Determination of Calcium Retention in the Rat by Whole-Body Liquid Scintillometry

Keyphrases □ Calcium retention—rats □ Liquid scintillometry, whole body—analysis □ Parathyroid extract effect—calcium retention

Sir:

In many cases pathologic conditions affecting the skeleton are difficult to diagnose. Clinical chemical methods and radiography can rarely detect skeletal disease until a critical stage has developed. Short-lived, bone-seeking radioisotopes present great potential for the diagnosis of skeletal pathology in the initial stages. Calcium-47, emitting a gamma ray of 1.3 Mev. and having a half-life of 4.53 days, has been used for the evaluation of skeletal calcification (1). Of the many methods available for the detection of ^{47}Ca, whole-body counting techniques have proven to be sensitive and practical in clinical situations (2, 3). The relative absorption of calcium from the gastrointestinal tract of normal and diseased individuals was studied by Lutwak and Shapiro (4) using continuous counting of radiation in a subject's arm with a large volume liquid-scintillation counter after oral administration of ^{47}Ca. A similar but more precise method to determine calcium absorption using ^{47}Ca has been reported by Curtis *et al.* (5).

The use of whole-body liquid-scintillation counting for determining changes in calcium levels has been briefly investigated in our laboratories. The instrument used was the Purdue University Small Animal Counter (PUSAC). The PUSAC is a 4-pi whole-body liquid-scintillation counter designed for use with small laboratory animals (6). Mature, male Holtzman rats weighing 145–250 g. were employed in the investigation. The animals were maintained on a daily ration of commercial animal chow and tap water *ad libitum*. Twelve rats were used for the first part of the study. Six of these rats received a subcutaneous injection of 50 units of parathyroid extract per 100 g. of body weight for 5 consecutive days. The remaining six rats served as controls and were given daily subcutaneous injections of physiologic saline. Immediately after the last dose of parathyroid extract, each of the 12 rats was injected intraperitoneally with 0.2 μc. of carrier-free ^{47}Ca. The time of ^{47}Ca administration was considered to be time 0. The whole-body radioactivity was determined in the PUSAC at time 0 and at 4, 8, 12, and 48 hr. after time 0.

Eight rats were used for the second part of the investigation. The animals were divided into a drug and control group of four animals each. The regimen of parathyroid extract and ^{47}Ca administration was the same as that used in the first part of the study except that each rat was given 1.25 μc. of ^{47}Ca. Cumulative urine samples were collected from these animals at 12-hr. intervals from time 0 to 96 hr. The radioactivity of the whole urine samples was determined in the PUSAC.

All counting data were corrected for background, instrument efficiency, and radioactive decay. The corrected data from the whole body counting experiment were then expressed as average percent ^{47}Ca retention for the control and drug animals at each time interval of counting. Time 0 was considered to be 100% retention of ^{47}Ca. The corrected data from the studies of ^{47}Ca in the urine were expressed as average cumulative counts per minute. The data from the whole body counting study and the urine activity study are illustrated in Figs. 1 and 2. The data from the drug and control animals were compared statistically using a Hotelling's T^2 test.

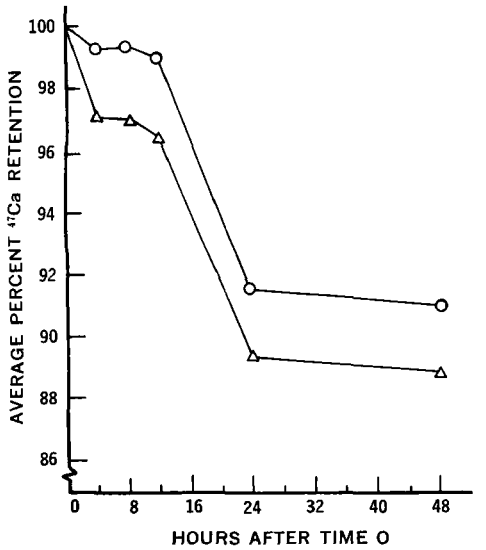

Figure 1—*Effect of parathyroid extract upon whole-body ^{47}Ca retention as determined by whole-body liquid scintillometry. Key: 0, control; Δ, parathyroid extract-treated.*

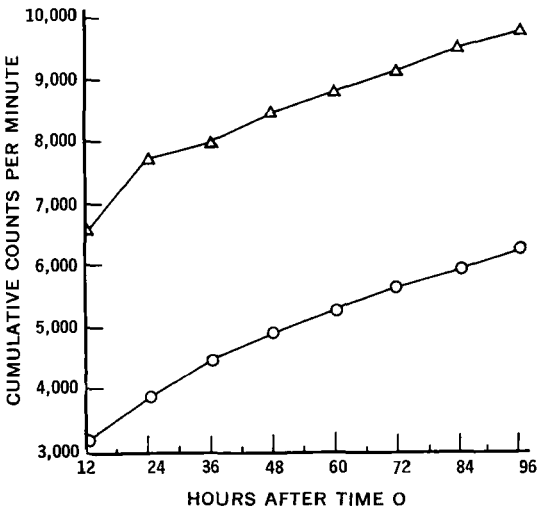

Figure 2—*Effect of parathyroid extract upon ^{47}Ca levels in the urine as determined by whole-body liquid scintillometry. Key: 0, control; Δ, parathyroid extract-treated.*

Rats receiving parathyroid extract were shown to have a statistically significant decrease in calcium retention as indicated by both the whole-body counting and urine-activity studies. The results of this initial investigation indicate that whole-body liquid-scintillation counting is a sensitive method for determining changes in calcium levels. Whole urine sample counting is also a useful means for detecting changes in calcium levels but the method requires a larger quantity of radioisotope. Further investigations are being conducted to compare whole-body liquid-scintillation counting methods with routine, clinical methods used for the evaluation of skeletal calcification.

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Received November 7, 1968.
Accepted for publication January 28, 1969.

Capacity-Limited Salicylurate Formation During Prolonged Administration of Aspirin to Healthy Human Subjects

Keyphrases ☐ Aspirin, prolonged administration—salicylurate formation ☐ Salicylurate formation—aspirin dose relationship ☐ Capacity-limited formation—salicylurate

Sir:

Man eliminates salicylate mainly by salicylurate formation, but this process has a very limited capacity (1, 2). Consequently, the elimination of salicylate is dose-dependent so that the time required to eliminate one-half of a single dose will increase from less than 3 hr. (for doses of less than 4 mg./kg. body weight) to 20–30 hr. when very large doses are given (1). This saturation effect has potentially serious toxicologic consequences since it may bring about drug accumulation to the point of intoxication during prolonged salicylate therapy (3). Indeed, it is known that the most severe salicylate poisonings in young children result from therapeutic use of aspirin (4), and that many more children die of therapeutic than of accidental aspirin poisoning (5). Pharmacokinetic studies of salicylurate formation have so far been carried out only following administration of single doses of salicylate (1, 2, 6) or after accidental salicylate intoxication in children (3), except that one group of investigators has used a regimen of six or seven 1-g. doses given every 6 hr., and studied the kinetics *after the last dose* (2, 7). It was therefore considered desirable to study salicylurate formation after prolonged administration of high doses of the drug. Unlike previous studies which relied on the determination of urinary excretion rates of salicylurate, the investigation to be described here consisted mainly of determinations of salicylurate and salicylate concentrations in the plasma.

Ten healthy male volunteers, 19 to 44 years old (average, 26.4 years), weighing 64.6 to 87.8 kg. (average, 77.5 kg.), received 2.4 g. aspirin daily for 8 days, and 7.2 g. daily for the next 8 days. The drug was administered in gelatin capsules, in two divided doses per day. Blood samples were obtained on Days 1, 4, 7, 12, and 15, 4 hr. after the morning dose. Urine collections of approximately 2 hr. were made in the morning of Days 7 and 15. Similar blood and urine collections were made in two subjects who received placebo capsules. All specimens were received for analysis labeled in code; the code was broken after the analyses had been completed. Salicylate in the plasma was determined by the spectrophotometric method of Brodie *et al.* (8); salicylurate in the plasma was measured fluorometrically as described by Schachter and Manis (9). Salicylate and its metabolites in the urine were determined according to the methods reported by Levy and Procknal (10). Blank values for plasma salicylate were 0.24 (±0.17) mg./100 ml., those for plasma salicylurate were 0.012 (±0.01) mg./100 ml. The recovery of salicylurate in the presence of a 160-fold excess of salicylate from initially drug-free plasma samples, to which known amounts of these substances had been added, averaged 92% (±6) in the concentration range of salicylurate encountered in this study. The experimental data were corrected accordingly.

The results of the study are summarized in Table I. The first day's blood sample was obtained 4 hr. after administration of the first dose of 1.2 g. aspirin and therefore yielded the lowest concentration of salicylate in the plasma. The average plasma concentration of salicylate then increased to about 12 mg./100 ml. in the first 8-day period, and to about 40 mg./100 ml. during the second 8-day period. One subject had close to average plasma salicylate concentrations from Day 1 to Day 12. Apparently, however, he discontinued the drug soon thereafter since his plasma concentration on Day 15 had decreased to less than 4 mg./100 ml. There is some indication that two other subjects may have reduced their salicylate intake in the last days of the study, since their salicylate levels had decreased to about 26 mg./100