ORGAN DISTRIBUTION OF ^{99m}Tc-AND ^{51}Cr-LABELED THYMOCYTES

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We have employed ^{99m}Tc as a radioisotopic label to study the organ distribution of murine thymocytes. The compartmentalization of ^{99m}Tc-labeled cells that had not been reduced by treatment with stannous chloride was similar to that of ^{51}Cr-labeled cells and was characterized initially by 48–50% uptake of the injected radioactivity by the lungs. Increased hepatic and splenic and decreased pulmonary localization were noted at 1 hr and these shifts were more pronounced by 4 hr. Technetium-99m-labeled cells reduced by stannous chloride had significantly different patterns of hepatic, pulmonary, and splenic localization and at 4 hr the lungs still retained 24% of the injected radioactivity compared with only 3% in the spleen. Size distribution studies revealed that unlabeled as well as unreduced and reduced ^{99m}Tc-labeled thymocytes were almost identical to one another so that differences in compartmentalization could not be attributed to this factor. Since reduction with stannous chloride did not alter the distribution of ^{51}Cr-labeled cells, this suggested some type of complex interaction between stannous ions and the labeling species of ^{99m}Tc. The in vivo localization of intravenously administered $Na^{99m}TcO_4$ and Na_2CrO_4 was markedly different from the corresponding radiolabeled cells thereby indicating that the distribution patterns that we observed truly represented cell-associated radioactivity. Although it may not necessarily be the proper reference point, the similarity in organ distribution of ^{99m}Tc- and ^{51}Cr-labeled cells should allow direct comparison of previously reported data employing this radionuclide and that obtained in future studies with ^{99m}Tc.

We have developed a method for labeling nucleated cells with ^{99m}Tc (*1,2*) and have described how this radionuclide can be used to study the migratory patterns of lymphocytes and tumor cells (*3*). Maximum splenic and hepatic localization of both syngeneic murine and xenogeneic human lymphocytes was noted 1 hr following intravenous administration to recipient mice and the number of gamma counts recorded in the spleen and liver was iinearly related to the number of cells injected.

We now present detailed data on the distribution of ^{51}Cr- and ^{99m}Tc-labeled murine thymocytes. The compartmentalization of ^{99m}Tc-labeled cells not reduced by treatment with stannous chloride was similar to that of ^{51}Cr-labeled thymocytes and was characterized by high initial uptake in the lungs with a subsequent shift to the liver and spleen. Technetium-99m-labeled cells reduced by stannous chloride, on the other hand, had very different patterns of hepatic, pulmonary, and splenic localization. Although the initial uptake was the same, migration of reduced cells from the lungs to the liver and spleen was considerably less than that of unreduced cells suggesting that treatment with stannous chloride produced an alteration of the cell surface membrane, the nature of which is yet to be determined. The organ distribution of free $Na^{99m}TcO_4$ and $Na_2{}^{51}CrO_4$ differed strikingly from that of the corresponding radiolabeled cells indicating that the distribution patterns that we have observed truly represented cell-associated radioactivity.

MATERIALS AND METHODS

Mice. BALB/c mice of either sex weighing approximately 18–20 gm were obtained from Carworth Farms, New City, New York or the Hall Mammalian Genetics Laboratory, University of Kansas, Lawrence, Kansas.

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Labeling of thymocytes with ^{99m}Tc. Thymic lymphocyte suspensions were prepared by mincing BALB/c thymuses, suspending the fragments in cold Hanks' Balanced Salt Solution (HBSS), and passing them through progressively higher gage needles attached to a 12-ml syringe. Sodium pertechnetate ($Na^{99m}TcO_4$) was eluted from a New England Nuclear molybdenum-technetium generator with 0.9% saline. Five microcuries of ^{99m}Tc and 100 μg of cold Na_2CrO_4 were added to a suspension containing 50 $\times$ 10^6 cells in 2 ml of HBSS and incubated for 10–15 min at 37°C. Following this the cells were sedimented by centrifugation at 400 $\times$ g for 15 min and the unbound radioisotope was removed by washing them three times in HBSS. The labeled cells, having a viability of 88–98% as determined by trypan blue exclusion (*4*), were adjusted to a final concentration of 4 $\times$ 10^7/ml. In some experiments the valence of ^{99m}Tc was reduced by the dropwise addition of 0.3 ml of a freshly prepared sterile solution of 0.2% $SnCl_2 \cdot 2H_2O$ dissolved in acid citrate dextrose (30 gm $Na_3C_6H_5O_7$, 0.15 gm NaH_2PO_4, and 2.0 gm dextrose per liter of distilled H_2O, pH adjusted to 7.4). After an additional 15 min incubation at 37°C, the cells were sedimented, washed three times in HBSS, and adjusted to a final concentration of 4 $\times$ 10^7/ml. Although reduction with stannous chloride increased the labeling efficiency approximately tenfold, it was not employed routinely for reasons that will be discussed later. The procedure described above has been modified recently. Thymic lymphocytes appear to label equally as well in the absence of Na_2CrO_4 carrier and with this method treatment of ^{99m}Tc-labeled cells with $SnCl_2$ does not result in the same degree of pulmonary entrapment that we observed with cells labeled in the presence of Na_2CrO_4.

Labeling of thymocytes with ^{51}Cr. Thymic lymphocytes were prepared as previously described. Labeling was carried out by adding 100 μCi of $Na_2{}^{51}CrO_4$ (specific activity 303 mCi/mg, Amersham/Searle, Chicago, Ill.) to a 12- $\times$ 75-mm plastic test tube containing 50–100 $\times$ 10^6 cells suspended in Eagle's Minimum Essential Medium supplemented with 10% fetal calf serum and allowing them to incubate for 30 min at 37°C with intermittent shaking. Following this, free ^{51}Cr was removed by washing the cells three times in phosphate-buffered saline pH 7.4 and then adjusting their final concentration to 4 $\times$ 10^7 cells per milliliter. In one set of experiments ^{51}Cr-labeled cells were treated with stannous chloride as previously described.

Size distribution. Sizing of unlabeled ^{99m}Tc and ^{51}Cr-labeled cells was carried out with a model ZBI Coulter Counter equipped with an H-4 Channelyzer®. This permitted volume discriminations in the range of ± 3 μ^3.

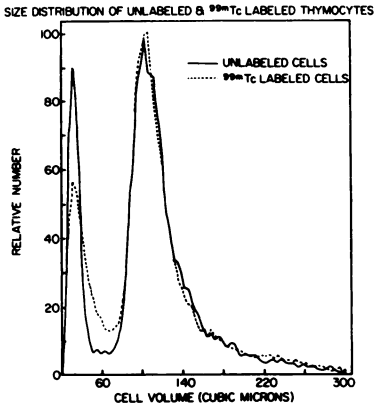

FIG. 1. Size distribution of unlabeled and ^{99m}Tc-labeled thymocytes. Sizing was carried out with model ZBI Coulter Counter equipped with H-4 Channelyzer. Two peaks were evident. One, consisting of particles having mean volume of 32 μ^3, was composed of cellular debris. Other peak, consisting of homogeneous population of intact cells, had mean volume of 100 μ^3.

Organ distribution experiments. Groups of four BALB/c mice were injected through the lateral tail vein with either 10^7 labeled cells suspended in 0.25 ml of HBSS, 1 mCi of $Na^{99m}TcO_4$, or 10 μCi of $Na_2{}^{51}CrO_4$. Animals were bled through the retro-orbital sinus immediately prior to killing by cervical dislocation, various organs and tissues were removed, and cell localization was determined by gamma scintillation counting for either ^{51}Cr or ^{99m}Tc. Decay correction was carried for ^{99m}Tc using a computer program by which each sample was corrected back to t_o, the time at which the first one of the series was counted. This program is available upon request. Gamma counts per minute recorded in each organ were converted to the percent injected dose of radioactivity by dividing by the total administered counts per minute and multiplying the quotient by 100.

RESULTS

Size distribution of unlabeled and ^{99m}Tc-labeled thymocytes. The size distribution patterns of ^{99m}Tc-labeled and unlabeled thymocytes were identical with one another (Fig. 1). Two peaks were evident. One, consisting of particles having a mean volume of 32 μ^3, was composed of cellular debris. The other, consisting of a homogeneous population of intact cells, had a mean volume of 100 μ^3. Chromium-51-labeled cells had a similar distribution pattern although the mean volume of the intact cells was somewhat less (90 μ^3).

Organ distribution of unreduced ^{99m}Tc and ^{51}Cr-labeled thymocytes. Groups of four BALB/c mice were injected intravenously with 10^7 ^{99m}Tc or ^{51}Cr-

TABLE 1. ORGAN DISTRIBUTION OF ^{51}Cr-LABELED BALB/c THYMOCYTES

Organ*	Percent injected dose of radioactivity†		
	10 min	1 hr	4 hr
Thymus	0.06 ± 0.02	0.04 ± 0.00	0.04 ± 0.00
Muscle	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Brain	0.89 ± 0.01	0.16 ± 0.02	0.06 ± 0.00
Skin	0.04 ± 0.00	0.27 ± 0.00	0.02 ± 0.00
Lymph nodes	0.08 ± 0.00	0.14 ± 0.02	0.22 ± 0.02
Heart	0.41 ± 0.08	0.40 ± 0.05	0.26 ± 0.03
Spleen	2.45 ± 0.17	16.16 ± 1.13	24.74 ± 1.68
Stomach	0.23 ± 0.02	0.18 ± 0.02	0.28 ± 0.05
Intestine	1.76 ± 0.14	2.34 ± 0.06	2.00 ± 0.02
Kidney	2.35 ± 0.26	2.29 ± 0.14	2.18 ± 0.18
Blood	12.28 ± 1.29	10.00 ± 0.09	5.96 ± 0.36
Liver	15.24 ± 1.38	25.32 ± 1.11	31.08 ± 0.72
Lung	50.57 ± 2.43	25.48 ± 1.68	4.60 ± 0.15
Total percent recoverable radioactivity	85.61	82.53	71.42

* BALB/c mice were injected intravenously with 10^7 ^{51}Cr-labeled BALB/c thymocytes and killed at the times indicated.
† Percent injected radioactivity was calculated by dividing the counts per minute of each organ or tissue by the total administered counts per minute and multiplying the quotient by 100.

labeled BALB/c thymocytes and killed after 10 min and 1 and 4 hr. Thymus, brain, lymph nodes, heart, spleen, stomach, intestine, kidneys, liver, lungs, skin (1 cm in diameter), muscle (~0.5 gm), and 0.1 ml of blood were removed for gamma counting. Counts per minute for total blood volume were calculated by multiplying by 24 (*5*). Unreduced ^{51}Cr and ^{99m}Tc-labeled cells were distributed primarily to the lungs, liver, blood, kidneys, spleen, and intestine (Tables 1 and 2). These organs accounted for 71–82% of the injected dose of ^{51}Cr and 57–79% of the ^{99m}Tc over a 4-hr interval. Ten minutes following intravenous administration, ^{99m}Tc and ^{51}Cr-labeled cells had similar patterns of distribution although shifts in compartmentalization were observed at 1 and 4 hr. The greatest differences were noted in the lungs which had 51% of the injected ^{51}Cr at 10 min, 25% at 1 hr, and 5% at 4 hr compared with 48% for ^{99m}Tc at 10 min, 21% at 1 hr, and 5% at 4 hr. Paralleling this decrease in the lungs, the liver and spleen showed comparable increases. Approximately 13% of the injected ^{51}Cr-labeled cells was recovered from the blood at 10 min and this decreased to 6% at 4 hr compared with 25% of the injected ^{99m}Tc-labeled cells at 10 min and 7% at 4 hr. The distribution of ^{51}Cr and ^{99m}Tc-labeled cells to the thymus, muscle, brain, skin, lymph nodes, heart, stomach, and kidneys was similar and without major change after 10 min.

Organ distribution of reduced ^{99m}Tc and ^{51}Cr-labeled thymocytes. The distribution of ^{99m}Tc-labeled cells that had been reduced by treatment with stannous chloride (Table 3) was markedly different from that observed with unreduced cells (Table 2). The greatest differences were noted in the lungs which had 38% of the injected ^{99m}Tc at 10 min, 22% at 1 hr, and 24% at 4 hr compared with 5% for unreduced cells at 4 hr. The spleen had 1% of the

TABLE 2. ORGAN DISTRIBUTION OF UNREDUCED ^{99m}Tc-LABELED BALB/c THYMOCYTES

Organ*	Percent injected dose of radioactivity		
	10 min	1 hr	4 hr
Thymus	0.08 ± 0.00	0.16 ± 0.02	0.16 ± 0.03
Muscle	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Brain	0.09 ± 0.00	0.06 ± 0.00	0.06 ± 0.00
Skin	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.00
Lymph nodes	0.28 ± 0.02	0.52 ± 0.02	0.53 ± 0.06
Heart	0.33 ± 0.04	0.38 ± 0.04	0.16 ± 0.03
Spleen	2.77 ± 0.25	12.14 ± 0.44	15.59 ± 2.35
Stomach	0.83 ± 0.05	1.79 ± 0.15	1.85 ± 0.25
Intestine	2.07 ± 0.19	4.53 ± 0.28	6.89 ± 0.34
Kidney	2.50 ± 0.22	2.43 ± 0.05	2.35 ± 0.18
Blood	24.78 ± 2.37	11.57 ± 0.64	6.62 ± 0.80
Liver	18.61 ± 1.20	27.64 ± 1.76	20.64 ± 2.39
Lung	47.52 ± 0.74	20.86 ± 0.64	5.35 ± 0.45
Total percent recoverable radioactivity	99.91	82.10	60.24

* BALB/c mice were injected intravenously with 10^7 ^{99m}Tc-labeled BALB/c thymocytes and killed at the times indicated. These cells had not been treated with stannous chloride.

TABLE 3. ORGAN DISTRIBUTION OF REDUCED ^{99m}Tc-LABELED BALB/c THYMOCYTES

Organ*	Percent injected dose of radioactivity		
	10 min	1 hr	4 hr
Thymus	0.16 ± 0.03	0.07 ± 0.01	0.17 ± 0.05
Muscle	0.06 ± 0.00	0.05 ± 0.01	0.13 ± 0.02
Brain	0.07 ± 0.01	0.05 ± 0.00	0.11 ± 0.00
Skin	0.08 ± 0.01	0.10 ± 0.01	0.08 ± 0.00
Lymph nodes	0.12 ± 0.01	0.13 ± 0.01	0.09 ± 0.01
Heart	0.30 ± 0.04	0.56 ± 0.29	0.59 ± 0.41
Spleen	1.34 ± 0.05	1.93 ± 0.38	2.76 ± 0.18
Stomach	0.48 ± 0.04	1.16 ± 0.13	2.94 ± 1.08
Intestine	2.06 ± 0.15	3.49 ± 0.50	5.26 ± 1.36
Kidney	3.84 ± 0.21	3.18 ± 0.75	3.88 ± 0.35
Blood	12.28 ± 1.54	4.83 ± 1.32	5.27 ± 0.22
Liver	15.27 ± 0.97	14.73 ± 2.99	15.92 ± 0.89
Lung	38.48 ± 3.27	22.33 ± 6.75	24.18 ± 2.23
Total percent recoverable radioactivity	74.57	52.62	61.39

* BALB/c mice were injected intravenously with 10^7 ^{99m}Tc-labeled BALB/c thymocytes and killed at the times indicated. These cells had been treated with stannous chloride.

TABLE 4. ORGAN DISTRIBUTION OF ^{51}Cr-LABELED BALB/c THYMOCYTES

Organ*	Percent injected dose of radioactivity		
	10 min	1 hr	4 hr
Thymus	N.D.†	N.D.	N.D.
Muscle	N.D.	N.D.	N.D.
Brain	N.D.	N.D.	N.D.
Skin	N.D.	N.D.	N.D.
Lymph nodes	0.02 ± 0.00	0.19 ± 0.01	0.27 ± 0.01
Heart	0.06 ± 0.00	0.07 ± 0.01	0.01 ± 0.00
Spleen	4.38 ± 0.20	14.66 ± 0.55	24.82 ± 0.64
Stomach	0.07 ± 0.01	0.08 ± 0.01	0.03 ± 0.00
Intestine	1.92 ± 0.08	2.60 ± 0.17	2.87 ± 0.19
Kidney	1.38 ± 0.10	2.11 ± 0.03	1.36 ± 0.05
Blood	8.22 ± 0.00	4.35 ± 0.00	2.27 ± 0.00
Liver	16.79 ± 0.58	35.94 ± 1.69	50.34 ± 2.84
Lung	64.58 ± 0.88	29.55 ± 1.83	5.73 ± 0.20
Total percent recoverable radioactivity	97.42	89.55	87.60

* BALB/c mice were injected intravenously with 10^7 ^{51}Cr-labeled BALB/c thymocytes and killed at the times indicated. These cells had been treated with stannous chloride.
† N.D. indicates that no radioactivity was detected.

injected ^{99m}Tc at 10 min, 2% at 1 hr, and 3% at 4 hr when reduced cells were given compared with 16% for unreduced cells. Contrasting with this decrease in the spleen at 4 hr, the liver had 16% of the injected ^{99m}Tc with reduced cells compared with 21% with unreduced cells. Total percent recoverable radioactivity for the reduced cells was 75% at 10 min, 53% at 1 hr, and 62% at 4 hr. Despite the lower total percent recoverable radioactivity with reduced compared with unreduced cells, the absolute counts per minute were approximately ten times higher with the former. The distribution of ^{51}Cr-labeled thymocytes that had been treated with stannous chloride (Table 4) was similar to that of untreated ^{51}Cr-labeled cells (Table 1). There was high initial uptake by the lungs followed by a shift to the liver and spleen. Hepatic uptake was 36% at 1 hr and 50% at 4 hr with stannous chloride-treated cells compared with 25% at 1 hr and 31% at 4 hr for untreated cells. This was associated with an equivalent increase in the total percent recoverable radioactivity. The distribution to all other organs was similar to that observed with unreduced cells. These data suggest that the altered distribution of ^{99m}Tc-labeled reduced cells was attributable to something more than a simple effect of stannous chloride on the cell surface.

Organ distribution of $Na^{99m}TcO_4$ and $Na_2{}^{51}CrO_4$. Groups of four BALB/c mice were injected intravenously with 1 mCi of ^{99m}Tc-labeled sodium pertechnetate or 10 μCi of ^{51}Cr-labeled sodium chromate and killed after 10 min, 1, 4, and 24 hr. Urine and feces were collected whenever possible. The organ distribution of the free radionuclides differed strikingly from that of radiolabeled cells. Technetium-99m localized primarily in the stomach, intestines, blood, and liver (Table 5). These organs accounted for 53% of the injected dose at 10 min, decreasing to 7% at 24 hr. Urinary excretion of ^{99m}Tc increased from 12% of the injected dose at 1 hr to 66% at 24 hr. The feces accounted for 3% of the injected dose at 4 hr and 33% at 24 hr. In contrast to the distribution of radiolabeled cells, the lungs had only 1% of the injected dose at 10 min and 0.03% at 24 hr. Chromium-51 localized primarily in the intestines, kidneys, blood, and liver (Table 6). The greatest difference in distribution of ^{51}Cr and ^{99m}Tc was noted in the stomach which had 22% of the injected ^{99m}Tc at 10 min, 32% at 1 hr, and 3% at 24 hr, compared with 1% of the injected ^{51}Cr over this same time interval. Decreased amounts of ^{51}Cr were detected in the intestines compared with ^{99m}Tc and this difference was greatest 4 hr following administration at which time they accounted for 5% of the ^{51}Cr compared with 25% of the ^{99m}Tc. Markedly decreased fecal excretion of ^{51}Cr paralleled the diminished gastrointestinal localization. Six percent of the injected ^{51}Cr was detected in the kidneys at 10 min and this remained constant thereafter while only 1% of the injected ^{99m}Tc was found in this organ. Urinary excretion of ^{51}Cr was 17% at 4 hr and 49% at 24 hr compared with 22% for ^{99m}Tc at 4 hr and 66% at 24 hr.

DISCUSSION

In the present series of experiments we have shown that ^{99m}Tc can be employed as a radioisotopic label to study the organ distribution of murine thymocytes. The in vivo localization of intravenously administered $Na^{99m}TcO_4$ (6) and $Na_2{}^{51}CrO_4$ was markedly different from that of the corresponding radiolabeled cells thereby indicating that the distribution patterns we have observed truly represented cell-associated rather than free radioactivity. The compartmentalization of unreduced ^{99m}Tc-labeled cells was similar to that observed with ^{51}Cr and, although this may not necessarily be the proper reference point, nevertheless it should allow direct comparison of previously reported data employing this radionuclide and that obtained in future studies with ^{99m}Tc. Increased hepatic and splenic and decreased pulmonary localization were noted at 1 hr and these shifts were more pronounced by 4 hr. The entrapment of stannous chloride-reduced ^{99m}Tc-labeled cells in the lungs was particularly striking and at 4 hr this organ still had 24% of the injected radioactivity.

TABLE 5. ORGAN DISTRIBUTION OF ^{99m}Tc-LABELED SODIUM PERTECHNETATE

Organ*	Percent injected dose of radioactivity			
	10 min	1 hr	4 hr	24 hr
Thymus	0.22 ± 0.02	0.13 ± 0.02	0.04 ± 0.00	0.00 ± 0.00
Muscle	1.45 ± 0.10	1.10 ± 0.09	0.52 ± 0.04	0.02 ± 0.00
Brain	0.15 ± 0.00	0.11 ± 0.00	0.04 ± 0.00	0.00 ± 0.00
Skin	0.21 ± 0.03	0.17 ± 0.05	0.03 ± 0.00	0.01 ± 0.00
Lymph nodes	0.81 ± 0.05	1.59 ± 0.19	0.39 ± 0.06	0.04 ± 0.00
Heart	0.63 ± 0.04	0.31 ± 0.04	0.09 ± 0.01	0.01 ± 0.00
Spleen	0.30 ± 0.05	0.24 ± 0.03	0.06 ± 0.00	0.00 ± 0.00
Stomach	21.96 ± 2.97	31.70 ± 2.14	12.57 ± 0.32	2.59 ± 0.37
Intestine	7.29 ± 0.80	14.83 ± 2.16	25.28 ± 3.42	2.84 ± 0.72
Kidney	1.29 ± 0.08	0.99 ± 0.09	0.45 ± 0.02	0.17 ± 0.01
Total blood	13.86 ± 0.73	10.17 ± 1.12	2.60 ± 0.34	0.41 ± 0.05
Liver	10.21 ± 0.80	8.69 ± 0.40	4.19 ± 0.50	1.24 ± 0.03
Lung	1.40 ± 0.11	0.89 ± 0.14	0.28 ± 0.04	0.03 ± 0.00
Urine	N.C.†	12.47 ± 0.00	21.56 ± 0.00	65.77 ± 0.00
Feces	N.C.	1.28 ± 0.00	3.43 ± 0.00	32.58 ± 0.00
Total percent recoverable radioactivity	59.78	84.67	71.53	105.71

* BALB/c mice were injected intravenously with 1 mCi of $Na^{99m}TcO_4$ and killed at the times indicated.
† N.C. indicates that none was collected.

These differences in the distribution of cells labeled in the absence of a formal reducing agent compared to reduced cells suggest that localization in part may be influenced by the chemical form of the radionuclide that has been used as a radioisotopic label as well as by cellular alterations that may result from the labeling procedure itself. Since unreduced and reduced ^{99m}Tc-labeled cells had similar viability as determined by trypan blue exclusion, this was not a tenable explanation for the differences that were observed. Nonviable cells preferentially localize in the liver (*7*), and in our studies similar amounts of ^{99m}Tc were detected in this organ irrespective of whether or not the cells had been reduced. Differences in size and sedimentation velocity may alter the migratory patterns of lymphoid cells. More rapidly sedimenting ^{51}Cr-labeled splenic or mesenteric lymph node cells have been reported to localize preferentially in the liver while less rapidly sedimenting cells localized in the spleen (*8*). Size dis-

TABLE 6. ORGAN DISTRIBUTION OF ^{51}Cr-LABELED SODIUM CHROMATE

Organ*	Percent injected dose of radioactivity			
	10 min	1 hr	4 hr	24 hr
Thymus	0.13 ± 0.01	0.12 ± 0.02	0.11 ± 0.02	0.05 ± 0.00
Muscle	0.07 ± 0.01	0.04 ± 0.00	0.06 ± 0.00	0.03 ± 0.00
Brain	0.12 ± 0.01	0.10 ± 0.00	0.06 ± 0.00	0.06 ± 0.00
Skin	0.07 ± 0.01	0.04 ± 0.00	0.04 ± 0.00	0.05 ± 0.00
Lymph nodes	0.39 ± 0.07	0.35 ± 0.04	0.33 ± 0.04	0.23 ± 0.01
Heart	0.58 ± 0.05	0.34 ± 0.11	0.38 ± 0.06	0.27 ± 0.05
Spleen	0.17 ± 0.01	0.24 ± 0.01	0.27 ± 0.03	0.18 ± 0.00
Stomach	0.77 ± 0.12	1.59 ± 0.20	0.62 ± 0.04	0.59 ± 0.04
Intestine	2.85 ± 0.16	6.08 ± 1.52	5.03 ± 0.20	4.80 ± 0.23
Kidney	6.19 ± 0.56	8.12 ± 0.63	7.91 ± 0.92	5.62 ± 0.82
Total blood	13.37 ± 1.82	12.80 ± 0.90	6.66 ± 0.57	5.75 ± 0.64
Liver	9.81 ± 0.84	12.02 ± 1.68	13.67 ± 1.15	7.33 ± 0.51
Lung	1.31 ± 0.12	1.05 ± 0.14	1.30 ± 0.22	0.57 ± 0.08
Urine	N.C.†	7.44 ± 0.00	17.26 ± 0.00	49.37 ± 0.00
Feces	N.C.	N.C.	1.80 ± 0.00	1.72 ± 0.00
Total percent recoverable radioactivity	35.83	50.33	55.55	76.62

* BALB/c mice were injected intravenously with 10 μCi of $Na_2{}^{51}CrO_4$ and killed at the times indicated.
† N.C. indicates that none was collected.

tribution patterns of unlabeled as well as unreduced and reduced ^{99m}Tc-labeled thymocytes were almost identical with one another so that the differences in compartmentalization could not be attributed to this factor. The chemical form and oxidation state of the labeling species of technetium in our system is unknown at the present time. It has been shown, however, that ^{99m}Tc forms chelates with organic molecules and proteins (*9–11*) and it can be postulated that this may be the mechanism by which subcellular organelles of nucleated cells are labeled (*12*). Since reduction with stannous chloride did not alter the distribution of cells labeled with ^{51}Cr or ^{99m}Tc in the absence of carrier Na_2CrO_4 (Barth and Singla, unpublished observations), this suggests some type of complex interaction between stannous cations and chromate anions, and a putative ^{99m}Tc chelate. The nature of this interaction and the concomitant alteration of the surface membrane of reduced cells remain to be determined.

On the basis of our own data on the distribution of ^{99m}Tc-labeled lymphoid cells in allograft recipients (*13*) and the effects of antilymphocyte serum on the localization of thymocytes (*14*) as well as on the recently reported use of ^{99m}Tc as a label to study platelet sequestration in patients with a variety of hematologic disorders (*15*), it appears that this radionuclide will have applicability to both experimental and clinical studies of cell migration.

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