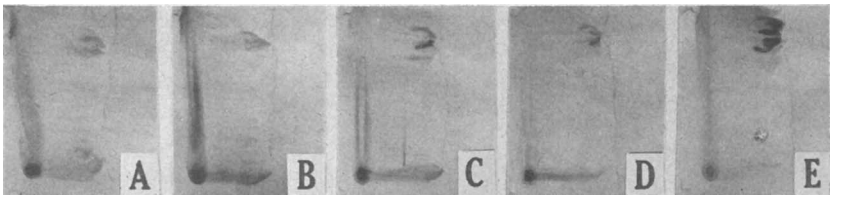

FIG 7. Snake venoms: A, cobra venom supplied by Haffkine Institute, Bombay, India; B, cobra venom supplied by Hynson, Westcott and Dunning, Inc., Baltimore, Md.; C, Russel viper venom supplied by Haffkine Institute, Bombay, India; D, Russel viper venom supplied by Wellcome Research Labs., Langley Court, Beckenham, England; E, *Agkistrodon piscivorus* supplied by Ross Allen's Reptile Institute, Silver Springs, Fla.

not and both proteins move in the second dimension. The amount of cytochrome *c* moving in the second dimension is proportional to the amount applied to the paper. The chromatographic patterns of purified proteins *e.g.* crystalline human serum albumin, α, β, and γ globulins, and fibrinogen differ from each other in a characteristic manner. The differences are more marked when the proteins are mixed with surface active agents with which they form complexes; and these complexes move at different rates on filter paper. Complexes of surface active agents with albumin move with greater ease along the first dimension than those formed with globulins; the latter tend to remain at the point of origin. Almost all the protein complexes, however, move in the second dimension. The pattern given with a blood plasma is a composite of the patterns of the individual protein fractions, the basal fractions being apparently due to globulin. The patterns of chromatograms of pathological cases (multiple myeloma, liver cirrhosis, cancer of the stomach, lupus erythematosis) differ significantly from the normal, and are often characterized by the presence of heavy basal fractions due probably to increase in the globulin content of the plasma.

The chromatograms of dried snake venoms (cobra, Russel viper, *Agkistrodon piscivorus*) show various distinctive features.

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Effect of Pretreatment with Cysteine on Survival of Mice Exposed to External and Internal Irradiation.* (18927)

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Patt, Tyree, Straube and Smith(1-3) reported that rats injected intravenously with cysteine, shortly before irradiation with lethal

* Carried out under Contract AT-(40-1)-269 with the Division of Biology and Medicine, United States Atomic Energy Commission.

1. Patt, H. M., Tyree, E. B., Straube, R. L., and Smith, D. E., *Science*, 1949, v110, 213.

2. Patt, H. M., Smith, D. E., Tyree, E. B., and Straube, R. L., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 18.

3. Smith, D. E., Patt, H. M., Tyree, E. B., and Straube, R. L., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 198.

doses of x-rays, survive considerably longer than irradiated, but not pretreated controls. Cronkite, Chapman and Brecher(4-6) obtained similar results in Swiss mice prepared by subcutaneous injection of another sulfhydril compound glutathione. We attempted, in experiments reported below, to analyze the mechanism of cysteine effect on irradiated mice by varying several factors of the experiment: (1) source of radiation; (2) doses of radiation; (3) dose and route of administration of cysteine; (4) strain of mice; (5) absence or presence of serous cellular exudate in the peritoneal cavity.

Material and methods. 1. *Animals.* Mice of dba and CFW strains, of 25 g weight, were used. In one series they were inoculated intraperitoneally 4 days before irradiation with 1 million Sarcoma 37 cells from serial peritoneal transfers(7). Total and differential counts of cells in their peritoneal fluid (accumulating as result of inoculation) were carried out repeatedly in irradiated mice and controls. The technic of inoculation, of fluid withdrawal and of cell counts was described elsewhere(7). 2. *Cysteine.* 2.5 to 10% solution (pH 1.0) was prepared a few minutes before injection and in several experiments adjusted to pH 6.0 or 6.5 with 8% NaOH solution. Doses of 0.1 or 0.2 cc of these solutions were injected into mice, intravenously, intraperitoneally or subcutaneously, about 10 to 15 minutes before irradiation. In a few preliminary control experiments the cysteine was injected 20 minutes after irradiation. 3. *Irradiation.* Doses of 300 to 800 of filtered x-rays (Filter: 1.2 mm Sn; 0.25 mm Cu; 1.0 mm Al; TSD 50 cm; HVL 3.27 mm Cu; 250 PKV) were used for external irradiation and doses of 0.8 mc of radioactive colloidal gold† for internal irradiation (intraperitoneal injections). For technical reasons, groups of not more than 10 mice were irradiated in each experiment and the results were pooled. 4. *Survival.* The total period of survival varied in different experiments, but for the period between 10 and 14 days the results were consistent in all experiments and therefore they could be pooled for recording.

Results. The preliminary experiments on after-treatment of irradiated mice with cysteine confirmed the observation of other authors(1-3) that this procedure does not influence survival of mice.

For easier reading and understanding the experiments reviewed in Table I are divided into 4 series illustrating (A) longer survival of x-irradiated mice pretreated with small doses of cysteine; (B) rapid death after irradiation, with x-rays of mice pretreated with high doses of cysteine; (C) failure of the procedure used in B in mice possessing large amount of peritoneal fluid; (D) lack of effect of pretreatment with cysteine in mice irradiated with radioactive gold.

Repeated examinations of peritoneal fluid in sarcoma inoculated mice were carried out in order to study the influence of x-ray treatment, with or without cysteine pretreatment on the following characteristics of the fluid: 1. Total number of cells (sarcoma cells and leukocytes) per cmm of fluid at various intervals after irradiation (Fig. 1). 2. Approximate volume of fluid in the peritoneal cavity. It was found that after x-ray treatment, with or without cysteine, the volume of peritoneal fluid increased in most mice of the series C 4 to 8 times within 48 hours. Differential counts showed that later increase in cell number in cysteine-x-ray group was paralleled by a rise in the percentage of lymphocytes and polymorphonuclears. Thus, there was at that stage, an absolute increase in cell number in the fluid and not only a relative increase due to partial loss of fluid. On the contrary in the peritoneal fluid of animals treated *i.p.* with radioactive gold, with or without cysteine, the predominant feature was the increase in cell concentration by progressive disappearance of fluid which became very scarce within 5 days after treatment.

4. Chapman, W. H., and Cronkite, E. P., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 318.

5. Cronkite, E. P., Brecher, G., and Chapman, W. H., PROC. SOC. EXP. BIOL. AND MED., 1951, v76, 396.

6. Cronkite, E. P., Chapman, W. H., and Brecher, George, PROC. SOC. EXP. BIOL. AND MED., 1951, v76, 456.

7. Goldie, H. and Felix, M. D., *Cancer Research,* 1951, v11, 73.

† Gold was obtained through Abbott Research Laboratories, Dr. D. L. Tabern, North Chicago, Ill.

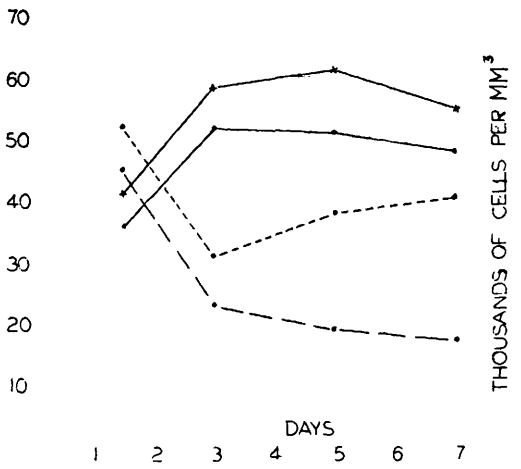

FIG. 1. Each graph represents average of 20 mice. x————x No treatment; more than 30% sarcoma cells. •————• Cysteine alone; more than 30% sarcoma cells. •– – – – – – – • X-rays alone; less than 15% sarcoma cells. ° — — — — ° Cysteine and X-rays; less than 15% sarcoma cells.

Tumor cells disappeared from the peritoneal fluid of gold treated animals, as it was expected(8). In all x-ray treated mice, there was a destruction of majority of tumor cells (as shown by comparison with untreated controls) and in some cases of all tumor cells, as demonstrated by negative results of implantation of large amounts (0.25 cc) of peritoneal fluid into new mice.

Discussion. The data of the series A experiments demonstrate that small doses (125 to 250 mg/kg weight) of cysteine injected before irradiation increased, at least temporarily, the survival of mice exposed to a lethal dose of x-rays. The importance of species, strain and several environmental or experimental factors in this type of experiments is demonstrated by: 1. great variations in survival of mice after the first two weeks following irradiation; 2. higher percentage of survivors, under the same experimental conditions, in dba mice than in CFW mice; 3. better protection obtained by similar technic in rats by Patt *et al.*(1-3). Decrease of cysteine (pH 1.0) activity after alkalinization to pH 6.0-6.5 (Table I) or to pH 7.0-7.5(1) was due evidently to partial denaturation of this amino acid by oxidation.

In new mice pretreated intraperitoneally with higher doses (more than 800 mg/kg) of cysteine (Series B, Table I), the irradiation even with lower (300 r) doses of x-rays provoked instantaneous death or agony lasting a few hours. The higher activity of cysteine at pH 1.0 was not due to its acidity, as shown by control experiments with saline solution at pH 1.0. It is obvious that either the biological effect of x-rays is potentiated by high doses of cysteine, or vice versa, the x-ray treatment enables blood cysteine to gain access promptly to some highly vulnerable body tissues. The first alternative is contradicted by well known facts that decrease of oxygen content in tissue by a reducing agent, such as cysteine, may attenuate but not enhance the effect of x-ray. There is more evidence in favor of the second alternative. Several authors(9-12) reported increased permeability of vessels and exudation following closely the irradiation. While in normal animals exposed to lethal dose of x-rays, there is a latent period of 1-2 hours before development of clinical symptoms(13), there is evidence(14) that functional changes (vasodilatation, exudation) and even histological changes(9) occur already during irradiation. In our experiments (Series C) in animals possessing peritoneal exudate, an increase in the amount of this fluid was noticeable within 2 hours after irradiation. The resistance of mice possessing copious peritoneal exudate to immediate lethal effect of high doses of cysteine (Series C) indicated that the exudate possesses some factor rapidly inactivating (oxidizing) cysteine. This conclusion was confirmed by the observation that

8. Goldie, H. and Hahn, P. F., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 638.

9. Bloom, W., *Histopathology of Irradiation*, McGraw-Hill, N. Y., Chapter 16, 1948 (R. P. Rhoades).

10. Warren, Shields, *Arch. Path.*, 1942, v39, 1070.

11. Wilder, H. C. and Maynard, R. M., *Am. J. Path.*, 1951, v37, 1.

12. Bigelow, R. R., Furth, J., Woods, M. C., and Storey, R. H., PROC. SOC. EXP. BIOL. AND MED., 1951, v76, 734.

13. Howland, J. W., and Warren, S. L., *Advances in Biol. and Med. Phys.*, N. Y., 1948, v1.

14. Curtis, H. J., *Adv. Biol. and Med. Phys.*, N. Y., 1951, v2.

TABLE I. Effect of Pretreatment with Various Doses of Cysteine on Survival of Mice, New or Inoculated i.p. with Sarcoma Cells, After Irradiation with X-rays or Radioactive Gold.

Mice		Pretreatment with cysteine			Irradiation		% of surviving mice	
Strain	No.	pH of solution	Dose, mg	Route	Source	Dose	Immediately after irradiation	After 10 to 14 days
Series A								
CFW	50	1	2.5	i.p.	filtered	600r	100	75
				i.v.	x-rays			65
				s.c.				50
	50	6	5	i.p.	"	600r	100	85
				i.v.				70
				s.c.				60
	25	Controls not pretreated			"	600r	100	40
	30	1	2.5	i.p.	Controls not irradiated		100	100
				i.v.				100
				s.c.				100
dba	50	1	2.5	i.p.	filtered	600r	100	85
				i.v.	x-rays			75
				s.c.				60
	50	6	5	i.p.	"	600r	100	90
				i.v.				75
				s.c.				70
	25	Controls not pretreated			"	600r	100	50
Series B	50	1	20	i.p.	"	300 to	0	0
CFW				i.v.		600r	50	0
	30	Untreated controls			"	300 to 600r	100	45
	50	1	20	i.p.	Controls not irradiated		100	50
				i.v.				
	30	Controls saline solution pH 1		i.p.	filtered	300 to	100	80
				i.v.	x-rays	600r		45
	20	6	40	i.p.	"	600r	60	60
				i.v.			70	70
	20	6	40	i.p.	Not irradiated controls		100	100
				i.v.				
Series C								
CFW mice inoculated with sarcoma	50	1	20 or 40	i.v.	filtered	300 to	100	40
				i.p.	x-rays	600r	100	35
Series D								
CFW	30	1	2.5	i.v.	radioactive colloidal gold	0.8 mc. i.p.	100	10
	30	6	5	i.v.	"	0.8 mc. i.p.	100	20
	20	1	2.5	i.v.	"	0.8 mc. i.p.	100	20
	20	6	5	i.v.	"	0.8 mc. i.p.	100	10
	30	Controls not pretreated			"	0.8 mc. i.p.	100	25

doses of 20 mg of cysteine mixed *in vitro* with 0.25 cc of peritoneal fluid and immediately injected into new mice were well tolerated. Presuming that the fluid transuded from the vessels by x-ray treatments possesses the ability of peritoneal exudate to inactivate cysteine, the lack of protective action of after-treatment with cysteine may be interpreted as inactivation of cysteine by the fluid transuded from irradiated vessels before cysteine injection.

Studies of peritoneal exudate in animals inoculated *i.p.* with sarcoma suggested an interpretation for "protective action" of small doses of cysteine, as compared with "potentiating action" of high doses. It was found that the total number of lymphocytes and polymorphonuclears decreases steadily after

treatment with x-rays alone, but in mice pretreated with cysteine this depression of leukocytes was only temporary and followed by increase in cell number. This increase was lacking in mice pretreated with high doses of cysteine. These results suggest that small doses of cysteine stimulate and high doses depress some cellular metabolic processes, perhaps, the activity of hematopoietic tissue as suggested by Cronkite(5,6). Since intraperitoneal route was most efficient for protection of mice by small doses of cysteine and for inducing their death by high doses, it may be presumed that some abdominal organs are the site of action of cysteine.

It follows from the experiments of the Series D that pretreatment with small doses of cysteine acted in internally (intraperitoneally) irradiated mice in a way similar to the effect of high doses in externally irradiated mice *i.e.*, it reduced their life span. It is reasonable to presume that this phenomenon is effected by a similar mechanism, *i.e.*, the increased vascular permeability of irradiated tissues enables cysteine to reach some vitally important tissues faster and in higher concentration.

Conclusions and summary. 1. CFW and dba mice were pretreated with small (125 and 250 mg/kg) doses of cysteine (pH 1.0 or 6.5) before irradiation with lethal doses of x-rays (600 r). It was found that 10 to 14 days after irradiation the percentage of surviving animals was consistently higher in cysteine pretreated group than in irradiated not pretreated controls. These results illustrate "protective" effect of cysteine against radiation. 2. In new mice prepared with high doses (more than 800 mg/kg) of cysteine the x-ray treatment was followed immediately by death or agony. In mice treated by one of these factors alone, the condition was affected only after several days. Thus, the rapid lethal effect of combined treatment with cysteine (high doses) and x-rays is interpreted as potentiation of one factor by the other. 3. This effect of high doses of cysteine was absent in x-ray treated mice possessing copious peritoneal exudate due to the growth of free S-37 cells in the peritoneal fluid. This failure of cysteine is attributed to inactivation (oxidation) of cysteine by some factor of the peritoneal fluid. In these mice with exudate pretreated with small doses of cysteine, the decrease in the cell number (in the exudate) induced by irradiation was followed by a steady increase, while in irradiated controls the decrease continued until their death. 4. Pretreatment with small doses of cysteine (intravenously) had unfavorable effect on survival of mice injected (*i.p.*) with radioactive colloidal gold. 5. An explanation of the above phenomenon as manifestation of cysteine penetration from blood stream into tissues after increase of blood vessels permeability by high doses of irradiation is offered.

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Use of N-Acetyl 4-Aminoantipyrine (NAAP)* in Measurement of Total Body Water. (18928)

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Previous studies have demonstrated that antipyrine is a satisfactory substance for use in the estimation of total body water(1). Antipyrine, however, does not entirely fulfill

* N-acetyl 4-aminoantipyrine may be purchased from the special chemicals division, Winthrop-Stearns, Inc., 1450 Broadway, New York City.

1. Soberman, R., Brodie, B. B., Levy, B. B., Axelrod, J., Hollander, V., and Steele, J. M., *J. Biol. Chem.*, 1949, v179, 31.