Tissue Distribution of ^{212}Bi in Rats* (33817)

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(Introduced by W. F. Bale)
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There is comparatively little information available as to the distribution of bismuth in animal tissues. A proposal to use radioactive ^{206}Bi (half-life = 6.4 days) adsorbed on charcoal particles for selective irradiation of the reticuloendothelial system has stimulated experimental studies in man (1) and rats (2). Experiments investigating the effects of inhalation of air containing radon or thoron and their short half-life daughters have yielded a certain amount of information (3–6) on the tissue distribution of bismuth when it occurs as a daughter of lead. Recent work in our laboratory on the kinetics of lead binding by erythrocytes suggested a method whereby the 60.5-min half-life ^{212}Bi could be conveniently separated from its 10.6-hr half-life parent ^{212}Pb. Using this method the separated ^{212}Bi was injected intravenously into rats and its fate was followed as a function of time yielding the results reported below.

Method. Thoron gas was swept from a dry source (7) by tank nitrogen passing into a 1-liter bottle at a flow rate of 87 ml/min. The outflow from the bottle passed through a Gelman Metricel filter Type VM-4 with a pore size 0.80 μ and was vented into a chemical hood. If complete mixing occurs in the bottle it is expected that about 90% of the 55.4 second half-life thoron would decay to ^{212}Pb. Approximately equal fractions of the ^{212}Pb formed are deposited in the bottle and on the filter. It was found most convenient to scavenge the lead from the bottle with 3–5 ml of physiological saline solution. After an elapsed time to permit the 60.5-min half-life ^{212}Bi daughter to grow to equilibrium 1.0 ml of the ^{212}Pb–^{212}Bi saline solution was added to 10 ml of freshly drawn heparinized human blood and this mixture was incubated in a rotating tonometer for 25 min at 37°. The blood was then centrifuged and the plasma was pipetted off. An aliquot of the plasma was measured for gamma activity in a NaI(Tl) well crystal detector with associated phototube and 100-channel analyzer. The average specific activity of the plasma preparations was 4.8 μCi of ^{212}Bi/ml with the average activity ratio of ^{212}Pb:^{212}Bi = 0.016. The reduction of ^{212}Pb in the plasma was caused by the selective binding of lead to the red cells.

Once prepared, 0.5 ml of the ^{212}Bi plasma solution was promptly injected into the tail vein of the unanesthetized rat. After injection the rats were placed in metabolism cages and held for sacrifice. The series includes four or five rats at each sacrifice time. Tissues were sampled as indicated in the tabular results. Routinely 2 or 3 g of tissues were placed in a test tube for gamma measurement of ^{212}Bi and ^{212}Pb in the well counter facility. In the case of bone and of marrow the sample size was about 0.3 g and 40 mg, respectively. Larger samples could have been obtained, but because of the relatively short half-life of ^{212}Bi, speed in sampling and in counting was important. Usually all samples were counted within 40-min postsacrifice. Sprague-Dawley rats were used with weights ranging from 245 to 367 g.

Measurement calibration and data reduction. The ^{212}Pb prepared as described above was taken up in 5 ml of 0.5 *N* HCl, and after an elapsed time of 6 hr, equal amounts were deposited on duplicate stainless steel planchets, heated to dryness, and counted on a 2 π alpha particle counter previously calibrated with a Ra D+E standard. Because of the condition of transient equilibrium, it is known that the beta disintegration rate of ^{212}Pb is equal to 0.907 times ^{212}Bi disinte-

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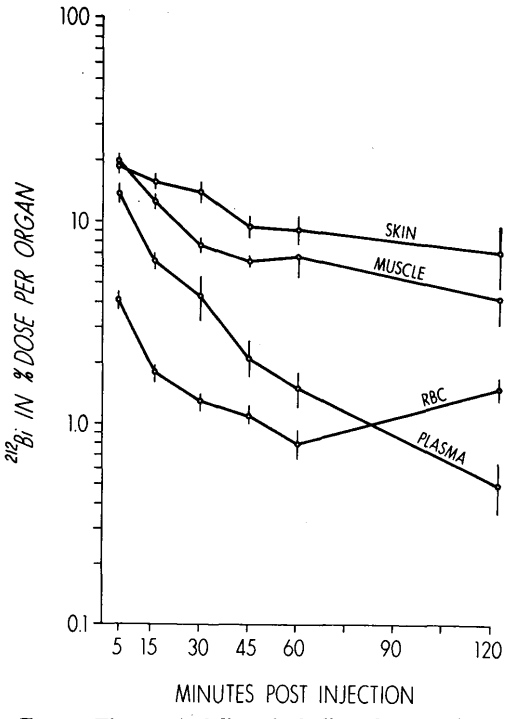

FIG. 1. The vertical lines including the experimental points indicate the standard error of the mean.

gration rate and that the latter is equal to the measured alpha disintegration rate. The calibrated stock solution counted in the well counter yielded a gamma calibration factor of 167 cpm/nCi for ^{212}Bi in the photon energy band selected and after appropriate allowance for Compton scatter, 518 cpm/nCi for ^{212}Pb in its photon energy band. The associated background counting rates were 60 and 50 cpm, respectively.

Since the experimental tissues samples varied somewhat in volume, an experiment was performed in which the same activity was placed in 0.5 *N* HCl solution of 1, 2, and 3 ml. Inasmuch as differences of less than 5% were found, no volume correction was applied to the sample counts.

All sample counts were adjusted to time of sacrifice by correcting for the decay up to time of measurement. For ^{212}Bi this involved a subtraction of the amount of bismuth (usually small) which had grown in from ^{212}Pb during the sacrifice–counting time interval, and then an adjustment of the net ^{212}Bi for physical decay during the same interval.

The corrected bismuth activities divided by sample weight yielded the activity concentrations. Relative rat organ weights were taken from the measurements of Caster and others (8) and the ^{212}Bi activity of the total organs was calculated. For this purpose the activity concentration in small intestine samples was taken as representative of the gastrointestinal tract. Exceptions occurred for the liver and kidney, the actual weights being used in these cases. The sum of the organ (or total tissue) activities plus the activity detected in the urine was taken as the total dose. This estimate neglects only 6% of the fat-free body mass. Samplings of brain, testis, spleen, and lymph nodes yielded relatively low activity concentrations ($=$ or $<$ the activity concentration for muscle) except for the spleen which accounted for 0.7–1.3% of the total activity.

Results and Discussion. Figures 1 and 2 present the change in the organ (or whole tissue) content of bismuth as a function of time and Table I lists changes in concentration. The plasma, red blood cells, muscle and

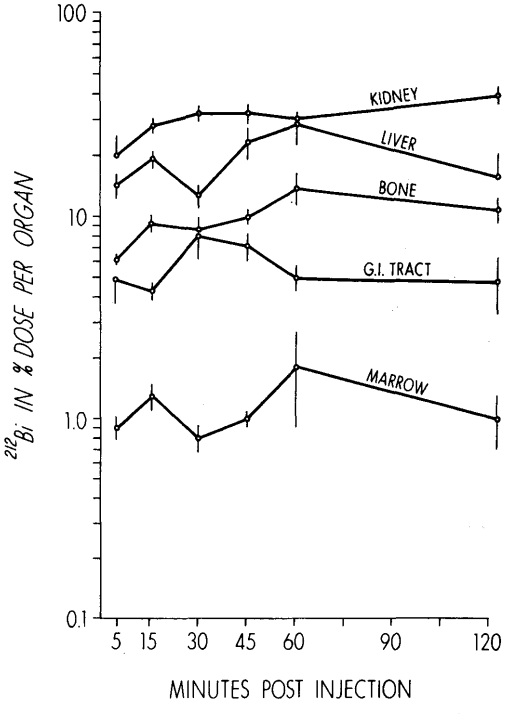

FIG. 2. The vertical lines including the experimental points indicate the standard error of the mean.

TABLE I. ^{212}Bi Concentration (% dose/g) as a Function of Sacrifice Time.

Time of sacrifice (min)	Red cells	Plasma	Kidney	Liver	Small intestine	Muscle	Bone	Marrow	Skin
5	0.45	1.99	7.27	1.40	0.56	0.14	0.35	0.82	0.32
16	0.18	1.00	11.5	1.91	0.40	0.09	0.54	1.13	0.27
30	0.13	0.64	14.1	1.30	0.73	0.053	0.50	0.69	0.24
45	0.13	0.39	14.6	2.40	0.74	0.046	0.71	1.02	0.18
60	0.10	0.23	13.9	2.86	0.50	0.05	0.82	1.68	0.17
122	0.18	0.07	17.0	1.78	0.45	0.03	0.65	0.88	0.12

skin (Fig. 1) lose bismuth over the 2-hr sampling period. The vertical lines passing through the individual points plot plus and minus the associated standard error of the mean. This statistic permits verification of the statement that the decrease from the 5-min average is significant ($> 2 \times$ the associated standard error) for all data points at later times except for the 15-min value for skin. However, in the majority of cases the large size of the error in terms of the bismuth content decrease from one sacrifice time to the next precludes the demonstration of a point by point decrease and makes further mathematical analysis of the curves (Fig. 1) of dubious value. However, the general trend is manifest and the data in no way conflict with the proposal that these tissues undergo a continuous loss of bismuth over the time interval sampled. The anomolous increase in the red blood cell content of bismuth from 1 to 2 hr is discussed in a later section of the paper. Figure 2 illustrates the results for 5 organs for which the bismuth content at 2 hr is either greater or not significantly different than the 5-min value. In consideration of the decrease in plasma content over the same time, it may be concluded that some mechanisms must exist in these tissues for binding bismuth. The magnitude of the decrease in bismuth content of the liver from 15 to 30 min is 3 times the associated standard error. The corresponding increase in the GI tract bismuth value is 2 times the standard error, as is the comparable decrease in the bone marrow. These variations may therefore be regarded as significant and as presumptive evidence that bismuth, as well as lead (9) may be transported via the bile to the gastrointestinal tract. Unfortunately the data are barely significant and the proposal is unsupported by evidence from routine sampling of gastrointestinal contents and other experimental devices which could provide more direct proof. The urinary recovery of bismuth (i.e., the sum of the contents of the bladder and that excreted in the cage) varied from an average of 0.7% dose for the 5-min sacrifice group to 13% for the 2-hr sacrifice group. Variation was high within a group and the possibility of loss during ether anesthesia was not controlled.

The results support the generalization that bismuth moves rapidly from the plasma into the kidney, liver, and other tissues. It is noteworthy that at times as short as 5-min postinjection, 82% of the activity has moved out of the blood. From the graph published by Coenegracht and Dorleyn (1) who injected ^{206}Bi citrate intravenously in man, it is possible to estimate that at 5 min about 77% or more of the bismuth had left the blood. The distribution of ^{212}Bi in the body tissues at 122 min as shown in Figs. 1 and 2 agrees with the tissue distribution found in rats by Eridani and others (2) at 48–120 hr after intravenous injection of ^{206}Bi acetate in the sense that the kidney shows the highest concentration and the liver and bone are major deposition sites in both studies. However, whereas the present study finds 30–40% of the dose in the kidney at times from 0.5 to 2 hr, Eridani and others (2) find only 1.5–2.5% of the dose (adjusted for physical decay) in the kidney at 48–120 hr. The liver and bone contents are likewise higher in the present study. It is reasonable to suppose that a portion of this difference is attributa-

ble to the excretion of ^{206}Bi over the 5-day experimental period. However, the authors (2) state that the sum of urinary plus fecal excretion up to 5 days is 35% of the dose. It is unclear from the context whether this finding applies only to the ^{206}Bi in charcoal suspension series or applies to the ^{206}Bi acetate injection series as well. If the latter is the authors' intent, there is approximately 60% of the dose which is held by organs other than the kidney, liver, bone, spleen, and lung. This appears improbable in the light of our more complete sampling data. It is rendered still more unlikely by reference to the results of Coenegracht and Dorleyn (1) who found in man that 47% of an intravenously injected dose of ^{206}Bi citrate is excreted in 24 hr and that 80–90% was recovered in the urine collected for 6 days.

For radiation dose calculations in the absence of distribution and metabolic data on a particular radionuclide, recourse is sometimes had to existing data on the stable isotope or on a longer-lived isotope, based on the premise that the chemical and consequently the biological behavior will be identical. It will be easily recognized that this approach may lead to error if the relative distribution of the element varies over a period of time long in relation to the half-life of the radioisotope for which the distribution is sought. In the comparison of the 10-hr half-life ^{212}Bi and the 6.4-day half-life ^{206}Bi, no change in relative tissue distribution occurred from 2 hr to 5 days. Another and somewhat special case where the stable isotope approach may be misleading is the circumstance in which the nuclide of unknown distribution occurs as the daughter of a longer-lived parent. When both parent and daughter are introduced into the body it is important to realize that the majority of the daughter atoms are not those introduced as such, but the ones formed in the body by decay of the parent. What influence does this have on the tissue distribution of the daughter? Bismuth is a case in point. In order to make a comparison of the distribution of ^{212}Bi administered in the two ways an ancillary experiment was performed in which 4 rats were injected with a steady state ^{212}Pb–^{212}Bi saline solution, sacrificed at 2 hr, and sampled as described in

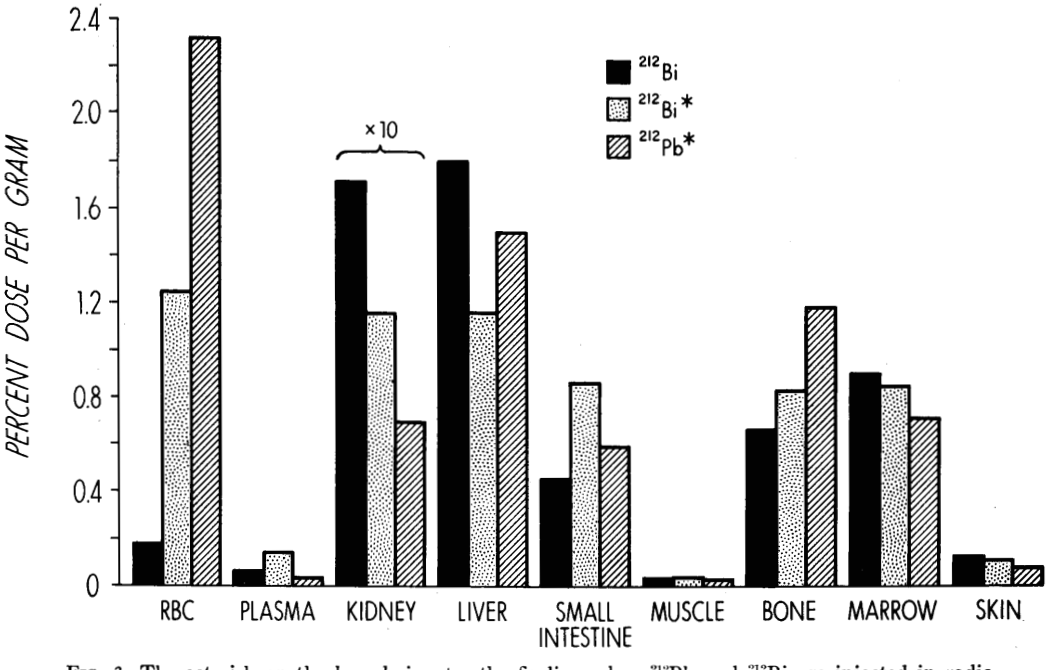

FIG. 3. The asterisk on the key designates the finding when ^{212}Pb and ^{212}Bi are injected in radioactive transient equilibrium. All values refer to sacrifice at 2-hrs postinjection.

"Method." Figure 3 compares the results of this experiment with the 2-hr data when ^{212}Bi was injected free of ^{212}Pb. The most marked difference is the increase in ^{212}Bi content of the red blood cells for the ^{212}Pb–Bi injected rats, caused by the strong binding of ^{212}Pb by the cells, and the relatively slow diffusion into the plasma of ^{212}Bi formed inside the cells. The apparently anamolous point for red cell content of bismuth at 2 hr as shown in Fig. 1 may now be cited as a further illustration of the same trend. The original data showed an average ^{212}Pb:^{212}Bi activity ratio for the samples equal to 1.2:1.0 accountable (i) in terms of the relative physical half-lives of bismuth and lead, and (ii) of the large fraction of the total body lead which is bound by the red cells. If the situation after the injection of ^{212}Pb–^{212}Bi in equilibrium may be approximated as a single compartment (the red cells) with an input of ^{212}Bi at the decay rate of ^{212}Pb and an output equal to the sum of ^{212}Bi decay rate plus a diffusion loss rate proportional to the number of ^{212}Bi atoms in the compartment, the data support a diffusion half-time of 1 hr. The shift of bismuth to the red cells is balanced by a decrease in the kidney content and to a lesser extent by a decrease in liver content. There occurs a small increase in the small intestine and in bone. The sum of the urine collected from the bladder and that excreted over the 2-hr period in the holding cage yielded an average of 8.8% dose for ^{212}Bi and 2.0% dose for ^{212}Pb for the ^{212}Pb–^{212}Bi-injected rats.

Summary. The ^{212}Bi, relatively free of its parent ^{212}Pb was injected intravenously into rats and the tissue distribution and was followed for a period of 2 hr postinjection. The kidney contained the highest concentration, equal to about 10 times that of the liver and spleen. A comparison was made of the ^{212}Bi distribution pattern at 2 hr postinjection when ^{212}Bi was introduced free of ^{212}Pb and when injected in transient equilibrium with ^{212}Pb. In the latter case the ^{212}Bi concentration in the red blood cells increased and in the kidney and liver decreased.

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