

# Combined chelation treatment for polonium after simulated wound contamination in rat

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**Abstract.** Contaminated puncture wounds were simulated in rat by intramuscular injection of  $^{210}\text{Po}$ . The aim of the study was to determine the effectiveness of chelation treatment as a function of time, dosage, and route of chelate administration. Ten newly synthesized substances containing vicinal sulphhydryl and carbodithioate groups were used and their effect was compared with that of chelators clinically applicable in man—BAL (2,3-dimercaptopropane-1-ol), DMPS (2,3-dimercaptopropane-1-sulphonate), DMSA (*meso*-2,3-dimercaptosuccinic acid), and DDTc (sodium diethylamine-*N*-carbodithioate). The results indicate first that complete removal of  $^{210}\text{Po}$  from the injection site is achieved by only two local injections of DMPS, beginning as late as 2 h after injection of  $^{210}\text{Po}$ . Second, many of the substances used merely induce translocation of  $^{210}\text{Po}$  from the injection site into other tissues. Third, a combined local treatment at the injection site with DMPS plus repeated systemic, subcutaneous, treatments with HOEtTTC (*N,N'*-di-(2-hydroxyethyl)ethylenediamine-*N,N'*-biscarbodithioate), a derivative of DDTc, results after 2 weeks in a reduction of the estimated total body retention of  $^{210}\text{Po}$  to about one-third of that in untreated controls. In the latter case the cumulative excretion of  $^{210}\text{Po}$  increased from 8 to 54%, mainly via the faeces.

## 1. Introduction

In our previous work we have shown that treatment of simulated wounds contaminated with transportable radioactive substances such as  $^{238}\text{Pu}$ ,  $^{239}\text{Pu}$ ,  $^{241}\text{Am}$  or  $^{234}\text{Th}$  nitrates becomes more effective when the chelators are injected locally, and suitable combinations of chelators are used in order to produce a synergistic response (Volf 1974, 1975, Peter-Witt and Volf 1984).

$^{210}\text{Po}$  is an important  $\alpha$ -emitter to study (Rencová *et al.* 1993, 1995), and it can be chelated by substances containing sulphhydryl groups such as those used for the treatment of heavy metal ions (Volf 1973, Rencová *et al.* 1993). For simulated wounds contaminated with  $^{210}\text{Po}$ , it was reported that it was possible to mobilize  $^{210}\text{Po}$  from the wound site but at the same time

accumulation in the kidney was increased (Ilyin *et al.* 1977). In preliminary communications we demonstrated that indeed most of chelators used mainly translocate  $^{210}\text{Po}$  from a simulated wound into other tissues. However, suitable combinations of chelators may also substantially reduce overall retention of  $^{210}\text{Po}$  in the body (Volf *et al.* 1993, Rencová *et al.* 1994).

Our present investigations were performed on rat with puncture wounds simulated by intramuscular injection of  $^{210}\text{Po}$ . The aim was to determine the effect of chelation treatment as a function of time, dosage and route of administration, using clinically acceptable chelators such as BAL, DMPS, DMSA or DDTc (for explanation see §2) as well as new chelating agents such as HOEtTTC, DMBD, MiADMS (Jones and Cherian 1991). We had already used some of the latter after intravenous injection of  $^{210}\text{Po}$  (Rencová *et al.* 1993-1995).

## 2. Material and methods

### 2.1. Animals

Young female Sprague-Dawley rats (90-110g) were purchased from Charles River Wiga (Germany). They received standard food pellets (Altromin, Lage, Germany or St 1, Velaz, Czech Republic) and tap water *ad libitum*. Intramuscular injections were given under light ether anaesthesia.

Rats were killed by bleeding under ether anaesthesia; blood samples were collected in heparinized tubes. The protocols used in the experiments conformed to the European Convention for the Protection of Vertebrate Animals Used in Experimental and Other Scientific Purposes (1986).

### 2.2. Chemicals

Carrier-free  $^{210}\text{Po}$  in 3 M nitric acid was purchased from Amersham-Buchler (Braunschweig, Germany) and diluted to 0.3 M nitric acid to give an injection solution containing about 11 kBq  $^{210}\text{Po}$  in 0.05 ml (i.e. about 110 kBq  $\text{kg}^{-1}$  body mass), which was injected

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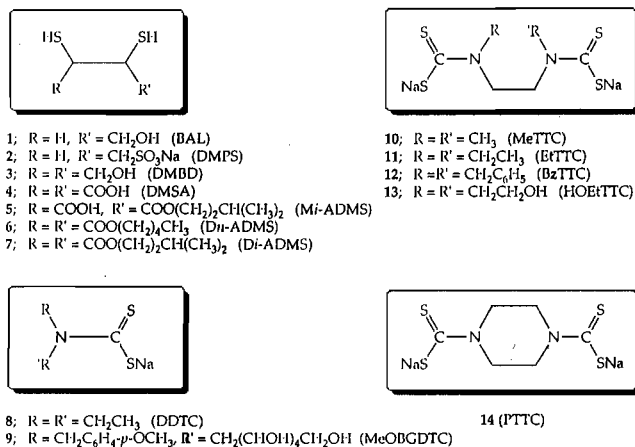


Figure 1. Structures of dithiols, and mono- and biscarbodithioates used for <sup>210</sup>Po decorporation.

intramuscularly. The technique for administering a small volume of radioactive solution into a reproducible depth of muscle has been described previously (Volf 1974).

Four chelators, i.e. 2,3-dimercaptopropane-1-ol (**1**, BAL), sodium 2,3-dimercaptopropane-1-sulphonate (**2**, DMPS), *meso*-2,3-dimercaptosuccinic acid (**3**, DMSA), and sodium diethylenediamine-*N*-carbodithioate (**4**, DDTC) (Figure 1), were purchased from Serva (Heidelberg, Germany). Ten other compounds (**5**–**14** in figure 1) were synthesized using the methods published previously. The reference is cited immediately after the compound number and the abbreviation: sodium *N*-(4-methoxybenzyl)-D-glucamine-*N*-carbodithioate (**9**, MeOBGDTC) (Jones *et al.* 1988); monoisoamyl *meso*-2,3-dimercaptosuccinate (**5**, Mi-ADMS) (Jones *et al.* 1992); di-*n*-amyl *meso*-2,3-dimercaptosuccinate (**6**, Dn-ADMS), and di-*i*-amyl *meso*-2,3-dimercaptosuccinate (**7**, Di-ADMS) (Singh *et al.* 1989a); 2,3-dimercaptobutanate-1,4-diol (**3**, DMBD), sodium *N,N'*-dimethylethylenediamine-*N,N'*-biscarbodithioate (**10**, MeTTC), sodium *N,N'*-diethylethylenediamine-*N,N'*-biscarbodithioate (**11**, EtTTC), sodium *N,N'*-dibenzylethylenediamine-*N,N'*-biscarbodithioate (**12**, BzTTC), sodium *N,N'*-di-(2-hydroxyethyl)-ethylenediamine-*N,N'*-biscarbodithioate (**13**, HOEtTTC), and sodium piperazine-*N,N'*-biscarbodithioate (**14**, PTTC) (Singh *et al.* 1989b).

The injection solutions of all compounds but BAL were prepared in distilled water. In cases where the resulting solutions were acidic, NaHCO<sub>3</sub> was used to adjust the pH to 7.4. BAL was diluted with peanut oil because of its lipophilicity. All the solutions were prepared under an inert atmosphere (N<sub>2</sub>) to avoid oxidation of the air-sensitive –SH and >NCS<sub>2</sub>Na groups.

## 2.3. Treatment

Rats were injected with <sup>210</sup>Po intramuscularly (i.m.) into the right thigh. Local treatment was administered i.m. (0.5 ml per injection) by infiltrating the tissue around the <sup>210</sup>Po injection site. Systemic treatment was administered by subcutaneous injection (s.c., 0.5 ml) on the back behind the neck, in the case of BAL and partly with DMPS by i.m. injection into the left thigh, i.e. into the thigh opposite to that with the <sup>210</sup>Po contaminated wound. For combined treatment the different chelators were administered in separate syringes. Details of the time, number of treatments, and period of observation, are indicated in the Tables.

## 2.4. Determination of radioactivity

Dissected tissues were digested with the mixture of perchloric acid and hydrogen peroxide and the radioactivity was measured by scintillation counting (Seidel and Volf 1972). The total radioactivity in the blood and muscles was calculated by counting known aliquots, assuming 6.5 ml blood 100 g<sup>-1</sup> body mass (Baker *et al.* 1980) and that muscles contribute 45% of the body mass (determined by J. R., unpublished data). Total radioactivity in the skeleton was assumed to be 20 times that of one femur, which is close to what was determined for other actinides (V.V., unpublished data). The sum of <sup>210</sup>Po measured or estimated for individual organs plus that remaining at the injection site was considered to be the total retention of <sup>210</sup>Po. The significance of the differences (*p* < 0.05) between the arithmetic means of the control and treated groups were evaluated statistically using Student's *t*-test.

## 3. Results

In a pilot experiment, the distribution pattern of i.m. injected <sup>210</sup>Po in the untreated control rat was followed up to 30 days (Figure 2). During the first 2 days about 60% of <sup>210</sup>Po was removed from the injection site. Then its release was slower; it followed a single exponential phase indicating a biological half-life of about 1 month. The greatest fraction of the translocated <sup>210</sup>Po appeared in the kidneys within 2 weeks, when almost the same amount was found in the liver plus skeleton. Accumulation of <sup>210</sup>Po in blood, skeleton and spleen proceeded at comparable rates (doubling time of about 2 weeks), which obviously reflects the high affinity of <sup>210</sup>Po for red blood cells at all stages of their development and circulation.

Figure 3 shows the concentrations of <sup>210</sup>Po in the body tissues of rat. These values, when corrected for radioactive decay of <sup>210</sup>Po (14% after 30 days), would be directly proportional to the radiation dose-rates and

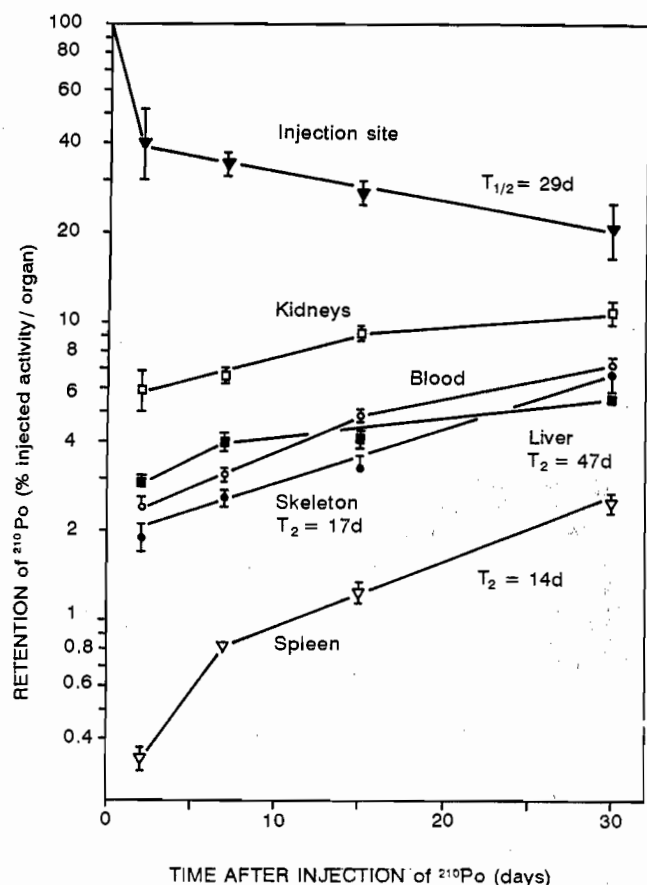


Figure 2. Retention of i.m. injected  $^{210}\text{Po}$  in the untreated control rat. Geometric means  $\pm$  SEM.  $T_{1/2}$  is biological half-time of  $^{210}\text{Po}$  translocation from the injection site.  $T_2$  is 'doubling' time of  $^{210}\text{Po}$  accumulation in body tissues.

thus indicate the respective levels of  $^{210}\text{Po}$  risk. The highest concentrations of  $^{210}\text{Po}$  were in the kidney and spleen (6% of injected activity  $\text{g}^{-1}$  after 30 days), whilst those in the liver, bone (femur), and blood were lower by an order of magnitude. The concentrations of  $^{210}\text{Po}$  in the kidney and liver remained reasonably constant within the first month after exposure, which indicates that the growth of these organs in the young rat compensates for the continuously increasing deposition of  $^{210}\text{Po}$  translocated from its injection site.

The first experiment on chelation of  $^{210}\text{Po}$  in simulated wounds was performed using different treatment schedules of BAL (Table 1). Only when BAL was injected locally 1 h and 4 days after  $^{210}\text{Po}$  did it reduce the radioactivity at the injection site appreciably to 19% of that in untreated controls. Translocated  $^{210}\text{Po}$  was partly deposited in the organs, mainly in the liver (a three-fold increase) but in spite of this the total retention of  $^{210}\text{Po}$  was significantly reduced (to 65% of control values). When BAL was injected repeatedly but only systemically, it did not reduce  $^{210}\text{Po}$  at the

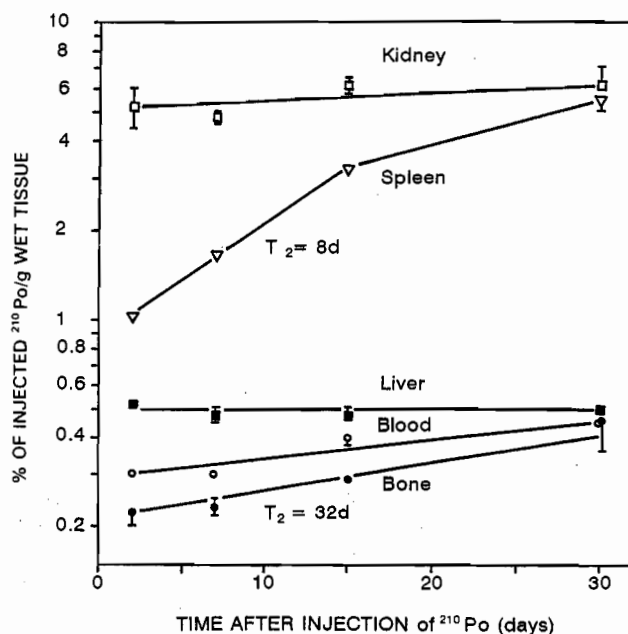


Figure 3. Concentration of  $^{210}\text{Po}$  in different body tissues after i.m. injection into the untreated control rat. For further explanations, see figure 2.

injection site, or in the liver, but only in blood, spleen, and skeleton.

Table 2 shows the results of the second experiment in which repeated local (3) and systematic (7–10) treatments were administered within 2 weeks after  $^{210}\text{Po}$ . The  $^{210}\text{Po}$  at the injection site was substantially reduced by MeOBGDTC but not by DDTC. The total retention of  $^{210}\text{Po}$  was reduced with MeOBGDTC to 62% of controls, i.e. a similar effectiveness to that found with BAL (Table 1).

Table 3 summarizes the results of the third experiment in which the effects of three derivatives of DMSA were compared with that of DMPS. After 2 weeks,  $^{210}\text{Po}$  at the injection site was reduced by DMPS to 12% and the retention in liver, spleen, muscle and skeleton was reduced to 14–40% of control values. However, the blood content was unchanged and deposition in the kidney increased to 340% of controls. Thus, the overall retention of  $^{210}\text{Po}$  in the organs was increased by about 60% and total retention of  $^{210}\text{Po}$  (including injection site) was reduced to only about 68% of control values. Similar reduction of total  $^{210}\text{Po}$  retention was achieved by Mi-ADMS; however, retention of  $^{210}\text{Po}$  at the injection site was greater, 31% than with DMPS, 12%, but deposition of  $^{210}\text{Po}$  in organs was not increased.

Table 4 shows data from the fourth experiment on the effect of protracted administration of large amounts of DMSA or DMPS. Essentially identical results were obtained at the highest dosage when the chelator was injected only systemically or systemically plus locally. In

Table 1. Effect of local and systemic treatment with BAL on the retention of i.m. injected  $^{210}\text{Po}$  in rat.

Treatment <sup>a</sup>				Retention of $^{210}\text{Po}$ at 7 days (% of control values) <sup>b</sup>							
Dosage (mmol kg <sup>-1</sup> )		Time		Injection site	Blood	Kidney	Liver	Spleen	Skeleton	All organs	Total
Local	Systemic	Local	Systemic								
1 × 0.5	—	1 h	—	95 ± 24	96 ± 5	80 ± 4	167 ± 23	101 ± 8	93 ± 7	98 ± 5	96 ± 13
1 × 0.5	—	4 d	—	71 ± 26	71 ± 7	86 ± 11	269 ± 42	102 ± 13	107 ± 7	135 ± 15	97 ± 9
2 × 0.5	—	1 h, 4 d	—	19 ± 10	89 ± 7	80 ± 4	277 ± 33	95 ± 5	107 ± 14	131 ± 7	65 ± 5
—	5 × 0.2	—	1 h, 1,2,3,4 d	103 ± 27	76 ± 5	58 ± 5	143 ± 19	61 ± 8	64 ± 7	88 ± 6	98 ± 15
1 × 0.5	4 × 0.2	4 d	1 h, 1,2,3 d	61 ± 13	73 ± 2	67 ± 3	190 ± 13	81 ± 4	79 ± 7	101 ± 4	78 ± 8

<sup>a</sup> Local, i.m. at  $^{210}\text{Po}$  injection site; systemic, i.m. into the opposite thigh.

<sup>b</sup> Arithmetic means ± SEM; five rats per group. Values in *italics* are statistically significant from controls ( $p < 0.05$ ). Control values (% injected activity): injection site, 31.0 ± 8.0; blood, 4.3 ± 0.2; kidney, 8.1 ± 0.9; liver, 4.8 ± 0.4; spleen, 0.83 ± 0.05; skeleton, 2.8 ± 0.2; all organs minus injection site, 20.8 ± 1.4; and total, 51.3 ± 6.3.

Table 2. Effect of local systemic treatment with DDTC and MeOBGDTC on the retention of i.m. injected  $^{210}\text{Po}$  in rat.

Treatment <sup>a</sup>					Retention of $^{210}\text{Po}$ at 14 days (% of control values) <sup>b</sup>							
Chelator	Dosage (mmol kg <sup>-1</sup> )		Time		Injection site	Blood	Kidney	Liver	Spleen	Skeleton	All organs	Total
	Local	Systemic	Local	Systemic								
DDTC	—	10 × 1.0	—	1 h, 1–4 d 7–11 d (daily)	75 ± 40	70 ± 1	68 ± 4	205 ± 39	56 ± 6	41 ± 5	99 ± 10	81 ± 21
DDTC	3 × 1.0	7 × 1.0	1 h, 4 d 9 d	1,2,3 d 7,8,10,11 d	51 ± 30	68 ± 4	67 ± 4	154 ± 68	63 ± 10	61 ± 8	87 ± 17	64 ± 17
MeOBG	3 × 1.0	7 × 1.0	1 h, 4 d 11 d	1,2,3 d 7,8,10,11 d	43 ± 19	77 ± 9	54 ± 9	193 ± 39	83 ± 7	62 ± 10	94 ± 7	62 ± 9

<sup>a</sup> Local, i.m. at  $^{210}\text{Po}$  injection site; systemic, s.c. on the back.

<sup>b</sup> Control values (% injected activity): injection site, 27.7 ± 2.4; blood, 5.0 ± 0.3; kidney, 8.0 ± 0.7; liver, 5.6 ± 0.7; spleen, 1.0 ± 0.1; skeleton, 2.1 ± 0.2; all organs minus injection site, 22.2 ± 1.3; and total, 52.4 ± 4.8.

For other explanations, see Table 1.

both cases the effectiveness of DMPS was superior to that of DMSA. The former mobilized about nine times more  $^{210}\text{Po}$  from the injection site than in untreated controls. In spite of this, less  $^{210}\text{Po}$  was deposited in organs; the total retention was reduced to 40% of controls, which is substantially better than in the three previous experiments. On the other hand, DMSA mobilized less  $^{210}\text{Po}$ , which was then deposited mainly in the kidney. Similarly, when DMPS was administered at lower dosage than that described above, less effect on  $^{210}\text{Po}$  was achieved because of the high accumulation of translocated  $^{210}\text{Po}$  by the kidneys (three times more than in controls). This effect was independent of whether DMPS was administered as 10 daily injections, as continuous infusion, or in drinking water.

In the fifth experiment (Table 5) the removal of  $^{210}\text{Po}$  from the wound site by repeated local injections of DDTC or 5 bismarckdithioates was compared with that

for DMPS and DMBD (the latter is an analogue of BAL). Only HOEtTTC and DMBD reduced  $^{210}\text{Po}$  at the injection site to 40% of controls and reduced its total retention to 65 and 75% of controls respectively. Although DMPS removed more  $^{210}\text{Po}$  from the injection site, the translocated radioactivity was mainly accumulated by the kidney, so that the overall retention of  $^{210}\text{Po}$  remained unchanged. After treatment with HOEtTTC and DMBD there was an increase of  $^{210}\text{Po}$  in brain and muscle respectively; both chelators reduced accumulation of  $^{210}\text{Po}$  by the kidney.

In view of the above results, the sixth experiment combined local injections of DMPS to remove  $^{210}\text{Po}$  from the wound, with systemic injections of the other chelators, to prevent accumulation of  $^{210}\text{Po}$  in tissues, mainly the kidney. Table 6 shows that after 1 week (experiment A) < 10% of the control  $^{210}\text{Po}$  was found at the injection site. The total retention of  $^{210}\text{Po}$  was

Table 3. Effect of local and systemic treatment with DMPS and derivatives of DMSA on the retention of  $^{210}\text{Po}$  in rat.

Chelator <sup>a</sup>	Injection site	Retention of $^{210}\text{Po}$ at 14 days (% of control values) <sup>b</sup>							Total
		Blood	Kidney	Liver	Spleen	Skeleton	Muscle	All organs	
DMPS	12 ± 1	115 ± 4	340 ± 19	43 ± 4	29 ± 1	20 ± 8	14 ± 8	157 ± 8	68 ± 3
Mi-ADMS	31 ± 12	146 ± 12	149 ± 5	77 ± 8	45 ± 4	25 ± 3	44 ± 14	99 ± 2	59 ± 6
Di-ADMS	85 ± 22	111 ± 8	91 ± 8	118 ± 4	108 ± 5	33 ± 5	90 ± 15	90 ± 3	87 ± 13
Do-ADMS	92 ± 26	70 ± 5	88 ± 7	91 ± 7	76 ± 9	22 ± 6	74 ± 16	74 ± 5	85 ± 15

<sup>a</sup> Local treatment (i.m.) at 1 h and 5 days; systemic treatment (s.c. except of DMPS i.m.) at 1, 7, 9, 12 days. Each dosage 0.75 mmol kg<sup>-1</sup>

<sup>b</sup> Five rats per group. Control values (% injected activity): injection site, 29.2 ± 2.6; blood, 2.3 ± 0.3; kidney, 8.0 ± 0.7; liver, 2.9 ± 0.3; spleen, 1.1 ± 0.1; skeleton, 2.8 ± 0.4; muscle, 3.4 ± 0.5; all organs minus injection site, 20.5 ± 1.9; and total, 49.7 ± 3.2.

For further explanations, see Table 1.

Table 4. Effect of local and systemic treatment with DMSA and DMPS on the retention of i.m. injected  $^{210}\text{Po}$  in rat.

Chelator (route of entry)	Treatment <sup>a</sup>				Retention of <sup>210</sup> Po at 15 days (% of control values) <sup>b</sup>								All Total
	Dosage (mmol kg <sup>-1</sup> )		Time										
	Local	Systemic	Local	Systemic	Injection site	Blood	Kidney	Liver	Spleen	Skeleton	organs		
DMSA (inj.)	—	10 × 1.0	—	1 h, 1,2,3,4,7 8,9,10,11 d	50 ± 15	79 ± 13	188 ± 13	55 ± 7	79 ± 7	124 ± 5	128 ± 5	87 ± 6	
DMSA (inj.)	3 × 1.0	7 × 1.0	1 h, 4 d 9 d	1,2,3,7, 8,10,11 d	44 ± 17	63 ± 4	184 ± 9	61 ± 7	64 ± 7	110 ± 5	122 ± 5	81 ± 8	
DMPS (inj.)	—	10 × 1.0	—	1 h, 1,2,3,4,7 8,9,10,11 d	12 ± 3	77 ± 13	107 ± 6	50 ± 2	21 ± 7	14 ± 2	73 ± 4	41 ± 3	
DMPS (inj.)	3 × 1.0	7 × 1.0	1 h, 4 d 9 d	1,2,3,7, 8,10,11 d	12 ± 4	73 ± 14	108 ± 17	36 ± 5	21 ± 7	14 ± 2	70 ± 8	40 ± 5	
DMPS (inj.)	—	10 × 0.4	—	1 h, 1,2,3,4,7 8,9,10,11 d	28 ± 6	89 ± 7	309 ± 7	48 ± 2	36 ± 7	38 ± 2	165 ± 3	94 ± 2	
DMPS (inf.)	—	0.2 d <sup>-1</sup>	—	0-15 d (cont.)	52 ± 21	91 ± 5	343 ± 11	52 ± 2	29 ± 7	33 ± 2	178 ± 5	112 ± 1	
DMPS (oral)	—	0.6 d <sup>-1</sup>	—	0-15 d (cont.)	73 ± 21	132 ± 9	347 ± 29	73 ± 2	36 ± 7	43 ± 5	193 ± 14	130 ± 6	

<sup>a</sup> Local, i.m. at  $^{210}\text{Po}$  injection site; systemic, s.c. on the back behind the neck. Time 0, immediately after  $^{210}\text{Po}$ . Inf., continuous infusion; oral, continuously in drinking water.

<sup>b</sup> Control values (% injected activity): injection site, 27.6 ± 4.7; blood, 5.6 ± 0.3; kidney, 10.8 ± 0.5; liver, 4.4 ± 0.1; spleen, 1.4 ± 0.2; skeleton, 4.2 ± 0.2; all organs minus injection site, 26.2 ± 0.7; and total, 54.0 ± 4.7.

For further explanations, see Table 1.

reduced by up to one-half of that in controls, although  $^{210}\text{Po}$  content was increased in several organs, mainly liver and kidney.

However, after 2-week treatment (Table 6, experiment B) using DMPS and HOEtTTC, the  $^{210}\text{Po}$  at the injection site and the retention of  $^{210}\text{Po}$  in the organs was reduced to 4 and 52% of controls respectively. Thus, total retention of  $^{210}\text{Po}$  in all tissues investigated plus the injection site decreased to 27% of controls. When only DMPS was used for local and systemic treatments, total retention of  $^{210}\text{Po}$  was reduced to 42% of controls, and there was still a significant increase of  $^{210}\text{Po}$  in the kidney. When local injections of DMPS were combined with systemic

administration of DDTC,  $^{210}\text{Po}$  in kidney, blood and brain increased so that the total retention of  $^{210}\text{Po}$  was twice as high as in rat treated with DMPS and HOEtTTC.

Because of the latter, a part of experiment B with combined treatment by DMPS and HOEtTTC was repeated and completed by a follow up of  $^{210}\text{Po}$  excretion. Figure 4 shows that the cumulative excretion of  $^{210}\text{Po}$  in faeces increased after treatment from 7 to 44%, and that in urine from 0.8 to 10% of the administered radioactivity. Thus, the total cumulative excretion of  $^{210}\text{Po}$  increased within 2 weeks from 8% of injected radioactivity in controls to 54% in treated rat, i.e. seven times.

Table 5. Removal of  $^{210}\text{Po}$  from simulated wounds in rat by local treatment with carbothioates and vicinal dithiols.

Retention of $^{210}\text{Po}$ at 7 days (% of control values) <sup>b</sup>										
Chelator <sup>a</sup>	Injection site	Blood	Kidney	Liver	Spleen	Skeleton	Muscle	Brain	All organs	Total
DDTC	66 ± 13	83 ± 4	105 ± 9	172 ± 32	79 ± 6	68 ± 5	166 ± 18	518 ± 73	124 ± 6	85 ± 9
MeTTC	67 ± 24	93 ± 8	57 ± 9	113 ± 27	69 ± 8	59 ± 7	74 ± 9	55 ± 9	79 ± 10	71 ± 18
EtTTC	105 ± 15	51 ± 2	50 ± 7	125 ± 13	61 ± 3	73 ± 4	81 ± 8	73 ± 5	76 ± 6	95 ± 10
HOEtTTC	41 ± 7	125 ± 15	50 ± 6	168 ± 34	122 ± 8	119 ± 8	121 ± 11	155 ± 9	110 ± 8	64 ± 6
PTTC	64 ± 13	111 ± 3	70 ± 9	146 ± 14	110 ± 15	100 ± 9	100 ± 5	91 ± 5	103 ± 8	77 ± 7
BTTC	117 ± 16	22 ± 4	25 ± 4	79 ± 8	62 ± 11	71 ± 9	58 ± 12	59 ± 9	50 ± 6	95 ± 10
DMBD	42 ± 11	84 ± 6	69 ± 7	189 ± 26	72 ± 8	85 ± 10	270 ± 33	155 ± 23	135 ± 15	73 ± 8
DMPS	14 ± 3	89 ± 5	616 ± 17	168 ± 71	127 ± 8	63 ± 3	87 ± 8	173 ± 18	271 ± 22	99 ± 8

<sup>a</sup> Local treatment (i.m.) at 1 h, and 2 and 4 days; each dosage 1 mmol kg<sup>-1</sup>.

<sup>b</sup> Ten controls, five rats per each treated group. Control values (% injected activity): injection site, 39.6 ± 4.9; blood, 2.9 ± 0.2; kidney, 6.3 ± 0.5; liver, 4.5 ± 0.7; spleen, 0.7 ± 0.1; skeleton, 2.1 ± 0.2; muscle, 3.3 ± 0.3; brain, 0.022 ± 0.002; all organs minus injection site, 19.6 ± 1.2; and total, 59.2 ± 4.4.

For further explanations, see Table 1.

Table 6. Effect of combined treatment with DMPS and carbodithioates on the retention of  $^{210}\text{Po}$  in rat.

Chelator <sup>a</sup> (route of entry) <sup>a</sup>		Retention of <sup>210</sup> Po (% of control values) <sup>b</sup>									
		Injection site	Blood	Kidney	Liver	Spleen	Skeleton	Muscle	Brain	All organs	Total
Experiment A.	Killed at 7 days										
DMPS +	(i.m.)	9 ± 3	116 ± 6	207 ± 24	226 ± 28	58 ± 4	69 ± 3	29 ± 8	69 ± 12	139 ± 10	57 ± 4
MeTTC	(s.c.)										
DMPS +	(i.m.)	7 ± 2	71 ± 3	98 ± 8	444 ± 24	45 ± 4	63 ± 4	27 ± 10	93 ± 5	133 ± 6	54 ± 2
EtTTC	(s.c.)										
DMPS +	(i.m.)	4 ± 2	115 ± 6	241 ± 14	151 ± 12	49 ± 4	54 ± 3	17 ± 6	116 ± 6	132 ± 6	51 ± 2
HOEtTTC	(s.c.)										
DMPS +	(i.m.)	9 ± 3	107 ± 6	119 ± 7	262 ± 39	100 ± 9	102 ± 9	183 ± 13	136 ± 7	160 ± 6	64 ± 2
DMBD	(s.c.)										
Experiment B.	Killed at 14 days										
DMPS +	(i.m.)	4 ± 1	93 ± 5	160 ± 8	49 ± 6	22 ± 3	23 ± 2	44 ± 1	71 ± 6	83 ± 4	42 ± 2
DMPS	(i.m.s.)										
DMPS +	(i.m.)	9 ± 2	126 ± 3	176 ± 6	102 ± 7	46 ± 2	22 ± 1	105 ± 8	328 ± 18	120 ± 3	63 ± 2
DDTC	(s.c.)										
DMPS +	(i.m.)	4 ± 1	156 ± 15	19 ± 3	90 ± 9	34 ± 3	36 ± 2	28 ± 2	63 ± 5	52 ± 4	27 ± 2
HOEtTTC	(s.c.)										

<sup>a</sup> Treatment in experiment A: i.m. at 2 h and 3 days (0.5 mmol kg<sup>-1</sup> each); s.c. at 2 h and days 1-4 (0.5 mmol kg<sup>-1</sup> each). Treatment in experiment B: i.m. at 2 h and 3 days (0.5 mmol kg<sup>-1</sup> each); s.c. at 2 h and days 3, 8, 10 plus days 1, 2, 4, 7, 9, 11 (0.5 plus 1.0 mmol kg<sup>-1</sup>, respectively). i.m., locally at Po injection site; i.m.s., systemically into opposite thigh.

<sup>b</sup> Control values (10 rats each; % injected activity) at 7 and 14 days: injection site, 35.7 ± 5.7, 29.7 ± 1.9; blood, 2.5 ± 0.3, 3.9 ± 0.2; kidney, 6.8 ± 0.4, 8.2 ± 0.2; liver, 3.6 ± 0.2, 3.1 ± 0.1; spleen, 0.7 ± 0.1, 1.3 ± 0.1; skeleton, 3.0 ± 0.2, 3.8 ± 0.3; muscle, 4.2 ± 0.5, 7.3 ± 0.1; brain, 0.033 ± 0.004, 0.083 ± 0.001; all organs minus injection site, 20.8 ± 1.5, 27.4 ± 0.6 and total, 56.5 ± 5.5, 57.1 ± 1.6.

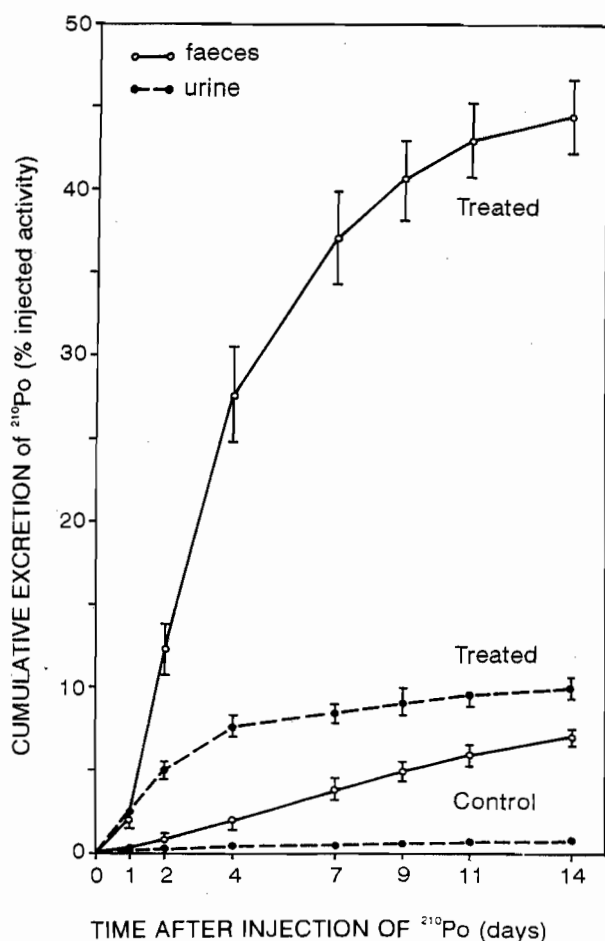


Figure 4. Enhanced excretion of i.m. injected  $^{210}\text{Po}$  in rats treated with local (i.m.) injections of DMPS and systemic (s.c.) injections of HOEtTTC. For treatment schedule and retention of  $^{210}\text{Po}$ , see Table 6, experiment B.

#### 4. Discussion

In these experiments with rat,  $^{210}\text{Po}$  was injected i.m. in a small volume of 0.3 M nitric acid to simulate a puncture wound in a human working with a solution of  $^{210}\text{Po}$ . For severe incorporation it would be desirable to complement surgical excision by suitable chelation treatment to enhance the translocation of residual  $^{210}\text{Po}$  from the wound and to prevent its deposition in body tissues.

The preliminary experiment on the biokinetics of  $^{210}\text{Po}$  in the untreated rat indicated that after a rapid decrease in wound retention within the first 2 days  $^{210}\text{Po}$  was translocated from an i.m. injection site rather slowly and accordingly there was a protracted accumulation and retention of  $^{210}\text{Po}$  in different organs. As to the former, our experience with chelation treatment of simulated wounds with  $^{239}\text{Pu}$  (Volf 1974) indicated that local treatment is more effective than systemic administration. This has been confirmed by others

(Harrison *et al.* 1978, 1980, Stradling *et al.* 1993). As to the accumulation of translocated  $^{210}\text{Po}$  in body tissues, our experience from experiments after i.v. injection of  $^{210}\text{Po}$  indicated that most chelators may merely induce redistribution of  $^{210}\text{Po}$ , rather than its enhanced excretion (Rencová *et al.* 1993, 1994).

While it was not known whether a single local chelate injection would be sufficient to remove  $^{210}\text{Po}$  from the wound, it was obvious that repeated systemic treatment will be necessary to bind  $^{210}\text{Po}$ , which is being continuously translocated into the blood. Last, we did not know if it was possible to remove  $^{210}\text{Po}$  from an injection site and prevent its deposition in body tissues when using the same chelator. Therefore, we performed pilot experiments with clinically acceptable chelators (BAL, DDTTC, DMSA, DMPS), which served as reference substances when their derivatives or analogues were used.

It has been demonstrated that DMPS can almost completely remove  $^{210}\text{Po}$  from simulated wounds (Volf *et al.* 1993). Our present data indicate that at least two large dosages have to be injected, but that the first treatment can be delayed for at least 2 h. Surprisingly, it seems unnecessary to give the first two injections of DMPS locally, but if treatment is continued then it is desirable to inject the ligand only i.m. since after several s.c. injections of high dosages of DMPS ( $1 \text{ mmol kg}^{-1}$ ) local tissue irritation develops. When DMPS is administered in smaller dosages, which do not cause irritation, accumulation of translocated  $^{210}\text{Po}$  by the kidney increases considerably, irrespective of whether DMPS is injected once a day or administered continuously by s.c. infusion or in drinking water. It seems therefore desirable to combine a local treatment by DMPS with systemic treatment by another chelator.

We compared the effectiveness of chelators that removed a significant fraction of  $^{210}\text{Po}$  from the injection site (Table 7). The upper half of Table 7 summarizes the results obtained after a week of treatment. In addition, about one-quarter to one-third of the injected  $^{210}\text{Po}$  was removed by local injections of HOEtTTC, DMBD, BAL and DMPS. When deposition of translocated  $^{210}\text{Po}$  increased above that in untreated controls, it was less than the  $^{210}\text{Po}$  fraction, which was additionally translocated by treatment, with one exception: after local treatment with DMPS virtually all  $^{210}\text{Po}$  additionally translocated from the simulated wound appeared in the kidney. This untoward side effect could be substantially diminished by additional systemic administration of MeTTC, EtTTC, HOEtTTC or DMBD.

Results obtained after 2 weeks of treatment are shown in the lower half of Table 7. Combined local and systemic treatment with DMSA, MeOBGDTC or Mi-ADMS mobilized an additional one-fifth of the

Table 7. Comparison of effectiveness of chelators which removed a significant fraction of  $^{210}\text{Po}$  from simulated wounds in rat.

Treatment <sup>a</sup>		Fraction of <sup>210</sup> Po (% injected activity)					
		Translocation of Po from wound site <sup>b</sup>		Retention of Po in all organs <sup>c</sup>		Increase in Po retention (organ)	
		Total	Increase after treatment	Total	Change after treatment		
Local	Systemic						
At 7 days after <sup>210</sup> Po							
Controls		62 ± 3	—	21 ± 2	—	—	
HOEtTTC	—	88 ± 2	26 ± 3	23 ± 2 <sup>d</sup>	—	brain	(0.03)
DMBD	—	87 ± 3	25 ± 4	28 ± 3 <sup>d</sup>	—	muscle	(8.9)
BAL	—	94 ± 3	32 ± 4	27 ± 2	+ (6 ± 3)	liver	(13.3)
DMPS	—	96 ± 1	34 ± 3	56 ± 5	+ (35 ± 5)	kidney	(38.8)
						brain	(0.04)
DMPS	DMBD	97 ± 1	35 ± 3	33 ± 1	+ (13 ± 2)	liver	(9.4)
						muscle	(7.7)
						brain	(0.05)
DMPS	MeTTC	97 ± 1	35 ± 3	29 ± 2	+ (8 ± 3)	kidney	(14.1)
						liver	(8.1)
DMPS	EtTTC	98 ± 1	36 ± 3	28 ± 1	+ (7 ± 2)	liver	(16.0)
DMPS	HOEtTTC	99 ± 1	37 ± 3	28 ± 1	+ (7 ± 2)	kidney	(16.4)
						liver	(5.4)
At 14/15 days after <sup>210</sup> Po							
Controls		71 ± 3	—	24 ± 1	—	—	
DMSA	DMSA	88 ± 5	17 ± 5	29 ± 1	+ (5 ± 1)	kidney	(19.9)
MeOBGDTC	MeOBGDTC	88 ± 5	17 ± 6	22 ± 2 <sup>d</sup>	—	—	
Mi-ADMS	Mi-ADMS	91 ± 4	20 ± 4	24 ± 1 <sup>d</sup>	—	kidney	(11.9)
						blood	(3.4)
DMPS	DDTC	97 ± 1	26 ± 3	29 ± 1	+ (5 ± 1)	kidney	(13.1)
						blood	(4.9)
						brain	(0.3)
DMPS	DMPS	99 ± 1	28 ± 3	20 ± 1	— (4 ± 1)	kidney	(13.1)
DMPS	HOEtTTC	99 ± 1	28 ± 3	12 ± 1	— (12 ± 1)	blood	(6.1)

<sup>a</sup> Local treatment i.m. at injection site; systemic treatment s.c. on back, DMPS i.m. into opposite thigh.

<sup>b</sup> 100 minus retention at injection site. Increase, treated minus control values. Values are arithmetic mean ± SEM (22 and 17 controls at 7 and 15 days respectively; five rats per each treated group).

<sup>c</sup> Sum of retention in all organs tested minus Po at injection site. Change, difference between treated and control rats.

<sup>d</sup> Not significantly different from controls. All other values are significantly different from respective controls ( $p < 0.05$ ).

injected amount of  $^{210}\text{Po}$ , without causing any substantial change in retention of translocated  $^{210}\text{Po}$ . Combined local treatment with DMPS and systemic treatment with DMPS or DDTC removed virtually all  $^{210}\text{Po}$  from the injection site without increasing retention of translocated  $^{210}\text{Po}$ . When DMPS was combined with HOEtTTC, only about 15% of the administered  $^{210}\text{Po}$  was estimated to remain in the body, compared with about 60% in untreated controls. Excretion data demonstrated that  $^{210}\text{Po}$  mobilized from the wound site was eliminated mainly in the faeces (Figure 4).

It is concluded from the present study that a combined local treatment with two dosages of DMPS ( $0.5 \text{ mmol kg}^{-1}$ ) and systemic treatment with 10

dosages of HOEtTTC ( $0.5 \text{ mmol kg}^{-1}$ ) was the most effective. In order to characterize further the treatment effect achieved, Table 8 shows the data from this experiment in terms of concentration of  $^{210}\text{Po}$  in various tissues. This, as stated above, indicates the levels of radiation risk due to incorporated  $^{210}\text{Po}$  (its decay within 2 weeks after its administration would be  $< 10\%$  and can be neglected). In most organs, the concentration of  $^{210}\text{Po}$  was reduced by the treatment to about one-third or less of the corresponding control values. With respect to the known affinity of  $^{210}\text{Po}$  for erythrocytes it is not surprising that the radioactivity for translocated  $^{210}\text{Po}$  in whole blood increased 40% above that in controls.

An examination of the variation in polonium-mobilizing effectiveness, as the chemical

Table 8. Effect of combined treatment with DMPS and HOEtTTC on tissue concentration of i.m. injected  $^{210}\text{Po}$  in rat.

Group	$^{210}\text{Po}$ at 14 days (% injected activity/g wet tissue) <sup>a</sup>						
	Kidney	Spleen	Lymph nodes	Liver	Blood	Femur	Muscle
Controls	6.5 ± 0.7	2.6 ± 0.1	1.7 ± 0.8	0.44 ± 0.06	0.38 ± 0.03	0.24 ± 0.02	0.06 ± 0.01
Treated	1.1 ± 0.2	0.64 ± 0.07	0.13 ± 0.01	0.24 ± 0.03	0.53 ± 0.05	0.08 ± 0.01	0.02 ± 0.01

<sup>a</sup> For treatment schedule, see Table 6, experiment B.

Values are arithmetic means ± SEM (five control and six treated rats).

structure of the chelating agent is altered, reveals some interesting trends which can serve as a guide in efforts to develop more effective agents. The problem of the chelate-induced redistribution of polonium is an obvious difficulty with individual chelating agents of almost any type (Table 7). The redistribution of polonium to the kidney is probably the result of reduced pH in the renal fluids with a consequent enhancement of chelating agent protonation and a reduction in the effective stability constant. In the presence of chelating agents with reducing powers such as DMPS and carbodithioates (or dithiocarbamates), polonium is probably present as  $\text{Po}^{2+}$ , which complexes with the unoxidized chelating agent. The subsequent fate of such complexes is determined in part by their preferred route of elimination. BAL and carbodithioates enhance the liver levels of polonium (Tables 1 and 2), a finding which suggests an interruption in the biliary excretion of the complex. DMPS, DMSA, and Mi-ADMS enhance renal polonium levels, as might be expected from the loss of polonium from complexes which were undergoing renal excretion. The suppression of such processes appears to require two chelating agents with rather different characteristics, as can be seen from the data in Table 7. The data in Figure 4 indicate that the major portion of the excreted polonium when DMPS and HOEtTTC are both given is removed via the bile, thus reducing the amount of polonium presented to the renal tubules for possible absorption.

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## References

- BAKER, H. J., LINDSEY, J. R. and WEISBROTH, S. H., 1980, Selected normative data. In: *The Laboratory Rat*, vol. 2. Edited by: H. J. Baker, J. R. Lindsey and S. H. Weisbroth (Academic Press, New York), p. 257.
- HARRISON, J. D., DAVID, A. J. and STATHER, J. W., 1978, Experimental studies on the translocation of plutonium from simulated wound sites in the rat. *International Journal of Radiation Biology*, **33**, 457–472.
- HARRISON, J. D., DAVID, A. J. and STATHER, J. W., 1980, The wound clearance and distribution of plutonium, americium and curium in rodents. *International Journal of Radiation Biology*, **37**, 505–512.
- ILYIN, L. A., IVANIKOV, A. T., BAZHIN, A. G., KONSTANTINOVA, T. P. and AITKHOVA, G. A., 1977, Intake of  $^{210}\text{Po}$  into the body through the damaged skin and efficiency of some methods in preventing its absorption. *Health Physics*, **32**, 107–111.
- JONES, M. M. and CHERIAN, M. G., 1991, New developments in therapeutic chelating agents as antidotes for metal poisoning. *Critical Reviews in Toxicology*, **21**, 209–233.
- JONES, M. M., SINGH, P. K., GALE, G. R., SMITH, A. B. and ATKINS, L. M., 1992, Cadmium mobilization *in vivo* by intraperitoneal or oral administration of monoalkyl esters of meso-2,3-dimercaptosuccinic acid in the mouse. *Pharmacology and Toxicology*, **70**, 336–343.
- JONES, S. G., SINGH, P. K. and JONES, M. M., 1988, Use of the Topliss scheme for the design of more effective chelating agents for cadmium decorporation. *Chemical Research in Toxicology*, **1**, 234–237.
- PETER-WITT, E. and VOLF, V., 1984, Efficacy of different DTPA treatment schedules for removal of  $^{234}\text{Th}$  from simulated wounds in rats. *International Journal of Radiation Biology*, **45**, 45–49.
- RENCOVÁ, J., VOLF, V., JONES, M. M. and SINGH, P. K., 1993, Relative effectiveness of dithiol and dithiocarbamate chelating agents in reducing retention of polonium-210 in rat. *International Journal of Radiation Biology*, **63**, 223–232.
- RENCOVÁ, J., VOLF, V., JONES, M. M. and SINGH, P. K., 1994, Decorporation of polonium from rats by new chelating agents. *Radiation Protection Dosimetry*, **53**, 311–313.
- RENCOVÁ, J., VOLF, V., JONES, M. M., SINGH, P. K. and FILGAS, R., 1995, Bis-dithiocarbamates: effective chelating agents for mobilization of polonium-210 from rat. *International Journal of Radiation Biology*, **67**, 229–234.

- SEIDEL, A. and VOLF, V., 1972, Rapid determination of some transuranic elements in biological material by liquid scintillation counting. *International Journal of Applied Radiation and Isotopes*, **23**, 1-4.
- SINGH, P. K., JONES, M. M., GALE, G. R., ATKINS, L. M. and SMITH, A. B., 1989a, The mobilization of intracellular cadmium by butyl and amyl esters of meso-2,3-dimercaptosuccinic acid. *Toxicology and Applied Pharmacology*, **97**, 572-579.
- SINGH, P. K., JONES, M. M., JONES, S. G., GALE, G. R., ATKINS, L. M., SMITH, A. B. and BULMAN, R. A., 1989b, Effect of chelating agent structure on the mobilization of cadmium from intracellular deposits. *Journal of Toxicology and Environmental Health*, **28**, 501-518.
- STRADLING, G. N., GRAY, S. A., MOODY, J. C., PEARCE, M. J., WILSON, I., BURGADA, R., BAILLY, T., LEROUX, T., RAYMOND, K. N. and DURBIN, P. W., 1993, Comparative efficacies of 3,4,3-LIHOPO and DTPA for enhancing the excretion of plutonium and americium from the rat after simulated wound contamination as nitrates. *International Journal of Radiation Biology*, **64**, 133-140.
- VOLF, V., 1973, Dekorporierung von Radionukliden (Untersuchung und Polonium). *Strahlentherapie*, **145**, 101-115.
- VOLF, V., 1974, Experimental background for prompt treatment with DTPA of  $^{239}\text{Pu}$  contaminated wounds. *Health Physics*, **27**, 273-277.
- VOLF, V., 1975, The effect of combinations of chelating agents on the translocation of intramuscularly deposited  $^{239}\text{Pu}$  nitrate in the rat. *Health Physics*, **29**, 61-68.
- VOLF, V., RENCOVÁ, J., JONES, M. M. and SINGH, P. K., 1993, Preliminary data on treatment of simulated wounds contaminated with polonium. *Plzeň Medical Report*, **68** (Suppl.), 59-61.