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Metabolic and toxicological studies on cobalt

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Abstract

In this study, the following in vitro and in vivo experiments were carried out: (a) Rats were exposed to the radionuclide $^{57}Co^{2+}$ ions in single intraperitoneal or intravenous doses (from 10 ng to 1 mg Co/rat) or to 50 ng Co/rat/day through drinking water for 109 days. The target tissue for cobalt depended on the dosage and route of administration (e.g. lung, kidney or bone). Excretion took place mainly through urine (i.p. and i.v. administration) or feces. At 24 h, testis of rats i.v. injected with 10 ng Co/rat contained 0.056% of the dose, with ~0.036% in the epididymus, and 0.08% in the deferens. No radioactivity was found in the germinal cells. (b) In vitro incorporation of cobalt in rat sperm. Cobalt enters the germinal cells suggesting that in vivo barriers against the incorporation of the element in sperms may occur. (c) Dose-effect relationships in BALB/3T3 cell cultures exposed to concentrations of cobalt from 1000 to 1 μM of Co^{2+}. Cobalt induced a dose dependent cytotoxic response. At 10 μM, cell growth was reduced to about 30%. No inhibition was found at 1 μM. Morphological transformation assays gave negative results when the cells were exposed to 1000 μM of cobalt.

Keywords: Cobalt; Metabolism; Rats; Cell cultures; Radiotracers

1. Introduction

High levels of exposure to cobalt containing dust may cause pulmonary effects as respiratory irritation, lung fibrosis and pneumoconiosis [1,2]. Increased levels of Co have been found in lung and lymph nodes of patients with hard metal lung disease [3]. Cobalt is considered to be the major causative factor for the observed disease, a tungsten carbide — cobalt mixture being more toxic to the respiratory tract than cobalt alone [4].

Cobalt compounds are also used in the production of various salts used in electroplating, in pigments and as additives in animal feeds. Allergic skin diseases caused by cobalt salts are well known [5] and myocardial effects have been observed in heavy beer drinkers when Co was added as a foam stabilizer [6]. Thus, cobalt can enter the body by different routes, mainly absorbed from the pulmonary and the gastrointestinal tract. Recently, considerable concentrations of Co were found also in pubic hair, toe nails and sperm of exposed workers [7], samples which could provide information on long-term exposure to the metal.

Cobalt is known as a component of vitamin B_{12} (cyanocobalamin). No other essential function for the element in humans is known [8]. Since the origin of the observed effects in persons exposed to cobalt is not yet well known, and which mech-

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anisms are involved, we undertook this study using in vivo and in vitro experiments to obtain more knowledge on the toxicokinetic and metabolic behaviour of low doses of Co after different routes of exposure and time.

2. Materials and methods

2.1. Chemicals and radiochemicals

All reagents used were of analytical grade. Stable $CoCl_2$ was supplied by Merck (Italy). Cyanocobalamin (vitamin B_{12}) was supplied by (Sigma Chemical Co., St. Louis, MO). Carrier-free ^{57}Co (>4 mCi/μg Co) as cobalt chloride was purchased from Dupont de Nemours (Germany) and was used to prepare the ^{57}Co-labelled solutions by dilution with stable $CoCl_2$ up to the desired final concentrations.

2.2. Radio-counting of ^{57}Co

The radioactivity of ^{57}Co in all samples was measured with a Philips Automatic Gamma Counter PW 4800 apparatus using the characteristic line of 122 keV. The amount of cobalt present in the samples was determined by comparison with ^{57}Co standard solutions of known specific radioactivity.

2.3. Animals and rat sperm

Male Sprague–Dawley rats, (Nossan, Milan, Italy) weighing 150–180 g (long-term experiments) or 200–220 g (short-term experiments) were used. They were maintained on a pellet diet and mineral water ad libitum under controlled conditions of humidity, temperature and light. Animals used for excretion studies were kept in metabolic cages for the separate collection of urine and feces.

Rat sperm for in vitro studies were obtained from two adult rats. They were sacrificed, the cauda epididymus excised and placed in a Petri dish containing 10 ml of Dulbecco's Ca- and Mg-free phosphate-buffered saline with 10 mg/ml of bovine serum albumin at 37°C [9]. The epididymus was minced and kept at 37°C before sperm cell counting.

2.4. BALB/3T3 cells

The embryo cell-line BALB/3T3 clone A 31-1-1 (passages 10) were cultured in Eagle's minimal essential medium (MEM) supplemented with 10% fetal calf serum (FCS, Gibco, Mascia Brunelli, Monza, Milan, Italy), penicillin (100 IU/ml) and streptomycin (100 ml/mg) (Sigma Chemical Co., St. Louis, MO).

The FCS was heat-inactivated at 56°C for 30 min. The cells were cultured in plastic Petri dishes or plate flasks (Costar) and maintained at 37°C in a humidified incubator with 5% CO_2 atmosphere as previously described [10]. Phosphate-buffered saline solution (PBS) was supplied by Gibco (Mascia Brunelli, Monza, Italy), and trypsin-EDTA from Sigma Chemical Co. (St. Louis, MO).

2.5. In vivo short-term experiments

They included the following studies: (i) distribution of Co in rat tissues, subcellular fractions and intracellular components (ii) excretion pattern.

(i) Four rats were injected i.v. with 10 ng ^{57}Co as $CoCl_2$ per rat in saline solution. After 24 h, the animals were anesthesized and sacrificed by cardiac puncture. The tissues were dissected out, washed and weighed. Blood was collected with heparinized syringes. All samples as well as urine and feces were counted for ^{57}Co radioactivity. Whole blood was centrifuged at 2500 × g for 15 min to separate RBC and plasma. The subcellular distribution of ^{57}Co in lung, spleen, liver, kidney and testis was carried out on the homogenized tissues in ammonium acetate buffer (10 mM, pH 7). The homogenates were then centrifuged at 700 × g for 10 min to sediment nuclei and cell debris, at 9000 × g for 15 min to obtain the mitochondrial fraction and at 30 000 × g for 25 min to seperate the lysosomal fraction. The supernatant was then centrifuged at 105 000 × g for 90 min to yield microsomes and the cytosol. The cytosols of lung, liver and kidney were chromatographed on Sephadex G 150 as well as on Sephadex G 25 resins. The resins were equilibrated with ammonium acetate buffer, 10 mM, pH 7 and calibrated with a set of proteins with known molecular weights. The UV profile of the eluate at 280 nm was recorded by a LKB Uvicord III instrument and the ^{57}Co radioactivity measured in all eluted fractions.

(ii) Ten rats were injected i.p. with 10 ng $^{57}Co^{2+}$

per rat and urine and feces collected separately every day for 7 days. At day 3 and day 7, the tissues were examined as described in the 24-h experiment. To determine the chemical form of cobalt, the urine after 1 day was passed through a Chelex 100 (100–200 mesh) column (BioRad, Milan, Italy) previously equilibrated with 10 ml 2 M HNO_3, 20 ml 1 M NH_4OH and water to a final pH of 6.6. The eluate contained the organic Co form, whereas the inorganic Co was retained on the resin. The eluate was then chromatographed on Sephadex G 25 resin as described above.

2.6. In vivo long-term experiments

They included the following studies: (i) distribution of Co in tissues and subcellular fractions (ii) incorporation of the element into components of the reproductive system.

(i) Two groups of 10 rats each were injected i.p. with a single dose of 5 μg $^{57}Co^{2+}$ per rat and 1 mg $^{57}Co^{2+}$ per rat, respectively and kept for 100 days with free access to food and water. After this time, the tissues were examined as in the previous experiments. In another study, 12 animals were given cobalt via drinking water. The approximate dose was 50 ng Co^{2+} per rat per day for 109 days. At different time intervals (72, 95, 107 and 109 days), the rats were sacrificed and tissues measured as before. From day 73–74, three rats were kept in metabolic cages to collect urine and feces. On day 109, subcellular distribution on the liver and kidney of the rats was carried out as described above.

2.7. In vitro experiments

They included the following experiments: (i) Uptake of Co by rat sperm cells. Three equal parts of rat sperm suspension containing $(2.37 \pm 0.37) \times 10^7$ cells/3 ml were each incubated separately with 10 ng, 5 μg and 1 mg, respectively of ^{57}Co as chloride for 2 h at 37°C in a water bath. The spermatozoa were counted, washed and measured for radioactivity [11]. (ii) Uptake, cytotoxicity and morphological transformation of Co in BALB/3T3 cell line.

Cytotoxicity and morphological transformation assays of Co in BALB/3T3 cells were performed as previously described [12]. Briefly, in cytotoxicity experiments, 200 cells were seeded into Petri dishes in complete culture medium (MEM) and maintained at 37°C for 24 h. Then, the medium was changed with one containing the selected Co concentration and the cells incubated for 24, 48 and 72 h. The medium was changed with a new one (without Co), the cells reincubated for 7 days, fixed and stained and the number of colonies counted by an inverted optical microscope, establishing the Colony Forming Efficiency (CFE). Percentages of the number of colonies of the exposed cultures were compared to those observed in unexposed controls.

In morphological transformation experiments, 10^4 cells (after incubation with the MEM for 24 h) were exposed for 72 h to cobalt-containing MEM, incubated for 5 weeks, fixed, stained and the number of type (III) foci (index of irreversible neoplastic transformation) determined per clonal survivor (spontaneous transformation frequency in BALB/3T3 cells under our experimental conditions was 10^{-5}).

Uptake and intracellular distribution of $^{57}Co^{2+}$ in BALB/3T3 cells was investigated by growing and exposing subconfluent cultures (T25 or T75 flask) to selected concentrations of ^{57}Co-labelled Co^{2+} solutions in 2 or 4 ml of MEM, which at the desired time, was removed. The adherent cells monolayers were then washed three times with PBS, trypsinated (by trypsin-EDTA solution), centrifuged ($600 \times g$, 2 min) and resuspended in 1.5 ml PBS. Total cells were counted using a haemocytometer, then sonicated and 1 ml of the homogenate centrifuged at $105\,000 \times g$ for 90 min at +4°C (TL-100 ultracentrifuge, Beckmann Instruments, Palo Alto, CA) in order to separate pellets (cell organelles) and supernatant (cytosol). ^{57}Co was counted in total cell suspension, in cell organelles and cytosol fractions by gamma-counting (see section on radio-counting of ^{57}Co).

3. Results and discussion

3.1. Short-term studies

Table 1 shows the tissue distribution 24 h after a single i.v. injection of 10 ng ^{57}Co per rat as chloride expressed as ng/g wet wt. The highest concentrations were found in lung and large intestine, followed by kidney, liver and spleen. This confirms

Table 1
Tissue distribution of Co 24 h after i.v. injection of 10 ng $^{57}Co^{2+}$ per rat as chloride

Tissue	Cobalt content (ng/g wet tissue)
Lung	0.05 ± 0.02
Kidney	0.04 ± 0.01
Liver	0.03 ± 0.01
Spleen	0.03 ± 0.01
Ribs	0.008 ± 0.003
Femur	0.006 ± 0.003
Skull	0.005 ± 0.003
Large intestine	0.05 ± 0.002
Small intestine	0.004 ± 0.001
Pancreas	0.004 ± 0.002
Heart	0.004 ± 0.002
Stomach	0.0035 ± 0.002
Skin	0.002 ± 0.001
Testis	0.0022 ± 0.0008
Epididymus	0.0021 ± 0.001
Vas deferens	0.0001 ± 0.00008
Blood (μg/ml)	0.003 ± 0.001
Plasma (% of whole blood)	89.9 ± 2.9
Red blood cells (% of whole blood)	10.1 ± 3.1

that Co is highly soluble [13,14] as seen by its presence in plasma, i.e. about 90% of the total blood, and then quickly distributed in the above mentioned organs. In the other tissues, the cobalt concentration was about 10 times lower. Among these, bones, such as ribs, femur and skull showed the highest values.

The intracellular distribution of cobalt in lung, spleen, liver and kidney is shown in Fig. 1. With the exception of the kidney, cobalt was present in the nuclear fraction between 55 and 70% of the homogenate. The 40% found in the cytosol fraction of the kidney represents a more soluble fraction which would be more easily excreted. Gel chromatography on G 150 resin of lung, liver and kidney cytosols (Fig. 2) showed that more than 50% of Co was eluted in one peak in the low molecular weight region. The elution profiles of the same cytosols on G 25 resin showed that in liver and kidney, these fractions were associated with molecular weights of about 1400 Da which corresponds to that of vitamin B_{12}. Autopsy studies on humans showed that the liver contained the highest Co concentration [15]. Since the total liver content of Co in the

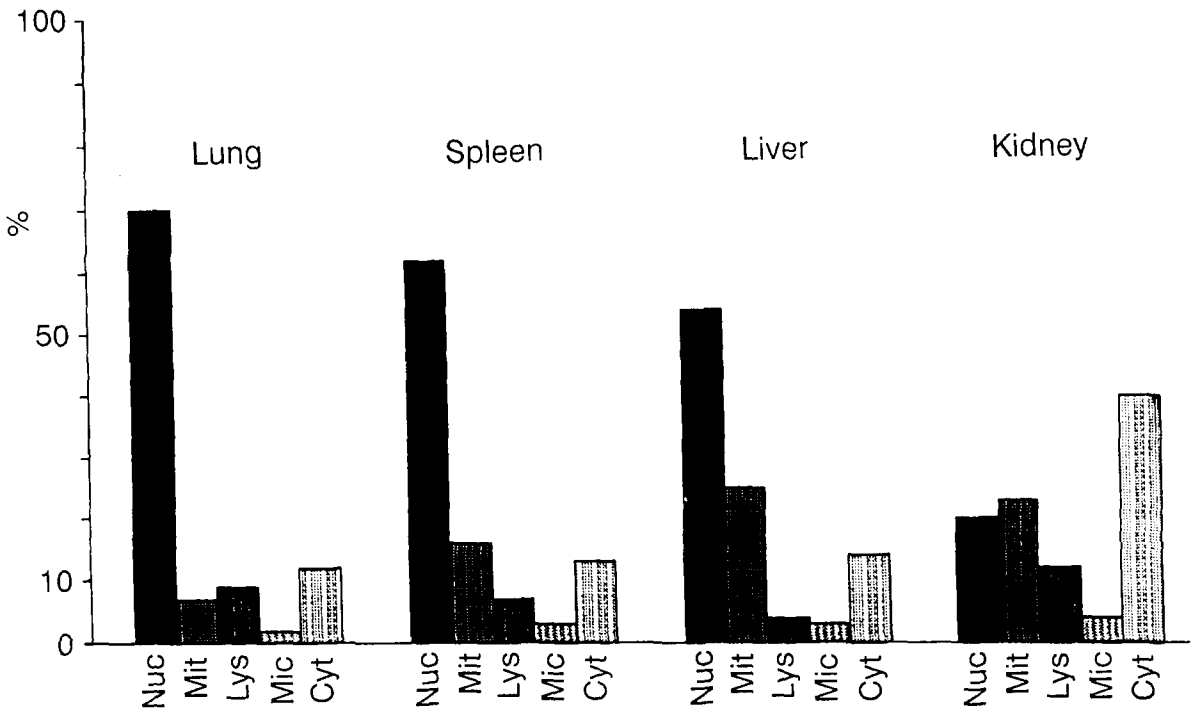

Fig. 1. Intracellular distribution of cobalt in lung, spleen, liver and kidney of rats 24 h after i.v. injection of 10 ng $^{57}Co^{2+}$ per rat as chloride.

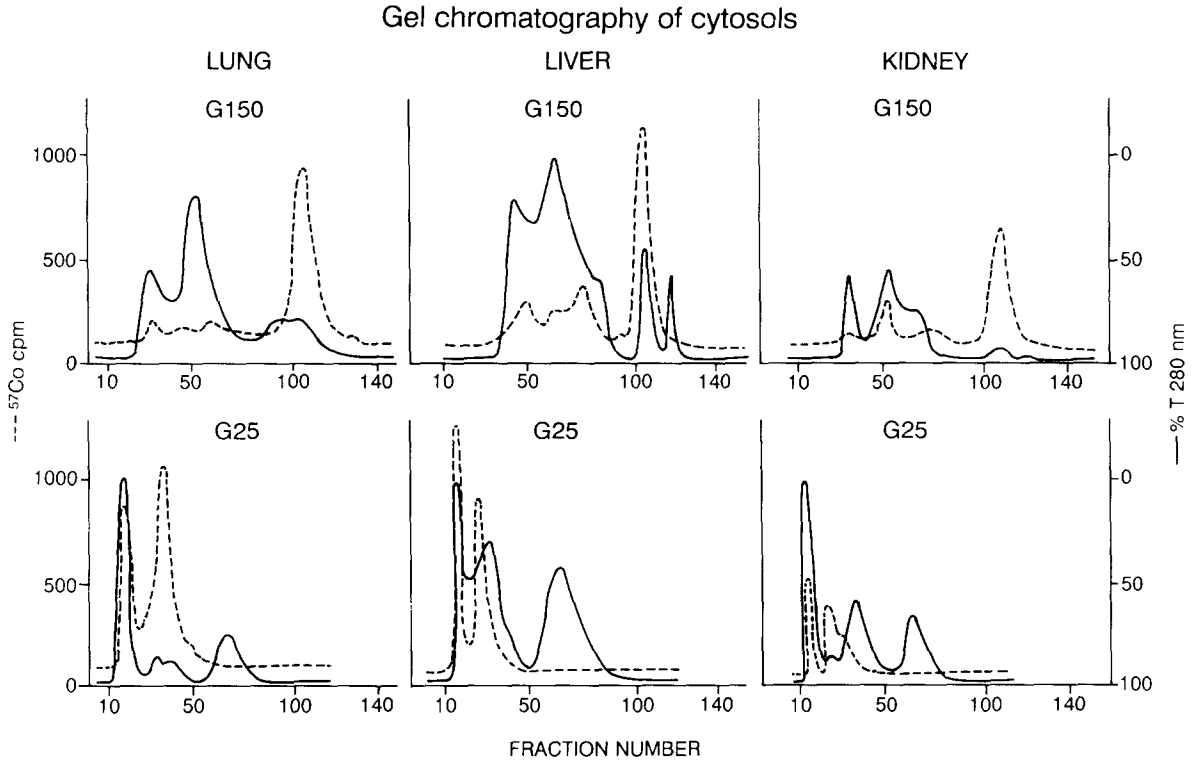

Fig. 2. Elution profiles from Sephadex G 150 and Sephadex G 25 columns of lung, liver and kidney cytosols 24 h after i.v. injection of 10 ng $^{57}Co^{2+}$ per rat as chloride.

form of vitamin B_{12} is estimated to be about 1.7 mg, the cobalt values found in human liver could be present mainly in the form of vitamin B_{12}. In the lung, the peak of ^{57}Co was present in the region of molecular weights of about 400 Da representing a mobile or diffusible cobalt form. The association of cobalt with high molecular weight components would be involved in the retention of the element.

3.2. Excretion

Fig. 3 shows the Co content in tissues 3 and 7 days after a single i.p. injection of 10 ng Co^{2+} per rat. At these times, cobalt was already cleared from the blood stream. After 3 days, the element was mainly present in the pancreas; about 12% of the dose/g $\times$ 10^3, followed by kidney (8% of the dose/g $\times$ 10^3), small intestine, liver, spleen and large intestine.

Seven days after treatment, this pattern changed and the highest concentration was found in the kidney; about 25% of the dose/g $\times$ 10^3, followed by liver, spleen and pancreas. This shows that, with time, Co concentrates in different organs. The high amount present in the kidney could be characteristic of an accumulation of Co, since at that time, the urinary excretion was very low (Fig. 4), showing two phases: about 2.5% of the dose was excreted during the first day decreasing to less than 0.1% on day 7. Cobalt in the urine of workers exposed by inhalation showed the same two phase behaviour [16]. The fecal excretion was about 10 times lower, being 0.2% of the dose on the first day, showing the same pattern observed in exposed humans. Both urinary and fecal excretion seem to be dose dependent, since other authors [17] found higher values in rats after i.v. injection of about 80 μg Co per rat.

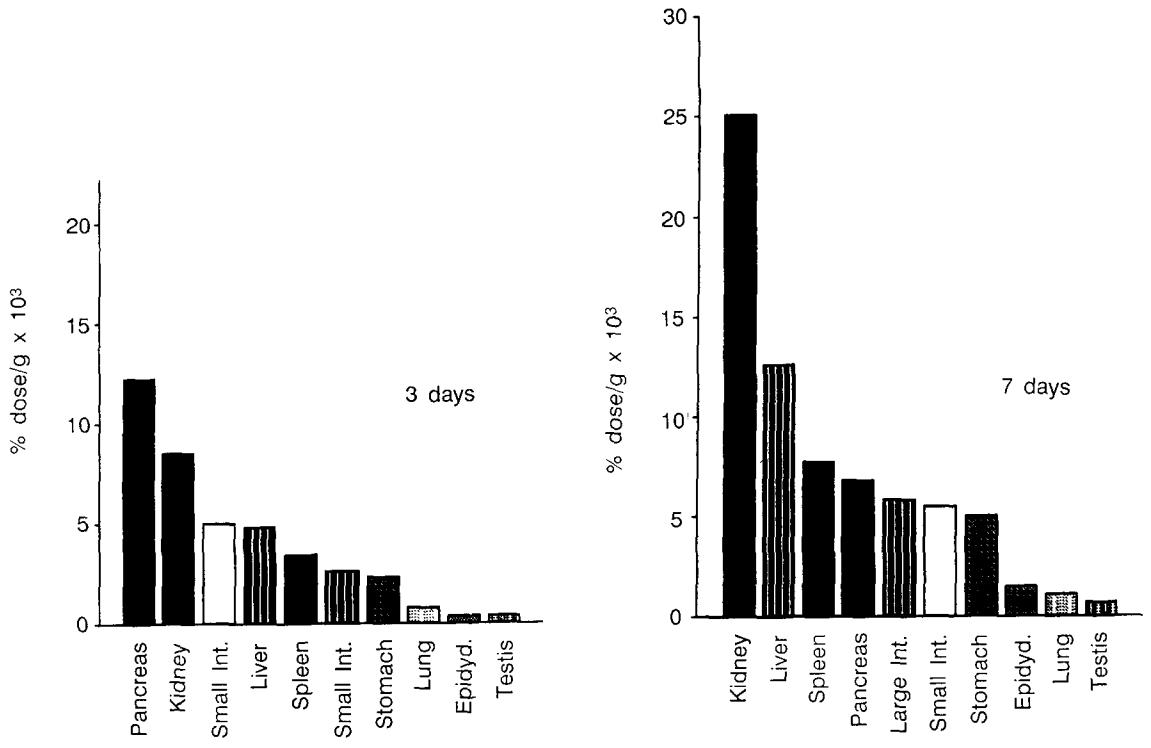

Fig. 3. Cobalt retention in rat tissues 3 and 7 days after i.p. injection of 10 ng $^{57}Co^{2+}$ per rat as chloride.

After submitting the 24-h urine to the Chelex 100 resin, about 50% of the ^{57}Co radioactivity was found in the eluate representing an organic Co form. This eluate was further chromatographed on Sephadex G 25. The profile is shown in Fig. 5.

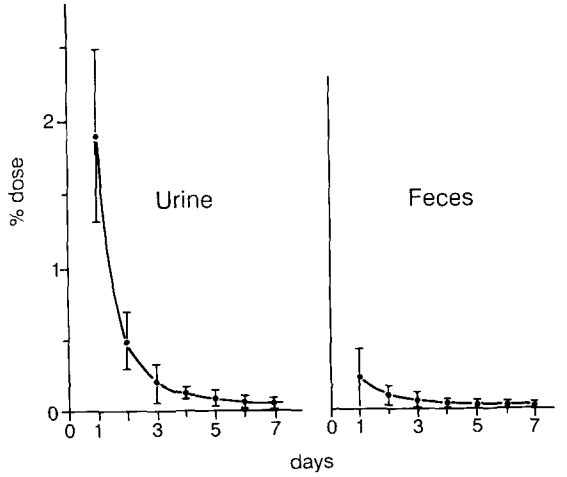

Fig. 4. Urinary and fecal cobalt excretion in rats during 7 days after a single i.p. injection of 10 ng $^{57}Co^{2+}$ per rat as chloride.

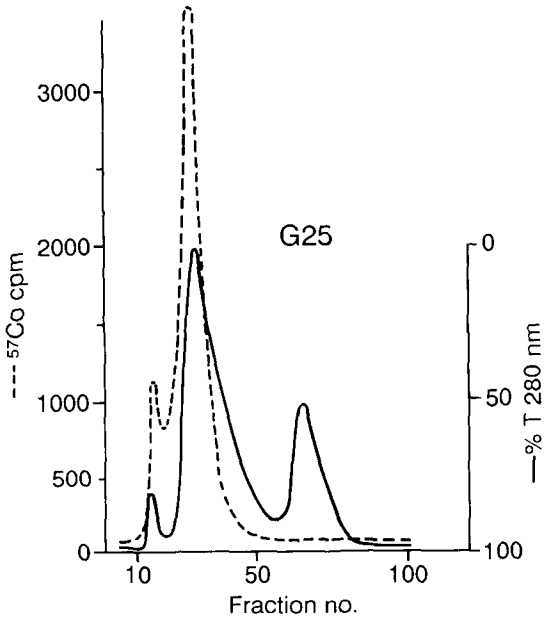

Fig. 5. Elution profile from Sephadex G 25 of the organic cobalt form present in urine 24 h after i.p. injection of 10 ng $^{57}Co^{2+}$ per rat as chloride.

Here, the ^{57}Co was eluted in two peaks, the first small one in the region of high molecular weight components, the second much higher corresponding to the molecular weight of cyanocobalamin, thus confirming that about 50% of the excreted urinary cobalt was present in an organic form.

3.3. Long-term studies after single doses

One hundred days after a single i.p. injection of 5 μg $^{57}Co^{2+}$ per rat and 1 mg $^{57}Co^{2+}$ per rat, respectively, again the tissue distribution of Co was different (Fig. 6). At the lower dose, the element was mainly present in spleen and pancreas followed by skull and femur. At the higher dose, the three bone samples showed the highest concentration indicating that, at these doses and/or with time, cobalt tends to accumulate in bone tissue. Interestingly, the cobalt amount present in heart was higher than after short-term and lower dose exposures which may be a sign of the cardiovascular effects caused by Co ingestion [18]. In testis and epididymus, cobalt was present only in very small concentrations suggesting that a strong barrier exists in vivo in rats under these experimental conditions.

3.4. Long-term studies via drinking water

When rats had been treated with 50 ng Co/rat/day via drinking water for 109 days, the tissue distribution was again different (Fig. 7). Liver, kidney and large intestine showed the highest concentration, whereas bone (femur) contained only very small amounts. This could indicate that the accumulation and retention of cobalt in the body when taken up orally is much lower. An exception might be the liver, as in humans [15], since in this tissue, cobalt concentrations increased from day 72 to day 109. Also the kidney showed a somewhat similar pattern. In spite of the daily Co intake during more than two cycles of spermatogenesis, the ^{57}Co radioactivity in testes and epididymus was negligible. Corrier et al. (1985) [19] found in earlier studies that 98 days after a daily administration of 4 mg Co with the food, the testes of the rats were dark, congested and reduced in size. In the present work, we observed none of these effects, perhaps due to the much lower dose used. The much higher amounts present in the large intestine with respect to the small intestine indicate that the route of excretion was mainly via feces. Also, when cobalt was given orally to humans,

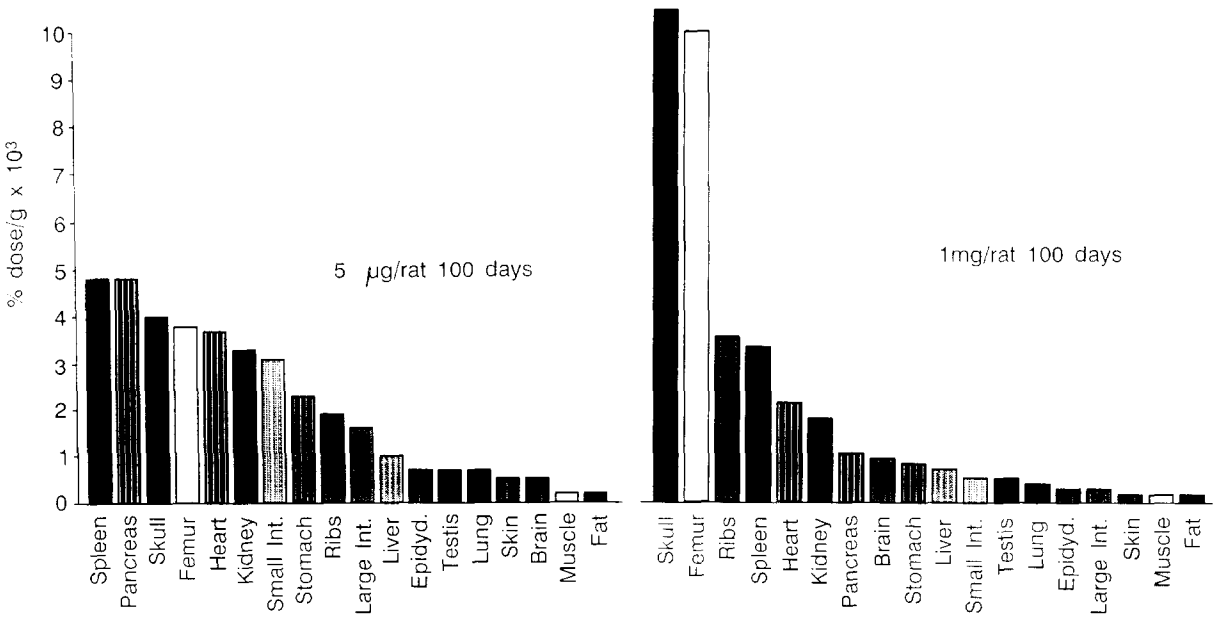

Fig. 6. Cobalt distribution in rat tissues 100 days after a single i.p. injection of 5μg or 1 mg $^{57}Co^{2+}$ per rat as chloride.

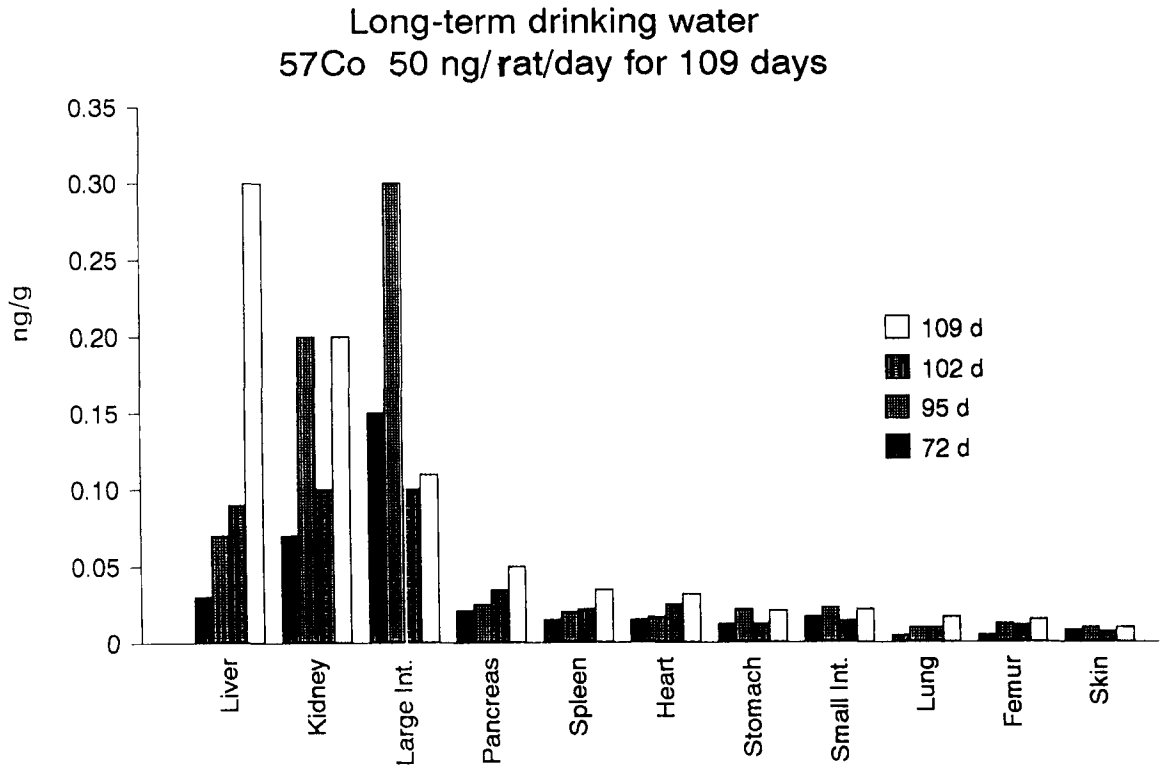

Fig. 7. Cobalt distribution in rat tissues 72, 95, 107 and 109 days after treatment with 50 ng $^{57}Co^{2+}$/rat/day via drinking water.

more than 40% was found in feces [15]. In our experiment on rats, the same values were found (Table 2). Studies on the biliary excretion of Co in rats after i.v. injection had shown that, with increasing doses, the percentage of the cobalt dose in bile increased also [17]. The considerable levels of cobalt-derived radioactivity in the intestine after intake with drinking water, however, could be due to the presence of physiological routes other than bile for the entry of the element into the gut. In urine, Co was present to about 15% in the organic form only, in contrast to that excreted 24 h after i.v. injection where this amount was about 50% (Fig. 5).

Table 3 shows the intracellular distribution of

Table 2
Excretion of Co in rats 109 days after administration of 50 ng $^{57}Co^{2+}$/rat/day as chloride via drinking water

Compartment	Co content (% of the dose)
Feces	44.5 ± 23.8
Urine:	
Total	1.43 ± 0.26
Inorganic form[a]	84.6 ± 6.5
Organic form[a]	15.4 ± 3.9

[a]Percentage of the total Co in urine.

Table 3
Intracellular distribution of Co in liver and kidney of rats after 109 days of administration of 50 ng $^{57}Co^{2+}$/rat/day as chloride via drinking water. Mean ± S.D.

Fraction	Co content (% of the homogenate)	
	Liver	Kidney
Homogenate	100	100
Nuclei	24.0 ± 4.3	19.5 ± 0.8
Mitochondria	15.5 ± 3.2	14.5 ± 1.6
Lysosomes	39.1 ± 2.1	21.1 ± 0.6
Microsomes	6.3 ± 1.8	3.5 ± 0.3
Soluble	15.1 ± 1.3	41.4 ± 2.3

Co in liver and kidney of rats after being exposed to Co via drinking water for 109 days. In the liver, the nuclei contained nearly 24% of the total Co content in the liver, half the amount of that present in this fraction 24 h after i.v. injection of the element (Fig. 1). In both organs, there was a marked increase of Co in the lysosome fractions. If this was due to lysosomal injury caused by the element, which has to be verified, then this could be an early sign of liver and kidney necrosis.

In Table 1, we report the values of Co present in testis, epididymus and vas deferens 24 h after i.v. injection of 10 ng Co per rat. No measurable Co was found in the sperm cells, indicating that no direct uptake of the metal occurs. The intracellular distribution in testis is shown in Fig. 8. 50% of the ^{57}Co radioactivity in the homogenate was present in the nuclear fraction and about 40% in the cytosol. In the latter, Co was mainly associated with low molecular weight compounds as seen in Fig. 9 which shows the elution pattern after gel chromatography of the testis cytosol on Sephadex G 150 resin. In this case, Co had a similar behaviour to the cytosols of lung, liver and kidney (Fig. 2) showing the poor capability of binding to proteins of higher molecular weights.

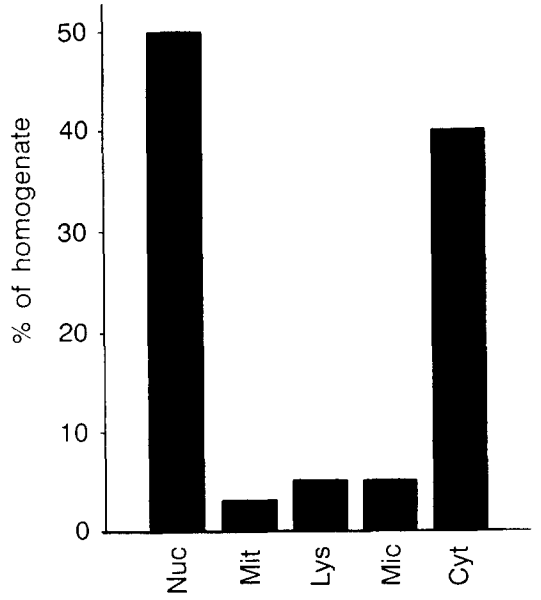

Fig. 8. Intracellular distribution of cobalt in testes of rats 24 h after i.v. injection of 5 ng $^{57}Co^{2+}$ per rat as chloride.

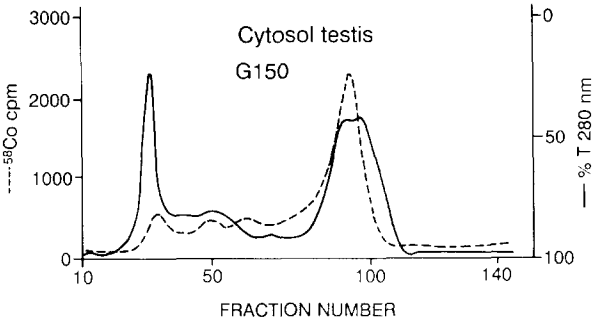

Fig. 9. Elution profile from Sephadex G 150 of testes cytosol 24 h after i.v. injection of 5 ng $^{57}Co^{2+}$ per rat as chloride.

3.5. *In vitro studies on rat sperm*

Since the amount of Co detected in testis and epididymus by in vivo studies (Table 1) was close to the detection limit, the direct uptake of Co in rat sperm cells was investigated in vitro. In this case, Co was able to enter the sperm cells in elevated concentrations with increasing doses (Table 4). It could be inferred that a strong blood-testis barrier for cobalt exists at the lower doses employed in our in vivo studies. Other workers [19] using much higher doses of the metal found degenerative effects on the reproductive system of rats.

3.6. *In vitro studies by BALB/3T3 cells*

Recently, soluble Co compounds have been classified by the IARC (International Agency of Research on Cancer) as a possible carcinogenic agent to humans [20]. Thus, studies on the carcinogenic potential of Co were investigated in vitro by using aneuploid cell lines of mesenchymal origin such as BALB/3T3 cells which are widely

Table 4
In vitro uptake of $^{57}Co^{2+}$ in rat sperm cells 2 h after incubation of $2.37 \pm 0.37 \times 10^7$ cells with 10 ng, 5 μg and 1 mg of $^{57}Co^{2+}$ as chloride

Dose exposure	Co content (Mean ± S.D.)	
	% dose	ng
10 ng	2.01 ± 0.81	0.2 ± 0.08
5 μg	0.25 ± 0.11	5.65 ± 1.3
1 mg	0.07 ± 0.02	683.3 ± 218.3

Table 5
Intracellular repartition of Co in BALB/3T3 cells after exposure to 10^{-6} M ^{57}Co-labelled Co^{2+} as chloride. Mean ± S.D.

Fraction	Co content (% of the uptake in whole cells[a])	
	24 h	72 h
Whole cells	100	100
Cytosol	96.1 ± 0.7	88.8 ± 1.3
Pellet	3.9 ± 0.7	11.2 ± 1.2

[a]13.3 and 4.4 pg of Co/10^6 cells/h at 24 h and 72 h, respectively.

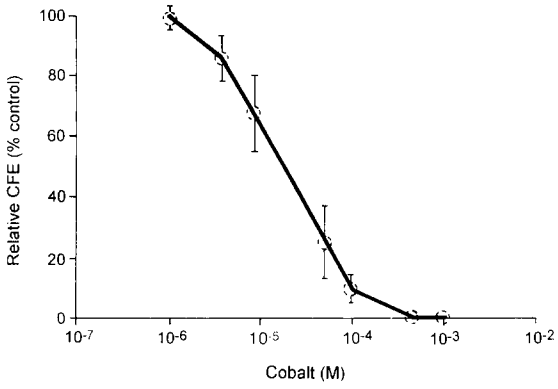

Fig. 10. Cytotoxicity of Co on the BALB/3T3 cells after exposure to doses of $^{57}Co^{2+}$-labelled Co^{2+} as chloride ranging from 1 to 1000 μM.

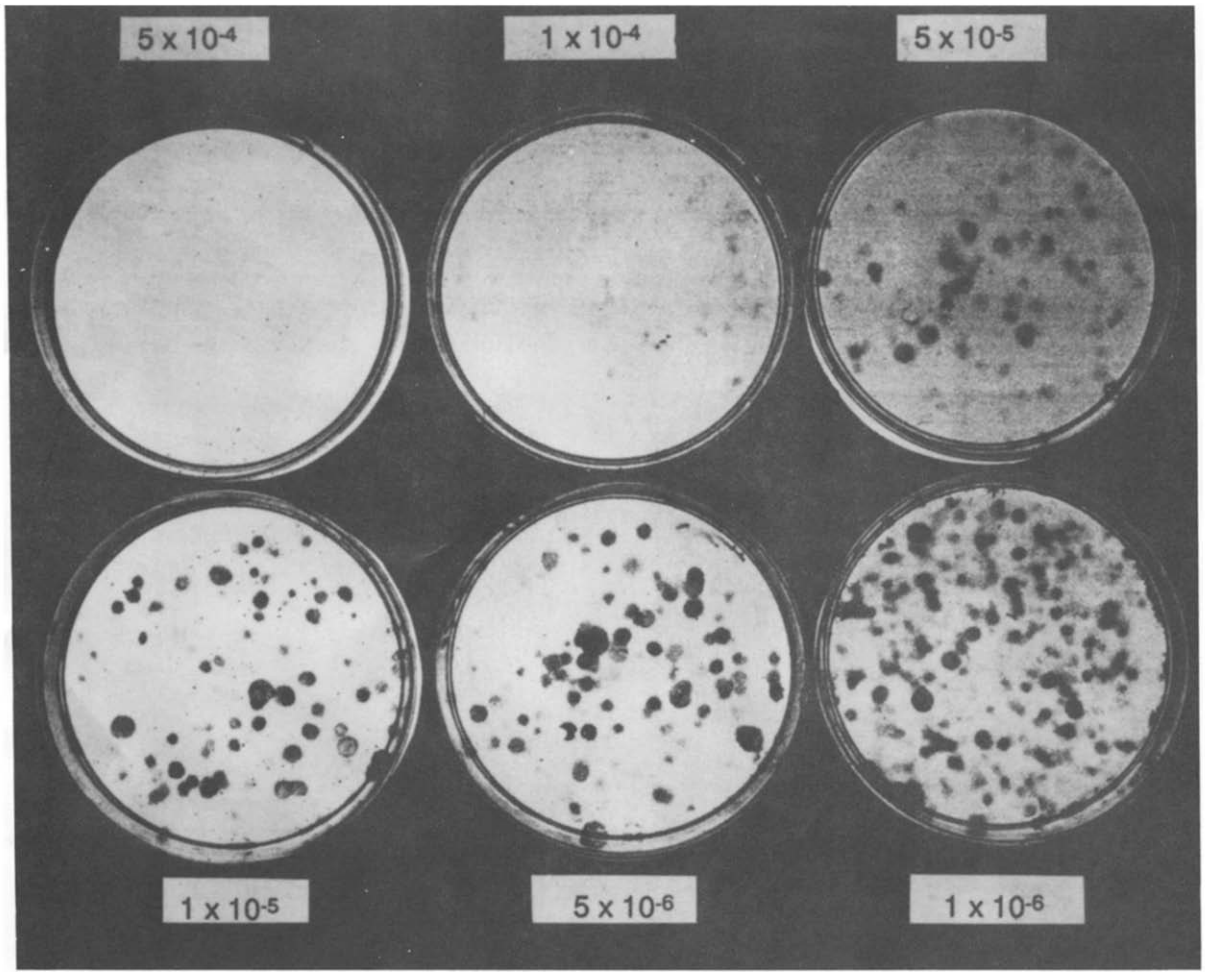

Fig. 11. Effects of $CoCl_2$ on the growth of BALB/3T3 cells.

Table 6
Concurrent cytotoxicity and morphological transformation in BALB/3T3 cells induced by cobalt chloride

Dose of exposure (M)	CFE (%)	No. of type III foci/ No. of dishes	No. of type III positive dishes/ No. of dishes	Transformation frequency (10^{-4})	P-value[b]
1×10^{-5}	70.8	0/18	0/18	0.00	NS
1×10^{-6}	102	1/18	1/18	0.05	NS
0.1% (v/v)[a]	100	1/18	1/18	0.05	NS

[a]control using distilled H_2O.
[b]38 degrees of freedom; NS = not significant.

used to study qualitative and quantitative dose-dependent responses to potential carcinogens, including trace metals [21–23]. The uptake of the cells after 24, 48 and 72 h of exposure to 1 μM of ^{57}Co-labelled Co^{2+} as chloride was in any case of the order of 1% of the dose (320 pg Co/10^6 cells or 0.32 fg Co/cell). Interestingly, the distribution of Co between cellular organelles and supernatant (Table 5) was different at 24 h and 72 h. In the first case, 96.1% of Co was recovered in the supernatant and only 3.9% was associated with the pellet fraction. At 72 h of exposure, the fraction of Co present in the pellet was about three times higher compared with the situation at 24 h suggesting that a redistribution of Co in the cell had occurred.

Figs. 10 and 11 show the cytotoxicity of Co on the BALB/3T3 cells after 72 h of exposure to different cobalt concentrations, and the effect of Co on cellular growth. The following conclusions can be drawn: (i) the cytotoxicity effect of Co was dose-dependent; (ii) increasing doses from 0.1 μM to 1000 μM caused a dose-related increase in cytotoxicity. The Colony Forming Efficiency (CFE) at 100 and 1 μM was 10% and 90% of the controls, respectively (Fig. 10); (iii) no effect of Co on cellular growth was observed at concentrations lower than 1 μM (Fig. 11).

The results of the concurrent cytotoxicity and transformation assay of Co on BALB/3T3 cells exposed to 10^{-5} and 10^{-6} M $CoCl_2$ are shown in Table 6. Cobalt was not significantly positive in the transformation assays (number of foci III formed) for the tested doses as shown by the transformation frequency calculated for both doses compared to the spontaneous transformation.

These findings tend to suggest that inorganic soluble salts of Co have a low degree of cytotoxicity and should not be considered as having a carcinogenic potential.

These conclusions are drawn from in vitro studies. However, the present in vivo experiments have shown the complexity of studies on Co metabolism which depends on route of administration and dose exposure. Thus, the in vitro cellular models used here could not mimic the real in vivo situation.

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5. References

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