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Comparative Protective Effect of Cysteine against Fast Neutron and Gamma Irradiation in Mice. (20586)

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There are numerous examples of protective effects by hypoxia and chemicals in radiation biology(1) and, for the most part, one is prone to interpret these phenomena in terms of the postulated energy transformations in irradiated water. Perhaps the most cogent evidence for this sort of mechanism is derived from comparison of the modifying influence of oxygen and of various substrates on the radiation-induced reactions in simple aqueous solutions and in living systems. The observations of Thoday and Read(2,3) and, more recently, of Giles *et al.*(4) are particularly significant. The former found a negligible effect of oxygen deprivation on growth reduction and chromosome aberrations in *Vicia faba* roots irradiated with alpha particles, while the latter observed an intermediate protective effect of hypoxia on aberration frequency in *Tradescantia* inflorescences exposed to fast neutrons. In each case, oxygen deprivation afforded considerable protection against the effects of gamma or X-irradiation. These findings parallel certain of the radiochemical reactions involving oxygen in aqueous solution and will be discussed in connection with the data presented here. The present experiments are concerned with a comparison of the protective efficiency of cysteine against the acute lethal effects of fast neutron and gamma irradiation in mice. Fast neutrons have been estimated to be some 4.5 times as effective as gamma rays for acute killing of mice(5). In view of the radiobiological importance of ionization density and of its contribution to chemical effects in water, useful information regarding radiation mechanisms may be gained from such comparisons of protective efficiency.

Methods. Female mice (CF1) 10 to 12 weeks of age and weighing 18-25 g were maintained on a diet of Rockland checkers and water *ad libitum*. Control and experimental animals were irradiated simultaneously, caged together in groups of 10 to 15 and otherwise handled similarly. Cysteine was administered as a 12.5% solution of the hydrochloride neutralized to pH 7 with 10 N sodium hydroxide. Only freshly prepared solutions were employed. The cysteine dose was 1200 mg/kg of body weight and was given intravenously. Control mice were injected with an equivalent volume of 5% sodium chloride. In general, cysteine was injected between 5 and 15 minutes before exposure. The exposure time varied from 80 to 90 minutes for gamma rays and 60 to 90 minutes for neutrons. The mice were exposed in the gamma-neutron radiation chamber recently described(6). The dose rate of gamma rays from the Co^{60} source was about 11-12 r per minute. Fast neutrons were produced by allowing the neutrons from the thermal column of the Argonne CP-3 reactor to impinge on uranium. Appropriate shielding devices prevented slow neutrons from reaching the animal exposure position. Several methods of dosimetry indicate that the gamma

TABLE I. Comparative Protective Effect of Cysteine.

Gamma irradiation						Fast neutron irradiation					
	Sodium chloride		Cysteine				Sodium chloride		Cysteine		
Dose, r	No. mice	% mortality 30 days	No. mice	% mortality 30 days	% dose reduction	Dose, rep	No. mice	% mortality 30 days	No. mice	% mortality 30 days	% dose reduction
961	24	50	24	12	15.7	197	21	33	21	14	8.3
968	40	75	40	45	9.8	205	35	46	36	25	6.8
992	23	61	23	17	15.9	212	15	53	15	33	6.2
1007	27	67	27	41	8.4	215	36	61	36	44	5.1
1020	30	90	30	10	29.1	239	39	85	39	64	7.3
1027	50	76	50	36	13.0	252	29	100	30	63	9.4
1054	30	77	30	40	12.0						
Mean, all groups		72		30	14.9±2.5*			66		43	7.2±.6

* Refers to stand. error of the mean.

All differences between sodium chloride and cysteine treated mice are significant ($p < .01$). Difference between mean % dose reduction for gamma and neutron irradiation is significant ($p < .05$).

contamination of the fast neutron beam is less than 10%. The dose rate of fast neutrons was 2-4 rep per minute. Animals were exposed in lucite cages previously standardized as to isodose groups for both gamma and neutron radiation. Equal numbers of cysteine and saline injected mice were included in each isodose group.

Results. The results, summarized in Table I, reveal that cysteine pretreatment affords significant protection ($p < .01$; t test) against the acute lethal effects of both fast neutron and gamma irradiation. The protection observed with fast neutrons is, however, about half of that seen with gamma irradiation. This difference in protective capacity is statistically significant ($p < .05$). When these effects are evaluated in terms of the dose-mortality curves for the two radiations, the dose reduction is 14.9% for gamma rays and 7.2% for fast neutrons. Cysteine appears to protect equally over the entire spectrum of mortality during the 30-day post-irradiation period. These findings, presented as the probability of death during each 3-day interval after exposure, are shown in Fig. 1. It may be noted that the maximum killing occurs around the 8th day with neutrons and 14th day with gamma rays. In this analysis, the individual experiments in a particular group, *e.g.*, sodium chloride-gamma irradiated or cysteine gamma-irradiated, were combined even though the radiation dosage differed from one experiment to another.

In the case of gamma irradiation there is little difference in the mean day of death over the dosage range employed. With neutrons, however, survival time decreases appreciably as the dosage is increased. In view of this dose dependence, it is necessary to examine the neutron experiments individually. The data in Fig. 2 indicate that the mean survival time (survivors counted as 30 days) is the same function of the 30-day

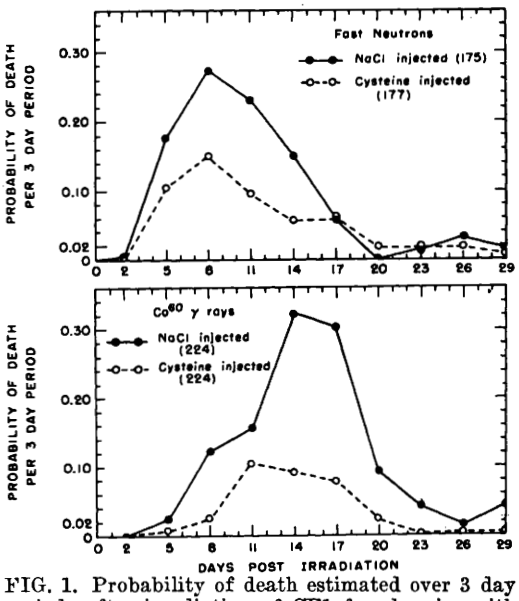

FIG. 1. Probability of death estimated over 3 day periods after irradiation of CF1 female mice with fast neutrons and Co^{60} gamma rays. (Estimates based on numbers alive at start of each interval. The figures in parentheses refer to No. of animals exposed.)

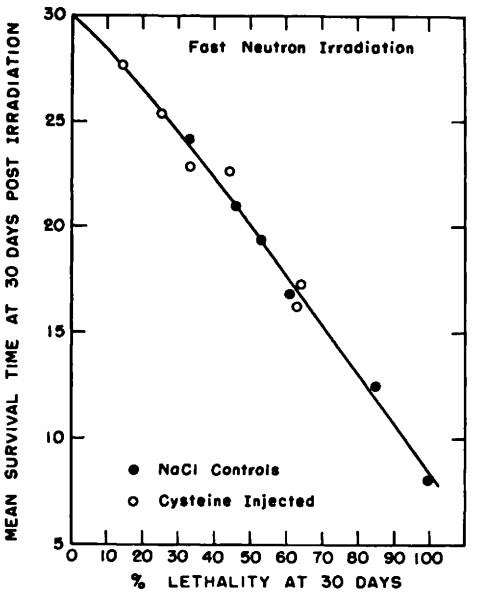

FIG. 2. Relationship between mean survival time and 30 day lethality. (Includes decedents and survivors, the latter counted as 30 days.)

mortality in both control and cysteine treated irradiated mice. This may be taken as further evidence of the general reduction in the effective neutron dose by cysteine rather than of selective protection of specific physiological systems.

Discussion. Cysteine has been shown to diminish many of the effects of X-irradiation in a variety of biological systems(1). The present experiments establish a protective effect against acute lethality in mice resulting from either gamma or fast neutron irradiation. It is noteworthy that the protection in this instance is considerably less than that reported previously for mice and rats after X-irradiation(7,8). In the case of gamma rays this may be attributed to the protracted exposure necessary to deliver a lethal dose with the facilities available. It will be recalled that the magnitude of the protection is a function of the interval between injection and irradiation as well as of the cysteine dose(7-9). A similar argument may be advanced for the neutron exposure, although this is only part of the story since the dose reduction is less for neutron than for gamma irradiation under identical conditions.

It may be noted that cysteine affords a reasonably uniform protection against a number of X-radiation sequelae in the mouse and that the efficiency of protection is apparently independent of radiation dosage(8). In general, the data presented for fast neutron and gamma irradiation are consistent with the concept of a true dose reduction in the sense that certain primary mechanisms are involved. Of particular significance is the finding that cysteine changes the mean survival time after neutron irradiation in the same manner as an equivalent reduction in radiation dosage. It follows, therefore, that the decisive protective action occurs at a common and presumably early stage in the sequence of events induced by irradiation. For the present, the most attractive mechanism involves an effect on the pathways of energy dissipation.

Since water must serve as the major repository of the initial energy transfer, it is reasonable to look for an effect on the reactions of irradiated water. Although our understanding in this area is incomplete, several salient facts may be noted: 1) irradiated water constitutes an oxidation-reduction system; 2) the number of altered water molecules per unit of energy absorbed is independent of ionization density; 3) the nature and fate of the breakdown products are dependent on ionization density, the presence of oxygen and other solutes. The high ion density radiations are generally less effective than the low ion density radiations in initiating chemical reactions in dilute aqueous solutions, whereas the reverse is true for many biological effects (10). It will be recalled that the protective effect of hypoxia decreases from gamma and X-rays to fast neutrons and is slight with alpha particles(2-4). Since the peroxide yield becomes less dependent upon oxygen with increase in ionization density, these results are suggestive of a greater role of H_2O_2 than of radical reactions *in vivo*. The greater dose rate dependence for most biological effects resulting from low ion density irradiation also parallels the situation for hydrogen peroxide formation in water(11). Other interpretations are possible, however, particularly when the matter of biological organization is considered.

In contrast to the picture in dilute aqueous

solution, it seems likely that OH radicals formed close together will have greater opportunities for reaction in the nonhomogeneous cell system with varying gradients of concentration and solubility and numerous interfaces exceeding the dimensions of a single compact radical track. Under these conditions, one might anticipate that the forward reaction to H_2O_2 would be decreased and that the radical reaction with targets in the immediate vicinity would be enhanced. The small or negligible oxygen effect with high ion density radiations is consistent with such a scheme. Since the OH radicals generated by gamma and X-rays are distributed over a larger area, these radiations should be less effective particularly when two or more radical reactions must occur in close proximity in space and time as, for example, in the production of chromosome interchanges. Hence, many types of effects with low ion density radiations may require radicals formed along two or more particle tracks, while a single particle track will suffice with high ion density radiations. The chances for interaction of hydrogen with oxygen to form HO_2 should be considerably greater with low ion density radiations, the result being an increase in effective radical concentration in the presence of oxygen with a corresponding increase in biological efficiency. The greater dose rate dependence of gamma and X-ray than that of neutron and alpha ray effects may also be interpreted in terms of an increase in effective radical concentration without regard to whether this is brought about by OH, HO_2 or H_2O_2.

The experimental findings in general are consistent with the assumption that cysteine serves as absorbing material for the oxidants formed in intracellular water. The decreased effectiveness with fast neutrons in contrast to gamma rays may then be attributed to a greater disparity in the concentration of radicals and peroxide along the particle tracks relative to that of cysteine. However, this interpretation is perhaps too naive for the biological system. Moreover, there is independent evidence that suggests that cysteine may act at least in part by diminishing the availability of oxygen(12-14). In this connection it may be noted that biological additivity of two briefly spaced X-ray doses in mice is a simple function of the cysteine dose preceding each exposure(8) and that a similar additivity obtains for the oxygen effect on broad bean roots(15). Differences in the magnitude of protection by cysteine against the effects of gamma and fast neutron irradiation in mice are in essential agreement with the inverse relationship between ionization density and the extent of the oxygen effect. Some oxygen effect would be anticipated with fast neutrons, its magnitude depending on the energy of the recoil protons.

It should be emphasized that there is no compelling reason to think of this sort of chemical protection only in terms of some interaction with, or interference in the production of, one or another of the primary radiotoxins derived from water. Protection of thymic cells by cysteine is dependent upon temperature both before and during the early post X-irradiation period(16). Thymic cells (14) and onion epidermis cells(17) are also partially protected when the amino acid is added immediately after X-irradiation. These findings suggest that at least some of the protective phenomena cannot be interpreted completely in terms of immediate oxidative reactions. It is necessary, therefore, to inquire about possible cysteine effects on the physico-chemical relationships of cell constituents and on other more delayed or secondary reactions as well. Such effects may be relatively less important with high ion density radiations owing to the distinctive distribution of the reactive intermediates.

Summary. Cysteine pretreatment has been shown to confer significant protection against the acute lethal effects of gamma (Co^{60}) and fast neutron (fission) irradiation in mice. The protection observed with fast neutrons is, however, about half of that found with gamma irradiation. The data presented support the concept of a true dose reduction in the sense that primary mechanisms are involved. The inverse relationship between protective efficiency and ionization density is comparable to that noted for the oxygen effect and, for the present, may best be interpreted in terms of

the spatial distribution of the radicals formed in water and their reactions.

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Effect of Carbonic Anhydrase Inhibitor (6063) on Arterial-Alveolar CO_2 Gradient in Man.* (20587)

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Inhibitors of carbonic anhydrase activity have been employed in the effort to block the *in vivo* reaction, $H_2O + CO_2 \rightleftharpoons H_2CO_3$. Various physiological mechanisms are dependent upon this reaction. Höber(1) first demonstrated that sulfonilamide, a carbonic anhydrase inhibitor, impairs the acidification mechanisms of the frog kidney. The use in the dog of stronger sulfonamide inhibitors, especially Diamox (6063 or 2-acetylamino-1, 3,4-thiadiazole-5-sulfonamide), has implicated carbonic anhydrase in the secretion of hydrochloric acid by the stomach(2) and of bicarbonate by the pancreas(3). It has been recently shown(4) that Diamox increases plasma bicarbonate concentration and pCO_2 in marine fishes, an effect possibly attributable to inhibition of erythrocyte carbonic anhydrase.

Roughton(5) showed that carbon dioxide exchange in unanesthetized humans was impeded when sulfonilamide was combined with exhaustive exercise. In anesthetized dogs(6), Diamox is reported to interfere with CO_2 transport, possibly by inhibition of the erythrocyte enzyme system. It was the purpose of this study to determine whether the carbonic anhydrase inhibitor, Diamox, in concentrations adequate to inhibit renal acid excretion, would interfere with the transport of CO_2 from pulmonary capillary blood into the alveoli in normal, non-anesthetized human subjects.

The subjects were 3 convalescent patients without cardiac, renal, or pulmonary diseases from the First (Columbia University) Medical Division of Bellevue Hospital. These pa-

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