

Distribution of Thallium-201 Injected into Rats Following Stress: Imaging, Organ to Plasma Uptake Ratios, and Myocardial Kinetics

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Male rats were stressed by forced swimming for 2 hr, after which 0.2 mCi Tl-201 were given i.v. to each rat. Some animals were imaged in vivo; when a pinhole collimator was used, the heart, liver, and kidneys could be distinguished. Animals were killed 30 min or 3 hr after injection for tissue sampling and in in vitro study of myocardial kinetics. Results for tissue sampling (kidneys, heart, adrenals, lungs, aorta, liver, spleen, thymus, seminal vesicles, muscle, testes, brain, fat, erythrocytes, and fur) are expressed as the ratio of activity in organ to that in plasma. Kidneys and heart showed the largest uptake; considerable uptake was also found in adrenals. At 3 hr kidneys, heart, and adrenals already showed a diminution of uptake as compared to 30 min. At 3 hr, muscle, brain, and fur showed a marked increase in uptake; testes and fat exhibited a dramatic increase. It appears that organ uptake is greater in stressed than in unstressed animals. In the myocardium study a three-compartment model (extracellular, intracellular, and subcellular) was found to describe adequately the kinetics of Tl-201. Transport rate constants (k_{ij}) and relative compartment sizes for Tl-201 distribution were determined by a continuous outflow method. In the intracellular compartments 2 and 3, each inflow k_{ij} was larger than the corresponding k_{ji} leaving from the same compartment, thus indicating that Tl-201 movement is directed predominantly into cellular structures. Values for k_{ij} furnish a norm that may be used comparatively to study quantitatively the kinetics of different tracers. The ratio of compartment sizes indicated that more than 70% of Tl-201 is in the intracellular compartments. For these kinetic studies there was no statistically significant difference between results obtained at 30 min and 3 hr after Tl-201 injection.

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Renewed interest in the distribution of thallous ion in body organs has arisen with increasing clinical use of Tl-201 as a radionuclide for myocardial imaging (1-3). Some useful information has been derived from studies on animals (4-6). Since in clinical situations the best imaging with Tl-201 is obtained following injection after some kind of stress (increased heart rate through pacemaker, treadmill exercise, etc.), it was thought that a study of several

aspects of Tl-201 distribution in the rat following stress might be of some scientific interest besides perhaps further delineating the clinical usefulness of imaging of heart and possibly other organs with this radionuclide. This paper describes: (a) rat in vivo

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imaging with Tl-201, (b) relative organ-to-plasma uptake of Tl-201, and (c) in vitro kinetics of Tl-201 distribution in myocardium.

MATERIALS AND METHODS

General procedure. Male Sprague-Dawley rats, 250–300 g each, were used. Rats were acclimatized in our animal quarters for approximately 10 days before the study. One the day of the experiment the rat was stressed by forcing it to swim in water at room temperature for 2 hr. The animal was then anesthetized with pentobarbital (35 mg/kg) intraperitoneally, the jugular vein was exposed surgically and about 0.2 mCi of $^{201}\text{TlCl}$ (specific activity greater than 200 mCi/mg) in 0.2 ml of 0.9% NaCl were injected. Nylon sutures were applied to close the skin incision. The animals were divided into two groups according to the time tissue sampling was carried out, i.e., 30 min or 3 hr after Tl-201 injection.

In vivo imaging. With the anesthetized animal lying in the posterior (dorsal) or 45° right posterior oblique position, images were obtained approximately 15 min after Tl-201 injection by counting to 200,000 counts with a gamma camera, with appropriate-high resolution or pinhole collimator with energy window set for 75 keV and 25% width. Images were recorded on Polaroid prints using a triple-lens camera. Because of time limitations, only those animals destined for tissue sampling at 3 hr after Tl-201 injection could be imaged.

Organ Tl-201 uptake at 30 min and 3 hr. At the respective times, rats were guillotined, bled, and thoracic, abdominal, and cranial cavities quickly opened. Organs were excised, placed in stoppered, previously tared flasks, and weighed on a laboratory balance. Samples of the organs were transferred to a small vial and inserted in a scintillation well detector. Appropriate number of counts per sample were recorded with a spectrometer attached to a printer. A specimen of blood was also collected, and an aliquot of it was centrifuged for plasma separation. Counts were then recorded for both whole blood and plasma.

In vitro kinetic studies in myocardium. After rapid removal of the heart from the thoracic cavity, special care was taken to effect prompt excision of the outer layer of the left ventricle. With the anterior coronary artery as a guide, an attempt was made to sample consistently the same region of the heart in different animals. Segments of 0.5 mm thickness were cut with a Stadie-Riggs microtome. Each thin segment (about 10×8 mm) was subjected to continuous outflow of radioactivity as described in detail earlier (7), in order to determine transport rate constants for Tl-

201 exchanges within the myocardium. The method consists of suspending the tissue in a tube where it is washed continuously with nonradioactive Krebs solution bubbled with 95% O₂ and 5% CO₂ in an apparatus specially built in the laboratory (7), which assures constant temperature (37.5 C) and flow (15 ml/s). The tube containing the myocardial segment was placed in a NaI well detector. Radioactivity was recorded with a spectrometer and printer every 10 sec for the 1-hr duration of the outflow experiment.

A compartmental analysis model was used to describe thallium kinetics in the myocardium. Such a model is specified by a number of compartments and by intercompartmental relationships. The latter are commonly referred to in the earlier literature as transfer rates, transport rate constants, transfer rate coefficients, fractional turnover rates or simply as rate constants. We will use the terminology and symbolism recommended by the Task Group on Tracer Kinetics of the International Commission on Radiation Units and Measurements (8). Thus transport rate constant, k_{ij} , is the fraction of compartment j that enters compartment i in unit time. A compartmental system is said to be solved when all transport rate constants have been numerically determined.

For the solution of the present compartmental system of thallium movements in the myocardium, we used a computer simulation procedure employing the SAAM (Simulation, Analysis and Modeling) program developed by Berman (9). The procedure has been reported previously in detail (7,10). It consists of entering the print-out counts together with the time intervals and an initial set of estimated values for k_{ij} as the data input for the SAAM program. Although data points were obtained every 10 sec, only those at 10, 20, 30, 60, 120, 240 sec, and each 4 min thereafter, were used for the computer solution in terms of the compartmental model. The reduced data set was considered adequate in defining the experimental records and it saved computer time.

Since the experimentally obtained curves were easily resolved into three exponentials using manual sequential curve peeling, it follows from compartmental analysis theory (11) that a system composed of at least three compartments is required to model Tl-201 kinetics adequately. The three-compartment model should be interpreted as consisting of an extracellular, an intracellular, and a subcellular (endo-intracellular) space (Fig. 1). The computer output for the outflow of thallium from the tissue is a double curve: (a) a theoretically calculated curve that is compared point by point with (b) the experimentally obtained curve. The computer program modifies iteratively the initial estimates of the k_{ij} to seek the

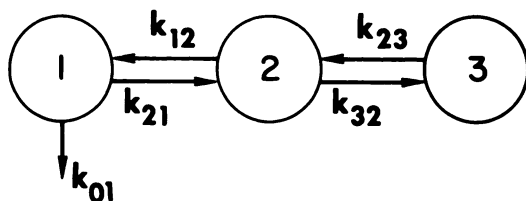


FIG. 1. Model of TI-201 distribution in myocardium consisting of three compartments: (a) extracellular, (b) intracellular, and (c) sub-cellular. Intercompartmental transport rate constants are symbolized by the k_{ij} .

best fit between the two curves. The iterative procedure is terminated when the rate of decrease in the sum of squares with respect to a previous iteration reaches a preassigned value, which in the reported results was arbitrarily established at 2% for all curve

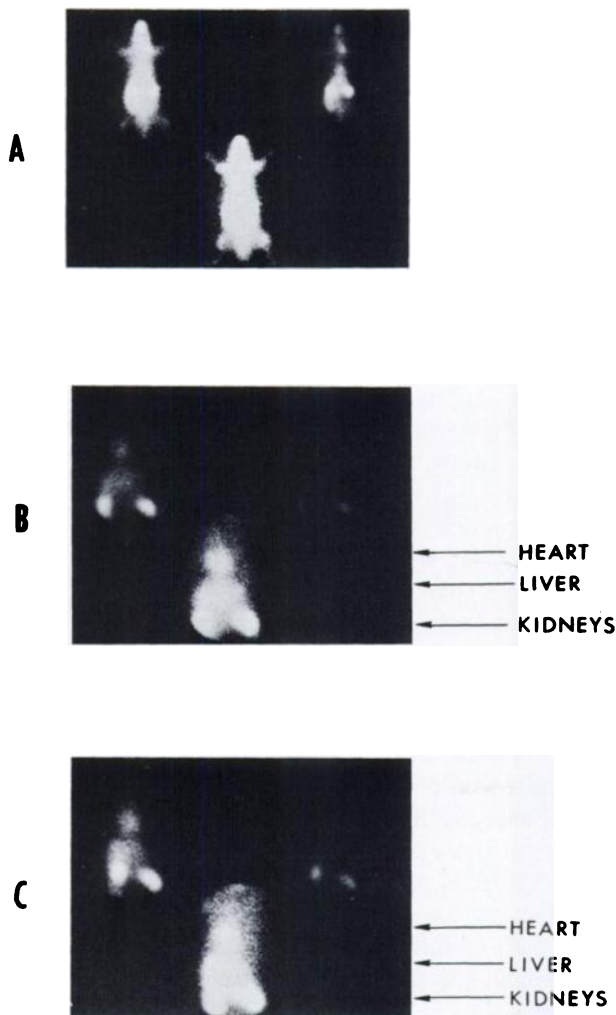


FIG. 2. Rat in vivo images started approximately 15 min after i.v. injection of TI-201 (A) anterior (ventral) view with parallel-hole collimator; (B) anterior view with pinhole collimator; (C) 45° left anterior oblique view with pinhole collimator. Heart, liver, and kidneys show distinctly in B and C.

comparisons. At this stage the set of k_{ij} is taken as final.

RESULTS

In vivo imaging. When the high-resolution, parallel-collimator was used (Fig. 2A), the general distribution of the radionuclide was visualized. When the pinhole collimator was used, the relative uptakes in heart, liver, and kidneys were visually distinguished in the anterior (ventral) view (Fig. 2B) and in the 45° left anterior oblique view (Fig. 2C).

Organ TI-201 uptake at 30 min and 3 hr. Although different calculations were made (total uptake per organ, activity per gram of organ, etc.), for the purpose of this study it was found that the easiest way to grasp results was as the ratio of activity per gram of organ (cpm/g) to the activity in plasma (cpm/g, assuming 1 g plasma/ml). This presentation emphasizes the TI-201 organ uptake relative to blood plasma and may provide some useful hints regarding organs that may be amenable to imaging as well as the optimal time for potential organ imaging. In addition, one can compare immediately the tissue-to-plasma radionuclide ratio exhibited by different organs.

The results for the 30-min and 3-hr groups are shown in the left-hand part of Table 1, where they are tabulated in decreasing order of 30-min uptake. As expected, kidney and heart headed the list. Quite unexpected was the finding of a considerable uptake in the adrenal glands, 115 times that of plasma.

The changes in uptake from the 30-min to the 3-hr period are of some interest (Table 1). The kidneys, heart, and adrenals showed a sizable and comparable diminution. Other organs showed increases at 3 hr: moderate in the spleen, thymus, and seminal vesicles; marked in skeletal muscle, brain, and fur; and dramatic in testes and fat. Some organs, especially muscle, fur, and fat exhibited considerable individual variation, as reflected in the rather large error estimates.

In vitro kinetics of TI-201 distribution in myocardium. Typical plots of tracer washout for the kinetic model and actual myocardium are shown in Fig. 3 (rat killed at 30 min) and Fig. 4 (rat killed at 3 hr). The plots illustrate the agreement between the experimental data and model-derived results calculated by computer iteration.

In Table 2 the values for the transport rate constants are compared. Referring to Fig. 1, note that the transport rate constants leading into intracellular compartments 2 and 3 (k_{21} and k_{32}) were larger than those leading out (k_{12} and k_{23}), which indicates that the movement of thallos ion is directed predominantly into the cellular structures. There was

TABLE 1. UPTAKE OF Tl-201 IN THE RAT, AS RATIO OF ORGAN-TO-PLASMA ACTIVITY* AT TWO DIFFERENT TIME INTERVALS AFTER INJECTION

Organ	Data from present study on stressed animals (mean \pm s.e.)			Data recalculated from unstressed animals (4)	
	30 min N = 9	3 hr N = 8	Percentage change 3 hr vs. 30 min	30 min N = 2	4 hr N = 3
Kidneys	222 \pm 26.5	150 \pm 19.6	-33	120	190
Heart	157 \pm 23.0	114 \pm 23.2	-27	41	23
Adrenals	115 \pm 19.9	71.2 \pm 11.6	-38	—	—
Lungs	50.4 \pm 7.4	50.2 \pm 10.1	-0.4	26	15
Aorta (arch)	48.0 \pm 7.5	40.8 \pm 8.6	-15	—	—
Liver	46.8 \pm 2.9	43.9 \pm 6.0	-6.2	17	15
Spleen	26.5 \pm 4.4	41.4 \pm 6.6	+56	20	18
Thymus	21.2 \pm 2.5	32.3 \pm 7.4	+52	—	—
Seminal vesicles	11.8 \pm 2.2	17.2 \pm 6.6	+45	—	—
Muscle (gracilis)	10.7 \pm 2.3	26.8 \pm 7.9	+150	11	16
Testes	3.47 \pm 0.65	15.6 \pm 3.5	+350	2.6	12
Brain (cortex)	3.10 \pm 0.49	6.03 \pm 1.7	+95	1.3	4.0
Fat (mesenteric)	3.10 \pm 1.1	16.2 \pm 7.0	+422	—	—
Erythrocytes	1.63 \pm 0.07	1.48 \pm 0.12	-9.2	—	—
Fur (nape)	1.46 \pm 0.45	3.44 \pm 0.70	+136	—	—

* Calculated as cpm/g of tissue divided by cpm/ml of blood plasma.

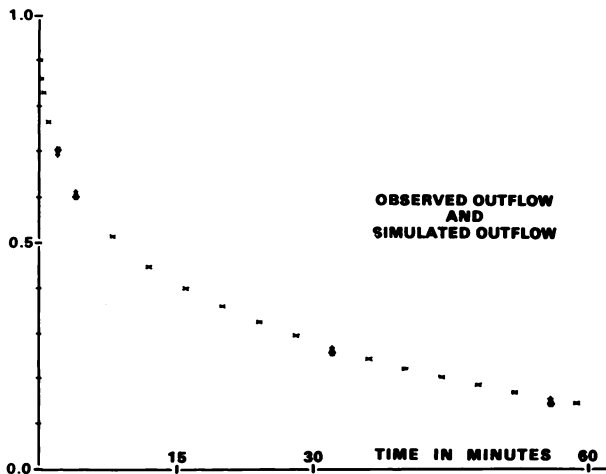


FIG. 3. Plot of kinetic model of Tl-201 exchange in rat myocardium. Ordinate: radioactivity as fraction of initial count. Abscissa: time in minutes. This is an illustrative record for a sample obtained 30 min after i.v. injection of Tl-201. Experimentally obtained outflow data (*) and computer-simulated outflow (+) are compared; coincidental points are printed as (x).

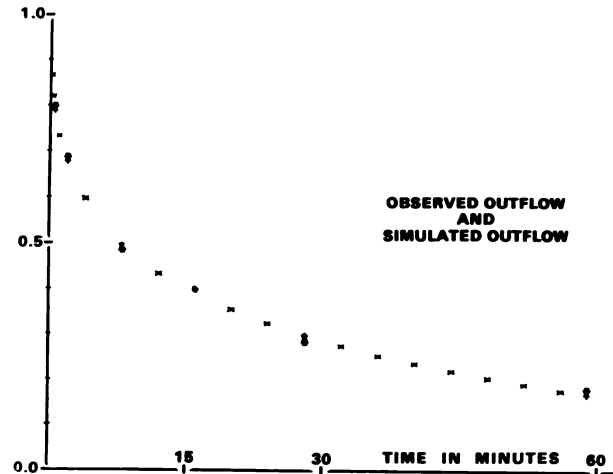


FIG. 4. Plot of kinetic model of Tl-201 exchange in rat myocardium. Ordinate: radioactivity as fraction of initial count. Abscissa: time in minutes. This is an illustrative record for a sample obtained 3 hr after i.v. injection of Tl-201. Experimentally obtained outflow data (*) and computer-simulated outflow (+) are compared; coincidental points are printed as (x).

no statistically significant difference between the transport rate constants found for 30 min and 3 hr after Tl-201 injection.

The relative compartment sizes may be calculated as ratios of appropriate k_{ij} as detailed elsewhere (7,12) and are tabulated in the lower half of Table 2. Note that the intracellular compartments (2 and 3) are both larger than compartment 1 (extracellular). The last row of the table shows the ratio of compartment 1 (extracellular, EC) to the sum of

compartments 2 and 3 (both intracellular, IC). This ratio indicates that more than 70% of Tl-201 is intracellular. Again, there was no statistically significant difference between the data for 30 min and 3 hr.

DISCUSSION

Some of the uptake data presented here for the stressed rat are chronologically comparable with those reported for a small sample of rats not sub-

TABLE 2. COMPARATIVE VALUES OF TRANSPORT RATE CONSTANTS* ($k_{ij} \times 10^{-3} \text{ s}^{-1}$) AND RELATIVE COMPARTMENT SIZES† (q_j/q_T) FOR Tl-201 IN MYOCARDIUM OF STRESSED RATS (mean \pm s.e.)

Parameter of interest	30 min (N = 9)	3 hr (N = 8)
k_{01}	29.1 \pm 3.71	32.6 \pm 4.88
k_{12}	3.07 \pm 0.52	2.52 \pm 0.27
k_{21}	4.90 \pm 0.95	4.19 \pm 0.47
k_{32}	0.59 \pm 0.046	0.52 \pm 0.039
k_{33}	0.83 \pm 0.13	0.74 \pm 0.15
q_1/q_T	0.22 \pm 0.018	0.22 \pm 0.023
q_2/q_T	0.34 \pm 0.016	0.35 \pm 0.035
q_3/q_T	0.44 \pm 0.030	0.43 \pm 0.049
$q_1/(q_2 + q_3) = \text{EC/IC}$	0.29 \pm 0.031	0.29 \pm 0.043

* Transport rate constant (k_{ij}) is the fraction of Tl-201 in one compartment (2nd subscript) that enters another (1st subscript) in unit time (see Fig. 1). Values of k_{ij} (which have the dimension sec^{-1}) were multiplied by 10^3 to facilitate comparison. Differences between the 30-min and 3-h columns are not statistically significant by Student's *t*-test.

† Relative compartment contents were calculated as ratios of appropriate k_{ijs} . Details are given elsewhere (7,12). Each q_j symbolizes the quantity of Tl-201 in its compartment and q_T the total quantity in tissue at the beginning of the outflow. Bottom row value has the meaning of extra-intracellular ratio (EC/IC).

jected to stress (4). We have recalculated these results as the ratio of organ-to-plasma activity and have listed them in two columns at the right of Table 1. Note the following differences between stressed and unstressed animals: Tl-201 uptake by the stressed heart was four times that of the unstressed heart at 30 min, and five times at 3 hr; and kidney uptake at 30 min, stressed, was about twice that in unstressed animals. In our experiments, however, the kidney uptake was already falling at 3 hr, whereas in the unstressed animals it was increasing at 4 hr.

We note particularly the very high uptake found in the adrenal glands—the third highest in our tabulation. There are no values for acute adrenal uptake of thallium reported in the literature. The very high uptake found in our experiments must reflect the energetic adrenal response to stress. (We recall that the rat swimming test was one of the pioneering methods of evaluating potency of adrenocortical extracts and compounds.) The moderately increased but protracted Tl-201 uptake by lungs and liver probably portrays the effects of stress on oxygenation and carbohydrate metabolism. Fur was studied because of the reported affinity of thallium for hair in chronic intoxication. Table 1 shows that the uptake in fur has already more than doubled at 3 hr.

The results of the kinetic study of myocardial seg-

ments indicate, as expected, that most of Tl-201 is intracellular. The fact that compartment 3 (subcellular) showed even greater thallous-ion accumulation (q_3/q_T in Table 2) than compartment 2 is consistent with the independent observation that heart mitochondria are capable of considerable thallous-ion uptake (13). Whether this is related to the extensive mitochondrial swelling occurring in the rat heart after intensive swimming (14) cannot be decided by the present data.

Note that the measurement of Tl-201 outflow kinetics in myocardial segments may not be directly translated to the situation *in vivo* where the tissue in its natural environment might be less permeable to the loss of Tl-201. Hence, the obtained value of 70% for intracellular Tl-201 most likely represents a least value and the actual *in vivo* myocardial intracellular concentration of Tl-201 may be higher.

Among the advantages of characterizing the movements of a substance throughout a tissue by numerical transport rate constants are that their values, under certain conditions, are independent of the total amount of substance and furnish a norm that may be used comparatively to study quantitatively the kinetics of different tracers.

The transport rate constants are independent of the total amount of tracer if the tissue under consideration has reached constant specific activity throughout its different compartments before the outflow phase of the experiment. Our present Tl-201 studies were carried out at 30 min and 3 hr after injection of the tracer. From data directly reported by other workers or calculable from their results, it is clear that the $t_{1/2}$ for disappearance of Tl-201 from blood is about 5 min in man (15), less than 1 min in the goat (5), and 2.9 min in the anesthetized dog (6). For Tl-204 it is about 1 min in the rat and about 1.8 min in the dog (4). These figures, therefore, provide evidence that at 30 min and 3 hr the myocardial uptake has reached equilibrium; hence, theoretically, the same transport rate constants should hold. This turned out to be the case experimentally.

Finally, we conclude that *in vivo* organ imaging with Tl-201 is feasible in the rat. Importantly, the heart appears reasonably well delimited from the liver (Figs. 2, B and C). This extends the observations of Bradley Moore et al. (5) in the rabbit. Rat heart imaging may be a useful adjunct to follow the development of acute experimental myocardial infarction by cauterization, which has been claimed (16) to be a correlative model of the human counterpart.

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