# The distribution in mice of radioactive cobalt administered by two different methods

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The short time distribution of $^{58}Co$ in mice that received a $^{58}CoCl_2$ solution intravenously or intraperitoneally was examined by means of scintillation measurements. The serum, kidney, pancreas, spleen, liver, and heart were analyzed. Statistically significant differences in the distribution pattern after intraperitoneal and intravenous administration were noted for serum, kidney, liver, and heart 1 h after injection and for kidney, liver, and heart 5 h after injection. Only the heart showed a significant difference between the two administration methods 24 h after injection. Intravenous injections gave less variation between animals of the $^{58}Co$ distribution pattern than intraperitoneal injections. □ *Metal distribution; scintillation measurements*

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Cobalt is a heavy metal with which we come into daily contact as it is a component of the stainless steel alloys. In medicine and odontology alloys containing cobalt are used in, for example, implants and removable partial dentures. Öwall & Nyquist (37) mentioned that during 1972 about 33,000 cast cobalt–chromium constructions for removable partial dentures were made in Sweden. The corresponding figure in 1979 was 44,000 constructions (21). Dental cobalt–chromium alloys contain up to 60% cobalt (27).

Corrosion of metallic constructions does occur in the oral milieu (27), and the migration of metal ions from metallic dental restorative materials into both soft and hard oral tissues has been demonstrated by, among others, Bergenholtz et al. (2) and Söremark et al. (31). Sensitivity reactions in patients who had received dental cobalt–chromium dentures have been described (12, 14, 20).

Many authors have demonstrated that cobalt can have certain toxic properties. Metabolic changes, mitotic disturbances, and a reduced rate of oxygen uptake of cobalt in cell cultures have been demonstrated in in vitro studies (4, 9, 16, 26, 36). Animal experiments have shown that cobalt has carcinogenic properties (17, 18, 24, 29, 35). Decreased iron absorption from the intestine (28), increased vascular permeability (11), and changes in blood composition (4, 5, 11, 30) were seen in experimental animals after the administration of cobalt compounds. In man a cardiomyopathy caused by cobalt in beer has been reported (1).

With regard to the distribution of cobalt, there have been several investigations using various experimental animals and administration methods, including oral (8, 15), intravenous (5, 6, 25, 32), intraperitoneal (19, 22, 34), subcutaneous (7, 23), and intramuscular (10). The distribution of cobalt is species-dependent (33). In future experiments on cobalt we plan to use the mouse, to facilitate application of whole-body autoradiographic technique.

Only limited information is available about potential differences between the cobalt distribution patterns after various administration methods in mice. Thus it was considered to be of value to study and compare the short-term tissue distribution of cobalt in mice after intravenous and intraperitoneal injections of radioactive cobalt.

## Materials and methods

The experimental animals used were 60 approximately 8-week-old female mice (N.M.R.I. strain) weighing about 20 g, from Anticimex, Stockholm, Sweden. They were housed in acrylic cages with stainless steel covers. The animals had free access to ordinary tap water and a conventional pellet diet (210 Anticimex).

Of the commercially available solutions of radiocobalt, $^{58}CoCl_2$ was considered best for this study because it has a suitable half-life (71 days) and has been used in earlier studies of cobalt distribution in experimental animals (7, 15, 19). From a stock solution of carrier-free $^{58}CoCl_2$ (2 mCi/μg Co) in 0.1 M HCl with an activity 2 mCi/ml (Radiochemical Centre, Amersham, England) 1 ml was taken and diluted with 3 ml physiologic saline solution. The 4 ml of solution obtained showed an activity of 500 μCi/ml. From this an injection solution was prepared by diluting 0.5 ml with 12 ml physiologic saline solution, which gave an injection solution with an activity of approximately 20 μCi/ml. Of this solution 0.25 ml was injected per mouse (about 5 μCi/mouse).

The experimental animals were divided at random into 2 groups of 30 mice. Group I was given $^{58}CoCl_2$ in physiological saline solution intraperitoneally, and group II was given $^{58}CoCl_2$ in physiological saline solution intravenously via a tail vein.

After being anesthetized with ether, 10 experimental animals from each group were decapitated 1, 5, and 24 h, respectively, after the injection. Sample collection was carried out as described by Bergman (3). Samples of serum, kidney, pancreas, spleen, liver, and heart were taken. The whole of the heart, spleen, and left kidney were taken. Those parts of the pancreas that were visible when the spleen was lifted were cut out, and a sample of the middle lobe was taken from the liver. The blood from the aorta was collected in a tube immediately after decapitation. After being centrifuged the serum was soaked up in a pipette. All samples were placed in weighed Pyrex tubes, and the wet weight was determined. The sample technique and weighing procedure are standard routines in our laboratory. The methodological errors have been found to be so small (3) that they can hardly have influenced the results of the present study.

Scintillation measurements were made with a 7.6 × 7.6 NaI (TI) scintillation detector (Picker Dual Channel Analyzer). A reference solution was prepared so that the injection solution that had been administered to each mouse (0.25 ml) was diluted to 25 ml with physiological saline solution in a retort. Of this solution 0.5 ml was used as a reference solution, corresponding to 1/50th part of the activity that was administered to each mouse. The samples were placed in the bottom of the tubes, to ensure a counting geometry as comparable as possible with the reference solution. Corrections were made for the background. The relative activity (RA) of $^{58}Co$ was defined as:

$$\frac{\text{cpm/gram wet weight of the sample}}{\text{cpm in injection solution/gram body weight}}$$

The data obtained were multiplied by 100 to obtain RA as a percentage. In all groups and for each post-injection survival time the mean value, standard deviation, and coefficient of variation were calculated with regard to RA × 100. It is possible with scintillation measurements that variations in the geometry of the samples may influence the numbers of impulses registered. In the present study the same operator took all the measurements, in accordance with a method mentioned by Bergman (3), in which the influence of the error due to geometry was analyzed. There was no significant influence in that study.

To estimate statistically whether $^{58}Co$ had similar distributions in the two groups, Wilcoxon's test for two independent random samples (based on rank sum; used interindividually) was used.

## Results

The results shown in Table 1 and Figs. 1 and 2 demonstrate that the RA generally showed the highest mean values 1 h after injection and the lowest values 24 h after injection,

Table 1. The relative activity (× 100) of $^{58}Co$ in some organs of mice 1, 5, and 24 hours after an injection, in group I with $^{58}CoCl_2$ in physiological saline solution intraperitoneally and in group II with $^{58}CoCl_2$ in physiological saline solution intravenously. Each value indicates the mean for 10 mice. $\bar{x}$ = mean value, S = standard deviation, CV = coefficient of variation (100 × S/$\bar{x}$)

| | | 1 h | | | 5 h | | | 24 h | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Group | Tissue | $\bar{x}$ | S | CV | $\bar{x}$ | S | CV | $\bar{x}$ | S | CV |
| I | Serum | 117.5 | 47.6 | 40.51 | 123.1 | 26.3 | 21.36 | 50.5 | 22.5 | 44.55 |
| | Kidney | 144.2 | 64.3 | 44.59 | 109.4 | 22.4 | 20.48 | 55.4 | 23.5 | 42.42 |
| | Pancreas | 184.3 | 72.6 | 39.39 | 135.0 | 37.3 | 27.63 | 50.8 | 29.6 | 58.27 |
| | Spleen | 66.5 | 32.0 | 48.12 | 50.9 | 8.4 | 16.50 | 17.1 | 7.6 | 44.44 |
| | Liver | 282.1 | 105.4 | 37.36 | 222.0 | 46.4 | 20.90 | 109.8 | 67.1 | 61.11 |
| | Heart | 42.4 | 18.5 | 43.63 | 48.0 | 7.6 | 15.83 | 34.3 | 12.6 | 36.73 |
| II | Serum | 193.4 | 53.4 | 16.75 | 144.6 | 24.2 | 16.74 | 69.6 | 17.4 | 25.00 |
| | Kidney | 298.4 | 53.0 | 17.76 | 184.1 | 19.6 | 10.65 | 89.5 | 23.0 | 25.70 |
| | Pancreas | 229.0 | 44.7 | 19.52 | 124.0 | 17.6 | 14.19 | 50.9 | 17.8 | 34.97 |
| | Spleen | 36.0 | 5.2 | 14.44 | 27.7 | 3.0 | 10.83 | 13.5 | 2.8 | 20.74 |
| | Liver | 480.7 | 121.5 | 25.28 | 296.8 | 44.3 | 14.93 | 122.7 | 28.5 | 23.23 |
| | Heart | 105.9 | 19.9 | 18.79 | 95.5 | 10.5 | 10.99 | 59.8 | 13.5 | 22.58 |

with a decreasing RA approximately similarly in each tissue studied. The heart and serum were the exceptions in the group injected intraperitoneally (Fig. 1), having the highest mean RA values 5 h after injection. After 24 h the spleen had the lowest mean values in both groups. The liver had the highest value at all three survival times in both groups.

The statistical analysis of the cobalt distribution for all tissues and survival times, comparing groups I and II visualized in Fig. 3, was based on 10 experimental animals per group and survival time, except for the group injected intraperitoneally, for which only 9 values have been used 1 and 24 h after injection ('outliers' taken away; see Discussion). Comparison of group II with group I (Fig. 3) showed that, out of the six tissues studied, serum, kidney, liver, and heart at 1 h after injection and kidney, liver, and heart at 5 h after injection displayed significantly ($p < 0.01$) higher RA values in the group intravenously injected. At 1 and 5 h, respectively, only the spleen showed a significantly ($p < 0.01$) higher RA value in the group intraperitoneally injected. Only one tissue, the heart, showed a significant difference 24 h after injection, with a higher RA value in the group intravenously injected.

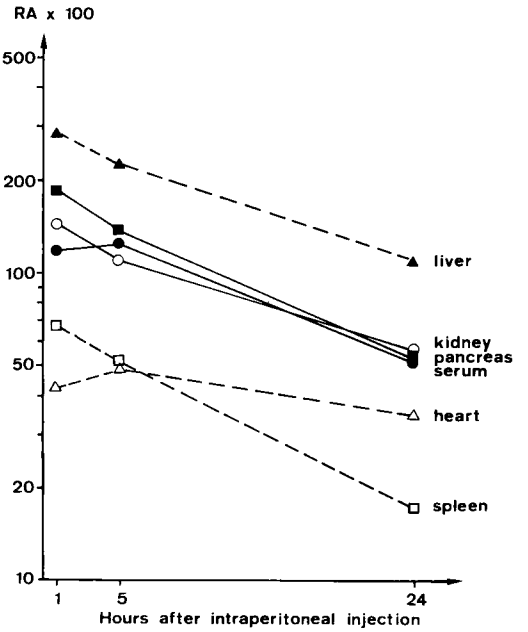


Fig. 1. Variation with time in the relative activity (RA × 100) of some organs of mice after *intraperitoneal* injection of *$^{58}CoCl_2$ in physiological saline solution.* Each value indicates the mean for ten mice. Log scale for RA.

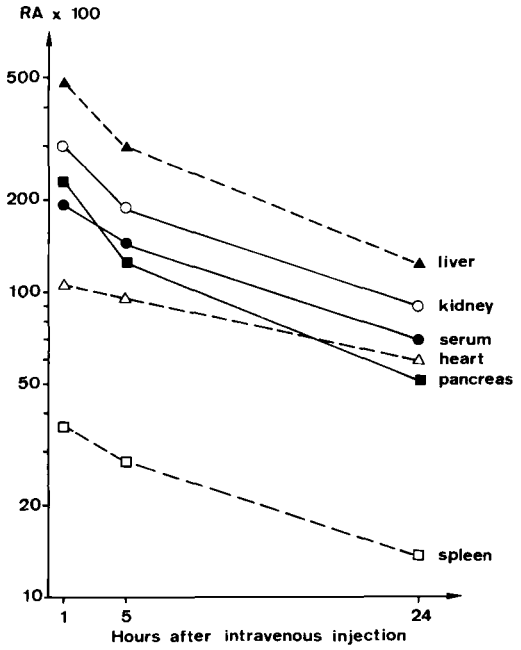


Fig. 2. Variation with time in the relative activity (RA × 100) of some organs of mice after *intravenous* injection of $^{58}CoCl_2$ *in physiological saline solution*. Each value indicates the mean for 10 mice. Log scale for RA.

| Tissue | Hours after injection | | |
|---|---|---|---|
| | 1 hour | 5 hours | 24 hours |
| Serum | $p < 0.01$ | $0.05 > p > 0.01$ | N.S. |
| Kidney | $p < 0.01$ | $p < 0.01$ | $0.05 > p > 0.01$ |
| Pancreas | N.S. | N.S. | N.S. |
| Spleen | $p < 0.01$ | $p < 0.01$ | $0.05 > p > 0.01$ |
| Liver | $p < 0.01$ | $p < 0.01$ | N.S. |
| Heart | $p < 0.01$ | $p < 0.01$ | $p < 0.01$ |

II > I
I > II
N.S.

Fig. 3. Comparisons of the distribution of $^{58}CoCl_2$ in physiological saline solution in group II (intravenously) and group I (intraperitoneally). The statistical treatment (Wilcoxon's test) is based on 10 mice, except for group I at 1 and 24 h after administration (mean values for 9 mice due to 'outliers'; see text). $p < 0.01$, significant difference; $0.05 > p > 0.01$, almost significant differences; $p > 0.05$, no significant difference; and N.S. = nonsignificant difference.

## Discussion

Two experimental animals in the intraperitoneally injected group killed 1 and 24 h, respectively, after injection showed strongly divergent RA values compared with the other animals in this group. An error in the sample technique would hardly explain this divergence, because all the tissues of the two animals showed low RA values. It is more probable that the reason is to be sought in the injection method. With intraperitoneal administration the operator works without direct observation and consequently he cannot determine with absolute certainty where the solution is placed. There is some risk that the solution may be deposited directly in some organ intraperitoneally. With intravenous injection via a tail vein the operator can observe the injection field directly and has the possibility of placing the injection needle correctly. The extreme values mentioned for the two experimental animals in the intraperitoneally injected group were regarded as 'outliers' for the statistical analysis.

In the intraperitoneally injected group all tissues studied, except the heart and serum, showed the highest RA values 1 h after injection and the lowest values 24 h after. In the intravenous group there was a continuous decrease of $^{58}Co$ from 1 to 24 h. The differing results obtained for the groups with regard to the serum, kidney, liver, and heart can be ascribed to the more rapid uptake of $^{58}Co$ in the blood system after an intravenous injection, whereas the intraperitoneally injected solution will be deposited among various abdominal tissues and taken up in the blood system later on. The different results obtained for the pancreas and spleen might depend on deposition of the $^{58}CoCl_2$ solution into or near these organs when injecting intraperitoneally.

The relatively high $^{58}Co$ uptake in the liver, kidney, and pancreas after a single intravenous injection of cobalt chloride shown in the present study is in agreement with results obtained in short-term studies in mice (13, 32) and in the rat (6, 25). There is no short-term tissue distribution study in mice after a single intraperitoneal injection of a cobalt solution to be found in the literature. However, the results obtained in the present study concerning the tissue distribution of cobalt after a single intraperitoneal injection are in accordance with results obtained in the rat (34).

In the present study it was found that after 24 h only the heart showed a statistically significant difference between the intravenously and intraperitoneally injected animals. This indicates that observed differences in distribution patterns of cobalt between the two administration methods will gradually disappear. Such an assumption is supported by results obtained in rodents after intravenous and intragastric administration (33).

Since the present study has shown that the short-term distribution pattern of cobalt may vary with the method of administration, it would be wrong to presume that the same distribution pattern would be obtained after release of cobalt from a partial denture or an implant as, for example, after intravenous or intraperitoneal injections of cobalt. Studies ought thus to be specially designed to elucidate the distribution of a potential dissolution of cobalt from an implant or a partial denture.

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