both the –SH and $-NH_2$ groups appears to be critical for protection[1]; moreover, a spatial separation between these groups of more than 3 carbon atoms causes a marked decrease in activity[2]. Although dithiothreitol (DTT)[3] bears no $-NH_2$ group, it came to our attention for its high erythrostimulating activity[4], its very low redox potential (– 0.33 V at ph 7) and the presence of its molecule of 2 free –SH groups. In addition, its high solubility in physiological medium and its very low rate of oxidation at room temperature make it a very suitable compound for recovery and radioprotection experiments[5].

Material and method. Male albino mice, Swiss strain, 8 weeks old, weight range 24–27 g, kept unlimited standard diet, were whole-body irradiated in groups of 20 in a 'Perspex' box lying on a wooden support to avoid backscattering. The animals were exposed to an X-ray dose of 625 R by a Siemens Stabilipan unit operating at 180 kV, 10 mA, filtration 0.5 Cu, 26 R/min. In these conditions only $5.6 \pm 3.5\%$ of the untreated animals survived irradiation, the effect being observed in 200 mice. The mortality-rate was checked daily up to the 30th day following X-ray exposure.

Dithiothreitol in the reduced form was purchased from Calbiochem (USA). The trial solution adjusted to pH 7.0 was injected i.p. in each group of 50 animals, at a constant volume of 0.2 ml/mouse. In the protection experiments, the injection was performed 15 min prior to exposure.

Results. Owing to drug toxicity, the highest dose of DTT in the reduced form that could be administered was 120 mg/kg. As shown in Figure 1, this dose causes a moderate survival percentage ($29 \pm 3.4\%$). A dose of 80 mg/kg, however, produces a survival percentage of $15 \pm 1.2\%$; while no radioprotection was detected when lower doses were administered.

In separate experiments (Figure 2), when the 120 mg/kg dose was injected 0.5, 2.5, or 24 h after irradiation, no significant difference in the survival rate could be observed. Only when the compound was administered 72 h after exposure, did survival decrease to $14 \pm 1.0\%$.

From these experiments it was evident that dithiothreitol had a low radioprotective activity and that the same effect could be detected when the drug was given within 24 h after exposure. Since, to our knowledge, no other free –SH groups bearing compound has this particular feature, a question was raised whether the oxidized form of DTT rather than the reduced one accounted for this effect. The former was easily obtained following Cleland's method, by oxidizing the –SH groups to form a disulphide bridge, i.e., producing a ciclization of the molecule.

As shown in Figure 1, when DTT in the oxidized form (200 mg/kg) was injected i.p. before X-ray exposure, survival at 30 days was increased up to $56 \pm 0.6\%$. A dose of 120 mg/kg gave 10% survival, while a higher dose (160 mg/kg) brought the survival to $34 \pm 6.2\%$. No different results were obtained in other experiments (Figure 2) when

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the drug (200 mg/kg) was administered 0.5–2.5 h after irradiation; injections at 24 and 72 h after X-ray exposure gave a survival of $46 \pm 4.6\%$ and $30 \pm 2.3\%$ respectively.

Both the very low redox potential of reduced DTT, and the high concentration of peroxy radicals in biological fluids during irradiation, make it hard to see how the effect ascribed to the higher dose of oxidized DTT may be due to its conversion in the reduced form.

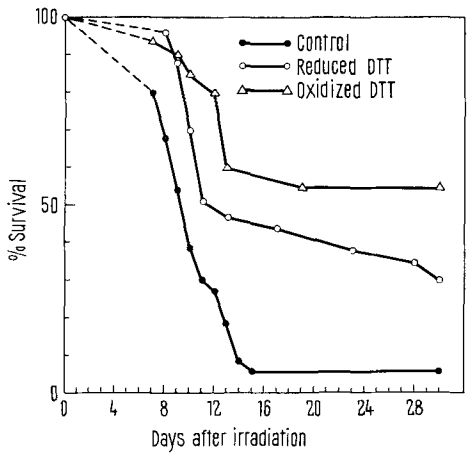

Fig. 1. Effect of reduced and oxidized dithiothreitol on the survival rate of irradiated mice. Data were obtained on groups of 50 animals given an X-ray dose of 625 R.

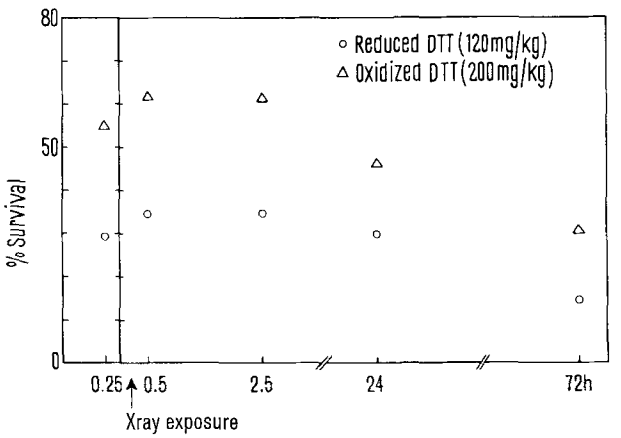

Fig. 2. Percentage of survival at 30 days in mice treated with reduced and oxidized dithiothreitol at different times before and after irradiation with 625 R. Each experimental point is the average of 50 animals.

In fact, when in separate experiments aqueous solutions of reduced DTT at a concentration of 3 mg/ml were exposed to a dose of 625 R in conditions identical to those used in the animal experiments, almost 90% of the compound, as shown by the increase in absorbancy at 268 nm, was converted to the oxidized form. On the other hand, no change in absorbancy was observed in control experiments using oxidized DTT at a concentration of 5 mg/ml.

Dicussion. We may therefore summarize the 3 major features of the effect of DTT on irradiated mice as follows: (1) the reduced form is able to bring about recovery from X-ray damage with the same effectiveness up to 24 h after irradiation, an effect which seems to be unique among sulphydryl compounds; (2) the oxidized form shows an appreciable radioprotection and recovery effect up to 72 h after exposure; (3) the oxidized form, used at a concentration twice as high as that of the reduced one, has greater effect (60%) on survival than reduced DTT (30%), in both protection and recovery experiments.

It has recently been reported that chromosomal aberrations in leucocyte cultures exposed to an X-ray dose of 200 R are decreased if the cultures are pretreated with 1–4 dithiothreitol[6]. The results obtained with reduced DTT in whole animals show a fair correlation with these obtained in vitro. MAISIN et al.[7] have shown that yeast RNA hydrolysate is able to recover mice from X-ray exposure, but the mechanism of the action of such effect is not yet fully understood.

The chemical structure of DTT does not resemble a nucleotide and in order to explain its effects as a recovery factor, some other mechanism should be invoked. Probably DTT acts as inducer molecule for repairing processes in irradiated cells.

Résumé. Le DTT réduit et oxydé, administré à des sourris irradiées par des doses mortelles de rayons X, provoque une survie notable; le meilleur effect est obtenu avec le DTT oxydé, administré indifféremment avant ou après l'irradiation.

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Unterschiede der ATPase-Hemmung durch Azid in weissen und roten Muskelfasern der Ratte

Man unterscheidet in der quergestreiften Skelettmuskulatur zwischen weissen und roten Fasern. Bei manchen Tierarten gibt es ganze Muskeln, die entweder weisse oder rote Fasern enthalten, wie zum Beispiel beim Kaninchen oder Huhn. Bei anderen Tierarten sind in verschiedenen Muskeln weisse und rote Fasern mehr oder weniger vermischt vorhanden. Bei der Ratte kann man in verschiedenen Muskeln weisse und rote Teile voneinander trennen. Weisse und rote Muskelfasern unterscheiden sich in ihren mechanischen, elektrischen und biochemischen Reaktionen in verschiedener Art. In den roten Fasern ist ein mehrfach höherer Gehalt an Mitochondrien vorhanden als in den weissen Fasern (PADYKULA und GAUTHIER[1]). Es lässt sich daraus folgern, dass an Mito-

[1] H. A. PADYKULA and G. F. GAUTHIER, in *Exploratoy Concepts in Muscular Dystrophy and Related Disorders* (Ed. A. T. MILHORAT; Excerpta medica foundation, International congress series, Elsevier, Amsterdam 1966), Nr. 147, p. 117.