Kinetics of Aspirin, Salicylic Acid, and Salicyluric Acid following Oral Administration of Aspirin as a Tablet and Two Buffered Solutions

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Abstract □ Twelve fasting normal volunteers received three aspirin dosage forms in a single-dose, complete crossover study; the plasma and urine levels of aspirin, salicylic acid, and salicyluric acid were measured for 10 hr. The three dosage forms were an unbuffered tablet, an effervescent solution with 16 mEq of buffer, and an effervescent solution with 34 mEq of buffer. Significant differences in the absorption rate were observed, with the solution having 16 mEq of buffer being fastest, the solution having 34 mEq of buffer being intermediate, and the tablet being slowest. These differences are attributed to gastric emptying rates and tablet dissolution. Urine pH and renal clearance for all three acid compounds are influenced by the buffer during the first 2 hr following dosing but not later. Area under the curve comparisons suggest that ~20% more aspirin reaches the general circulation intact following the tablet but that the total amount of salicylate absorbed is not different. Further studies are required to select the optimal buffer content to provide rapid absorption with minimal sodium dose and urine alkalinization.

Keyphrases □ Aspirin—tablet and two buffered solutions, kinetics following oral administration, salicylic acid and salicyluric acid levels and kinetics □ Salicylic acid—kinetics following oral administration of aspirin as tablet and two buffered solutions □ Salicyluric acid—kinetics following oral administration of aspirin as tablet and two buffered solutions □ Absorption kinetics—aspirin in tablet and two buffered solutions □ Buffer—effect on aspirin kinetics

Aspirin is the drug of choice when a mild analgesic–antipyretic is required, and it is also a primary agent in the chronic management of rheumatic fever, rheumatoid arthritis, and osteoarthritis. Following oral administration, rapid absorption is necessary to provide rapid onset of effects and to reduce the contact time with the gastric mucosa.

Table I—Mean Plasma Aspirin Data and Computed Parameters

Parameter	Mean ± *SD*, μg/ml[a] T	S-16	S-34
Time, min			
5	0.8 ± 1.0[c]	2.5 ± 3.1[c]	1.7 ± 2.2[c]
10	2.9 ± 3.3[c]	7.5 ± 6.2[c]	4.8 ± 4.5[c]
15	4.8 ± 4.2[c]	12.0 ± 7.3[d]	8.4 ± 6.6[cd]
20	5.9 ± 4.0[c]	13.0 ± 5.8[e]	9.5 ± 6.1[d]
30	8.8 ± 3.8[c]	9.5 ± 2.8[c]	9.6 ± 2.2[c]
45	7.0 ± 2.8[c]	5.1 ± 1.8[c]	5.8 ± 2.0[c]
60	4.9 ± 1.3[d]	2.7 ± 0.9[c]	2.9 ± 1.1[c]
90	2.9 ± 1.2[d]	0.8 ± 0.4[c]	0.9 ± 0.4[c]
120	1.3 ± 0.7[d]	0.3 ± 0.2[c]	0.3 ± 0.2[c]
Area under curve to 120 min, (mg min)/liter	518.0 ± 104.8[d]	494.0 ± 85.3[d]	450.0 ± 75.4[c]
Time of maximum concentration, min	35.0 ± 12.7[e]	22.1 ± 9.5[c]	27.5 ± 9.7[d]
Maximum concentration, mg/liter	9.6 ± 3.7[c]	16.4 ± 4.7[e]	12.6 ± 4.5[d]
Area under curve to infinity, (mg min)/liter	582 ± 86[d]	506 ± 89[d]	465 ± 78[a]
MRT, min	63 ± 16[c]	38 ± 9.9[d]	41 ± 9.0[d]
MAT[b], min	38 ± 13[c]	15 ± 10[d]	19 ± 8.1[d]
VRT, hr	29 ± 16[c]	13 ± 6.6[d]	13 ± 7.1[d]
VAT[b], hr	20 ± 15[c]	4.6 ± 5.8[d]	4.6 ± 7.0[d]

[a] A common letter following the standard deviation indicates no significant difference ($p < 0.05$) (17). [b] The *MAT* defined in the discussion corresponds to the MRT_g in Ref. 13, and *VAT* corresponds to the VRT_g.

BACKGROUND

A recent review (1) indicated that prolonged and repeated contact of aspirin alters the gastric membrane to allow hydrogen-ion back-diffusion, which results in irritation, bleeding, and, possibly, ulceration. As the pH of the stomach contents is raised, gastric irritation and bleeding decrease. This finding is consistent with reports (2, 3) that administering aspirin in sufficient buffer to reduce the acidity of gastric fluids reduces occult blood loss.

Other studies (4–6) showed that aspirin absorption through the stomach is negligible at alkaline pH and increases (to ~11% of the dose) when administered in an unbuffered or acid solution. Reducing the acid content of the stomach speeds gastric emptying, which reduces the residence time for aspirin in the stomach (7). Thus, administration of aspirin as a buffered solution provides more rapid delivery to the primary absorption site (*i.e.*, the intestine) while reducing the potential for adverse effects on the gastric membrane. Martin (8) reviewed aspirin kinetics and the influence of formulation factors on aspirin absorption.

It is desirable to optimize the amounts of buffer components to provide rapid absorption and to reduce irritation with minimal alkalinization of urine and sodium intake. Since previous studies (2, 3, 9) measured the total salicylate in plasma, the efect of buffering on the bioavailability of unhydrolyzed aspirin is not known. In this study, plasma and urine aspirin, salicylic acid, and salicyluric acid were quantitated by high-pressure liquid chromatography (HPLC) following aspirin administration in two different effervescent buffered solutions and as unbuffered tablets to 12 fasting normal volunteers.

EXPERIMENTAL

Dosage Forms—Three commercially available dosage forms were used to provide approximately equal doses of aspirin. They were two plain tablets[1], each containing 325 mg of aspirin (T); one effervescent tablet[2] containing 640 mg of aspirin, 1.825 g of sodium bicarbonate, and 1.079 g of citric acid (16 mEq of buffer) (S-16); and two effervescent tablets[3], each containing 324 mg of aspirin, 1.904 g of sodium bicarbonate, and 1.0 g of citric acid (34 mEq of buffer) (S-34).

Subjects—Twelve healthy male volunteers, 21–29 years old and 61.4–81.4 kg, were screened by a comprehensive physical examination, complete blood chemistry, urinalysis, and complete blood count and differential. All were free of any active disease, such as flu, and from the use of any medication for 14 days prior to the study. None of the subjects had a history of GI disease or surgery.

Method—A Latin-square design for three treatments in 12 subjects over three consecutive Saturdays was employed. A 10-hr fast preceded dosing and continued 4 hr postdosing except for water. At approximately 7 am, predose urine and blood samples were obtained, and a single dose of aspirin with 240 ml of water was administered. The effervescent tablets were dissolved in 140 ml of water 3 min prior to dosing, and then the mixture was swallowed. The glass was rinsed with 100 ml of water, which also was swallowed. Following dosing, 100 ml of water was administered at 1, 2, and 3 hr, and a uniform meal was served after the 4-hr sample. Subjects remained standing or sitting through the day, and exercise was limited to walking about the room.

[1] Bayer aspirin, Glenbrook Laboratories, Division of Sterling Drug, New York, N.Y.
[2] Aspirvess, Miles Laboratories, Elkhart, Ind.
[3] Alka-Seltzer, Miles Laboratories, Elkhart, Ind.

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Table II—Mean Plasma Salicylic Acid Data

Parameter	Mean ± SD, μg/ml[a] T	S-16	S-34
Time			
5 min	0.7 ± 0.6[b]	2.5 ± 3.0[b]	2.3 ± 2.3[b]
10 min	2.7 ± 2.9[b]	9.4 ± 8.4[b]	7.5 ± 6.6[bc]
15 min	6.2 ± 5.4[b]	18.0 ± 12.4[b]	15.3 ± 11.9[c]
20 min	9.1 ± 6.0[b]	25.9 ± 13.9[b]	21.6 ± 14.3[c]
30 min	17.9 ± 7.7[b]	34.5 ± 11.9[b]	33.0 ± 12.2[c]
45 min	27.3 ± 11.3[b]	37.2 ± 7.3[b]	39.1 ± 7.3[c]
1 hr	31.6 ± 12.8[b]	38.3 ± 7.2[b]	40.0 ± 6.3[c]
1.5 hr	37.7 ± 10.1[b]	36.1 ± 6.2[b]	38.2 ± 5.0[b]
2 hr	38.0 ± 8.1[b]	34.8 ± 5.6[b]	34.6 ± 3.7[b]
3 hr	33.9 ± 6.9[c]	29.7 ± 5.1[b]	29.8 ± 4.9[b]
4 hr	30.2 ± 6.5[c]	25.2 ± 5.5[b]	25.6 ± 4.8[b]
6 hr	21.4 ± 6.4[c]	17.1 ± 4.8[b]	18.1 ± 5.3[b]
8 hr	14.2 ± 6.2[c]	10.6 ± 4.7[b]	11.5 ± 4.6[b]
10 hr	8.4 ± 4.7[c]	6.7 ± 3.9[b]	7.4 ± 3.7[bc]
Area under curve to 10 hr, (mg hr)/liter	230 ± 56.6[c]	210 ± 44.9[b]	216 ± 40.9[bc]
Time of maximum concentration, hr	1.9 ± 0.4[c]	1.0 ± 0.5[b]	1.0 ± 0.4[b]
Maximum concentration, mg/liter	39.8 ± 8.9[b]	40.3 ± 6.8[b]	41.2 ± 6.0[b]
Area under curve to infinity, (mg hr)/liter	282 ± 104[c]	244 ± 71.8[b]	253 ± 63.2[b]

[a] A common letter following the standard deviation indicates no significant difference ($p < 0.05$) by ANOVA and least-significant difference method (17).

Blood was drawn into chilled vacuum containers[4] *via* an indwelling catheter at 5, 10, 15, 20, 30, and 45 min and at 1, 2, 3, 4, 6, 8, and 10 hr. Plasma was separated by centrifugation at 1764×*g* within 20 min of collection. All urine was collected at 2-hr intervals over 10 hr, the pH and volume were measured, and an acidified aliquot was saved for analysis. All samples were frozen at −30° and assayed within 2 weeks of collection by the HPLC method described previously (10).

RESULTS

As shown in Tables I–III, plasma aspirin, salicylic acid, and salicyluric acid levels rose more rapidly following the solutions than following the tablet; the solution containing 16 mEq of buffer was fastest. The rank order and profile projected in the tables are the same in 11 of the 12 subjects, except that the two tablets occasionally showed two or three maxima.

Urine pH ranged between 5.8 and 7.4, and the urine flow rate averaged 108 ± 58 ml/hr for the 170 samples collected at 2-hr intervals. Over 10 hr, urinary excretion of aspirin, salicylic acid, and salicyluric acid (in aspirin equivalents) accounted for 1.06–1.52, 5.4–10.9, and 55.2–56.9% of the dose (Table IV). Projected to infinite time, salicyluric acid accounted for 64–69% of the aspirin dose, which is consistent with an earlier report (11). The mean renal salicylurate clearance of 35.7 liters/hr (595 ml/min) is somewhat higher than that reported earlier (11, 12), which may be due to the young subjects whose prestudy creatinine clearances all exceeded 120 ml/min.

Significant ($p < 0.05$) differences in urine pH and the renal clearance of aspirin and salicylic acid occurred only during the first 2 hr, and salicyluric acid clearance was not significantly affected. Multiple linear regression analysis provided a negative correlation of aspirin and salicylic acid renal clearance with the urine hydronium-ion concentration and no significant correlation with the urine flow rate or plasma drug concentration. Salicylurate renal clearance did not correlate with urine hydronium-ion concentration, urine flow rate, or plasma salicylurate or plasma salicylate concentrations.

Statistical moments as described by Yamaoka *et al.* (13) were used to estimate the mean residence time in the body and GI tract (MRT and MRT_g) and the corresponding variances (VRT and VRT_g) for aspirin. These parameters were computed for each dose for each subject, assuming first-order elimination from a single compartment.

Linear regression of the natural logarithm of the last three measured plasma concentrations of each curve was used to estimate the apparent half-life and to compute the area to infinite time. For each subject, the smallest apparent half-life was taken as an estimate of the one-compartment elimination half-life and used for subsequent statistical moment computations. This approach resulted in an elimination half-life of 15.6 ± 2.7 (mean ± *SD*) min for the 12 subjects, which closely approximates the 14.9-min half-life reported for intravenous aspirin (14). Averages of the mean residence times and variances are presented in Table I. These parameters provide a quantitative estimate of the relative absorption rate without reference to an absorption model and also reflect the more consistent plasma curve obtained following ingestion of solutions in comparison to the tablet.

[4] Vacutainer BD, 278-069, 7.0 ml containing 14 mg of potassium oxalate and 17.5 mg of sodium fluoride.

Table III—Mean Plasma Salicyluric Acid Data

Parameter	Mean ± SD, μg/ml[a] T	S-16	S-34
Time			
5 min	0.0 ± 0.0[b]	0.0 ± 0.0[b]	0.0 ± 0.0[b]
10 min	0.0 ± 0.1[b]	0.2 ± 0.2[b]	0.2 ± 0.2[b]
15 min	0.2 ± 0.2[b]	0.4 ± 0.3[b]	0.4 ± 0.4[b]
20 min	0.4 ± 0.2[b]	0.7 ± 0.4[b]	0.6 ± 0.4[b]
30 min	0.7 ± 0.3[b]	1.0 ± 0.4[b]	0.9 ± 0.5[b]
45 min	1.1 ± 0.4[b]	1.4 ± 0.6[b]	1.2 ± 0.6[b]
1 hr	1.2 ± 0.4[b]	1.5 ± 0.8[b]	1.4 ± 0.7[b]
1.5 hr	1.4 ± 0.5[b]	1.6 ± 0.8[b]	1.5 ± 0.6[b]
2 hr	1.5 ± 0.5[b]	1.7 ± 0.9[b]	1.5 ± 0.6[b]
3 hr	1.5 ± 0.5[b]	1.6 ± 0.9[b]	1.4 ± 0.4[b]
4 hr	1.4 ± 0.5[b]	1.5 ± 0.7[b]	1.3 ± 0.4[b]
6 hr	1.3 ± 0.3[b]	1.3 ± 0.7[b]	1.2 ± 0.2[b]
8 hr	1.0 ± 0.2[b]	0.9 ± 0.6[b]	0.8 ± 0.2[b]
10 hr	0.6 ± 0.1[b]	0.6 ± 0.4[b]	0.6 ± 0.1[b]
Area under curve to 10 hr, (mg hr)/liter	11.5 ± 2.9[b]	12.3 ± 6.4[b]	10.9 ± 2.9[b]
Time of maximum concentration, hr	2.9 ± 1.6[b]	1.7 ± 0.4[b]	2.4 ± 1.3[b]
Maximum concentration, mg/liter	1.6 ± 0.5[b]	1.8 ± 0.9[b]	1.5 ± 0.6[b]

[a] A common letter following the standard deviation indicates no significant difference ($p < 0.05$) (17).

DISCUSSION

The combination of a specific HPLC analysis with sample handling and extraction procedures allows a detailed evaluation of plasma and urine aspirin, salicylic acid, and salicyluric acid levels following a tablet and two effervescent solutions. The solution with 16 mEq of buffer was the most rapidly absorbed, with the solution containing 34 mEq of buffer and the tablet following in that order.

The gastric emptying rate was measured[5] for the two solutions following a similar protocol by recovery of the gastric contents, and gastric emptying was observed with both to be about five times faster than occurs when the same volume of water is taken in the fasting state (7).

There are several possible methods of analysis for the aspirin absorption rate. One could utilize the Wagner–Nelson equation (15) to analyze the plasma aspirin or salicylate concentration–time curves. However, this approach presumes that a single-body compartment model applies to these data. Both compounds exhibit biexponential disposition, and salicylate–salicylurate data indicate that the salicylate is eliminated by one or more capacity-limited processes. A further analysis of the apparent percent absorbed–time curves, which could be obtained from such an analysis, requires certain presumptions, *e.g.*, that either first- or zero-order absorption kinetics apply. An alternative is to compare the peak time or concentrations. However, this approach leads only to a rank correlation and is open to serious errors due to difficulties in accurate estimation of the peak time and/or concentration from the available data.

Therefore, the application of the mean residence time approach recently introduced by Yamaoka *et al.* (13) was explored. It was shown[6] recently that relative mean absorption time (MAT) values can be calculated from oral data using the terminal rate constant in the calculation. In this approach, a drug exactly exhibiting one-compartment first-order elimination would have an MRT_{iv} equal to:

[5] J. N. Hunt, School of Medicine, Baylor University, Houston, Tex., personal communication.

[6] S. Riegelman and P. Collier, School of Pharmacy, University of California, San Francisco, Calif., personal communication.

Table IV—Mean Urine pH and Renal Clearance of Aspirin, Salicylic Acid, and Salicyluric Acid [a]

Period, hr	Treatment	pH, mean ± *SD*	Renal Clearance, liters/hr: Aspirin, mean ± *SD*	Salicylic Acid, mean ± *SD*	Salicyluric Acid, mean ± *SD*
Predose	T	5.93 ± 0.28	—	—	—
	S-16	5.93 ± 0.24	—	—	—
	S-34	5.91 ± 0.31	—	—	—
		NS[b]			
0–2	T	5.72 ± 0.44[c]	0.84 ± 0.48[c]	0.054 ± 0.071[c]	33.6 ± 6.5[c]
	S-16	5.89 ± 0.48[cd]	1.21 ± 0.54[c]	0.141 ± 0.138[cd]	32.7 ± 8.6[c]
	S-34	6.42 ± 0.58[d]	1.50 ± 0.69[a]	0.304 ± 0.243[d]	37.2 ± 7.3[c]
2–4	T	6.01 ± 0.58[c]	—	0.113 ± 0.155[c]	36.2 ± 5.0[c]
	S-16	6.48 ± 0.63[d]	—	0.204 ± 0.180[c]	34.4 ± 8.5[c]
	S-34	6.59 ± 0.48[d]	—	0.199 ± 0.160[c]	37.5 ± 4.5[c]
4–6	T	5.93 ± 0.50[c]	—	0.086 ± 0.080[c]	33.5 ± 7.4[c]
	S-16	6.24 ± 0.48[c]	—	0.156 ± 0.095[c]	34.4 ± 9.4[c]
	S-34	6.31 ± 0.52[c]	—	0.116 ± 0.104[c]	37.7 ± 6.3[c]
6–8	T	6.31 ± 0.46[c]	—	0.276 ± 0.289[c]	35.1 ± 6.3[c]
	S-16	6.52 ± 0.56[c]	—	0.260 ± 0.149[c]	36.2 ± 8.9[c]
	S-34	6.53 ± 0.51[c]	—	0.234 ± 0.185[c]	36.8 ± 4.2[c]
8–10	T	6.30 ± 0.41[c]	—	0.211 ± 0.140[c]	37.5 ± 5.0[c]
	S-16	6.69 ± 0.42[c]	—	0.342 ± 0.159[c]	36.6 ± 9.6[c]
	S-34	6.77 ± 0.30[c]	—	0.310 ± 0.167[c]	36.4 ± 9.7[c]
Aspirin equivalents excreted in 10 hr, mg	T	—	6.87 ± 3.64	34.8 ± 29.8	364.8 ± 89.5
	S-16	—	9.69 ± 4.34	6.4 ± 68.7	353.3 ± 85.8
	S-34	—	9.91 ± 5.11	70.6 ± 65.7	368.7 ± 110
Percent of aspirin dose in 10 hr	T	—	1.06 ± 0.56	5.4 ± 4.6	56.3 ± 13.8
	S-16	—	1.51 ± 0.67	10.1 ± 10.7	55.2 ± 13.4
	S-34	—	1.52 ± 0.78	10.9 ± 10.1	56.9 ± 16.9

[a] A common letter following the standard deviation indicates no significant difference ($p < 0.05$) (17). [b] NS = not significant.

$$MRT_{iv} = 1/k \qquad \text{(Eq. 1)}$$

When administered orally with a first-order absorption rate constant of k_a, the drug would exhibit an MRT_{oral} of:

$$MRT_{oral} = \frac{1}{k_a} + \frac{1}{k} \qquad \text{(Eq. 2)}$$

The mean absorption time could be estimated as:

$$MAT = MRT_{oral} - MRT_{iv} \qquad \text{(Eq. 3a)}$$

or:

$$MAT = \left(\frac{1}{k_a} + \frac{1}{k}\right) - \frac{1}{k} = \frac{1}{k_a} \qquad \text{(Eq. 3b)}$$

if one could estimate the elimination rate constant from the fall-off curve.

Since absorption disposition after intravenous administration probably exhibits two-compartment body kinetics (*2CBM*), a small error is involved in the *MAT* estimate. Riegelman and Collier[6] showed that the MRT_{iv} value for a drug exhibiting two-compartment body kinetics exactly equals:

$$(MRT_{iv})_{2CBM} = \frac{1}{\alpha} - \frac{1}{k_{21}} + \frac{1}{\beta} \qquad \text{(Eq. 4)}$$

Thus, when one calculates the *MAT* values using Eq. 1, one obtains a value equivalent to:

$$MAT_{exact} = MAT_{uncorr} + \left(\frac{1}{\alpha} - \frac{1}{k_{21}}\right) \qquad \text{(Eq. 5)}$$

For aspirin, the magnitude of the correction assuming two-compartment body kinetics can be estimated from the intravenous studies of Rowland and Riegelman (14) to be 4.5 min on the average. If one presumes that disposition does not change significantly between studies, this negative correction should be added to the relative or uncorrected *MAT* values listed in Table I. The resultant *MAT* values are not dependent on any particular input kinetics but probably represent the time for 63% of the dose to be absorbed.

The computed mean residence times for aspirin in the GI tract (*MAT* values) of 15 ± 10, 19 ± 8.1, and 38 ± 13 min for S-16, S-34, and T, respectively, indicate a statistically significant increased MRT_g value for the tablet. These results probably reflect the rate at which aspirin leaves the stomach and enters the intestine and the continued dissolution of the tablets. With the more acid gastric environment, the tablet is emptied more slowly into the intestine, and the potential for gastric absorption and irritation is greater than with the two buffered solutions. Although *in vitro* experiments showed the aspirin tablets to be ~90% dissolved in 5 min, *in vivo* some solid aspirin particles may exist for a longer time and even be emptied into the intestine.

Pharmacokinetic studies showed that aspirin solutions are absorbed completely and that ~60% reaches the general circulation unhydrolyzed (16). In the present study, two findings suggest that the amount of total salicylate (*i.e.*, aspirin plus salicylic acid) absorbed is equivalent for the three dosage forms. First, the total salicyluric acid excreted over 10 hr was essentially equal for all dosage forms (Table IV). Second, the areas under the plasma salicylate curve following the three treatments were within an 11% range, which may be attributed to the differences in renal clearance. The area under the curve for aspirin was 20% higher following the tablet than following the solution with 34 mEq of buffer, a difference not attributable to the small difference in renal clearance of aspirin associated with the two dosage forms. Thus, following the tablet, ~20% less aspirin was hydrolyzed prior to and/or during absorption when compared to the buffered solutions. If ~60% of the aspirin reaches the general circulation unhydrolyzed following the tablet, then ~50% does so following the effervescent solutions. This difference may result from a partial hydrolysis at the elevated pH associated with the buffered solution. Alternatively, part of the aspirin from the tablet may escape metabolism in the intestinal mucosa by being absorbed through the stomach.

An interesting observation is that the effervescent solution with 34 mEq of buffering agent and 648 mg of aspirin in a two-tablet dose contains more buffering than is required for rapid aspirin absorption in the fasting subject. Since this dosage form is commonly employed to treat the combined symptoms of headache and gastric hyperacidity, the 34 mEq of buffering may not be excessive. The effects of food on the absorption rate from buffered solutions are currently under study and may reveal different buffering requirements than were observed in the fasting stomach. However, for patients requiring only an analgesic effect, it may be advantageous to employ a solution with less buffering to obtain rapid absorption with a low sodium dose.

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Anthracycline Assay by High-Pressure Liquid Chromatography

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Abstract □ A general method of analysis of anthracycline concentrations was developed. Drug is extracted from plasma with organic solvent and separated from metabolites by high-pressure liquid chromatography on an aminocyanosilica column. Detection and quantitation are by the endogenous fluorescence of compounds having an intact tetracyclic ring structure. Limits of sensitivity are 5, 1, and 5 ng/ml of plasma for doxorubicin, carubicin, and marcellomycin, respectively. The assay can be used for studying the aldo–keto reductase and reductive glycosidase reactions with anthracyclines as the substrates and for the evaluation of the clinical pharmacology or pharmacodynamics of various doxorubicin analogs.

Keyphrases □ Anthracyclines—doxorubicin, carubicin, daunorubicin, and marcellomycin, high-pressure liquid chromatographic analysis, dog plasma □ High-pressure liquid chromatography—analysis of doxorubicin, carubicin, daunorubicin, and marcellomycin in dog plasma □ Antineoplastic activity, potential—high-pressure liquid chromatographic analysis of anthracyclines in dog plasma, doxorubicin, carubicin, daunorubicin, and marcellomycin

Doxorubicin (adriamycin) is the best known of several hundred characterized anthracyclines. It has a broad spectrum of activity, so it is widely used in cancer therapy. However, because of significant acute and chronic toxicities, there is a constant search for active anthracyclines with less severe or less frequent adverse effects.

Several anthracycline analogs have reached the clinical stage of development. Daunorubicin (daunomycin, cerubidine), for example, was shown to be useful in the treatment of leukemia (1) and is now commercially available. Carubicin (carminomycin), an anthracycline that was developed and clinically tested in the Soviet Union (2), is being evaluated in the United States and Europe. Aclacinomycin A, a drug from Japan, is being evaluated in Europe (3) and tested clinically in the United States. Another anthracycline, marcellomycin, appears to be relatively nontoxic to white cells in animals (4) and is now undergoing toxicity studies in preparation for clinical testing.

A simple, rapid, inexpensive method of analysis of anthracycline concentrations in biological fluids is needed not only for comparative studies of pharmacokinetics and pharmacodynamics but also for the clinical monitoring of patients receiving doxorubicin analogs. A method for determining parent drug concentrations for various anthracyclines including doxorubicin, daunorubicin, carubicin, and marcellomycin has been developed. The method employs drug extraction with an organic solvent followed by high-pressure liquid chromatography (HPLC) with fluorescence detection. *In vitro* enzyme studies and *in vivo* pharmacokinetic investigations can be performed using this assay.

EXPERIMENTAL

Reagents and Drugs—Purified doxorubicin[1], daunorubicin[1], carubicin[2], marcellomycin[2], rudolphomycin[2], and aclacinomycin A[2] were used for assay development. Clinical grade doxorubicin[3] and carubicin[2] were used in animal studies. Adriamycinol and daunorubicinol were obtained by reduction of the parent compound with aldo–keto reductase according to the procedure of Felsted *et al.* (5). Carminomycinol was prepared by borohydride reduction according to a modified procedure of Povarov *et al.* (6).

Aglycones were prepared by acid hydrolysis at elevated temperature (7). Deoxyaglycones of doxorubicin and adriamycinol were produced by reaction of the parent compound with microsomal enzymes in the presence of reduced nicotinamide-adenine dinucleotide phosphate (NADPH) under anaerobic conditions (8). Solvents were reagent grade or better, and degassing was not necessary. Water was glass distilled.

Instruments—Volume measurements were made with automatic pipets[4]. The high-pressure liquid chromatograph included an injection port[5] and a single-piston pump[6] with electronic damping. The column (25 cm × 4.6 mm i.d.) was packed with 10-μm aminocyanosilica[7]. The detection unit consisted of a filter-type fluorometer[8] with an emission filter of bandwidth 560–570 nm and an excitation filter of bandwidth

[1] Adria Laboratories, Dublin, Ohio.
[2] Bristol Laboratories, Syracuse, N.Y.
[3] Adriamycin, Adria Laboratories, Dublin, Ohio.
[4] Digital Pipetman, Rainin Instrument Co., Brighton, Mass.
[5] Model 7210, Rheodyne, Berkeley, Calif., or model U6K, Waters Associates, Milford, Mass.
[6] Model 110, Altex, Rainin Instrument Co., Brighton, Mass.
[7] Partisil-10 PAC, Whatman, Clifton, N.J.
[8] Spectra-Glo, Gilson Medical Electronics, Middleton, Wis.

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