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The Path of Cobalt-60, Ionic or Chelated, in Albino Rats after Intravenous Administration.*† (27661)

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Previous investigation in this laboratory has shown that simultaneous administration of chelating agents can considerably alter the tissue distribution of cobalt-60 in albino rats (1). While nearly 80% of the radioactive cobalt, injected alone, is excreted within 24 hours, mainly through urine, most of the chelating agents cause decreased excretion and preferential localization in various organs. Cysteine and ethylenediaminetetraacetic acid (EDTA) considerably enhance excretion of the radioisotope, excretion with EDTA amounting to 96.3%; with cysteine, the excretion is 88.1%. As a first step towards understanding the mechanism of these changes, the exact state in which the cobalt-chelate exists in the blood after intravenous administration has been studied(2). The results suggest that cobalt-60, injected alone or with any one of the chelating agents, is partly bound to serum proteins (mostly to albumin and α-globulin) and partly free, except that the EDTA-cobalt chelate exists as a separate soluble entity in the blood, being free and completely dialysable. The 1-nitroso 2-naphthol chelate in the blood has been found to be completely nondialysable, probably colloidal, but not completely protein-bound. Continuing these experiments, the movement of the tracer, ionic or chelated, after it leaves the vascular compartment has been investigated through time-distribution studies. The results are presented here. While the distribution of radioactive cobalt has been studied extensively in a number of species, most of the work relates to orally-administered cobalt over a period of days(3,4,5). Greenberg and coworkers observed that parenterally-administered cobalt is excreted mainly through urine(6).

Materials and methods. Cobalt-60, obtained from the Radiochemical Centre, Amersham, U.K., was as an aqueous solution of cobaltous chloride, containing about 35 μg cobalt per mc. The solution was suitably diluted with isotonic saline prior to injection. The chelating agents used, 1-nitroso 2-naphthol, sodium diethyldithiocarbamate (SDDC), rubeanic acid, 2:3 dimercapto propanol-1 (British Antilewisite, BAL), EDTA as its disodium salt, and L-cysteine HCl · H_2O, were all of high grade purity.

The tissue distribution of cobalt-60, injected alone or with a chelating agent, at various intervals after administration was studied. Four hundred adult male albino rats, weighing 150-250 g, divided into groups of 5, were used. The experimental procedure for administration of the isotope in the ionic or chelated form and for subsequent assay of radioactivity in the various tissues is the same as described earlier for studying the 24-hour distribution(1). In the present investigation, in addition to the 24 hours, the distributions at various other times from 5 minutes to 72 hours, were also determined by sacrificing the

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TABLE I. Distribution of Cobalt-60 (ionic form) at Different Times After Injection.
(Values expressed as % of injected activity.)

Tissue \ Time	5 min.	10 min.	15 min.	30 min.	1 hr	3 hr	5 hr	16 hr	24 hr	48 hr	72 hr
Blood	24.1	23.3	13.3	17.1	17.3	9.9	8.4	3.3	2.3	1.0	.8
Liver	29.0	25.6	27.3	25.8	24.2	16.8	15.2	10.0	9.3	6.6	4.7
G I T	7.5	7.2	8.3	6.7	6.5	7.3	8.4	7.6	7.6	2.6	1.1
Kidneys	5.1	5.1	5.5	5.3	4.0	3.8	3.1	1.8	1.1	.7	.8
Excretion	1.3	2.0	7.8	14.0	22.9	40.1	52.2	71.1	74.5	79.4	81.5
Urine								67.9	68.8	67.2	71.2
Feces								3.2	5.7	12.2	10.3
Carcass	35.5	45.0	38.0	35.7	16.8	17.2	13.8	8.9	8.8	6.6	7.7
Skin & hair	19.1	19.4	19.3	18.9	8.9	7.0	3.8	4.3	1.9	1.3	1.4
Bones	8.8	17.7	13.6	8.8	7.4	6.9	4.7	4.2	2.7	1.8	2.0
Muscles	7.6	7.9	5.1	8.0	1.3	3.3	5.3	2.4	4.2	3.5	4.3

G I T = Gastro-intestinal tract.

appropriate groups of rats at the specified times.

In some of the earlier experiments, the skin, bones and muscles were not separated, but treated together as "the carcass". But, when the carcass was found to contain a high percentage of injected activity in some cases, activity in each tissue was determined individually.

Results. In Table I are presented data concerning distribution of cobalt-60, administered in the ionic form, at various times after injection. There is a rapid removal of radioactivity from the vascular compartment, more than 75% disappearing within the first 5 minutes. Simultaneously, there is considerable deposition in the liver, skin and bones, so that at 5 minutes, these 3 tissues, along with blood, account for most of the radioactivity injected. Subsequently, there is a gradual decrease of radioactivity in all these tissues and a rapid increase in the urinary excretion, which accounts for over 80% of the activity at 72 hours. This conforms with earlier observations of Greenberg and coworkers(6).

The 1-nitroso 2-naphthol chelate is even more rapidly removed from the blood stream, only about 15% remaining in blood at the end of 5 minutes (Table II). During this time, more than 70% of the complex concentrates in the liver. The accumulation in the liver reaches a peak of about 85% in 15 minutes, then falls, with a simultaneous increase in the activity in the skin, bones and muscles. These tissues account for over 50% of the activity at about 16 hours. These observations suggest that there is a definite transfer of cobalt-60 from the liver to these tissues before it is excreted. The excretion, initially urinary, becomes predominantly fecal as time

TABLE II. Distribution of Cobalt-60 ~ 1-Nitroso 2-Naphthol Chelate.
(Values expressed as % of injected activity.)

Tissue \ Time	5 min.	10 min.	15 min.	30 min.	1 hr	3 hr	5 hr	16 hr	24 hr	48 hr	72 hr	120 hr
Blood	18.3	15.5	6.4	5.9	5.2	4.7	4.7	1.9	1.4	1.4	.5	.3
Liver	73.4	76.7	83.5	76.6	56.9	41.8	26.2	9.9	7.2	4.8	3.4	2.0
G I T	.4	.6	.7	1.5	4.5	5.8	8.8	13.3	10.9	6.8	3.2	2.8
Kidneys	.3	.3	.3	.6	1.1	1.3	1.5	.9	.7	.6	.4	.4
Excretion	.4	.2	.1	.4	.5	1.8	3.7	14.6	22.9	40.9	41.5	50.1
Urine								11.5	13.4	20.6	20.0	21.6
Feces								3.1	9.6	20.3	21.5	28.5
Carcass	0	2.0	4.5	8.5	19.7	38.7	49.0	49.3	50.5	39.2	32.2	28.3
Skin	0	1.0	1.3	2.3	5.2	13.5	10.8	14.3	15.1	16.0	9.8	10.6
Bones	0	1.0	3.2	6.1	9.5	19.0	17.4	15.2	15.2	11.9	6.8	5.6
Muscles	0	0	0	0	5.0	6.2	20.8	20.3	20.3	11.3	15.6	12.1

G I T = Gastro-intestinal tract.

TABLE III. Distribution of Cobalt-60 ~ SDDC Chelate.
(Values expressed as % of injected activity.)

Tissue \ Time	5 min.	10 min.	15 min.	30 min.	1 hr	3 hr	5 hr	16 hr	24 hr	48 hr	72 hr
Blood	14.8	8.4	6.3	7.7	8.2	6.3	4.3	1.8	1.7	1.2	.7
Liver	58.4	65.4	65.0	44.8	42.7	31.6	27.9	21.9	10.2	7.8	5.9
Carcass	17.8	13.2	32.0	30.2	25.5	22.9	20.0	15.5	12.4	11.0	8.2
Kidneys	1.13	.6	.5	.8	.8	.9	.7	.5	.5	.4	.3
G I T	3.8	3.6	3.6	14.5	21.0	28.1	30.1	9.8	15.2	3.4	3.7
Excretion	.1	.1	.3	.6	1.2	9.0	10.4	45.1	58.7	77.8	89.3
Urine								19.0	30.5	34.5	38.1
Feces								26.1	28.2	46.4	51.2
G I T + excretion	3.9	3.7	3.9	15.1	22.2	37.1	40.5	54.9	73.9	81.2	93.0

G I T = Gastro-intestinal tract.

progresses. Even at the end of 120 hours, nearly 40% of the radioactivity is retained in the body, mainly in the skin, bones and muscles.

The data concerning distribution of the SDDC-complex with time are presented in Table III. This chelate behaves in a qualitatively similar manner to the 1-nitroso 2-naphthol complex, though to a slightly lesser degree. An initial accumulation in the liver followed by a small concentration in the carcass is noted. The increased radioactivity in the gastrointestinal tract (GIT) observed at some stages has been traced to the contents of the intestines rather than to the intestinal loop. The excretion is again predominantly through the feces.

The cobalt-rubeanic acid chelate also accumulates in the liver initially, the blood and the liver accounting for most of the radioactivity during the first 60 minutes (Table IV). Activity in these tissues decreases comparatively slowly, so that even at the end of 48 hours, the liver still contains about 20% of the injected cobalt. On the other hand, this chelate does not concentrate to an appreciable extent in the skin, bones and muscles, the activity in 'the carcass' being always about 10% or less. These 3 tissues are probably of minor significance in the metabolism of this chelate.

With BAL, immediately after administration, the radioactivity is present mainly in the blood, the liver and the carcass, as it is with ionic cobalt. But, while the activity in the blood and carcass diminishes rapidly, the loss of radioactivity from the liver is very slow, just as with rubeanic acid. Excretion of radioactivity is about equally divided between urine and feces.

In sharp contrast to the behaviour of others, less than 3% of the EDTA - chelate accumulates in the liver at any time (Table V). A conspicuous deposition of radioactivity in the carcass, notably in the skin and bones, is observed within 5 minutes after injection. The carcass accounts for over 50% of the radioactivity during the first 10 minutes. The excretion, mainly through urine, is very rapid,

TABLE IV. Distribution of Cobalt-60 ~ Rubeanic Acid Chelate.
(Values expressed as % of injected activity.)

Tissue \ Time	5 min.	10 min.	15 min.	30 min.	1 hr	3 hr	5 hr	16 hr	24 hr	48 hr	72 hr
Blood	41.0	36.0	26.9	32.0	33.8	20.1	22.8	7.1	6.1	2.3	2.0
Liver	42.1	47.1	44.9	45.0	36.0	39.4	39.3	24.5	22.0	20.5	12.5
G I T	4.4	5.5	5.5	4.9	5.1	5.6	6.1	6.1	5.4	3.0	2.1
Kidneys	2.5	3.5	2.5	2.7	2.6	1.9	1.9	1.4	1.2	.7	.7
Excretion	.5	3.4	6.1	6.1	7.8	19.8	38.7	44.9	57.1	62.9	64.2
Urine								38.1	46.5	46.1	49.0
Feces								6.8	10.6	15.8	15.2
Carcass	0	11.0	9.1	10.2	9.5	4.5	8.6	8.7	8.7	6.5	6.1

G I T = Gastro-intestinal tract.

TABLE V. Distribution of Cobalt-60 ~ EDTA Chelate.
(Values expressed as % of injected dose.)

Tissue \ Time	5 min.	10 min.	15 min.	30 min.	1 hr	3 hr	5 hr	16 hr	24 hr	48 hr	72 hr
Blood	33.2	21.1	21.2	11.0	4.8	.5	.3	.1	0	0	0
Liver	2.6	2.0	1.4	1.0	.8	.5	.4	.3	.2	.2	.1
G I T	3.1	3.3	2.0	1.2	1.2	4.4	1.9	2.6	1.6	1.2	.6
Kidneys	7.7	5.9	4.2	2.7	2.0	.7	.4	.2	.1	.1	0
Excretion	5.6	22.3	26.7	32.5	76.2	81.3	97.0	97.0	100.0	99.6	100.0
Urine								88.5	90.2	90.0	89.0
Feces								8.5	9.8	9.6	11.0
Carcass	54.3	50.5	40.2	28.9	12.8	2.2	1.3	1.1	1.2	.8	1.0
Skin	28.2	25.1	25.6	16.6	7.8	1.0	.8	.6	.7	.5	.5
Bones	20.1	18.1	11.6	11.5	3.8	1.0	.4	.4	.4	.3	.3
Muscles	6.0	7.3	3.0	.8	1.2	.2	.1	.1	.1	0	.2

G I T = Gastro-intestinal tract.

over 75% being excreted within an hour.

Cysteine affects the distribution of cobalt-60 more or less in the same way as EDTA (Table VI). As with EDTA, a marked deposition of the radioactivity in the skin and the bones with a comparatively low concentration in the liver is noted. The excretion, also mainly urinary, is less rapid.

In all these experiments, the spleen, lungs, heart, brain and hair have been observed to contain very low amounts of radioactivity, the activity decreasing steadily with time.

Discussion. The data presented so far suggest that, in the rat, the liver, skin, bones and muscles play a vital role in cobalt metabolism, whether administered in the ionic or chelated form. It is interesting to note that, with ionic cobalt and with some of the chelates, there is considerable accumulation of radioactivity in the liver initially, even though subsequent distribution varies widely. The liver thus seems to collect the cobalt, ionic or chelated, from the blood and route its subsequent distribution depending upon the nature of the chelating agent. This possibility is definitely indicated in the case of 1-nitroso 2-naphthol chelate, where there is unequivocal evidence for the pathway Blood → Liver → Skin, Muscles and Bones → Excretion. But this sequence is very unlikely with the EDTA - chelate which exhibits a marked apathy for the liver, but preferentially concentrates in the skin and the bones. The rapid accumulation of this chelate in the skin and its subsequent removal is particularly noteworthy, since skin, unlike the liver, is not usually considered to be metabolically important. With zinc-65 and iron-59 also, EDTA causes an increased deposition

TABLE VI. Distribution of Cobalt-60 ~ Cysteine Chelate.
(Values expressed as % of injected activity.)

Tissue \ Time	5 min.	10 min.	15 min.	30 min.	1 hr	3 hr	5 hr	16 hr	24 hr	48 hr	72 hr
Blood	28.8	19.5	16.7	10.2	5.0	3.1	2.8	.9	.9	.6	.2
Liver	7.1	7.1	8.9	9.5	9.2	9.2	6.7	3.3	3.3	2.5	1.6
Kidneys	11.0	11.7	11.0	12.5	9.5	5.2	4.0	1.8	1.6	1.2	1.1
G I T	6.2	5.2	4.7	4.2	8.3	5.1	5.3	3.0	4.4	1.8	.6
Excretion	.2	12.9	13.0	32.2	34.7	66.1	65.4	79.5	80.0	83.2	85.5
Urine								75.3	72.7	77.0	75.2
Feces								4 2	7.3	6.2	10.3
Carcass	52.5	46.7	48.8	29.4	18.3	11.7	10.0	4.2	4.4	2.8	2.5
Skin	20.8	26.9	25.2	14.3	9.5	5.6	3.9	2.0	2.0	1.2	1.2
Bones	30.7	17.1	16.0	10.2	6.6	4.9	5.7	1.8	1.9	1.3	.9
Muscles	1.0	2.7	7.6	4.9	2.2	1 2	.4	.4	.5	.3	.4

G I T = Gastro-intestinal tract.

of radioactivity in the skin§. The wide variations observed in the choice of various forms of cobalt for preferential localization among the body tissues, the different times at which such localizations occur, and also the vast differences in rate and preferred mode of excretion followed by these chelates, all suggest that the cobalt travels from one tissue to another in the form in which it is administered, perhaps in combination with some body constituents (for example, blood proteins), but never splitting up into the ionic form. This possibility is being investigated by studying the state in which the cobalt-60 exists in the various tissues and in excretion. Investigations on the uptake of cobalt-60 by liver slices *in vitro* indicate that cobalt is taken up by a passive process, which is enhanced by SDDC, but inhibited by most of the other chelating agents, EDTA being the most potent inhibitor.§

Summary. The path of cobalt-60, administered alone as cobaltous chloride or with any one of the chelating agents 1-nitroso 2-naphthol, SDDC, rubeanic acid, BAL, EDTA or cysteine, is traced by studying the tissue distribution at various times after intravenous administration to albino rats. With ionic cobalt and with a number of chelates, an initial accumulation of radioactivity in the liver is noted, even though subsequent distribution varies widely. The 1-nitroso 2-naphthol chelate follows the route Blood → Liver → Skin, Bones and Muscles → Excretion. EDTA- and cysteine-chelates exhibit a marked apathy for the liver, but preferentially concentrate in the skin and bones. Quantitatively, the liver, skin, bones and muscles are the chief tissues of physiological importance in cobalt metabolism.

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Characterization and Classification of ECHO 28-Rhinovirus-Coryzavirus Agents. (27662)

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Several viruses or groups of viruses have been recovered in tissue culture from cases of common cold. The first of these were the 2060 and JH viruses discovered independently by Pelon *et al.*(1) and by Price(2) and now designated ECHO virus type 28(3). Additionally, there are the rhinoviruses described by Tyrrell, Taylor-Robinson and coworkers(4) and shown to consist of more than 6 immunologically different strains, certain of which grew only in human cell cultures (H strains) while others were propagated in monkey renal (M strains) as well as human cells; the coryzaviruses described by Hamparian *et al.*(5) and shown to comprise at least 4 distinct groups; viruses recovered by Johnson *et al.* (unpublished) and stated to consist of 6 distinct serotypes of the general enterovirus family; and viruses recovered by Hamre *et al.* (6) and by Hobson *et al.*(7).

The present report describes the isolation and immunologic differentiation of 20 distinct