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DISTRIBUTION OF ^{35}S IN MICE AFTER ORAL ADMINISTRATION OF α -DITHIOACETAMIDIUM CHLORIDE

An autoradiographic investigation

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The beneficial effects of radiation protectors are often limited by toxic properties of the substances. WESTLAND *et coll.* (1972) presented a number of compounds of which one had promising radiation protective properties, when administered orally and in small amounts. That compound (2,2'-Dithiobis{N-[(1-adamantyl)-methyl]-acetamidine}-dihydrochloride), in the following shortened to S-75, was previously investigated by OUSAVAPLANGCHAI *et coll.* (1978), who found an increase in the relative weight and haemopoiesis of the spleen of roentgen irradiated mice, indicating a protective capacity of S-75 more specifically directed to this organ than was found for cysteamine hydrochloride.

The distribution of ^{35}S labelled S-75 in different organs by means of whole body autoradiography has now been investigated and the results compared with those by NELSON & ULLBERG (1960) concerning cysteamine.

Material and Methods

Eight inbred male CBA mice, aged about 85 days, were used. [^{35}S]-thiophosphoryl chloride (193 mg, 185 MBq (5.0 mCi)/mmol) purchased from the Radiochemical Centre, Amersham, England, was hydrolysed with 1.7 ml of 14% sodium hydroxide according to PIPER & JOHNSTON (1967). Recrystal-

lization and dehydration was carried out in essentially the same manner as described by ÅKERFELDT (1960) giving 188 mg (92%) of trisodium ^{35}S -phosphorothioate. Following the method of BARTON & BAUER (1969) the labelled sodium salt was dissolved in water and reacted with 0.26 g of N-(1-adamantylmethyl)-2-chloroacetamidine hydrochloride. The formed phosphorothioate was subsequently acid hydrolysed in an aqueous 2-propanol solution, yielding 235 mg (91%) of [^{35}S]-N-(1-adamantylmethyl)-2-mercaptoacetamidine hydrochloride.

The mercapto compound was then dissolved in 5 ml of methanol and 5 ml of water and oxidized by the addition of 85 μl of 30% hydrogen peroxide. After one hour at 60°C, 15 ml of water was added to the reaction mixture, which was cooled and the precipitated [^{35}S]-2,2'-Dithiobis{N-[(1-adamantyl)-methyl]-acetamidine}dihydrochloride collected, giving 188 mg (80%) with m.p. 235–39°C (decomp.; WESTLAND *et coll.*, m.p. 245–50°C). The substance was suspended in a solution of 0.5% Modocoll (Mo & Domsjö, Sweden) in distilled water, to give a 1% solution of S-75.

Of the suspension 0.2 ml, corresponding to 2 mg ^{35}S labelled S-75 per animal, was administered orally by means of syringe and stomach tube with a diameter of 0.5 mm. This amount of the suspension cor-

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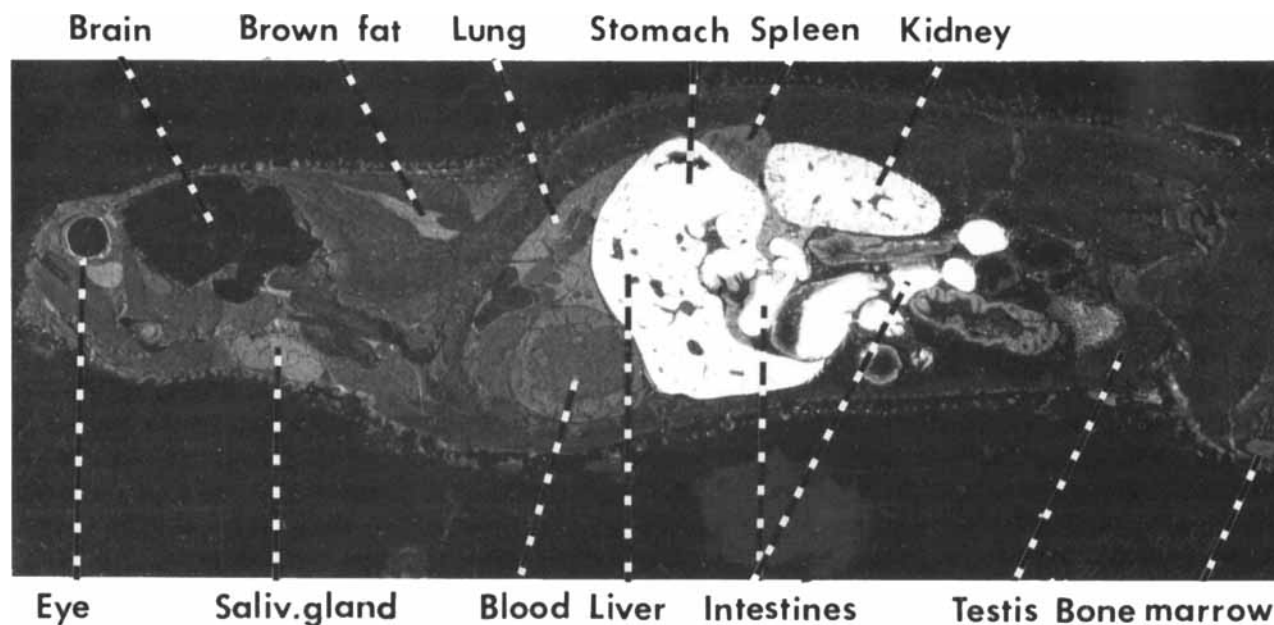


Fig. 1. Distribution of ^{35}S in male mouse 45 min after oral administration of ^{35}S labelled S-75. Highest concentration in liver and kidney and in gastro-intestinal tract.

responded approximately to an activity of 1 295 kBq ($35 \mu\text{Ci}$) ^{35}S .

The animals were euthanized by chloroform anaesthesia 10, 20, 30, 45, 60, and 90 min, and 6 and 24 h, respectively, after administration of the suspension. They were immediately mounted in an aqueous gel of carboxymethyl cellulose and frozen in n-hexane cooled with solid carbon dioxide (-78°C). Sagittal, $20 \mu\text{m}$ thick whole-body sections from different levels of the body were collected onto tape in a cryostat microtome (ULLBERG 1954, 1977). After having been freeze-dried, the sections were apposed to films (Industrex C, Kodak), which were developed three weeks later. Selected sections were stained in hematoxylin eosin before being mounted in Euparal (Flatters-Garnett Ltd., Manchester, England).

Results

Already 10 min after administration, activity was found in the small intestine and in the liver. At 20 min, the activity was distributed throughout the body. The highest concentration was found in the liver and kidneys, but a moderate activity was also found in the red pulp of the spleen, in the bone marrow, lungs, salivary glands, pancreas, brown fat,

and intestinal walls. The concentration in the blood and muscles was lower. A certain uptake was found in the uvea of the eye (iris, ciliary body, and choroid). The uptake in the brain and testes was low. Already at this time, activity was excreted in the kidneys (high activity in the pelvis), but the concentration in the gallbladder was comparatively low. The distribution was relatively unchanged at 30, 45, and 60 min (Figs 1, 2).

At 90 min and 6 h the general distribution throughout the body was clearly increased. In the spleen, the concentrations of the red and white pulp had become the same and were at a moderate level (Fig. 3) but somewhat higher than the blood concentration. A high activity was found in the gall and urinary bladders. An accumulation was also found in the uvea of the eye (particularly at 6 h).

At 24 h after injection, the total body concentration had decreased considerably and the distribution had changed in some respects. The uptake in the uvea of the eye now dominated, together with that in the gastro-intestinal tract and in the urinary bladder. A comparatively high concentration was recorded in the cartilage. Also the brown fat now showed a higher relative concentration than earlier. The concentration in the spleen was very low and the distribution was even (Fig. 4).

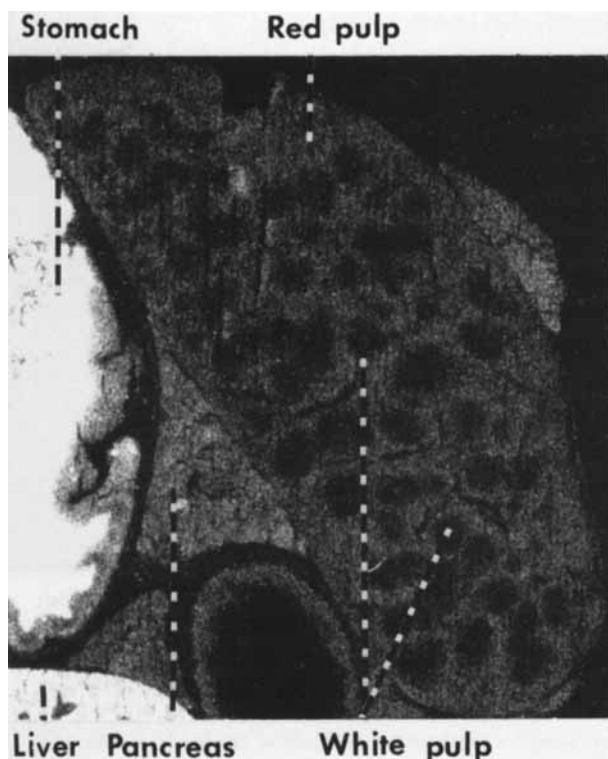


Fig. 2. Detail from Fig. 1. Distribution of ^{35}S in the spleen. Higher concentration in the red pulp than in the white.

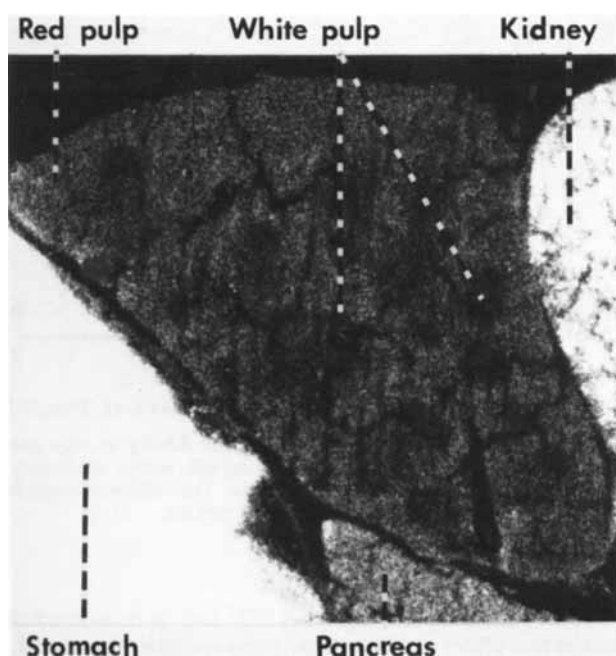


Fig. 3. Distribution of ^{35}S in the spleen 90 min after oral administration of ^{35}S labelled S-75. The concentration differences between the red and white pulp now greatly diminished.

Discussion

The distribution of ^{35}S labelled S-75 differs from that of ^{35}S labelled cysteamine (NELSON & ULLBERG), the latter substance having a more general distribution in the body. S-75 is mainly distributed in the liver, kidneys, red pulp of the spleen, bone marrow, lungs, salivary glands, pancreas, brown fat, and uvea of the eye (the melanin containing structures). NELSON & ULLBERG found high concentration of cysteamine in other organs as well, such as the central nervous system, pituitary, skin, thymus, adrenals, lymph nodes, both red and white pulp of the spleen, Harder's gland, retina, sclera, and cornea, but not in the uvea.

^{35}S labelled S-75 appears to be more rapidly excreted than ^{35}S labelled cysteamine. The latter substance is excreted mainly via the bile (NELSON & ULLBERG) while ^{35}S labelled S-75 has a marked excretion via the kidneys.

The intervals between administration of S-75 and killing of the animals were chosen according to previous survival experiments. In these experiments CBA males were roentgen-irradiated with 8.6 Gy at different intervals within the range of 5 min and 2 h after an oral administration of 2 mg S-75 per animal. The death rate during a 30-day observation period was plotted versus time (Fig. 5; RÖNNBÄCK, unpublished results).

Whether the observed distribution in the present experimental series represents intact S-75 or is due to ^{35}S containing metabolites has not been analysed. The uptake in cartilage at long survival intervals indicates a slow release of ^{35}S from the parental compound.

The differences found in the distribution between S-75 and cysteamine support the findings of OUSAVAPLANGCHAI *et coll* and CARFAGNINI *et coll.* (1981), indicating that cysteamine has more general protective properties than S-75. In addition, the protective properties of S-75 may be directed more towards the haemopoiesis of the red pulp of the spleen.

CARFAGNINI *et coll.* observed in the S-75 protected mice an increased mortality rate in the irradiated and splenectomized animals when compared with the mice similarly treated but not splenectomized. This difference was not found in the cysteamine protected mice.

The red pulp of the spleen is considerably involved in haemopoiesis in mice (SCHMERER 1967,

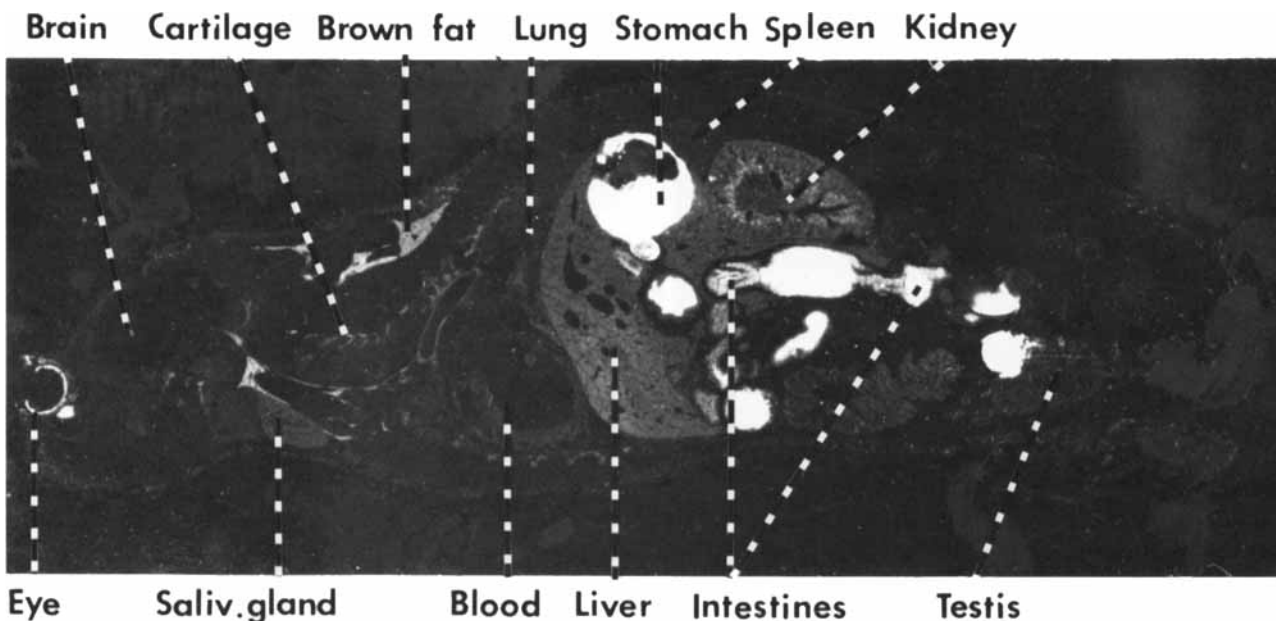


Fig. 4. Distribution of ^{35}S in male mouse 24 h after oral administration of ^{35}S labelled S-75. High concentration of activity still

found in the gastro-intestinal tract. Accumulation in the uvea of the eye and in cartilage.

BOGGS et coll. 1969). The importance of splenic haemopoiesis in irradiated mice has been pointed out by JACOBSSON et coll. (1949 a, b) and GURNEY & ROSETT (1968).

The findings of ^{35}S labelled S-75 in the red pulp of the spleen (Fig. 2) during the time when S-75 has its best protective ability, i.e. 45 min (Fig. 5) is thus notable. Cysteamine, on the other hand, is found in both the red and white pulp of the spleen. Both substances are found in the bone marrow. However, it has been noted that the regenerative capacity of the bone marrow in the cysteamine protected animals was better than that S-75 protected animals. The opposite situation is prevalent in the spleen (OUSAVAPLANGCHAI et coll.).

The radiation protective ability of S-75 ought to be tested in other species which do not have such a marked splenic haemopoiesis as the mouse.

SUMMARY

The distribution of ^{35}S in mice has been investigated by whole-body autoradiography after oral administration of the ^{35}S labelled S-75 (2,2'-Dithiobis[N-[(1-adamantyl)-methyl]-acetamidine]dihydrochloride). The substance was rapidly absorbed, and the highest concentration occurred in the liver and kidneys. During the highest radiation protective activity (after about 45 min) a substantial concentration was found in the red pulp of the spleen. This

Survival (per cent)

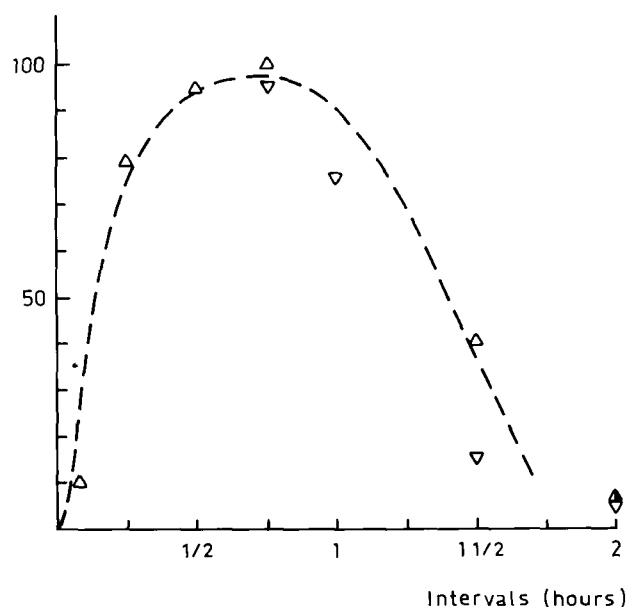


Fig. 5. Mortality rate after irradiation with 8.6 Gy in mice pretreated with 2 mg S-75 at different intervals before irradiation. Every point corresponds to 20 animals. The different symbols represent data pooled from several experiments.

supports previous findings that S-75 has its most marked protective effect on the splenic haemopoiesis. Previously, it was shown that cysteamine is more evenly distributed in the body than S-75, which is in agreement with its more generalized protective effect than S-75.

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