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DISTRIBUTION AND CHEMICAL FORMS OF DIETHYLDITHIOCARBAMATE AND TETRAETHYLTHIURAM DISULPHIDE (DISULFIRAM) IN MICE IN RELATION TO RADIOPROTECTION

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Abstract—The distribution and the chemical nature of the radioprotective thiol, diethyldithiocarbamate, and the corresponding disulphide, disulfiram, were studied in mice, using ^{35}S-labelled compounds. The results showed that the metabolism of these two compounds essentially follows the same pathways, since the disulphide immediately upon resorption becomes converted to the thiol. A similar relative distribution between organs was found with diethyldithiocarbamate and disulfiram. There was no particular accumulation in organs known to play an important role in the radiation syndrome. Ten to 20 times higher total concentrations of metabolites were obtained with diethyldithiocarbamate than with disulfiram, a finding which alone explains why, under the conditions used, only the former possesses radioprotective properties. Moreover, diethyldithiocarbamate gave rise to high concentrations of free, unmetabolized, thiol, whereas no free thiol was found after the injection of disulfiram. When labelled diethyldithiocarbamate as well as disulfiram had been injected, measureable amounts of ^{35}S was recovered bound to proteins. The protein-bound radioactivity derived from diethyldithiocarbamate, however, did not make up for more than 1–3 per cent of the total present, and only a fraction of this appeared to be bound by mixed disulphide linkages. The results, which are discussed in relation to chemical protection, suggest the necessity of free diethyldithiocarbamate in the organism in order to obtain radioprotective effect.

IN 1953 Bacq *et al.*[1,2] reported that sodium diethyldithiocarbamate offers protection to mice against the lethal effect of ionizing radiation. They found this compound to be as good as cysteamine,[3] one of the best chemical protectors known. The radioprotective properties of diethyldithiocarbamate has later also been demonstrated in chicks[4] as well as in isolated rat thymocytes.[5] In 1956 Van Bekkum published an extensive study on the relation between chemical structure and protective action of various derivatives of dithiocarbamic acid.[6] Ammonium dithiocarbamic acid, sodium dimethyldithiocarbamate and sodium diethyldithiocarbamate gave the best results, while further substitution at the N- or the S-position decreased the radioprotective properties. The corresponding disulphide of diethyldithiocarbamate, tetraethylthiuram disulphide (disulfiram), as well as tetraethylthiuram monosulphide did not have any radioprotective action.

Whereas the metabolism and chemical nature of various sulphur-containing radioprotectors such as cysteamine and aminoethylisothiuronium bromide (AET) have been

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subjected to detailed study during the past 10 yr, relatively little information has appeared on these relationships in the case of the radioprotective dithiocarbamates. However, recently Strømme[7] has presented a study on the metabolism of diethyldithiocarbamate and disulfiram in rats. It was found that the disulphide immediately upon resorption becomes converted to the corresponding thiol. Diethyldithiocarbamate, either formed *in vivo* from disulfiram or administered as such, was found to be further metabolized at a high rate, the main end products being the S-glucuronide of diethyldithiocarbamate, inorganic sulphate and carbon disulphide.

In view of the finding in rats that diethyldithiocarbamate is an obligatory intermediate in the metabolism of disulfiram, it appeared to be contradictory that only the former compound possesses radioprotective properties. On this background, and possibly to shed new light on the obscure mechanism underlying the protective action of diethyldithiocarbamate,[8] we have studied the metabolism of this compound and the parent disulphide in mice. Experimental conditions were chosen as identical as possible to those used in the previous protection experiments of Van Bekkum.[6] This was done in order to permit a comparison of the results obtained with the known radioprotective effect of these sulphur-containing compounds.

EXPERIMENTAL

Materials

^{35}S-labelled diethyldithiocarbamate was obtained from The Radiochemical Centre, Amersham, Bucks, England. ^{35}S-labelled disulfiram (tetraethylthiuram disulphide) was prepared by oxidizing radioactive diethyldithiocarbamate with alcoholic iodine as previously described.[9] Highly cross-linked dextran, "Sephades G-25", particle size 100–200 mesh (block polymerized), was purchased from AB Pharmacia, Uppsala, Sweden. Scintillators and 1 M hyamine in methanol were obtained from Packard Instrument Co., Inc., La Grange, Ill., U.S.A. Crystalline albumin from bovine serum was obtained from Sigma Chemical Company, St. Luis, Missouri, U.S.A., and crystalline human ceruloplasmin, lyophilized human γ-globulin and fibrinogen from AB Kabi, Stockholm, Sweden. Nucleoproteins from calf thymus were prepared as described by Jellum,[10] and highly polymerized DNA was isolated from calf thymus by the method of Hurst.[11]

Methods

Animal procedure. Ten to 14 weeks old mice of White label strain, kindly supplied by the Institute of General and Experimental Pathology, Rikshospitalet, Norway, weighing 20–28 g were used. The animals were maintained on an ordinary stock diet until the beginning of the experiments.

^{35}S-labelled sodium diethyldithiocarbamate (specific activity about 37 μc/mmole S) dissolved in 0·5 ml water was administered intraperitoneally in doses of 15 mg. The water-insoluble ^{35}S-labelled disulfiram (specific activity about 131 μc/mmole S) was finely ground in arachis oil and administered subcutaneously in doses of 60 mg suspended in a volume of 0·5 ml. Groups of 2–5 mice were used in each experiments and the plasma and tissues were pooled. This was done in order to obtain a large enough volume for further tests, and to reduce individual variations.

The mice were killed by decapitation. The blood from the neck vessels was collected in a tube containing 100 I.U. heparin. Samples of liver, spleen, kidney, testis, lung, heart, brain and intestinal mucosa were immediately excised. Samples of bone marrow were obtained by blowing air through the femurs with the aid of a syringe. The marrow extruded was directly collected on a tared watch-glass which was kept in a humidity chamber. About 3 mg bone marrow was obtained from each mouse.

Separation of ^{35}S-metabolites and determinations of radioactivity. The identification as well as the quantitative determinations of the various ^{35}S-metabolites of disulfiram and diethyldithiocarbamate in plasma and in the supernatants of liver homogenates were carried out as previously described in details.[7]

In the tissue-distribution study the various organs and tissues, while kept at 0°, were homogenized in a Potter–Elvehjem homogenizer in a medium of 10^{-2} M metal-free EDTA buffer, pH 8·5. The buffer was prepared as previously described.[12] The homogenates were subsequently sonified for 2 × 1/2 min at 20000 c/s and 3 A, using a Branson sonifier. Samples for the measurements of the total ^{35}S-compound present were prepared by taking a suitable aliquot (250–500 μl) of the homogenates into counting vials containing 1 ml M-hyamine in methanol. After complete dissolution of the proteins, the scintillation mixture[12] (10 ml) was added, and the radioactivity measured in a Tri Carb Liquid scintillation spectrometer (Packard Instrument Co., Inc.). Samples of plasma (50–100 μl) were directly taken into the hyamine solution followed by the addition of scintillation mixture. Corrections for quenching were made by the internal standard technique.

Determinations of ^{35}S bound to sub-cellular particles. Ten min after the intraperitoneal injection of 15 mg ^{35}S-diethyldithiocarbamate (specific activity 447 μc/mmole S), the mice were killed by decapitation, the liver and spleen excised, rinsed in water and immersed into a weighed beaker containing an ice-cold solution of 0·25 M sucrose in 10^{-2} M metal-free EDTA buffer, pH 8·5. After weighing, the organs were homogenized in about 10 volumes of the sucrose-EDTA buffer with the aid of a Potter–Elvehjem homogenizer. The sub-cellular particles both of liver and spleen were separated in a nuclear, a mitochondrial and a microsomal fraction by centrifugation at 0–4° as follows:

The 10 per cent homogenate (about 1 g liver or 0·5 g spleen) was centrifuged at 775 *g* for 8 min. The sediment was re-homogenized in 5 ml sucrose-EDTA buffer and, recentrifuged. This was repeated twice. The pellet, containing nuclei and some cell debris ('nuclear fraction'), was suspended in a small volume of 10^{-2} M metal-free EDTA buffer, pH 8·5 and sonified with the aid of a Branson sonifier to achieve disruption of the particles.

The pooled supernatants (about 20 ml) from the preceding centrifugations were centrifuged at 5000 *g* for 15 min. The sediment was re-homogenized in 10 ml sucrose-EDTA buffer, and both the supernatant and the re-homogenate were centrifuged under the same conditions. The two pellets were homogenized and pooled in 10 ml sucrose-EDTA buffer and subjected to a final centrifugation at 6600 *g* for 15 min. The sediment containing mainly mitochondria ("mitochondrial fraction") was suspended in a small volume of 10^{-2} M metal-free EDTA buffer with the aid of ultrasonic vibrations.

The pooled supernatants (about 40 ml) from the above centrifugations were centrifuged at 105000 *g* for 1 hr in a Spinco Model L preparative ultracentrifuge. The sediment was re-homogenized in 40 ml sucrose-EDTA buffer and recentrifuged.

The pellet, containing the microsomes ("microsomal fraction"), was suspended in a small volume of the 10^{-2} M EDTA buffer with the aid of ultrasonic vibrations.

The concentration of total proteins in these cellular fractions was determined by the biuret method, and the radioactivity measured as described above. To estimate the fraction of the radioactivity which was firmly anchored to the particles, and to get a clue as to how much of the latter was bound through mixed disulphide linkages, the suspensions were subjected to the following dialysis. One aliquot of each fraction was dialysed against 100 volumes of pure metal-free EDTA buffer, and another aliquot against 100 volumes of the buffer which in addition contained 10^{-2} M cysteamine as well as thiolated sephadex (about 100 mg dry gel per 10 ml buffer). The latter material which was prepared by the method of Eldjarn and Jellum,[13] has been shown efficiently to prevent autoxidation of thiols.[14] The dialysing solvents were renewed after 2 and 4 hr, and the dialysis was carried out at room temperature for a total of 20 hr. The surplus of free thiol in the solvents containing cysteamine was ascertained by regularly checking its presence with the aid of the nitroprussid spot test. The protein concentrations and the radioactivity of the dialysed samples were determined, and the amounts of radioactivity expressed as μmoles S/g protein.

RESULTS

Van Bekkum[6] found that in mice the optimal protective effect of diethyldithiocarbamate was offered when administered in intraperitoneal doses of about 15 mg. The irradiation was given during the first 15–20 min after the injections. No protection was found with the chemically related disulfiram in subcutaneous doses of 60 mg, the mice in this case being exposed to X-rays 4 and 20 hr after the injections. In the present study of the metabolism of these two sulphur-containing compounds, we have chosen identical experimental conditions with respect to dose, administration and time of sampling.

Organ distribution

Table 1 shows the distribution of diethyldithiocarbamate and disulfiram in mice. Ten minutes after the intraperitoneal administration of ^{35}S-labelled diethyldithiocarbamate in optimal protective doses (15 mg), highest amounts of the radioactive sulphur were found in plasma, liver and kidney, whereas the lowest amount was found in brain. Organs which play an important role in the radiation syndrome such as bone marrow, spleen and intestinal mucosa showed intermediate amounts.

Four and 20 hr after the subcutaneous administration of disulfiram (60 mg), the sulphur of this compound showed a relative distribution between the organs quite similar to that when using the thiol, except that in this case the intestinal mucosa appeared to be among the organs containing the highest amounts. In the case of disulfiram, however, the absolute amounts were much lower, most likely due to a slow resorption of this water-insoluble disulphide. In fact, a slow resorption is indicated by the finding that higher concentrations were present in the various organs 20 hr after the injections than after 4 hr (Table 1).

Chemical nature of diethyldithiocarbamate and disulfiram in plasma and liver

Four low molecular weight metabolites could be identified in plasma and in the supernatant of liver homogenates from mice, i.e. free diethyldithiocarbamate, the

TABLE 1. ORGAN DISTRIBUTION OF DIETHYLDITHIOCARBAMATE-^{35}S (ASH) AND DISULFIRAM-^{35}S (ASSA)

Tissue	ASH (15 mg i.p.)	ASSA (60 mg s.c.)	
	Time after dose		
	10 min	4 hr	20 hr
Plasma	6·62	0·26	0·79
	8·66	0·32	0·59
	8·29	—	—
Liver	5·76	0·25	0·43
	6·83	0·19	0·40
Kidney	5·11	0·25	0·52
	5·47	0·39	0·45
	5·01	—	—
Heart	1·27	0·06	0·14
	2·09	0·07	0·09
	1·83	—	—
Lung	2·69	0·08	0·36
	3·69	0·19	0·28
	3·21	—	—
Brain	0·72	0·02	0·05
	1·07	0·03	0·04
	0·75	—	—
Testis	1·38	0·05	0·18
	1·83	—	0·13
	1·52	—	—
Spleen	2·36	0·14	0·19
	2·81	0·10	0·16
Bone marrow	4·73	—	—
	2·21	—	—
Intestinal mucosa	2·39	0·21	1·03
	1·22	0·22	0·78

Two mice were used in each experiment. All amounts are expressed as μmole sulphur per g tissue (wet weight) or per ml plasma.

glucuronic acid conjugate of the thiol, inorganic sulphate and the volatile carbon disulphide. In addition a small amount of the sulphur was found to be bound to proteins. It was not possible to detect any disulfiram in the disulphide form even after its administration in toxic amounts, indicating an immediate and complete *in vivo* reduction of this disulphide. These results are in agreement with those previously obtained in rats.[7]

Figure 1 shows the amounts of the metabolites present in plasma during the first 40 min after the i.p. administration of diethyldithiocarbamate in optimal protective doses. Initially high concentrations of free, unmetabolized, thiol was found. During the following 40 min the concentrations decreased rapidly. Concommitantly, the concentration of the S-glucuronide of diethyldithiocarbamate and, to a much smaller extent, the concentrations of sulphate and protein-bound metabolites increased. In the liver (Fig. 2) the amount of free thiol was markedly less than that simultaneously found in plasma. On the other hand, a more rapid increase in the amount of the S-glucuronide occurred, probably reflecting the fact that the glucuronic acid conjugation takes place mainly in the liver.[7]

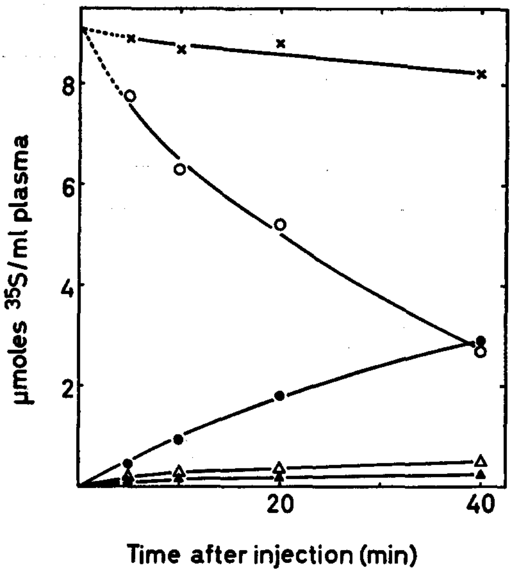

FIG. 1. ^{35}S-metabolites present in plasma of mice during the first 40 min after the injection of labelled diethyldithiocarbamate (15 mg i.p.). Groups of five mice were used in each experiment.
× —— ×, total ^{35}S; ○ —— ○, diethyldithiocarbamate-^{35}S; ● —— ●, glucuronic acid conjugate of diethyldithiocarbamate-^{35}S; △ —— △, sulphate-^{35}S; ▲ —— ▲, protein-bound ^{35}S.

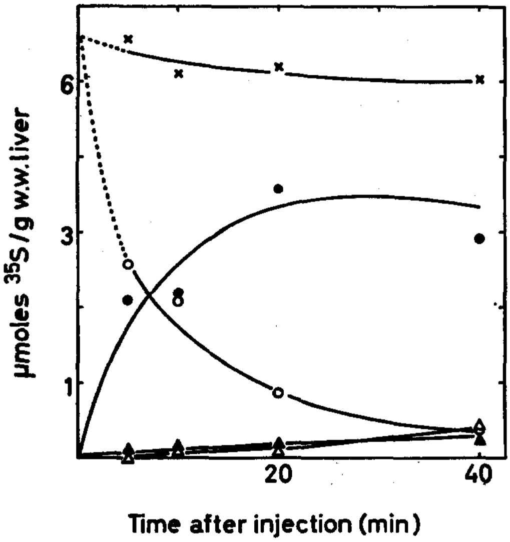

FIG. 2. ^{35}S-metabolites present in supernatant of liver homogenates of mice during the first 4 min after the injection of labelled diethyldithiocarbamate (15 mg i.p.). Groups of five mice were used in each experiment.
× —— ×, total ^{35}S; ○ —— ○, diethyldithiocarbamate-^{35}S; ● —— ●, glucuronic acid conjugate of diethyldithiocarbamate-^{35}S; △ —— △, sulphate-^{35}S; ▲ —— ▲, protein-bound ^{35}S.

In the case of disulfiram (Table 2) which appears to be inactive with respect to radioprotection, no free thiol could be found in the organism on examinations at 4 and 20 hr after its administration. The radioactive sulphur was identified as the S-glucuronide of diethyldithiocarbamate, sulphate and protein-bound sulphur. Thus, as compared to the results obtained with diethyldithiocarbamate (Figs. 1 and 2), the striking difference, aside from the much lower total amounts of radioactive sulphur, was the complete absence of free thiol after the disulphide injections.

TABLE 2. ^{35}S-METABOLITES IN PLASMA AND SUPERNATANT OF LIVER HOMOGENATE FROM MICE GIVEN LABELLED DISULFIRAM (ASSA, 60 mg s.c.)

System	Time after dose (hr)	Total ^{35}S	Protein bound ^{35}S	ASH*	AS-glucur.	Sulphate	Identified (% of total ^{35}S)
Plasma	4	262	26	0	26	214	101
	4	320	—	—	—	—	—
	20	785	42	0	78	574	88
	20	594	51	0	42	461	92
Supernt. of liver homogenate	4	151	27	0	43	59	84
	4	186	39	0	31	96	89
	20	351	68	0	54	182	86
	20	312	38	0	62	161	84

* Diethyldithiocarbamate.

Three mice were used in each experiment. The amounts are expressed as m-μmoles sulphur (^{35}S) per g liver (wet wt.) or per ml plasma.

When diethyldithiocarbamate as well as when disulfiram had been administered, small amounts of radioactivity were recovered bound to the plasma proteins and to the soluble proteins of liver. Although the absolute amounts of the ^{35}S attached to the proteins were about 2–3 times higher after the administration of thiol, the values nevertheless were within the same order of magnitude (50–150 mμmoles S/ml plasma or g wet weight liver) when compared to the large difference in the amounts of free thiol. Regarding the type of linkages involved, one would expect a predominance of mixed disulphide bonds since this has previously been observed in rats.[7] However, using the same technique of identification as used in the rat experiments,[7] we could only account for less than 50 per cent of the sulphur-protein linkages as mixed disulphides in mice.

In vivo *binding of* ^{35}*S to intracellular constituents*

Table 3 shows the amount of radioactive sulphur present in the nuclear, the mitochondrial and the microsomal fractions of liver and spleen from mice which had been injected 10 min before with 15 mg ^{35}S-diethyldithiocarbamate. It appears that a considerable fraction of the sulphur was loosely bound to the particles, since a significant amount of the radioactivity left in all the final preparations could be removed by subsequent dialysis against pure buffer. This amount was greater than would have been expected from the dilution steps in the preparation procedure, and may indicate that some type of concentration of diethyldithiocarbamate occurs either in or on the formed elements of the cells.

TABLE 3. ^{35}S PRESENT IN SUB-CELLULAR FRACTIONS OF LIVER AND SPLEEN FROM MICE GIVEN LABELLED DIETHYLDITHIOCARBAMATE (15 mg)

Organ	Sub-cellular fraction	Before dialysis	After dialysis against: Buffer only	10^{-2} M cysteamine
Liver	Nuclei	3·56 (8·5)	0·55 (1·3)	0·33 (0·8)
	Mitochondria	2·54 (6·0)	0·44 (1·0)	0·30 (0·7)
	Microsomes	3·45 (8·2)	1·36 (3·2)	1·04 (2·5)
Spleen	Nuclei	0·92 (5·5)	0·37 (2·2)	0·32 (1·9)
	Mitochondria	0·56 (3·3)	0·35 (2·1)	0·32 (1·9)
	Microsomes	0·69 (4·1)	0·44 (2·5)	0·29 (1·7)

For experimental details, see the text. The total ^{35}S was in whole liver 42·0 μmoles/g protein and in whole spleen 16·7 μmoles/g protein. The amounts of ^{35}S are expressed as μmoles per g protein, and the figures within brackets represent per cent of total ^{35}S.

The amounts of non-dialysable sulphur did not make up for more than 1–3 per cent of the total radioactivity present in liver or spleen. If the buffer contained 10^{-2} M cysteamine, and additional fraction of radioactive sulphur was regularly removed by dialysis, which suggests the presence of some mixed disulphides on the subcellular particles. The amounts liberated by cysteamine, however, were small, constituting only a fraction of a percentage of the total ^{35}S present in the respective organs. Because of the instability of diethyldithiocarbamate on the acid side,[12] the cell fractionation was carried out at pH 8·5, which may have facilitated the liberation of diethyldithiocarbamate residues from the mixed disulphide bonds. Still the results are taken to indicate that only a low degree of mixed disulphide formation occurs on the cell particles.

A somewhat greater amount of ^{35}S was found in the microsomal fraction of liver than in the two other liver fractions, but apart from this no particular accumulation was found either in the liver or in the spleen. The higher amount of ^{35}S bound to the liver microsomes may be related to the fact that conjugation with glucuronic acid, which is one of the main routes by which diethyldithiocarbamate is metabolized, is a microsomal process.[7]

Binding of diethyldithiocarbamate to proteins in vitro

It has repeatedly been proposed that the radioprotective substances or some of their metabolites have to be localized on or around the cellular target molecules in order to exert their action.[15–19] The formation of mixed disulphides,[20] thioester bonds[21, 22] and of non-covalent salt-like linkages[10, 18] are possible ways of bringing about such a local accumulation of the active agent.

In the case of diethyldithiocarbamate we have shown that this thiol becomes extensively, but reversibly, adsorbed to the proteins of human plasma.[12] It appears to be of interest to see whether this radioprotective agent becomes adsorbed to the cell proteins in general. In these experiments the previously described gel-filtration technique was used.[12] Throughout, 10^{-2} EDTA buffer, pH 8·5, was used as the solvent and as the effluent buffer, and the experiments were carried out at room temperature with diethyldithiocarbamate in concentrations from 2·5 to 5×10^{-4} M. The results, however, did not reveal any binding or adsorption of diethyldithiocarbamate to either the proteins of mice-liver homogenate (10 g wet wt./100 ml) or to the nucleoproteins (1 g/100 ml) and the polymerized DNA (0·1 g/100 ml) of calf thymus. Of the various proteins of plasma, the ability of adsorbing diethyldithiocarbamate seemed to be restricted to albumin as shown by the fact that crystalline bovine albumin (1 g/100 m) was the only one of the plasma proteins tested (albumin, γ-globulin, fibrinogen) which behaved like full plasma. However, crystalline ceruloplasmin (1 g/100 ml) interacted with and partly bound this strong metal chelating agent. A simultaneous change in the colour of the ceruloplasmin solution from blue to yellow indicated that the copper of this protein was involved in the reaction. Our results with crystalline ceruloplasmin stand in contrast to the previous claim that diethyldithiocarbamate does not react with the copper of ceruloplasmin in human serum.[23, 24]

DISCUSSION

Data has been presented on the metabolism, the distribution and the chemical nature of the radioprotective thiol, diethyldithiocarbamate, and the corresponding disulphide, disulfiram, in mice. The mice were examined during a period of time when it is known from the radiation experiments of Van Bekkum[6] that the animals injected with diethyldithiocarbamate were effectively protected against ionizing radiation whereas those injected with disulfiram were not protected.

The metabolism of these two sulphur-containing compounds in mice seems to follow essentially the same pathways as those previously found in rats. Even after high doses of disulfiram, no free disulphide could ever be detected. Apparently disulfiram upon resorption immediately becomes converted to diethyldithiocarbamate. This reduction most probably involves mixed disulphides on biological -SH groups as intermediates. The metabolism of diethyldithiocarbamate, either formed *in vivo* from disulfiram or administered as such, appears to proceed at a considerable rate along three main routes: (1) Conjugation with glucuronic acid, (2) oxidation to sulphate, (3) decomposition to diethylamine and the volatile carbon disulphide, this being eliminated via the lungs.

In agreement with results of distribution studies with other radioprotective sulphur-containing compounds such as cysteamine and aminoethylisothiuronium bromide (AET),[18, 25–28] a marked difference in the organ distribution was found also in the case of diethyldithiocarbamate. On the other hand, we were not able to show any particular accumulation in organs known to be most vulnerable to ionizing radiation such as bone marrow, spleen and intestinal mucosa.

The relative distribution between organs of the sulphur derived from the inactive disulfiram was quite similar to that of the radioprotective diethyldithiocarbamate. However, at the times of the radiation exposures used in the experiments of Van Bekkum,[6] disulfiram gave rise to total concentrations of radioactive sulphur which were only 1/10th to 1/20th that found with diethyldithiocarbamate. This may easily explain why disulfiram in contrast to the corresponding thiol does not show radioprotective properties. Moreover, with disulfiram no free diethyldithiocarbamate was found at the times of radiation exposure, whereas with diethyldithiocarbamate free thiol was present in plasma in concentrations ranging from 5×10^{-3} M to 10^{-2} M. Obviously, the resorption of the water-insoluble disulfiram is so slow that the organism is capable of metabolizing this compound at a rate sufficiently high to prevent any measureable accumulation of either the unchanged disulphide or the intermediate thiol. Higher rates of resorption may be obtained by more suitable ways of administering the disulphide.[7] However, it is doubtful if the rate will ever be high enough to obtain protective concentrations of the active metabolite.

There seems as yet no definite evidence available which explains the mode by which diethyldithiocarbamate offers radioprotection, although several hypotheses have been suggested.[8] Eldjarn and Pihl[20, 29] have suggested that mixed disulphide formation on biological sulphydryl and disulphide groups is of importance in the protective action of many sulphur-containing compounds. Diethyldithiocarbamate, however, is one of the substances which has been difficult to incorporate into this group of chemical protectors, mainly because *in vitro* studies so far have failed to demonstrate mixed disulphide formation of diethyldithiocarbamate with biological small molecular weight disulphides[20] and with SS-groups of proteins.[12] *In vivo* on the other hand, the present data as well as those previously obtained in rats[7] suggest that some mixed disulphide formation occurs both on the soluble and on the structural proteins. Probably this is brought about by an initial oxidation of the thiol to its disulphide disulfiram, which is known to have a great ability for oxidizing and forming mixed disulphides with -SH groups.[9, 12, 30] It may be questioned, however, whether the mixed disulphides formed *in vivo* is of any significance in the radioprotective action. Thus, in contrast to the considerable amounts of protein-bound sulphur recovered from mice treated with other radioprotective sulphur-containing compounds,[20, 21, 28, 31, 32] the amounts detected after injection of diethyldithiocarbamate represent only a minute fraction of the total sulphur given. This fact, held together with the finding that disulfiram under conditions when it has failed to show radioprotective action, gives rise to about the same amount of protein-bound sulphur as that obtained with diethyldithiocarbamate, makes it unlikely that the protein-bound metabolites play any important role in its radioprotective action.

Of the various metabolites present, the free thiol seems to be the most likely immediate active agent. Thus its presence in high amounts during the period of effective protection following its administration as such, and its complete absence after the administration of the inactive disulfiram, constitutes the only striking difference in the results obtained with these two compounds. The present data does not offer any explanation as to the way by which the free thiol exerts its protective action. As mentioned it appears justifiable to assume that the thiol must be accumulated at or around the target molecules, and, in fact, we have shown that diethyldithiocarbamate becomes adsorbed to the albumin of plasma. Apart from this, however, no accumulation was found to the various purified proteins tested. Still, the relatively high amounts of dialysable radioactivity present in the subcellular fractions, may suggest that some type of concentration takes place *in vivo*. It may therefore prove rewarding to examine further the problem of diethyldithiocarbamate adsorption.

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