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IRRADIATION OF MICE PRE-TREATED WITH RADIATION PROTECTIVE SUBSTANCES

A pathologic and haematologic investigation

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Great interest has been attached to the problem of finding a substance which combines a high irradiation protection with a low toxicity. A number of substances with irradiation protecting abilities have been produced but have not been applicable as they physiologically incapacitate the 'protected' individuals (CRIBORN & RÖNNBÄCK 1971). However, WESTLAND et coll. (1972) presented a compound (2,2'-Dithiobis{N-[(1-adamantyl)methyl]acetamidine}dihydrochloride) with low toxicity and promising irradiation protection properties when administered orally.

The present investigation aimed at comparing the protective capacity of this substance and that of cysteamine-hydrochloride.

Material and Methods

The experiment was performed with CBA male mice, 75 ± 5 days old. The animals were kept in groups of 7, in separate cages, in the same room, and were fed on a standard diet and water ad libitum during the observation period of 30 days following the irradiation.

The irradiation was performed with a Müller MG-300 roentgen apparatus,

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operated at 260 kV and 11 mA, inherent filtration 4 mm Al and with a distance between the tube and the object of approximately 60 cm, resulting in a dose rate of 7.7 mGy/s (48 R/min) measured in air. The mice were irradiated in groups of seven in a plastic 'wheel' and were then returned to their cages. After the irradiation the animals were transferred from the National Defence Research Institute to the National Veterinary Institute, where they were housed during the observation period.

The protective substance, here shortly named S-75, was synthesized at the Division of Applied Chemistry, National Defence Research Institute. It was administered orally as a suspension in distilled water corresponding to 2 mg per animal and was given 45 min before start of the irradiation. This dose was the highest one that did not cause any deaths within the first three days after irradiation of the treated animals.

Cysteamine-hydrochloride was used as a reference substance and was administered intraperitoneally with 6 mg per animal 15 min before irradiation. Under such circumstances this substance has shown a dose reduction factor of approximately 1.8 (ÅKERFELDT et coll. 1967).

The intervals between injection of the substance and the start of irradiation (45 and 15 min, respectively) were chosen according to earlier knowledge of the duration of protection of the substances.

Experimental schedule (14 animals in each irradiation group)

Substance	Dose	Irradiation dose
S-75 Cysteamine-HCl Unprotected controls	2 mg orally 6 mg intraperitoneally	7.1 8.6 10.0 11.4 12.8 Gy 750 900 1 050 1 200 1 350 R

When possible, one animal from each of these groups was killed at 7, 10, 13 and 17 days after irradiation, and the remaining animals were used to determine the irradiation mortality rate of each group. The survivors were killed after an observation period of 30 days. All animals included in the experiment were submitted to gross pathologic and microscopic examination. In addition, one animal from each group was, if possible, examined haematologically, as were the animals killed at the four occasions given.

The experiment was performed as a blind test, and thus all cages were given code numbers immediately after the irradiation. This code was not revealed until all examinations were finished.

Complete autopsy was made of both killed (atlanto-occipital dislocation) and spontaneously dead animals. In addition, the weight of the body, liver, spleen, heart and kidneys of each animal was noted.

Table 1

Mortality in different groups (percentages in parentheses)

Substance	Irradiation dose				
	7.1	8.6	10.0	11.4	12.8 Gy
	750	900	1 050	1 200	1 350 R
S-75	1/10 (10)	1/10 (10)	5/10 (50)	5/10 (50)	11/11 (100)
Cysteamine	0/10 (0)	1/10 (10)	0/10 (0)	3/10 (30)	10/10 (100)
Controls	11/11 (100)	12/12 (100)	13/13 (100)	13/13 (100)	14/14 (100)

Stomach, duodenum, liver, spleen, mesenteric lymph nodes and sternum were fixed in Stieve's solution, embedded in paraffin, cut 5 μ thick and stained with Harris' haematoxylin and eosin stain. The blood samples were smeared and stained according to May–Grünwald–Giemsa. Differential leukocyte count and microscopy of the bone marrow was performed.

Results

In all irradiated groups the deaths occurred between the 4th to 24th day after irradiation, i.e., a mortality period well known from earlier experiments within the same dose range. The mortality rate is given in Table 1 and these figures have been plotted in Fig. 1 to give a rough estimation of the LD_{50} for the two protected groups of animals. In the unprotected group the mortality rate was 100 per cent, which was unexpectedly high, according to previous experiments (RÖNNBÄCK, unpublished observations).

Pathology

Macroscopic appearances. In animals from all groups haemorrhages were found in numerous organs (the average degree of which appears in Fig. 2). In doses of 7.1 to 11.4 Gy the frequency and magnitude of bleedings was considerably higher for unprotected than for protected animals, but more or less equal for all groups when 12.8 Gy was administered. In the dose range 7.1 to 11.4 Gy cysteamine turned out to diminish the degree of heamorrhages better than did S-75.

In Table 2 is given the body weight and the weight of some visceral organs of animals killed 30 days after irradiation. It is notable that the mean body weight of S-75 treated animals was significantly lower than that of the cysteamine treated animals after doses of 8.6 to 11.4 Gy. The observed differences concerning the organ weights were not significant. In S-75 treated animals the spleen weight indicated an increase, positively correlated to the radiation dose within the range of 7.1 to 11.4 Gy. In cysteamine treated mice the spleen weight sharply decreased when the animals had received a dose of 11.4 Gy after an earlier increase in the lower dose range (Tables 2, 3).

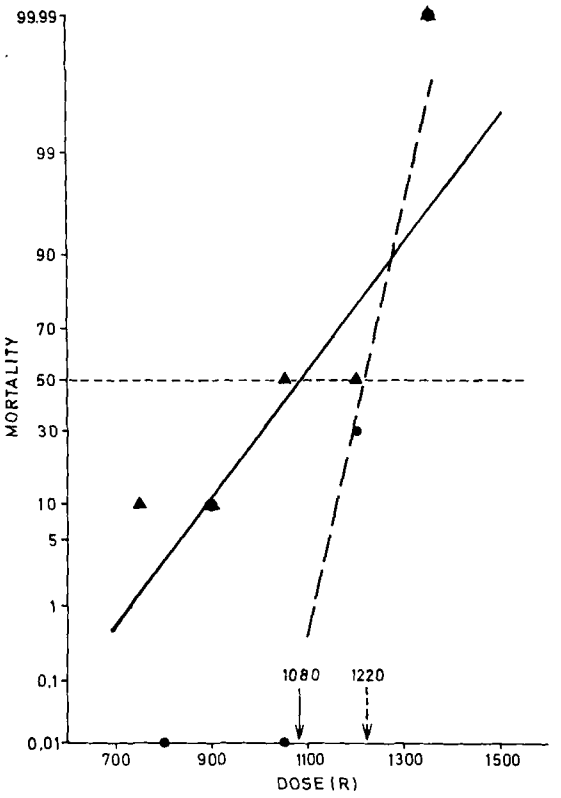

Fig. 1. Mortality in protected, irradiated groups. Arrows indicate approximate LD_{50}. ▲ S-75. ● Cysteamine-HCl.

Microscopy. In the stomach hyperemia, mucosal or subserosal haemorrhages and vacuolar degeneration of the secretory cells of the gastric gland were found in the irradiated mice of all groups (Fig. 3).

In the intestine no evidence of hyperemia was present in animals given S-75 or cysteamine, but appeared in a small degree in mice only irradiated. Haemorrhages were insignificant. Atrophy of the stroma of villi was a constant finding (Fig. 4).

The main lesions observed in the liver were hyperemia, localized haemorrhages, vacuolar degeneration, centrilobular necrosis and hyperplasia of Kupffer cells (Fig. 5).

In the spleen hyperemia, hypoplasia of the white pulp, extramedullary haematopoiesis, shrinkage and haemosiderosis were remarkable lesions. Haematopoiesis was prominent in the protected animals (Fig. 6).

The lesions in the mesenteric lymph nodes were hyperemia and haemorrhages, lymphatic hypoplasia, fibrosis, oedema and polymorphic infiltration (Fig. 7).

In the bone marrow the most marked lesions were haemosiderosis and cellular depletion varying from hypoplasia to aplasia (Fig. 8). The protective properties of cysteamine appeared to be somewhat better than those of S-75. In all protected mice

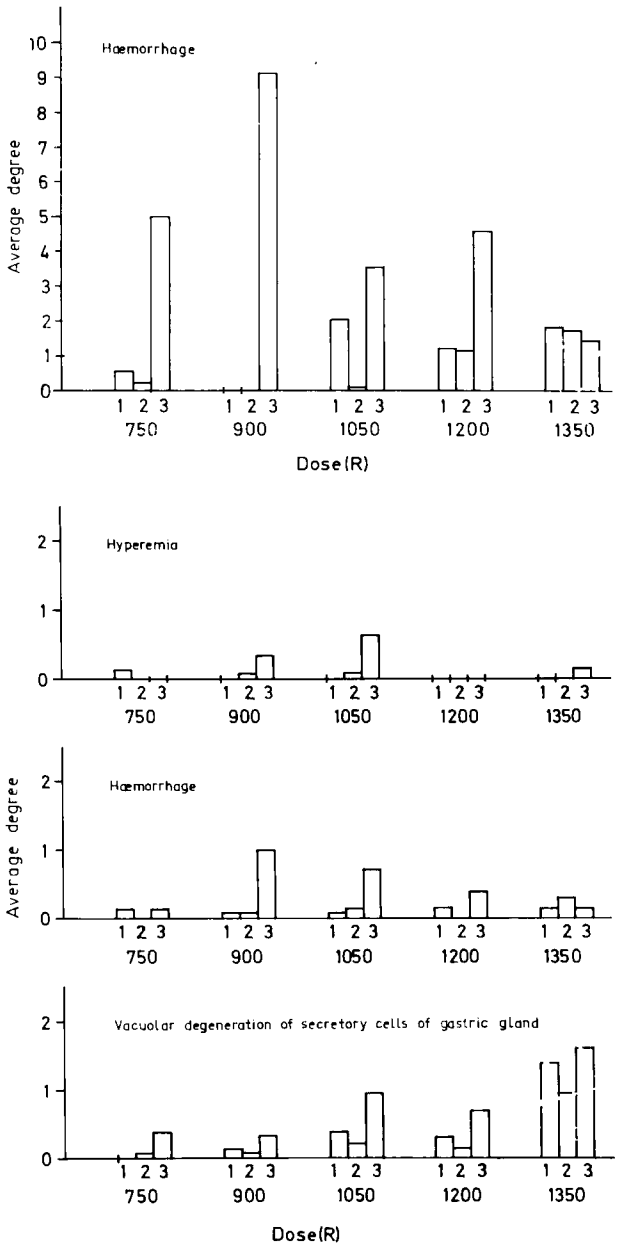

Fig. 2. Gross pathology. Average degree of haemorrhage in the stomach, intestine, lymph nodes, brain, meninges, heart and urinary bladder. The average degree is calculated by adding the degree of lesions (0 = no lesion, 1 + = slight, 2 + = moderate, 3 + = severe) of each organ in the group and dividing the sum with the number of animals in that group. The degree of lesion was classified according to an arbitrary scale. The experiment groups were indicated: (1) S-75-treated 45 min before irradiation (2) cysteamine-HCl-treated 15 min before irradiation (3) irradiated only.

Fig. 3. Microscopy of stomach. Average degree (cf. Fig. 2) of hyperemia, mucosal and subserosal haemorrhage and vacuolar degeneration of secretory cells.

the bone marrow showed a distinct regeneration of a very uneven distribution and a heterogeneous cellular appearance. Some marrow compartments were regenerated, while others in the same sternum showed complete aplasia (Fig. 9). Some regenerative marrows contained mainly one kind of cells such as megakaryocytes.

Table 2

Body and organ weights of animals killed at the end of observation period (in g). Mean ± SE

		7.1 750	8.6 900	10.0 1 050	11.4 1 200	12.8 Gy 1 350 R
Body weight	S-75	29.66 ±0.49	26.20 ±0.73	23.24 ±1.51	23.21 ±0.97	—
	Cysteamine	27.11 ±0.30	28.18 ±0.33	29.87 ±0.45	28.72 ±0.56	—
Heart	S-75	0.122 ±0.003	0.118 ±0.008	0.111 ±0.006	0.113 ±0.010	—
	Cysteamine	0.119 ±0.003	0.128 ±0.005	0.131 ±0.004	0.132 ±0.004	—
Liver	S-75	1.881 ±0.087	1.305 ±0.032	1.534 ±0.181	1.314 ±0.125	—
	Cysteamine	1.198 ±0.017	1.341 ±0.045	1.969 ±0.100	1.706 ±0.049	—
Spleen	S-75	0.139 ±0.012	0.163 ±0.012	0.173 ±0.044	0.185 ±0.020	—
	Cysteamine	0.168 ±0.020	0.183 ±0.018	0.192 ±0.014	0.147 ±0.005	—
Kidney	S-75	0.483 ±0.010	0.387 ±0.013	0.402 ±0.035	0.394 ±0.025	—
	Cysteamine	0.433 ±0.010	0.463 ±0.019	0.497 ±0.014	0.460 ±0.014	—

Table 3

Relative weight of organs from mice killed at the end of observation period ($\times 10^{-3}$)

		7.1 750	8.6 900	10.0 1 050	11.4 1 200	12.8 Gy 1 350 R
Heart	S-75	4.11	4.50	4.77	4.87	—
	Cysteamine	4.39	4.54	4.39	4.60	—
Liver	S-75	63.42	49.81	66.01	56.61	—
	Cysteamine	44.19	40.24	65.92	59.40	—
Spleen	S-75	4.69	6.22	7.44	7.97	—
	Cysteamine	6.20	6.49	6.43	5.12	
Kidney	S-75	16.29	14.77	17.30	16.98	—
	Cysteamine	15.97	16.43	16.64	16.02	—

Haematology. Rather few blood samples could be collected from the unprotected, only irradiated mice, as those animals died before the day determined for killing. Some blood smears were due to scarcity of leukocytes unsuitable for differential count. The relation between dose, kind of protective substance, duration after

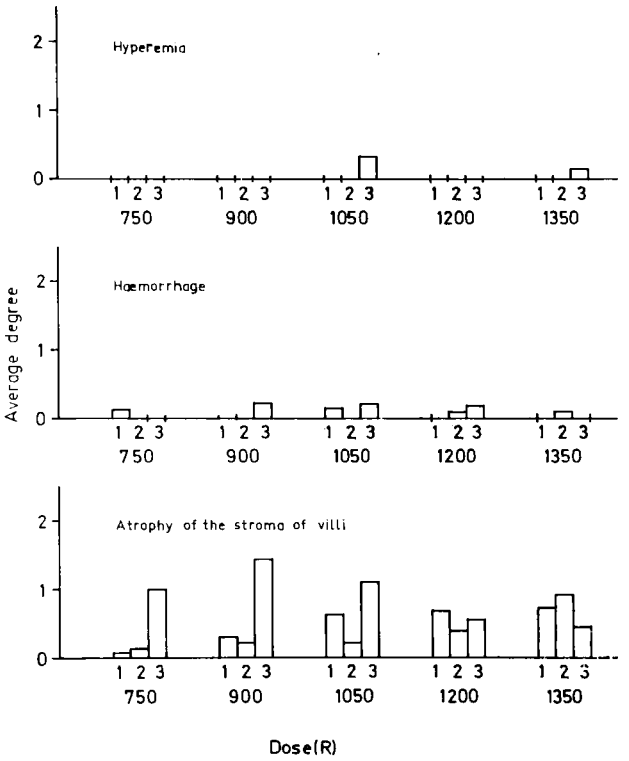

Fig. 4. Microscopy of duodenum. Average degree of hyperemia, mucosal haemorrhage and atrophy of stroma of villi.

irradiation and the number of blood samples which were countable, uncountable and uncollectable are presented in Fig. 10. Blood samples from protected, irradiated animals showed a tendency to relative neutrophilia, anisocytosis and polychromasia.

Discussion

Both the substances tested had protective properties. The dose reduction factor for cysteamine was previously reported to be 1.8 (ÅKERFELDT et coll. 1967). The mice in the present series seemed to be more sensitive to irradiation than in previous experiments, possibly due to stress (transport post irradiation; all unprotected mice died after 7.1 Gy. The true LD_{50} was therefore recalculated in extra groups of unprotected mice and gave a dose reduction factor of 1.6 for cysteamine and 1.4 for S-75. Cysteamine thus appeared to be slightly more effective than S-75, which, however, has the advantage of being effective after oral administration in small doses. In addition, the duration of protection by S-75 is about three times that of cysteamine and the optimum effect during these circumstances occurred about 45 min after administration (RÖNNBÄCK, unpublished results). From a pharmacologic

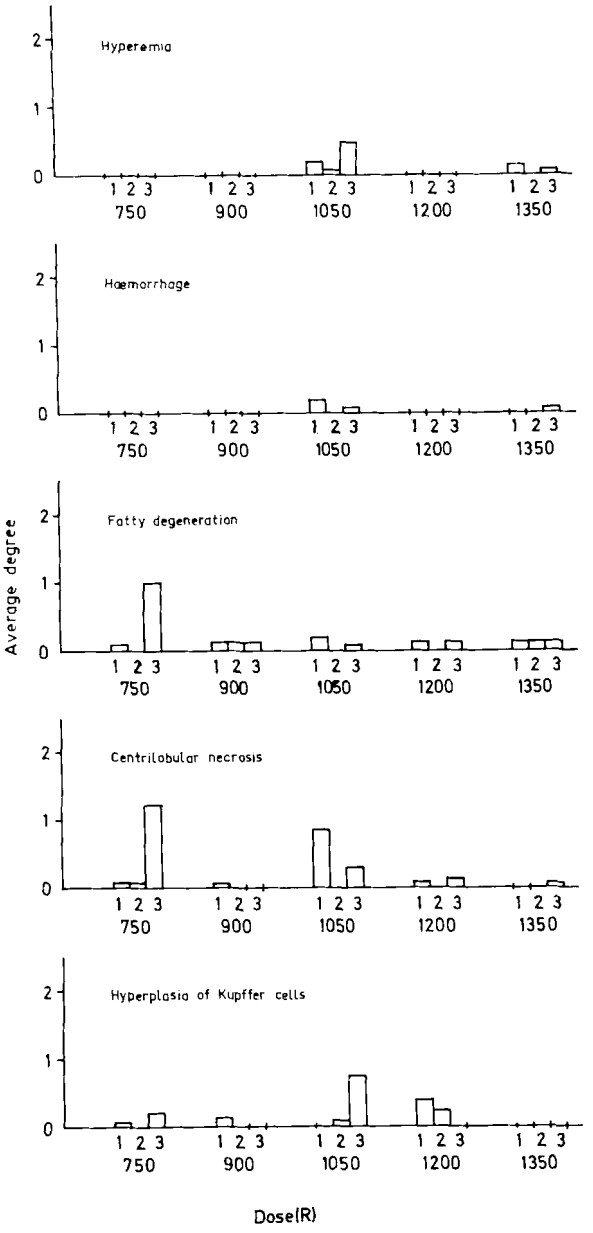

Fig. 5. Microscopy of liver. Average degree of hyperemia, localized haemorrhage, vacuolar degeneration, centrilobular necrosis and hyperplasia of Kupffer cells.

point of view, S-75 therefore seems to be the more promising substace for practical use.

In the dose range from 7.1 to 11.4 Gy haemorrhages were significantly more prominent in the unprotected than in the protected groups of mice. In the latter, cysteamine turned out to inhibit bleedings more effectively than did S-75. At the

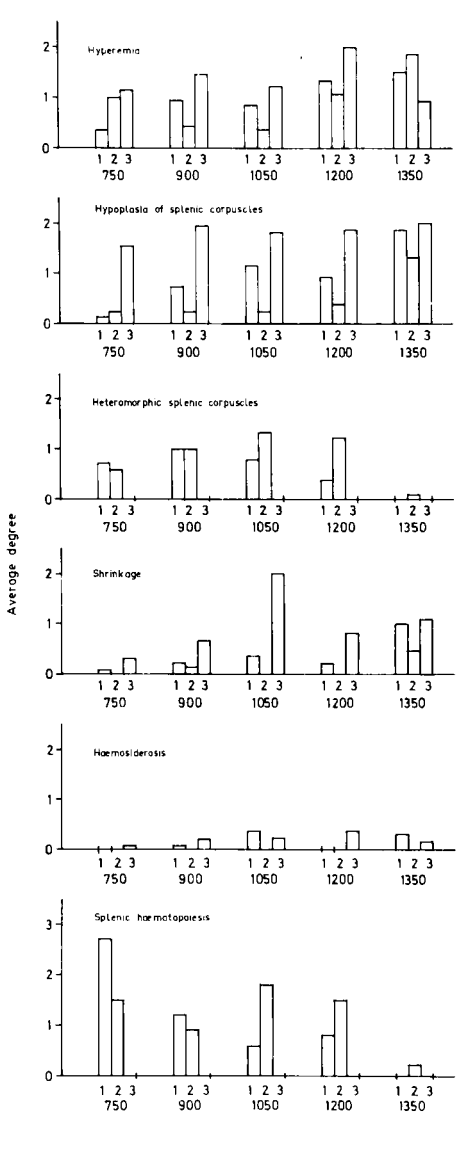

Fig. 6

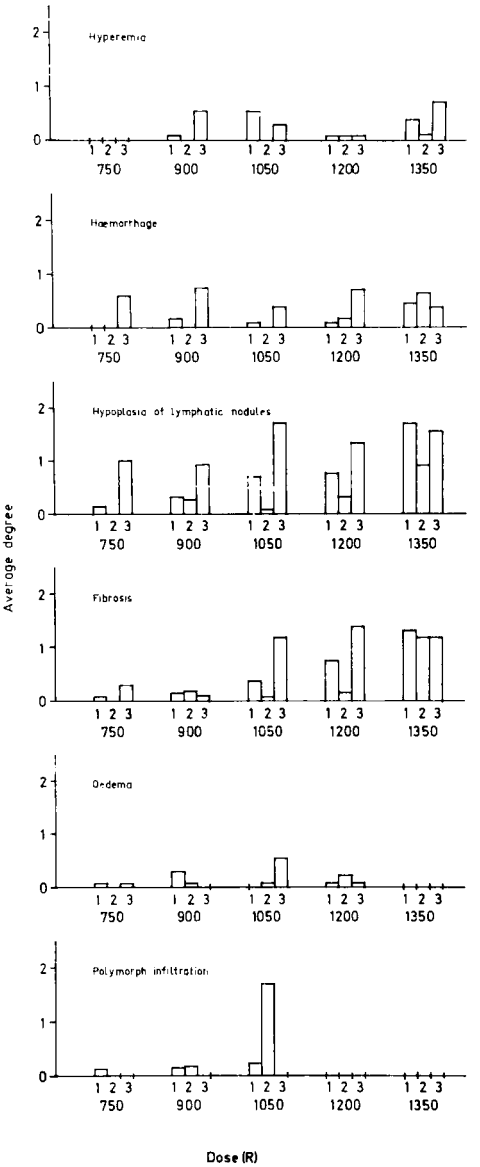

Fig. 7

Fig. 6. Microscopy of spleen. Average degree of hyperemia, hypoplasia of splenic corpuscles, heteromorphic splenic corpuscles, shrinkage, haemosiderosis and splenic haematopoiesis.

Fig. 7. Microscopy of mesenteric lymph nodes. Average degree of hyperemia, diffuse or peripheral haemorrhage, hypoplasia of lymphatic nodules, fibrosis, oedema and polymorph infiltration.

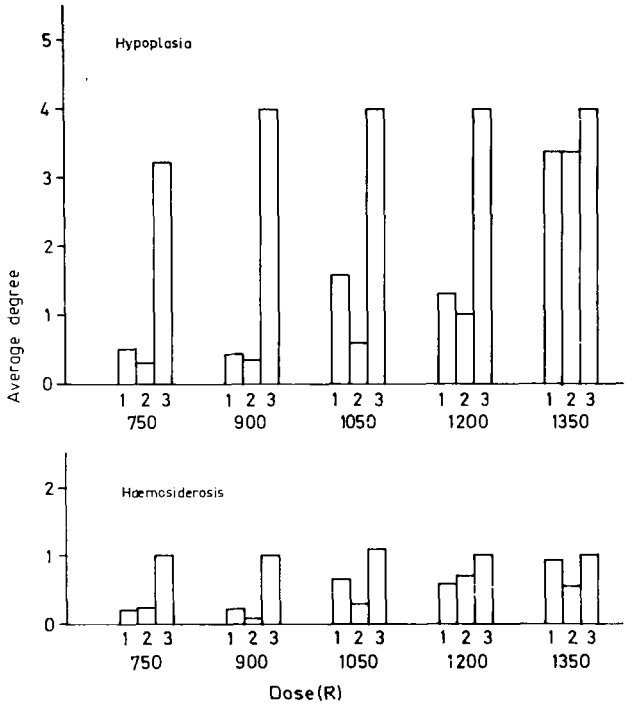

Fig. 8. Microscopy of bone marrow. Average degree of hypoplasia up to aplasia and haemosiderosis.

highest dose level (12.8 Gy) the frequency of bleedings was about the same in all groups, whether the mice were protected or not.

In the liver, after 7.1 Gy, unprotected mice showed only minor evidence of injury, such as a slight vacuolar degeneration and centrilobular necrosis, while the protected ones showed practically no changes. The lesions were apparently indirectly produced as a consequence of a severe anemia in the unprotected animals.

In unprotected mice hypoplasia of splenic corpuscles was very prominent as well as shrinkage, fibrosis and haemosiderosis. No evidence of heteromorphic splenic corpuscles and splenic haematopoiesis occurred in the unprotected animals but it was very marked in protected mice (Fig. 6), indicating an ability of S-75 and cysteamine to protect lymphoid cells from irradiation injury. Both substances appeared to have a beneficial effect upon the recovery mechanism of the haematopoietic system corresponding to HARTWEG's (1957) observations with cysteine when given to irradiated Albino rats. The strong splenic hyperemia in the unprotected mice largely seemed to depend upon destruction of the splenic corpuscles and a slight capacity of regeneration.

The increase in weight of the spleen (Tables 2, 3) and the magnitude of haematopoiesis and of heteromorphic splenic corpuscles in S-75 protected animals may point towards a protective and regenerative capacity of S-75 more directed towards this organ compared to the effect of cysteamine.

Severe hypoplasia up to aplasia was found in the bone marrow of unprotected

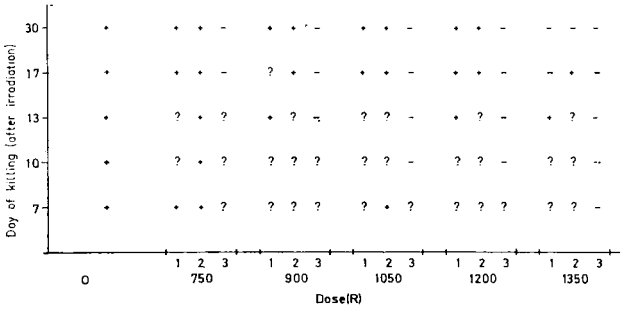

Fig. 10. Blood samples taken during the observation period being countable, uncountable or uncollectable (the animals died before the day they were to be killed).
+ = differential leukocyte count possible
? = too few leukocytes to be counted
– = no blood samples taken
O: untreated control
1: S-75-treated
2: cysteamine-HCl-treated
3: irradiated only

mice (Fig. 8). Regeneration of myeloid tissue occurred only in the protected mice, and cysteamine was somewhat more effective in protection of the bone marrow than was S-75. The stimulative effect of both substances seemed to be absent or low and thus in accordance with previous results (STOKKE 1968).

A non-uniform regeneration and a heterogeneous cellularity appeared in the bone marrow of protected animals. It is likely that both protective substances were able to protect haematopoietic tissue but not all types of cells to the same degree. Thus regeneration occurred from a few selected cells resulting in an abnormal appearence of an unproper cellularity of the regenerating marrow which mostly consisted of one kind of cells (Fig. 9). Furthermore, as circulation is not equal in all bone marrows, some areas might have had a low oxygen tension at the moment of irradiation, contributing to an increased resistance to radiation. In protected mice some haematopoietic cells probably survived in marrows with a low oxygen tension and regeneration took place as mentioned.

Haemosiderosis of the bone marrow and the spleen indicates destruction of erythrocytes, which was more extensive in unprotected mice. The lymph nodes seemed to be better protected by cysteamine than by S-75. However, both substances reduced the degree of hypoplasia and fibrosis compared to unprotected animals (Fig. 7).

Unfortunately, most of the unprotected mice died before the day determined for killing. Therefore, only a few blood samples were obtained from these animals, all uncountable on account of a severe leukopenia. Of the protected animals more countable blood samples were obtained from cysteamine treated animals than from S-75 treated animals (Fig. 10).

The sensitivity of the lymphocyte and the very slow recovery of the lymphoid tissue apparently resulted in a relative neutrophilia of the countable blood samples. Anisocytosis and polychromasia which occurred in protected mice mirrored the erythropoietic response to anemia. A rather late effect in the protected mice was a chronic anemia which appeared in concordance with the slow progress of erythropenia associated with the 20 to 40 day life span of the erythrocytes (SCHALM et coll. 1965) and a lower sensitivity of the erythrocytes to irradiation.

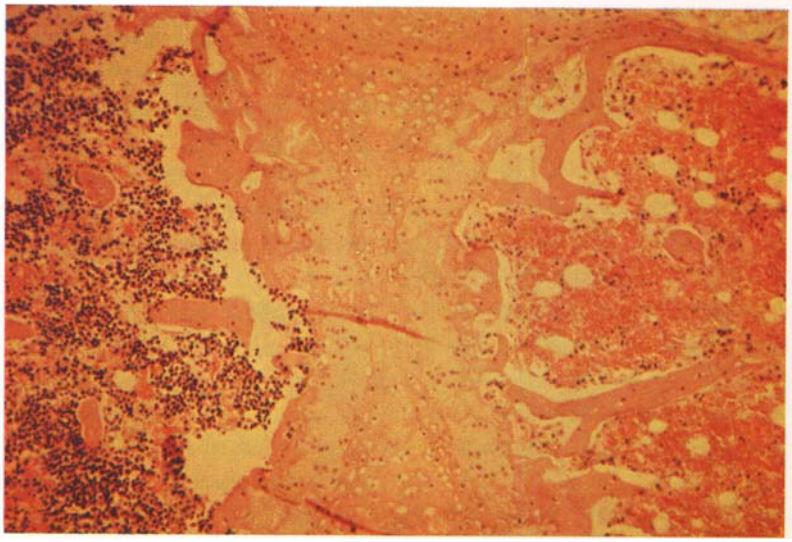

Fig. 9. Unproper cellularity of regenerating bone marrow. To the left regenerating marrow, to the right aplastic marrow. Cysteamine protected animal, irradiation dose 10.0 Gy, killed 30 days after irradiation. H & E stain. × 80.

Acknowledgement

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SUMMARY

CBA male mice were irradiated with single doses of 7.1, 8.6, 10.0, 11.4 or 12.8 Gy, respectively. A protective substance, 2,2-Dithiobis{N-[(1-adamantyl)methyl]acetamidine}-dihydrochloride, here called S-75, was administered orally, 45 min before start of irradiation. Cysteamine-HCl was used as a reference protective substance. Pathologic and haematologic examination of irradiated animals was performed. Cysteamine had somewhat better protective abilities than did S-75, but the latter had some other properties which indicate its possible usefulness in practice.

ZUSAMMENFASSUNG

Männliche CBA Mäuse wurden mit Einzel-Dosen von 7,1, 8,6, 10,0, 11,4 und 12,8 Gy bestrahlt. Eine Schutzsubstanz, 2,2-Dithiobis{N[(1-adamatyl)methyl]acetamidine}dihydrochloride, hier S-75 bezeichnet, wurde 45 Minuten vor Beginn der Bestrahlung oral verabfolgt. Cysteamine-HCl wurde als Referenz-Schutzsubstanz verwendet. Pathologische und hämatologische Untersuchungen der bestrahlten Tiere wurden vorgenommen. Cysteamine zeigte etwas bessere Schutzeigenschaften als S-75, die letztere Substanz hatte jedoch andere Eigenschaften, welche auf dessen mögliche praktische Anwendbarkeit hinweisen.

RÉSUMÉ

Des souris mâles CBA ont été irradiés par des doses uniques de 7,1, 8,6, 10,0, 11,4 ou 12,8 Gy. Une substance protectrice, le 2,2-Dithiobis {N-[(1-adamantyl)methyl]acetamidine}-dihydrochloride, appelée ici S-75, a été administrée par voie orale 45 minutes avant le début de l'irradiation. Le cystéamine-HCl a été utilisé comme substance protectrice de référence. Des animaux irradiés ont subi un examen anatomo-pathologique et hématologique. La cystéamine a un effet protecteur un peu meilleur que le S-75 mais ce dernier a quelques autres propriétés qui montrent qu'il peut être utile en pratique.

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