she noted that Egorov erroneously decided that Glebovskaja and Zaspelova referred the species *incognita* to *Ellesmeria* Tolmachoff, 1926, and consequently transferred the species to his new genus *Mossolovella* Egorov, in litt. Polenova then proceeded to describe and illustrate only one species as *Ellesmerina philippovae* Egorov in litt.

There are two ways of interpreting this case: (i) *Ellesmerina* Polenova, 1953 is a monotypic genus with the type species *E. philippovae* Polenova, 1953 (Article 68c) because *Ellesmerina incognita* Glebovskaja and Zaspelova in Polenova, 1953 is a *nomen nudum*, or (ii) *Ellesmerina* and *Mossolovella* are objective synonyms because they have the same type species (Article 61b).

Shaver (*10*, p. Q386) arrived at the second interpretation and indicated that they are objective synonyms. He assumed that Egorov's statement in the discussion of *Mossolovella* (*8*, p. 46), "The genotype of the new genus . . . was mistakenly assigned by E. M. Glebovskaja, and later by E. M. Glebovskaja and V. S. Zaspelova (1948) to *Ellesmeria* Tolmachoff . . . ," indicated a publication not available in the United States. On this basis he recognized *Ellesmerina* Glebovskaja and Zaspelova in Glebovskaja, 1948, and relegated *Mossolovella* Egorov, 1953 to objective synonymy.

Zanina and Polenova (*11*, p. 342, Fig. 874) considered *Ellesmerina* Zaspelova, 1953 as a junior synonym of *Mossolovella* Egorov, 1953, and illustrated *M. philippovae* Egorov, 1953. They, however, erred in citing the type species as *M. incognita* (Glebovskaja and Zaspelova, 1953), and also in crediting *Ellesmerina* to Zaspelova. The type species of *Mossolovella* is by original designation *M. incognita* Egorov, 1953; Zaspelova, 1953 (*12*) to whom they referred (*11*, p. 414) recorded without describing or illustrating *Ellesmerina incognita* Gleb. et Zasp., by definition a *nomen nudum*.

According to the Code, *Mossolovella* Egorov, 1953 (type species by original designation *M. incognita* Egorov, 1953) and *Ellesmerina* Polenova, 1953 (type species by monotypy *E. philippovae* Polenova, 1953) are objective synonyms. The valid name of the taxon is the oldest available name applied to it (Article 23). Because both reports are dated 1953, it is necessary to determine from the records of the publishing houses which paper was published first, in order correctly to record this taxon.

The above examples should convince researchers that premature citation of zoological names and the use of names in unpublished reports serve only to confuse zoological nomenclature.

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13. I am grateful to C. W. Sabrosky and E. L. Yochelson for constructive criticism. Publication authorized by the director, U.S. Geological Survey.

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## Cysteamine: Differential X-ray Protective Effect on Chinese Hamster Cells During the Cell Cycle

Abstract. *Cysteamine present during x-irradiation protects synchronized Chinese hamster cells in culture against lethal damage at all stages of the cell cycle. The effect is greatest for cells irradiated at sensitive stages such as $G_1$ and least for resistant cells; for example, late S (dose-modifying factors 4.2 and 2.7, respectively). The effect of 50 millimolar cysteamine is to render almost invariant the normally variant x-ray age response for lethality. This suggests that there are two components of x-ray damage, only one of which is age dependent, and it is against this component that cysteamine protects the cell. Cystamine, however, has no protective effect upon these cells at any stage of the cell cycle.*

Cysteamine (2-mercaptoethylamine) protects mammalian systems in vivo (*1*) and in vitro (*2*) against the lethal effects of x-radiation. Cysteamine has also been shown recently to exert a differential action on the length of division delay following x-irradiation at different parts of the cycle (*3*). While the mechanism of protective action is still in doubt (*1, 2, 4*), it may be related to effects of the compound upon cellular constituents the concentrations of which may vary throughout the generation cycle of mammalian cells. Support for this possibility comes from studies by Sakai and Dan (*5*) which show variations in the amount of sulfhydryl per protein nitrogen during the cell cycle of sea urchin eggs and tetrahymena. Experiments with cysteamine on cells irradiated at different stages of the cell cycle may cast some light not only on the action of the agent but also, perhaps more importantly, on our understanding of the variation of x-ray response through the mammalian cell cycle (*6*).

Synchronous cells of a Chinese hamster subline V79-S171 obtained by selection for dividing cells (*6*) were inoculated onto plastic petri dishes containing EM-15 medium (*7*). Cell cultures were then incubated at 37°C in a humid atmosphere of 2 percent $CO_2$ and air. Synchronous cells progress through $G_1$ in 2 to 2½ hours, S in 6 to 7 hours, and $G_2$ in about 1½ hours, and mitosis lasts about ¾ hour, the population desynchronizing with time. Cultures were irradiated with 250-kv (peak) x-rays (half-value-layer 0.9 mm Cu, exposure rate 105 r/min, absorbed dose 0.945 rad/r) at room temperature. Freshly dissolved cysteamine (*8*) was added to the medium of treated cultures to yield the desired concentration immediately prior to irradiation. These cultures were rinsed and fresh medium was added immediately after irradiation. Unirradiated cultures were divided into two groups, one untreated and the other receiving cysteamine to determine its toxicity. All cultures were handled ex-

actly alike and were subsequently incubated for 8 to 10 days. The resulting colonies were scored as a measure of the reproductive integrity of the treated cells.

The results of experiments with a single dose of x-radiation (710 rads) and two concentrations of cysteamine (5 and 50 m*M*) are shown in Fig. 1. (The data for two experiments were pooled. Additional later experiments show very similar results.) Even the higher concentration of cysteamine was not toxic during the 15- to 20-minute period required for addition, irradiation, and removal of the agent. Curve A in Fig. 1 shows the normal variation in x-ray response after 710 rads for untreated cells. Five-millimolar cysteamine (curve B) provides significant protection and some leveling out of the age response; 50 m*M* cysteamine (curve C) provides greater protection, and the age response through the cycle is almost invariant. The approximate lengths of subdivision of the cycle, as determined by pulse labeling with $H^3$-thymidine, are also shown on Fig. 1.

Additional experiments with concentrations of cysteamine between 1 and 50 m*M* were undertaken. The variation

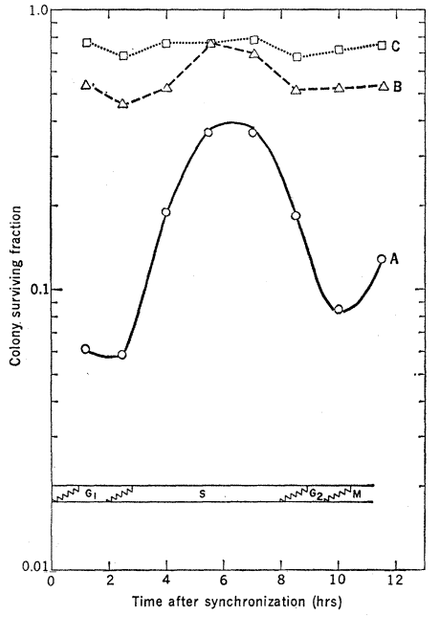


Fig. 1. Survival of synchronous Chinese hamster cells irradiated at different stages of the cell cycle with 710 rads of 250-kv (peak) x-rays. (The data points represent the means of results for two similar experiments. The standard error of each data point is approximately equal to the size of the symbols.) Curve A, no cysteamine; curve B, 5 m*M* cysteamine present during irradiation; curve C, 50 m*M* cysteamine present during irradiation.

in survival for $G_1$-early S cells (2.5 hours), curve A, and S cells (7 hours), curve B, with cysteamine concentration is shown in Fig. 2. These curves indicate that 50 m*M* cysteamine affords maximum protection for 7-hour cells and near maximum protection for 2.5-hour cells. At some concentration above 50 m*M* (~75 m*M*) survival for cells at both ages will presumably be identical. The age response will then be invariant at about 0.79 survival after 710 rads.

Survival curves for cells at different cell ages (1.5, 3.5, 7, and 9 hours) were determined in the presence and absence of 50 m*M* cysteamine. The results of the data for 1.5 hours, 3.5 hours, and 9 hours were quite similar, and results for 1.5 hours (curves A and C) and 7 hours (curves B and D) only are presented in Fig. 3. Curves A and B are without cysteamine and curves C and D are with 50 m*M* cysteamine present during irradiation. If doses for the same survival levels throughout the first decade are compared, dose-modifying factors for cysteamine, which include the effect on both shoulder and slope of the curve, are determined. These are found to be 4.2 for $G_1$ cells and 2.7 for S cells, which agree well with values between 3 and 4 found for asynchronous populations in vitro (*2*).

The most significant feature of these results so far is the leveling off of the age response as a result of cysteamine protection. This indicates that at least part of the reason for variations in x-ray survival normally evident during the cell cycle is the level of naturally occurring radioprotective constituents which the cell possesses at different ages. The nature of these cellular constituents is not known but they may perhaps be found among the precursor pools for nucleic acids and proteins, since survival falls when DNA synthesis is completed and RNA and protein synthesis decline in these cells (*9*). They are not uniquely concerned with these macromolecules, however, since the latter are not the only factors governing x-ray response during the cell cycle (*10*). Whatever their nature, it is clear that these constituents are not present in saturation quantities at any stage of the cell cycle since cysteamine, which presumably supplies these or similar constituents in excess, provides protection even at the most resistant part of the cell cycle. These results suggest that there are two components of x-ray damage, only one of which is

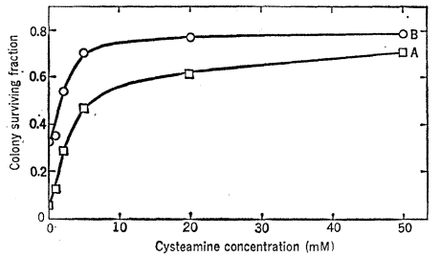


Fig. 2. Survival of 2.5-hour ($G_1$-early S) cells (curve A) and 7-hour (late S) cells (curve B) after 710 rads, 250-kv (peak) x-rays with different concentrations of cysteamine present during irradiation.

age dependent, and it is against this component that cysteamine protects the cell.

Regarding the action of cysteamine, this result, together with the fact that the age response for survival has the same form for both fully oxygenated and hypoxic cells (*11*) provides further evidence that the protective action of cysteamine is not dependent upon the state of oxygenation of the cell. In addition, when cystamine [bis-(2-aminoethyl)disulfide] in similar concentrations (1 to 50 m*M*) was employed, no protective action resulted, the x-ray response in the presence of the agent being the same as in its absence at all stages of the cell cycle. Thus the presence of the free sulfhydryl group of cysteamine appears to be vital for the protection of Chinese hamster cells, while the disulfide of cystamine is ineffective. This suggests

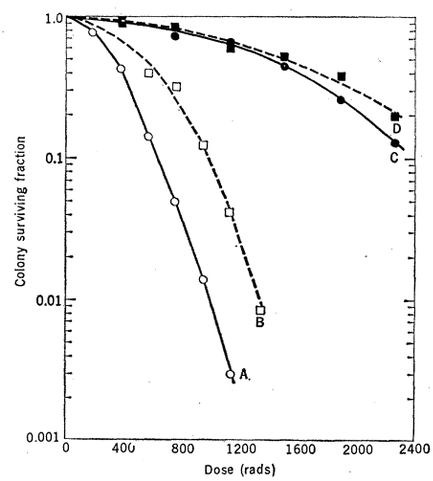


Fig. 3. Survival curves for $G_1$ (1.5-hour) and late S (7-hour) cells with and without cysteamine. Curves A ($G_1$) and B (late S), no cysteamine; curves C ($G_1$) and D (late S), with 50 m*M* cysteamine present during irradiation. Dose-modifying factors throughout the first decade are 2.7 for late S cells and 4.2 for $G_1$ cells.

that sulfhydryl groups should vary during the cell cycle of mammalian cells. Variations in sulfhydryl groups during the cell cycle have already been observed in other organisms (*5*).

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## Reinforcement Reduction: A Method for Training Ratio Behavior

Abstract. *By use of intracranial stimulation as reinforcement, fixed-ratio performance could be established directly from continuous reinforcement by gradual reduction of the train duration of the stimuli contingent on all but the terminal response in the desired ratio. Furthermore, stimuli of subreinforcement strength enhanced ratio performance when introduced after training by the conventional method.*

The usual method of training behavior under fixed- and variable-ratio schedules of reinforcement is to gradually increase the response-to-reinforcement ratio from a CRF (continuous reinforcement) schedule until the rate of response is stable under the desired ratio. When one generalized to extralaboratory situations it has generally been assumed that behavior under intermittent schedules of reinforcement is established and maintained according to this procedure.

An alternative method for the acquisition of ratio behavior is proposed: Instead of one withholding reinforcement completely and gradually increasing the response-to-reinforcement ratio, the desired ratio can be established directly from CRF by gradual reduction of the magnitude of the reinforcements contingent on all but the terminal response in the ratio chain. For instance, to establish a fixed-ratio 50 : 1 contingency from CRF, each 50th response is reinforced at optimal level while the mediating 49 responses are continuously reinforced at progressively decreasing magnitudes until behavior is stable under the ratio.

To test this proposed procedure, electrical intracranial stimulation was used as a reinforcer since it allows precise variation in magnitude of reinforcement along several parameters such as amplitude and pulse-train duration.

Adult male Long-Evans rats were permanently implanted with bipolar electrodes aimed at the anterior lateral hypothalamic area (*1*). The rats were tested for intracranial self-stimulation in a Skinner box, and their behavior was monitored with a cumulative recorder. Intracranial reinforcement consisted of 100-cy/sec a-c sine waves, with pulse-train duration kept constant at 0.5 second; current, adjusted individually for each animal, ranged from 200 to 450 $\mu$a.

After practice under CRF, training was begun under fixed-ratio schedules in the manner described above. Current levels were kept constant for all reinforcements, while the duration of the pulse trains was gradually decreased to zero for all but the terminal reinforcement in each ratio chain. Care had to be taken not to strain the schedule by premature reductions in pulse train. Generally, the greater the reduction in duration of the train, the more responses the animals had to be allowed at each level before further reduction was possible. Sometimes speed of response increased as the train durations became shorter. Five animals were trained in this manner: two under fixed ratio 16 : 1 and three under fixed ratio 32 : 1; in each instance the training was completed in less than 3 hours of one session, including the initial practice under CRF. Instead of pulse-train duration, amplitude could have been used to vary the magnitude of reinforcement, but use of amplitude was not attempted. but use of amplitude was not attempted.

Two more rats were trained to fixed ratio 32 : 1 by the traditional method of gradually increasing the size of the ratio; train durations were set at 0.5 second. After training, stimuli of 0.03-second duration were made contingent on all responses in the ratio, in addition to the optimal reinforcements already contingent on each 32nd response; in both instances the rate of response increased as performance became less strained. Furthermore it was possible to increase the ratio directly to fixed ratio 50 : 1 without affecting performance. When the optimal reinforcement was then completely withheld, leaving only the stimuli having 0.03-second train duration, all animals extinguished after a few hundred responses. This finding indicated that intracranial stimuli of subreinforcement magnitude can affect behavior, or function as reinforcers (perhaps conditioned reinforcers) when administered in the context of reinforcing stimuli.

The method of reduction in reinforcement may have practical application for training under intracranial reinforcement, and at least conceptual applications to reinforcement schedules, in and out of the laboratory, using traditional rewards. As a training method it allows direct acquisition under a particular ratio without requiring practice under smaller ratios; this feature could be of advantage in studies in which practice under smaller ratios would contaminate performance or extinction under the desired ratio.

With regard to human behavior, it is conceivable that verbal and other types of social reinforcement can be and are administered in a similar manner to establish and maintain ratio behavior.

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#### Reference and Notes

1. The stereotaxic coordinates used were reported for the anterior lateral hypothalamus by J. Bogacz, J. St. Laurent, J. Olds, *Electroencephalog. Clin. Neurophysiol.* **19** 75 (1965); the electrode placements were confirmed histologically.
2. I thank A. W. Mills and Benjamin Pressman.

25 August 1967