ALTERATIONS IN TISSUE DISTRIBUTION OF ^{99m}Tc-PERTECHNETATE IN RATS GIVEN STANNOUS TIN

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A dose of 8 mg Sn(II)/kg produces significant mortality and gross kidney damage in the rat. The tissue distribution of ^{99m}Tc-pertechnetate is altered in rats which have previously received Sn(II). Minimal changes are noted with a dose of 0.02 mg Sn(II)/kg. It should be noted that most kits for human use contain less than 1 mg of Sn(II) per kit and result in a dose less than 0.007 mg Sn(II)/kg. Changes are still present 13 weeks after a dose of 8 mg Sn(II)/kg. It is apparent that the effects of Sn(II) on ^{99m}Tc-pertechnetate are not limited to in vitro reaction. The alterations in tissue distribution of ^{99m}Tc-pertechnetate are most likely due to the chemical reduction and fixation of the ^{99m}Tc-pertechnetate to tissue components due to the continuing presence of Sn(II) in vivo.

Stannous ion is widely used in the preparation of ^{99m}Tc-labeled radiopharmaceuticals (*1–3*). There are little data on the toxicity or metabolic effects of Sn(II) (*4,5*). During investigation of the toxicity of Sn(II), it was observed that the tissue distribution of ^{99m}Tc-pertechnetate was altered in rats which had previously received Sn(II) by intravenous injection. The duration of the changes in tissue distribution of ^{99m}Tc-pertechnetate produced by toxic doses of Sn(II) has been studied and the minimum dose of Sn(II) which produces a measurable effect has been determined.

MATERIALS AND METHODS

$SnCl_2 \cdot 2H_2O$ [0.04–6 mg Sn(II)/ml] was freshly dissolved in 10% sodium gluconate solution and injected into the tail veins of male Sprague-Dawley rats (Horton Laboratories, Berkeley, Calif.). Experimental groups received 0.02, 0.05, 0.1, 0.2, 2.0, 8.0, and 12 mg Sn(II)/kg. The rats ranged in weight from 200 to 300 gm at the start of the experiment and were fed *ad libitum*. Subgroups of rats were injected intravenously with 1–3 mCi ^{99m}Tc-pertechnetate at times ranging from 1 hr to 13 weeks after the Sn(II) injection. Control animals were given ^{99m}Tc-pertechnetate intravenously. Experimental and control rats were sacrificed 1 hr after the injection of ^{99m}Tc-pertechnetate. Animals were sacrificed under ether anesthesia by bleeding via the abdominal aorta, withdrawing a minimum of 8 ml of blood to drain the organs of their blood pools. Gross pathological changes were noted. The organs and one femur were removed, the rats skinned, and as much muscle as possible was dissected from the skeleton. Microscopic sections stained with hematoxylin and eosin were prepared from the kidneys. Red cells were prepared for counting by adding 11 ml of physiological saline to 1 ml of whole blood, centrifuging, and removing the supernatant. The activity in red cells, plasma, femur, liver, kidneys, stomach, intestine, muscle, skin, and carcass was measured in comparison with appropriate standards under identical geometric counting conditions. The percent of injected activity present in the skeleton and muscle was calculated assuming that the skeleton represents 8.2% of the body weight. Rats which had received 4 and 8 mg Sn(II)/kg in gluconate solution were held in metabolic cages for complete urine collections for measurement of δ-aminolevulinic acid and coproporphyrin to see if the Sn(II) induced changes in heme metabolism (*6,7*).

RESULTS AND DISCUSSION

There was a 50% mortality within 2 weeks in rats receiving 12 mg Sn(II)/kg. These rats had edema in the retroperitoneal tissue and pale enlarged

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TABLE 1. DISTRIBUTION OF ^{99m}Tc ACTIVITY IN RATS PREVIOUSLY GIVEN 8 Mg Sn(II)/Kg IN GLUCONATE

Time between Sn(II) and TcO_4^-	1 ml blood	RBC*	Liver	Kidneys	Stomach	Intestine	Femur	Skeleton	Muscle	Skin
1 hr (3)†	3.46	1.22	6.28	7.15	0.49	6.32	0.26	11.96	9.46	6.09
24 hr (3)	5.18	4.83	11.09	2.80	1.18	6.16	0.23	8.03	8.24	4.92
4 day (3)	6.68	6.30	9.31	1.10	1.45	4.28	0.26	7.51	9.26	4.52
1 wk (15)	5.47	5.11	11.60	2.93	1.77	5.25	0.40	12.33	15.10	6.87
2 wk (12)	1.08	0.96	7.90	5.83	5.39	7.66	0.40	10.28	26.19	11.97
4 wk (6)	0.22	0.13	5.46	2.68	10.10	7.24	0.39	13.06	25.15	21.82
8 wk (3)	0.24	0.093	3.4	1.6	15.0	6.10	0.33	12.11	18.39	23.6
13 wk (3)	0.30	0.03	3.93	1.39	17.21	6.08	0.29	11.99	13.54	26.16
Controls (15)	0.53	0.06	4.25	1.02	17.07	7.16	0.19	7.29	11.33	27.24

* Red blood cells from 1 ml whole blood.
† Number of animals in parentheses.

kidneys and some had retroperitoneal and intrapleural hemorrhages. One rat that received 12 mg Sn(II)/kg survived for 10 months, at which time it appeared pale and ill. Hemoglobin was 7.5 gm% and blood urea nitrogen was 126 mg%. At sacrifice the kidneys appeared pale and granular. Several rats had renal function evaluated by the injection of ^{99m}Tc-DTPA (*3*) and ^{99m}Tc-caseidin (*8*). Glomerular filtration was absent in rats 6 days after 12 mg Sn(II)/kg and no renal uptake of ^{99m}Tc-caseidin was detected in the rat dying at 10 months. Rats that received 8 mg Sn(II)/kg had severe weight loss during the first week with a 10% mortality by the second week. Rats given 2 mg Sn(II)/kg or less showed no observable ill effects.

The distribution of ^{99m}Tc activity after the injection of ^{99m}Tc-pertechnetate in control rats and in rats previously given 8 mg Sn(II)/kg is shown in Table 1 and Fig. 1. One hour after ^{99m}Tc-pertechnetate injection was chosen as the time of sacrifice since the tissue distribution of the ^{99m}Tc activity was similar in normal rats sacrificed at 45 min, 1 hr, and 1½ hr. The results are expressed as percent of injected ^{99m}Tc activity per organ and in 1 ml of blood or plasma.

The fraction of activity bound to red cells from 1 ml of blood at 1 hr was 1.2% and by 24 hr had increased to 4.0% with a maximum binding at 4 days of 6.3%, then falling to 1% at 2 weeks and to the control level at 13 weeks. The activity in 1 ml of plasma at 1 hr was 3.9% compared with a control value of 0.63% but at all subsequent times the experimental groups had lower than normal activity in the plasma fraction. Assuming a blood volume of 64.1 ml/kg of body weight (*9*), it can be calculated that most of the activity was in the blood up to 1 week following Sn(II) injection.

The liver activity showed a pattern similar to the blood (Fig. 1). The animals were bled but not perfused, and blood activity could explain part of the observed changes in the liver activity. The fluctuations in kidney activity, on the other hand, did not

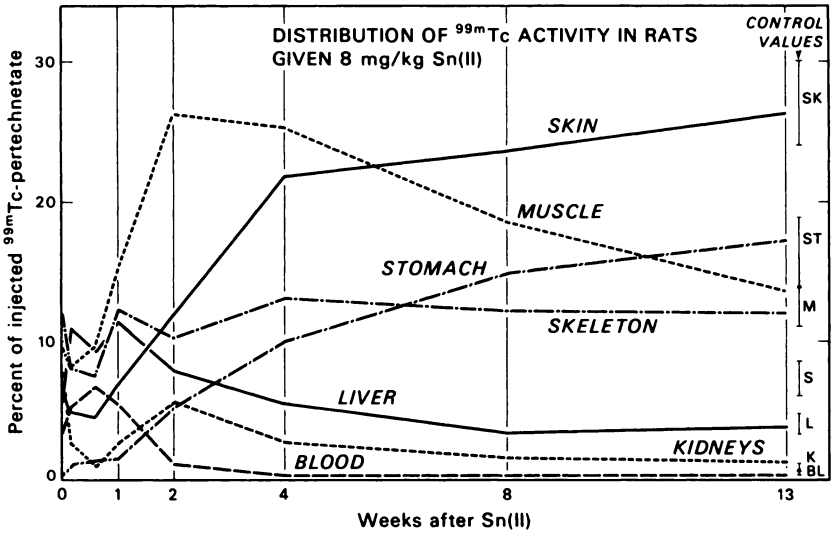

FIG. 1. Tissue distribution of ^{99m}Tc activity (percent of dose) in rats at times in weeks after injection of 8 mg Sn(II)/kg in gluconate solution.

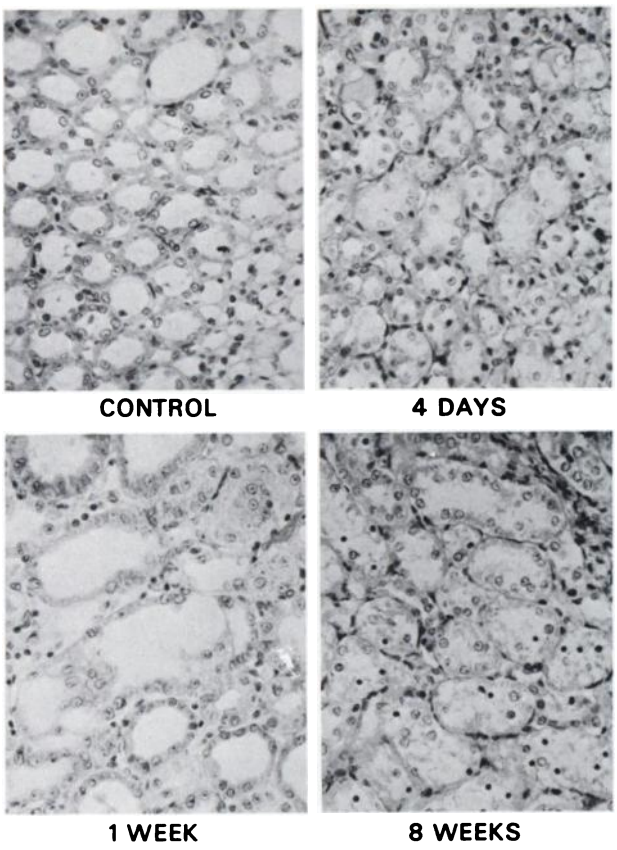

FIG. 2. Micrographs (465 × magnification) of rat kidneys showing effects of Sn(II).

follow the blood activity. The kidneys of the rats in the 1-hr group contained 7% of the injected activity when the blood activity was 3.5%. At 1 day kidney activity was 2.9% although blood activity had increased to 5%. Kidney activity was 1.1% at 4 days, the time of peak blood activity. As the blood activity fell, kidney activity increased to 5.2% at 2 weeks. These changes may be due to changes in blood flow occurring in the kidney as a consequence of damage produced by Sn(II). The kidneys appeared grossly normal in the 1-hr subgroup of rats but were pale at 1 day and enlarged and pale at 4 days. At 1 week the kidneys weighed 4 gm compared with the normal weight of 2.2 gm and were still pale in color. The kidneys remained enlarged and paler than usual at 13 weeks. Microscopic changes culminating in tubular epithelial necrosis occurred within the first week (Fig. 2). The necrosis was followed by epithelial regeneration of the tubules. The regenerated epithelium was lower than normal and the tubular lumen were markedly dilated. The cortical interstitial tissue was edematous and contained occasional small foci of lymphocytes.

The activity in the stomach was greatly reduced in the subgroups of animals sacrificed at 1 hr to 4 weeks and was within the normal range at 8 and 13 weeks. Presumably ^{99m}Tc-pertechnetate did not enter its normal metabolic pathway in the stomach because it was chemically reduced and bound in other tissues. The stomach and small and large intestines were grossly normal in the 1-hr subgroup. At 1 day the stomachs were distended with food and the intestines appeared empty. At 4 days the stomachs were normal in size and the vessels of the intestines were dilated with an overall inflamed appearance. By 1 week stomach and intestines appeared normal. These gross changes resemble those described in lead poisoning where the stomach appears normal and the intestines are inflamed (*10*). The activity found in the intestines showed little change in any of the experimental subgroups.

The percent activity in the skin rose from 5% at times prior to 1 week to 12% of the injected activity at 2 weeks. It measured 22% at 4 weeks and levels were little different from the control value of 27% at the later times.

The calculated muscle activity in normal rats was 11.3% of the injected activity. The muscle activity was slightly below the control level through Day 4. At 1 week the fraction of activity in muscle was 15%, rising to a level of 26% at 2 weeks and decreasing again to 13% at 13 weeks, the latest time of study.

Calculated skeletal activity and the activity in one femur were increased above the control levels in all experimental groups except the animals sacrificed on Day 4 when red cell activity accounted for the major fraction of the injected activity. The skeletal activity remained around 12% from 1 week to 13 weeks compared with the control level of 7.3%.

Comparable changes in ^{99m}Tc-pertechnetate distribution were seen in rats which received 8 mg Sn(II)/kg dissolved in water, saline, 5% dextrose, and citrate buffer pH 6.2 as in rats given the gluconate solutions (Table 2).

To explore the dose response curve of the effect of Sn(II) on ^{99m}Tc-pertechnetate metabolism, rats were injected with amounts of Sn(II) ranging from 0.02 mg/kg to 2 mg/kg and ^{99m}Tc-pertechnetate distribution was studied at 24 hr and 2 weeks (Fig. 3). At 24 hr, in rats which received 0.02 mg Sn(II)/kg, the red cells from 1 ml of blood contained 1.4% of the injected activity compared with the control level of 0.06%. The red cell activity increased with the larger doses. Likewise, the activity in the liver and kidneys was increased above the control level at the lowest dose (0.02 mg/kg) with greater changes being induced by the larger doses. The activity in the stomach and skin decreased as the amount of Sn(II) injected was increased. At 2 weeks the activity in the kidneys of rats receiving 2 mg Sn(II)/kg was 3.7% (control 1%) and in the mus-

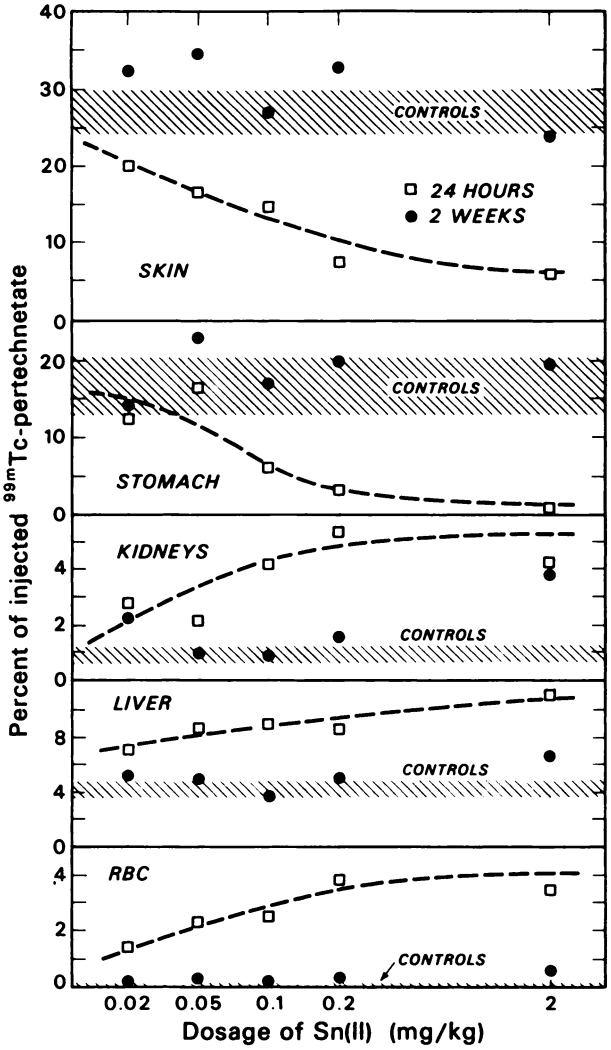

FIG. 3. Tissue distribution of ^{99m}Tc activity (percent of dose) 24 hr and 2 weeks after varying doses of Sn(II)/kg in gluconate solution to show threshold for effect on ^{99m}Tc distribution. Line drawn through 24 hr points to emphasize trend.

cle and skeleton combined was 36% compared with the mean figure of 18.5% in the control group. The distribution of activity at the other dose levels was within the control range. Preparation of liver colloid (Medi+Physics, Inc., Emeryville, Calif.), DTPA (*3*), and EHDP (*11*) without ^{99m}Tc-pertechnetate produced similar but small changes in ^{99m}Tc-pertechnetate distribution when given to rats in amounts resulting in a dose of Sn(II)/kg 70 times the amount of Sn(II)/kg which a patient would receive.

The chemical form of the ^{99m}Tc activity in lysed red cells and liver homogenates from rats given Sn(II) has been investigated using paper chromatography and Agl-X8 anion exchange (BioRad Labs, Richmond, Calif.) and Sephadex G-25 columns (Pharmacia Fine Chemicals, Piscataway, N.J.). Ninety-nine percent of the ^{99m}Tc activity in the lysate of washed red cells from a 4-day rat (Table 1) remained at the origin in an ascending paper chromatogram using 85% methanol as the solvent. No pertechnetate fraction was detected in the eluate from the Sephadex column, the activity being confined to the early fractions which were red in color. Ten percent of the activity was retained on the anion exchange column. Similar analysis of a liver homogenate of the animal showed that 80% of the ^{99m}Tc activity was not in the form of pertechnetate. It is proposed that the ^{99m}Tc-pertechnetate is reduced in the tissues of animals previously given Sn(II) and becomes fixed to tissue components such as hemoglobin (*2*).

The rats maintained in metabolic cages for 1 week following the injection of 4 and 8 mg Sn(II)/kg did not show an elevation of coproporphyrin in the urine nor was δ-aminolevulinic acid excretion increased. Although tin occurs in the same group in the periodic table of elements as lead and produces many of the toxic manifestations of lead poisoning, these results indicate that tin does not influence heme metabolism.

A single tracer experiment was performed in which ^{113}Sn(II) was administered with carrier $SnCl_2 \cdot 2H_2O$ in 10% sodium gluconate. Rats received between 2 and 16 mg Sn(II)/kg. This amount of carrier Sn(II) in gluconate solution had no effect on the tissue distribution of the tracer ^{113}Sn(II). In animals sacrificed at 1 hr, approximately 2% of ^{113}Sn was in one femur, 3% in the liver, 5% in the kidneys, 0.5%/ml whole blood, and 0.03%/gm of muscle.

TABLE 2. 8 Mg Sn(II)/Kg

$SnCl_2 \cdot 2H_2O$ dissolved in:	Time after Sn(II)	1 ml blood	RBC	Liver	Kidneys	Stomach	Intestine	Femur
10% Na gluconate	1 week	5.47	5.11	11.6	2.93	1.77	5.25	0.26
5% dextrose	1 week	4.52	4.30	11.6	2.71	1.83	5.13	0.27
Physiological saline	1 week	3.56	3.50	14.60	3.84	3.04	5.08	0.24
Citrate buffer pH 6.2	1 week	4.84	4.34	19.2	2.86	2.1	5.65	0.28
Water	4 days	3.91	3.69	7.0	1.25	2.27	3.63	0.23

A second group of animals was sacrificed at 10 days; 2% of the activity was in one femur, less than 1% in the liver and kidneys, 0.02%/ml of whole blood, and muscle activity was less than 0.01%/gm. It can be seen that the relative distribution of ^{113}Sn is different from that of ^{99m}Tc activity in rats which had received Sn(II).

The changes in tissue distribution of ^{99m}Tc-pertechnetate with time must be related to factors such as blood flow and sites of first contact with tissues containing Sn(II) and to the continued presence of tin as Sn(II) in some tissues for longer periods of time than in others. Alternatively Sn(II) may induce a biochemical process in some tissues with the capacity to reduce ^{99m}Tc-pertechnetate. Regardless of the exact mechanism, the ^{99m}Tc activity becomes associated with large molecules within the cell.

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