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

Table I—Salicylate and Salicylurate Concentrations in the Plasma of Ten Healthy Men During Prolonged Aspirin Administration

Day	Aspirin Dose, g./day	Av. Plasma Concn., mg./100 ml. Salicylic Acid	Salicyluric Acid
1	2.4	6.80 (0.93)[a]	0.163 (0.039)
4		12.1 (2.2)	0.189 (0.062)
7		11.2 (2.1)	0.228 (0.052)
8			
9	7.2		
12		38.8 (4.5)	0.160 (0.054)[c]
15		41.8 (9.5)[b]	0.188 (0.051)[d]

[a] Standard deviation in parentheses. [b] Excluding one subject (No. 1) who apparently stopped taking aspirin some time after Day 12. [c] Excluding one subject (No. 9) with zero salicylurate concentration. [d] Excluding Subjects 1 and 9. Average for all 10 subjects 0.166 mg./100 ml.

ml. on Day 15. They were motivated probably by the occurrence of typical symptoms of salicylism, which were noted by many of the subjects during the second week of the study.

Four hours after the initial dose of 1.2 g. aspirin, plasma salicylurate levels averaged 0.163 mg./100 ml. and remained practically constant throughout the study (*i.e.*, ranging in average from 0.160 to 0.228 mg./100 ml.). These levels were similar to those observed by Schachter and Manis after administration of a single large dose of salicylate (9). In the same period of time, salicylate concentrations in the plasma increased from an average of 6.8 to almost 42 mg./100 ml.

One subject with close to average plasma salicylate levels throughout the study, and close to average plasma salicylurate levels from Day 1 to Day 7, surprisingly had essentially zero salicylurate levels on Days 12 and 15. His urinary salicylurate output on Day 15, however, was similar to that of the other subjects. Incubation of his 12- and 15-day plasma samples with plasma containing salicylurate gave no evidence of the presence of an enzyme capable of degrading this metabolite. Since all analyses were carried out in duplicate, with the second analysis made 1 or 2 weeks after the first, it is unlikely that these observations are due to an assay error.

Of interest also are the relatively high salicylurate levels on Day 7, which were, again, verified by duplicate assays carried out on different days. Since the subjects received the same breakfast on every day of the study, a possible dietary cause for these higher salicylurate levels is not readily apparent.

It was technically impossible to obtain accurately timed urine samples during the study, but urines representing approximately a 2 hr. output were obtained once during the low and high dosing period, respectively. Urine samples collected on Day 7 contained 67% (±12%) of total salicylate as salicylurate, while the samples obtained on Day 15 contained 40.4% (±16%) of total salicylate as salicylurate. This difference is statistically significant ($p < 0.01$), and similar to the relative decrease in salicylurate excretion with increasing doses of salicylate observed in previous studies (1, 2). Urine pH did not differ significantly in the two sets of samples.

The results of this study are entirely consistent with and strongly support previous evidence that salicylurate formation occurs at an essentially constant rate practically independent of the amount of salicylate in the body when this amount is in the usual therapeutic range (1–3, 6). These observations have added significance in that they were made during prolonged administration of salicylate in amounts comparable to those which have been used in conditions requiring very high daily doses, *i.e.*, acute rheumatic fever (12). The plasma salicylate concentrations observed during the second week of the study exceed levels usually obtained during intensive salicylate therapy, and are the highest levels that can be attained safely. The amount of salicylate in the body on Day 15 probably averaged about 9 g. since similar plasma salicylate concentrations are obtained shortly after intravenous injection of 10 g. sodium salicylate (11). The essentially constant salicylurate concentration in the plasma, when the amount of salicylate in the body ranged from about 1 g. (Day 1) to about 9 g. (Day 15), and the significant decrease in the fractional excretion of salicylurate in the urine when the daily dose of salicylate was tripled, are striking evidence of the limited capacity of man to form salicylurate.

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Received October 30, 1968.
Accepted for publication December 2, 1968.
Supported in part by Grant No. 1R01 DS 00021 from the U. S. Public Health Service.