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Injection of Cells and Monoclonal Antibodies into Mice: Comparison of Tail Vein and Retroorbital Routes[1] (41955)

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Abstract. Organ distribution and blood concentration profiles were compared following injection of mice with radiolabeled test agents via the lateral tail vein or retroorbital venous sinus. Monoclonal antibodies directed against B16 melanoma of C57BL/6 origin were labeled with iodine-125. Thymocytes from BALB/c mice and B16 melanoma cells were labeled with technetium-99m sodium pertechnetate (Na $^{99m}TcO_4$). Animals were injected with 5 μCi of iodinated antibody, 5×10^5 syngeneic thymocytes, 2.5×10^5 melanoma cells, or 10 μCi Na $^{99m}TcO_4$ in 0.2 ml saline via either route. In non-tumor-bearing C57BL/6 mice radiolabeled monoclonal antibody was found primarily in the gastrointestinal tract, liver, and blood. Na $^{99m}TcO_4$ localized in the gastrointestinal tract, ^{99m}Tc-labeled thymocytes in the spleen and liver, and ^{99m}Tc-labeled B16 melanoma cells in the liver and lungs. Pharmacokinetic analysis of blood samples taken 4, 8, and 12 min following injection of the labeled agents suggested that the iodinated antibody had less vascular permeability than Na $^{99m}TcO_4$ and that thymocytes and B16 melanoma cells were trapped in the pulmonary vasculature as they passed through the lungs. It is noteworthy that no biologically significant differences in organ distribution patterns or blood decay profiles were found between lateral tail vein and retroorbital routes. The data clearly indicate that these routes can be used interchangeably with one another for intravenous injections.

The lateral tail vein has been a principal route by which a wide variety of agents have been injected intravenously into mice (1). An alternative injection technique via the retroorbital venous sinus has been described (2). The latter method is often quicker and easier to use than injection via the tail, especially with heavily pigmented mice. Retroorbital injections have been used infrequently, primarily because of the uncertainty as to whether the two routes were similar.

The purpose of the present study was to compare intravenous injections via the lateral tail vein and retroorbital sinus by studying organ distribution patterns and blood concentration profiles of four test agents: anti-B16 melanoma monoclonal antibodies, BALB/c thymocytes, C57BL/6 B16 melanoma cells, and ^{99m}Tc sodium pertechnetate. Monoclonal antibodies were labeled with iodine-125 and injected by either route into iodine-loaded C57BL/6 mice. Cells were labeled with technetium-99m and were similarly injected into syngeneic mice. Differences between the routes were studied by comparing the amount of radioactivity localizing in various organs and blood samples. Each agent had distinctive distribution patterns. No biologically significant differences were found in the blood concentration and organ distribution patterns when the two intravenous routes were compared, indicating that they can be used interchangeably.

Materials and Methods. *Preparation and labeling of monoclonal antibody.* Rat monoclonal antibodies were produced against B16 murine melanoma as previously described (3, 4). One monoclonal antibody, designated IB16-6, was selected for its specific binding to B16 melanoma cells. Ascites containing monoclonal antibody was harvested from hybridoma-bearing rats and purified with two ammonium sulfate precipitations and one sodium sulfate precipitation. The monoclonal

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antibody was purified further by passage over a G-100 Sephadex column (Pharmacia, Piscataway, N.J.). Purified antibody was iodinated using the iodogen method (5), and antibody concentration was adjusted to approximately 25 μg purified protein per milliliter (or 25 μCi ^{125}I) with Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline (PBS) containing 10% heat-inactivated calf serum (GIBCO, Grand Island, N.Y.) and 1 g D-glucose per liter of solution.

Labeling of cells with ^{99m}Tc. The method used to label thymocytes and B16 F10 melanoma cells was similar to a previously described technique (6). Thymocytes were obtained by passing a BALB/c thymus through an 80 mesh screen and then suspending the cells in 5 ml of cold Ca^{2+} and Mg^{2+} free PBS supplemented with D-glucose. B16 F10 melanoma cells, kindly provided by Dr. Erling Jensen, Mason Research Institute, Worcester, Massachusetts, were propagated as a continuous cell line in culture flasks with Dulbecco's modified Eagle's medium supplemented with 50 m*M* sodium pyruvate, 10% heat-inactivated newborn calf serum, 1% L-glutamine, 1% penicillin–streptomycin solution (GIBCO, Grand Island, N.Y.), 1% nonessential amino acids (MA Bioproducts, Walkersville, Md.), 8 μg insulin, and 1 m*M* oxalacetic acid (Sigma Chemical Co., St. Louis, Mo.). Cells were harvested from the flasks by rinsing once with PBS and treating them for approximately 15 min with 0.25 ml ethylenediamine-tetraacetic acid (EDTA) in PBS.

The thymocyte and the B16 cell suspensions were sedimented at 1500 rpm, washed three times with PBS/glucose, resuspended in 5 ml of cold Hanks' balanced salt solution (HBSS), (GIBCO, Grand Island, N.Y.), and their concentration adjusted to 2 to 5 ml HBSS. One-half milliliter of freshly prepared 0.2% $SnCl_2 \cdot 2H_2O$ in acid citrate dextrose buffer (30 g $Na_3C_6H_5O_7$, 0.15 g NaH_2PO_4, and 2.0 g dextrose per liter distilled H_2O; pH 7.4) was added to the cell suspension in order to serve as a reducing agent. The suspensions were incubated for 15 min at 37°C; and the cells were sedimented, washed twice with HBSS, and resuspended in 5 ml HBSS.

^{99m}Tc sodium pertechnetate was eluted from a molybdenum–technetium generator (Cintichem Incorp., Tuxedo, N.Y.) with 0.9% saline. One millicurie was added to the thymocytes, 5 mCi was added to the B16 melanoma cells, and both were incubated another 15 min at 37°C, sedimented, and washed three times in HBSS. The labeled thymocytes were suspended in enough HBSS to give a final concentration of 2.5×10^6 cells per milliliter solution. The B16 F10 cells were adjusted to a final concentration of 1.25×10^5 cells per milliliter. Viability, as determined by trypan-blue exclusion, ranged from >95% for thymocytes to >86% for B16 cells.

Injection of mice and determination of organ distribution patterns and apparent total blood clearance. Mice were divided into groups of six or eight animals, half of which were injected via lateral tail vein and the other half via the retroorbital venous sinus. Tail vein injections were carried out by warming the mice under a heat lamp for approximately 10 min and then injecting 0.2 ml of the test agent into either the right or left lateral tail vein. Injections into the retroorbital sinus were carried out by firmly grasping the mouse behind the head and inserting a 27-gauge needle into the lateral canthus of the right eye with the bevel facing medially. Groups of female C57BL/6 mice (Charles River, Portage, Mich., or Frederick Cancer Research Center, Frederick, Md.) approximately 15 weeks old and weighing 23 g were injected with 5 μCi ^{125}I-labeled monoclonal antibody (approximately 5 μg protein) or 2.5×10^5 of B16 melanoma cells in 0.2 ml of HBSS. Mice injected with ^{125}I-labeled antibody received iodinated drinking water (two drops of Lugol's solution per 100 ml) 5 days prior to injection. Female BALB/c mice (Charles River, Portage, Mich.), approximately 15 weeks old, received 0.2 ml of either 10 μCi of ^{99m}Tc sodium pertechnetate or 5×10^5 labeled thymocyte suspension in HBSS. For pharmacokinetic studies, groups of mice had 0.1 ml blood taken from the left venous sinus at 4, 8, and 12 min. Another 0.2 ml of blood was taken from the left venous sinus immediately prior to killing by cervical dislocation.

Organs and tissues were removed and placed into 12 × 75-mm tubes containing 2 ml of 10% buffered Formalin. The organs and blood samples were counted for ^{125}I or ^{99m}Tc by means of a gamma scintillation

counter (Tracor Analytic, Elk Grove Village, Ill.). Counts were corrected for decay by a computer program that corrected sample counts back to the time of injection. Values for blood were multiplied by 6.3 to adjust samples to total blood volume of 1.3 ml for 23-g mice (7). Gamma counts per minute per organ were divided by the counts per minute of the injected dose and multiplied by 100% to obtain the percentage injected dose of radioactivity. Values for the first blood samples taken from mice receiving ^{99m}Tc-labeled agents were subjected to one compartment pharmacokinetic analysis by NONLIN (8) computer fitting the data, using a weighting term of $1/y^2$.

Results. The organ distribution patterns of ^{99m}Tc sodium pertechnetate, ^{99m}Tc-labeled thymocytes and B16 melanoma cells, and IB16-6 monoclonal antibody are summarized in Tables I–IV. Data are expressed as the means ± SEM. For most of the organs and tissues, the mean ± SEM of the retroorbital and lateral tail vein routes overlapped with one another. In only a few instances, most notably the 4- and 24-hr values of liver for B16 cells, and the 24-hr values of lungs for both thymocytes and B16 cells, were differences greater than 2 SEM units noted between the two routes in one series of experiments (Tables II and III). When the experiments using ^{99m}Tc-labeled agents were repeated, however, no major differences were observed. (Data not included.)

The characteristic localization patterns of each agent appeared to be independent of the route of injection. In mice injected with ^{99m}Tc pertechnetate (Table I), most of the recoverable radioactivity was localized in the gastrointestinal tract. For example, after 1 hr, 22.1% of the injected ^{99m}Tc was found in the stomach and intestines of mice injected via retroorbital sinus, compared to 25.1% for mice injected via tail vein. Thymocytes localized mainly in the liver and spleen (Table II). For mice injected via retroorbital sinus, 11.3% of the injected radioactivity was found in the liver and 5% in the spleen at 24 hr. Corresponding values for mice injected via tail vein were 12.4% and 4.0%, respectively. Most of the injected radioactivity for B16 melanoma cells (Table III) was found in the lungs and liver for both retroorbital and tail

TABLE I. PERCENTAGE INJECTED DOSE OF SODIUM PERTECHNETATE[a,b]

Organ	1 hr		4 hr		24 hr	
	Retroorbital	Tail vein	Retroorbital	Tail vein	Retroorbital	Tail vein
Thymus	0.16 ± 0.02	0.21 ± 0.04	0.14 ± 0.02	0.13 ± 0.03	0.01 ± 0.00	0.01 ± 0.00
Muscle	0.21 ± 0.03	0.20 ± 0.04	0.18 ± 0.04	0.24 ± 0.03	0.01 ± 0.00	0.01 ± 0.00
Skin	0.34 ± 0.07	0.51 ± 0.13	0.25 ± 0.07	0.30 ± 0.11	0.03 ± 0.01	0.04 ± 0.02
Lymph nodes	0.33 ± 0.09	0.16 ± 0.05	0.17 ± 0.03	0.10 ± 0.01	0.01 ± 0.00	0.01 ± 0.00
Heart	0.28 ± 0.02	0.23 ± 0.03	0.21 ± 0.03	0.14 ± 0.01	0.01 ± 0.00	0.01 ± 0.00
Spleen	0.21 ± 0.00	0.24 ± 0.02	0.17 ± 0.02	0.16 ± 0.01	0.01 ± 0.00	0.01 ± 0.00
Stomach	17.35 ± 1.83	20.08 ± 2.22	8.77 ± 0.47	8.44 ± 1.27	0.76 ± 0.18	0.49 ± 0.08
Intestine	4.75 ± 0.52	4.99 ± 0.93	11.46 ± 1.26	10.55 ± 1.05	0.45 ± 0.03	0.92 ± 0.09
Kidney	0.87 ± 0.07	0.95 ± 0.09	0.91 ± 0.04	0.69 ± 0.04	0.14 ± 0.03	0.15 ± 0.03
Liver	3.58 ± 0.52	4.06 ± 0.56	3.14 ± 0.19	2.52 ± 0.09	0.27 ± 0.06	0.33 ± 0.06
Lung	0.52 ± 0.03	0.61 ± 0.12	0.38 ± 0.04	0.36 ± 0.02	0.02 ± 0.00	0.02 ± 0.00
Femur	0.14 ± 0.00	0.18 ± 0.03	0.12 ± 0.01	0.10 ± 0.01	0.01 ± 0.00	0.01 ± 0.00
Blood	9.11 ± 0.70	10.25 ± 1.14	7.21 ± 0.57	5.38 ± 0.25	0.13 ± 0.02	0.19 ± 0.02
Total percentage recoverable radioactivity	37.86	42.69	33.11	29.12	1.85	2.15

[a] Means ± SEM.
[b] $N = 4$ mice.

TABLE II. PERCENTAGE INJECTED DOSE ^{99m}Tc-LABELED THYMOCYTES[a,b]

Organ	1 hr		4 hr		24 hr	
	Retroorbital	Tail vein	Retroorbital	Tail vein	Retroorbital	Tail vein
Thymus	0.06 ± 0.00	0.09 ± 0.01	0.38 ± 0.03	0.41 ± 0.07	1.14 ± 0.17	0.80 ± 0.03
Muscle	0.09 ± 0.02	0.07 ± 0.01	0.29 ± 0.02	0.43 ± 0.01	0.82 ± 0.03	1.02 ± 0.04
Skin	0.17 ± 0.01	0.11 ± 0.04	0.45 ± 0.01	0.43 ± 0.02	1.02 ± 0.12	0.92 ± 0.10
Lymph nodes	0.10 ± 0.02	0.13 ± 0.01	0.34 ± 0.04	0.43 ± 0.06	1.00 ± 0.11	0.91 ± 0.09
Heart	0.18 ± 0.02	0.15 ± 0.01	0.48 ± 0.10	0.44 ± 0.15	1.01 ± 0.16	1.13 ± 0.08
Spleen	2.29 ± 0.35	1.73 ± 0.70	3.80 ± 0.49	4.93 ± 0.17	5.02 ± 1.14	4.85 ± 0.15
Stomach	1.68 ± 0.37	1.81 ± 0.25	1.80 ± 0.25	2.00 ± 0.17	0.98 ± 0.07	1.31 ± 0.18
Intestine	3.26 ± 0.26	3.11 ± 1.53	3.39 ± 0.92	5.15 ± 0.85	1.36 ± 0.12	1.71 ± 0.23
Kidney	3.74 ± 0.16	4.11 ± 0.11	3.78 ± 0.07	3.87 ± 0.37	2.32 ± 0.23	2.73 ± 0.17
Liver	10.15 ± 1.09	11.94 ± 3.45	10.06 ± 2.05	14.31 ± 2.13	11.26 ± 1.11	12.37 ± 1.55
Lung	2.10 ± 0.71	2.64 ± 0.93	1.72 ± 0.51	3.86 ± 1.14	1.90 ± 0.03	5.12 ± 2.77
Femur	0.15 ± 0.01	0.17 ± 0.02	0.47 ± 0.04	0.56 ± 0.03	0.98 ± 0.14	1.13 ± 0.17
Blood	2.71 ± 0.25	3.16 ± 0.19	4.17 ± 0.25	4.61 ± 0.38	7.46 ± 0.86	7.53 ± 1.14
Total percentage recoverable radioactivity	26.67	29.21	31.13	41.25	36.26	41.52

[a] Means ± SEM.
[b] $N = 3$ mice.

TABLE III. PERCENTAGE INJECTED DOSE OF ^{99m}Tc-LABELED B16 F10 MOUSE MELANOMA[a,b]

Organ	1 hr		4 hr		24 hr	
	Retroorbital	Tain vein	Retroorbital	Tail vein	Retroorbital	Tail vein
Thymus	0.11 ± 0.09	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.00	0.03 ± 0.01	0.03 ± 0.00
Muscle	0.01 ± 0.00	0.04 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.05 ± 0.02
Skin	0.03 ± 0.01	0.07 ± 0.02	0.03 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
Lymph nodes	0.02 ± 0.00	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.02 ± 0.00
Heart	0.47 ± 0.23	0.70 ± 0.39	0.14 ± 0.02	0.24 ± 0.13	0.13 ± 0.01	0.20 ± 0.06
Spleen	0.50 ± 0.06	0.64 ± 0.19	1.01 ± 0.03	1.24 ± 0.13	1.75 ± 0.33	2.07 ± 0.11
Stomach	0.88 ± 0.22	0.96 ± 0.24	1.08 ± 0.27	0.90 ± 0.21	0.49 ± 0.09	0.40 ± 0.08
Intestine	1.24 ± 0.14	0.98 ± 0.30	2.35 ± 0.43	2.18 ± 0.16	1.02 ± 0.18	1.27 ± 0.11
Kidney	1.87 ± 0.18	2.20 ± 0.39	2.15 ± 0.20	2.53 ± 0.02	1.81 ± 0.28	2.05 ± 0.15
Liver	8.12 ± 1.56	8.51 ± 1.10	8.23 ± 1.19	13.16 ± 1.21	11.00 ± 1.13	17.68 ± 1.07
Lung	14.08 ± 2.00	16.99 ± 2.52	22.46 ± 2.10	22.70 ± 4.32	9.60 ± 0.56	16.86 ± 3.57
Femur	0.04 ± 0.01	0.05 ± 0.01	0.07 ± 0.01	0.11 ± 0.01	0.14 ± 0.03	0.12 ± 0.02
Blood	1.45 ± 0.19	1.77 ± 0.32	1.14 ± 0.06	1.45 ± 0.02	0.51 ± 0.06	0.57 ± 0.06
Total percentage recoverable radioactivity	28.82	32.99	38.58	44.65	26.58	41.37

[a] Means ± SEM.
[b] $N = 4$ mice.

vein routes at 1, 4, and 24 hr. In contrast to this, most of the monoclonal antibody (Table IV) localized in the gastrointestinal tract (6.8% for retroorbital, 5.5% for tail vein), liver (6.4% retroorbital, 6.9% tail vein), or remained in the blood (42.88% retroorbital, 44.02% tail vein) at 1 hr.

The total recoverable radioactivity was consistently less than 100% and ranged from 64.35% for iodinated antibody injected via retroorbital sinus at 1 hr to 1.9% for Na $^{99m}TcO_4$ injected via the retroorbital route at 24 hr. This has been shown previously to be due to either localization in the carcass of the animals or alternatively excretion (9). In the present study, the carcass represented approximately 70% of the total body weight of a mouse.

The disappearance profiles of injected agents from the blood did not vary significantly between the two routes. Blood concentrations were determined for samples taken 4, 8, and 12 min after injection. Blood concentration data at the corresponding time points showed no significant differences, ($P < 0.05$), as determined by Student's t test, between retroorbital and tail vein routes. The combined averages of these values are presented graphically in Fig. 1, and were used for the pharmacokinetic analysis.

The data for the first 12 min after injection exhibited monoexponential decay, characteristic of apparent one compartment first-order elimination of a single species. Thus, blood concentration values were NONLIN computer-fitted to a one compartment open model system with first-order elimination and intravenous bolus administration. Injected material half-life ($t_{1/2}$), apparent volume distribution (V_d), and apparent total body blood clearance (Cl), are listed in Table V, and were calculated with the NONLIN computer program. The V_d and Cl values for the thymocytes and B16 melanoma cells were larger than the corresponding values for the other two agents. The larger apparent volumes of distribution and clearances probably were due to first-pass trapping of cells in the lungs. Since the sampling period of 12 min may represent only the initial disposition phase of these agents, the pharmacokinetic analysis should not be construed as definitive but rather as comparative.

TABLE IV. PERCENTAGE INJECTED DOSE OF ^{125}I-LABELED IBL6 MONOCLONAL ANTIBODY[a,b]

Organ	1 hr		4 hr		24 hr	
	Retroorbital	Tail vein	Retroorbital	Tail vein	Retroorbital	Tail vein
Thymus	0.50 ± 0.04	0.17 ± 0.02	0.25 ± 0.04	0.16 ± 0.02	0.07 ± 0.02	0.08 ± 0.02
Muscle	0.14 ± 0.01	0.09 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.05 ± 0.00	0.03 ± 0.00
Skin	0.25 ± 0.04	0.19 ± 0.02	0.34 ± 0.04	0.29 ± 0.07	0.17 ± 0.03	0.13 ± 0.01
Lymph nodes	0.15 ± 0.03	0.12 ± 0.02	0.21 ± 0.03	0.16 ± 0.01	0.06 ± 0.01	0.05 ± 0.00
Heart	1.13 ± 0.18	1.27 ± 0.11	0.67 ± 0.10	0.68 ± 0.11	0.32 ± 0.04	0.22 ± 0.05
Spleen	1.76 ± 0.20	1.54 ± 0.02	1.07 ± 0.06	1.15 ± 0.12	0.25 ± 0.02	0.25 ± 0.03
Stomach	3.02 ± 0.27	2.46 ± 0.24	3.69 ± 0.48	3.68 ± 0.70	0.39 ± 0.10	0.46 ± 0.06
Intestine	3.79 ± 0.38	3.08 ± 0.31	3.65 ± 0.30	2.81 ± 0.25	0.72 ± 0.04	0.61 ± 0.03
Kidney	2.22 ± 0.09	2.07 ± 0.11	1.44 ± 0.10	1.33 ± 0.08	0.35 ± 0.04	0.31 ± 0.01
Liver	6.35 ± 0.54	6.92 ± 0.30	4.31 ± 0.33	3.43 ± 0.25	1.01 ± 0.02	0.89 ± 0.08
Lung	1.34 ± 0.13	1.47 ± 0.21	1.10 ± 0.17	1.42 ± 0.37	0.26 ± 0.02	0.26 ± 0.02
Femur	0.84 ± 0.13	0.66 ± 0.05	0.70 ± 0.07	0.76 ± 0.06	0.30 ± 0.05	0.26 ± 0.03
Blood	42.88 ± 3.29	44.02 ± 3.35	25.87 ± 0.69	22.52 ± 0.38	6.58 ± 0.13	6.95 ± 0.44
Total percentage recoverable radioactivity	64.35	64.06	43.43	38.51	10.52	10.50

[a] Means ± SEM.
[b] $N = 4$ mice.

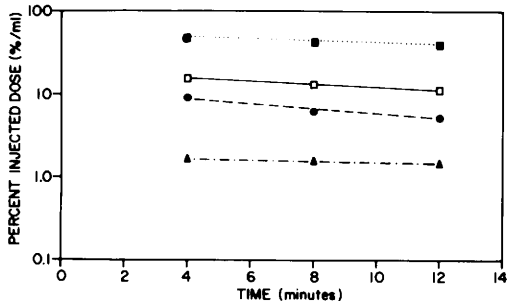

FIG. 1. Blood decay profiles of ^{125}I-labeled monoclonal antibody (■), technetium-99m sodium pertechnetate (□), thymocytes (●), and B16 melanoma cells (▲) after intravenous injection. Points on the graph represent the mean percentage injected dose of the combined tail vein and retroorbital disappearance rates.

Discussion. The results of our experiments showed that the organ distribution patterns and blood decay profiles for the two routes of iv injection were almost identical. This may indicate that differences in systemic intravenous routes do not affect localization patterns. In each case, the agent would travel to the right heart and from there to the pulmonary circulation without encountering the portal system or any other capillary network. Possible differences between the two routes could have been the total amount of the injected agent actually getting into the vascular compartment, or the time the agent would have taken to reach a specific organ site. However, the variability that occurred from animal to animal would have obviated the significance of these differences.

The organ distribution patterns and blood decay profiles reflected the differences in molecular or cellular size of the test agents. ^{99m}Tc sodium pertechnetate was quickly excreted from mice, as evidenced by the small total percentage recoverable radioactivity at 24 hr. ^{125}I-labeled monoclonal antibody persisted in the vascular compartment at higher levels than any of the other test agents, as would be expected of a high-molecular-weight serum protein. Thymocytes and melanoma cells were apparently trapped in the lungs and liver. Thymocytes also appeared to localize in the spleen. F10 melanoma cells showed a striking propensity to localize in the lungs, as might be predicted from their adhesive properties (10).

Differences in blood concentration between the two routes at 4, 8, and 12 min following injection were not statistically significant ($P < 0.05$). Pharmacokinetic analysis performed on the average data of both routes of administration indicated a higher degree of first-pass trapping of thymocytes and B16 cells in the pulmonary vasculature. These differences were probably due in part to differences in the size of the four test agents, and gave rise to large V_d and Cl values for thymocytes and melanoma cells. The small Cl and V_d values for the monoclonal antibody suggest that unlike the ^{99m}Tc sodium pertechnetate, ^{125}I-labeled monoclonal antibody has less vascular permeability which would be consistent with its larger molecular size.

Our experiments also demonstrated the variability that occurred among mice injected with the same agent administered by the same route. The organ receiving the largest amount of radioactivity occasionally varied. Much of this was attributed to mouse to mouse variation. On the other hand, the differences between the 4- and 24-hr values of liver for B16 cells were attributed to sporadic intergroup variation.

The similarity of data between the two routes indicates that retroorbital injections

TABLE V. HALF-LIFE, VOLUME OF DISTRIBUTION, AND CLEARANCE OF ^{99m}Tc SODIUM PERTECHNETATE AND ^{99m}Tc-LABELED CELLS

Parameter	^{125}I-monoclonal antibody	Na $^{99m}TcO_4$	^{99m}Tc-thymocytes	^{99m}Tc-B16 melanoma
$t_{1/2}$ (min)[a]	30.5	18.4	10.3	44.3
V_d (ml)[b]	1.93	5.59	8.65	55.22
Cl (ml/min)[c]	0.0439	0.210	0.582	0.864

[a] $t_{1/2}$ (half-life) = 1n2/Ke, where Ke = −slope of lines in Fig. 1.
[b] V_d (apparent volume of distribution) = dose/Cp^0, where Cp^0 is the concentration of agent at $t = 0$.
[c] Cl (apparent total body blood clearance) = $K_e V_d$.

can be given in many experiments where tail vein injections were previously employed. Retroorbital injections have the advantages of being faster and easier to administer than tail vein injections. They are usually successful on the first attempt without prewarming the mice; this can reduce injection time and stress placed on the animals and minimize the loss of the agent to be injected. In some instances, however, injection via tail vein may be preferable. For example, in a preliminary experiment C57BL/6 mice were injected via the retroorbital sinus with B16 cells in order to determine the pattern of metastases. In some animals, tumors developed at the site of the injection. These grew into the orbit, invaded the brain, and killed the mice before the metastatic nodules developed. Tail vein injections would have obviated this particular problem.

In some experiments neither route may be acceptable, particularly if there is excessive trapping of agents in the lungs. Retroorbital and tail vein injections of cells are associated with a high degree of first-pass trapping in the lungs. This could be reduced if the cells were injected intraarterially or directly into the left ventricle. Before any injection route is selected, however, the specific experimental requirements should be considered so that the route chosen meets the needs of the experiment.

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