Table I—Reproducibility of Colored Complex of Replicate Homatropine Methylbromide Samples Containing 0.5 mg/ml

Solution	Absorbance
1	0.421
2	0.427
3	0.424
4	0.423
5	0.422
6	0.426
7	0.425
8	0.421
9	0.422
Average	0.423
CV	0.52%

of 0.005 and standard errors of the estimate of the intercept and slope of 0.05 and 0.84, respectively. The colored complex in chloroform was stable for at least 5 hr.

The precision of the assay procedure was determined by running replicate studies on nine 2.0-ml aliquots of a standard homatropine methylbromide solution containing 0.5 mg/ml. Each solution was assayed by the proposed procedure. The coefficient of variation for the nine replicate samples was 0.52% (Table I). A blank sample, containing tropinium methylbromide and mandelic acid equivalent to 100% hydrolytic degradation of homatropine methylbromide, assayed by the proposed procedure yielded no absorbance at 365 nm, demonstrating that the method is stability indicating.

Results obtained by applying the procedure to commercially available homatropine methylbromide dosage forms are presented in Table II. Comparison of the experimental data with those obtained using an ion-exchange method (12) shows a good correlation.

Table II—Results of Analysis of Commercially Available Homatropine Methylbromide Dosage Forms

Dosage Form	Amount Claimed, mg/Tablet or mg/ml	Percent of Claim Found[a]			
		Proposed Method	±*SD*	Ion-Exchange Method	±*SD*
Tablet[b]	5	98.4	0.47	98.5	0.14
Elixir[c]	1	99.6	0.36	98.8	0.30
Tablet[d]	5	97.8	0.58	97.5	0.88
Elixir[e]	1	101.0	0.71	101.2	1.90
Tablet[f]	5	95.1	0.11	95.1	0.23
Tablet[g]	2.5	91.3	0.83	91.9	0.46
Drops[h]	0.27	104.0	0.57	102.6	0.40
Tablet[i]	2.5	104.0	0.75	103.5	0.11

[a] Average of three assays. [b] Mesopin tablets, Endo Laboratories Pharm. [c] Mesopin elixir, Endo Laboratories Pharm. [d] Mesopin PB tablets, Endo Laboratories Pharm. [e] Mesopin PB elixir, Endo Laboratories Pharm. [f] Cholan V tablets, Pennwalt. [g] Cholan HMB tablets, Pennwalt. [h] Sedadrops, Merrell-National. [i] Gustase Plus tablets, Geriatric Pharmaceutical Corp.

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Physical Form as a Determinant of Effect of Buffered Acetylsalicylate Formulations on GI Microbleeding

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Abstract □ During a 48-day period, 12 male dogs received four buffered sodium acetylsalicylate formulations, quantitatively virtually identical (a homogeneous disintegrating swallow tablet, a swallow tablet in which the sodium acetylsalicylate was contained in a dissolving core, an encapsulated powder, and an aqueous suspension), at 650-mg aspirin equivalent doses twice daily during four 7-day treatment periods (each preceded by a 5-day period of no treatment) in a complete changeover fashion. Mean daily fecal blood losses of 0.75, 1.37, 1.43, and 2.89 ml were observed in the 12 dogs during treatment with the aqueous suspension, the homogeneous tablet, the encapsulated powder, and the core tablet, respectively. These findings indicate that the physical form of buffered acetylsalicylate formulations is a critical factor in the effect of such formulations on GI microbleeding.

Keyphrases □ Sodium acetylsalicylate—buffered formulations, various dosage forms, effect on GI microbleeding, dogs □ Aspirin, sodium salt—buffered formulations, various dosage forms, effect on GI microbleeding, dogs □ GI microbleeding—buffered sodium acetylsalicylate, effect of various dosage forms, dogs □ Dosage forms—buffered sodium acetylsalicylate, effect on GI microbleeding, dogs □ Analgesics—sodium acetylsalicylate buffered formulations, various dosage forms, effect on GI microbleeding, dogs

Gastric mucosal irritation, reflected in increased fecal occult blood loss, is a common side effect of orally administered aspirin. Levy (1) suggested that the degree of gastric mucosal erosion following the oral administration of aspirin is related to two factors: the concentration of aspirin in solution and the duration of contact of the so-

Table I—Description of the Buffered Sodium Acetylsalicylate[a] Formulations Employed

Formulation Code	Physical Form	Buffer Component, mg/Unit Dose			Calculated Excess Buffer Capacity, mEq
		Calcium Carbonate	Magnesium Carbonate	Tricalcium Phosphate	
A2	Aqueous suspension of powder[b]	215	182	48	8.98
B2	Encapsulated powder	215	182	48	8.98
C2	Homogeneous, disintegrating swallow tablet	246	211	—	6.77
D2	Dissolving core swallow tablet	215	182	48	8.98

[a] Unit doses of the four formulations contained 365 mg of sodium acetylsalicylate, equivalent to 325 mg of aspirin. [b] Aqueous suspensions were prepared just prior to administration. Powder equivalent to two unit doses was placed in a 150-ml beaker, 75 ml of distilled water was added, and the mixture was stirred manually for a time adequate to give a uniform suspension. The resulting suspension, followed by a 25-ml distilled water rinse, was administered by gavage.

lution with the mucosa. He noted that particles of solid aspirin adherent to the moist mucosal surface are surrounded by a saturated solution of aspirin; as dissolved unionized drug is absorbed into the mucosa, the solid particle acts as a reservoir and provides additional aspirin to the solution. Anderson (2) agreed with this view and noted that, with insoluble aspirin preparations, there was good correlation between the number of aspirin particles in the stomach and the number of focal erosions.

In recent years, Leonards and Levy (3–5) demonstrated conclusively that the effect of aspirin on the gastric mucosa can be minimized by administration of the analgesic either in dilute aqueous solution or as rapidly dissolving tablets buffered to maintain the acetylsalicylate in its ionized form. Phillips *et al.* (6) described a homogeneous swallow tablet formulation[1] containing sodium acetylsalicylate and 6.77 mEq excess base in the form of a nonabsorbable buffer system, which did not elicit GI microbleeding in humans; unfortunately, the formulation ultimately proved to be unstable. A second similar formulation[1] (containing more buffer), except that the sodium acetylsalicylate was contained in a dissolving core surrounded by the buffer ingredients, was prepared in an attempt to achieve adequate stability. While the second formulation proved to be stable, it also increased GI microbleeding significantly in humans[2].

It was hypothesized that the differential effect of the two formulations (C2 and D2, Table I) on GI microbleeding was attributable to the fact that the sodium acetylsalicylate component of the second formulation was contained in a surface-eroding, dissolving core. During the period following disintegration of the buffer shell, when the core is dissolving, the solid core may become adherent to the gastric mucosa. In that case, significant reconversion to the acid could take place.

The present study was undertaken to test this hypothesis; it illustrates the importance of physical form as a determinant of aspirin-induced GI microbleeding and supports the concepts of Leonards and Levy (3–5).

EXPERIMENTAL

Animals—Twelve male beagle dogs[3], 6.7–10.5 kg, were employed. The dogs were immunized previously against distemper and hepatitis and received a vermifuge on at least two occasions. The animals were caged individually and had free access to drinking water; approximately 300 g of dry food[4] was offered to each dog for 2 hr at about 10:00 am daily. Two weeks prior to the start of the experiment (Day −15), each dog received 50 μCi of ^{59}Fe-labeled ferrous sulfate[5] intravenously.

Sample Handling—GI blood loss was determined by measurement of the iron-59 content of 24-hr stool collections. Details regarding the collection, preparation, and counting of 24-hr stool and weekly whole blood samples and calculation of the whole blood content of 24-hr stool collections were described previously (7).

Acetylsalicylate Formulations—Four formulations (two swallow tablets, an encapsulated powder, and a powder suspended in water) were employed in the present study. They were coded at random for convenience and are described in Table I. Unit doses of the four formulations contained the equivalent of 325 mg of aspirin and had similar total buffer capacities.

Experimental Design—The dogs were assigned to three 4 × 4 Latin squares in such a manner as to place the lightest four in one square and the heaviest four in a second square. The four treatments were then assigned to rows within the squares in such a fashion as to balance the design to permit detection of residual effects of the treatments; the dogs were assigned at random to columns within the squares.

Study Days 0 through 5, 13 through 17, 25 through 29, and 37 through 41 were designated as control periods. Days 6 through 12, 18 through 24, 30 through 36, and 42 through 48 were designated as treatment periods. During these treatment times, two tablets (or the equivalent of two tablets as suspended or encapsulated powder) of the appropriate formulations were administered orally twice daily, at 8:00 am and 4:00 pm, in complete changeover fashion.

Statistics—The experimental observations were defined as the average daily fecal blood volume observed in each animal during each treatment period. Average daily fecal blood volumes observed in the 12 dogs during the four treatment periods were subjected to analysis of variance. When this analysis revealed that there were significant differences among the direct effects of the four treatments, the significance of differences between treatments was determined using the Student–Newman–Keuls test (8). Details regarding statistical evaluation of the data were described previously (6).

RESULTS

The average daily fecal blood losses observed in the 12 dogs during the four control and four treatment periods are summarized in Table II. The analysis of variance of the average daily fecal blood loss observed during the four treatment periods indicated that there were significant differences between the direct effects of the four treatments. The significance of differences between the means of average daily fecal blood loss observed during treatment with the four formulations is also summarized in Table II. The results indicate that the homogeneous tablet formulation C2 and the encapsulated powder B2 caused less GI microbleeding than the dissolving core tablet formulation D2 but more than the suspended powder A2.

The analysis of variance also revealed that there were: (*a*) significant differences between squares to which dogs had been assigned on the basis

[1] Prepared by the Pharmacy Research Department, Consumer Products Division, Miles Laboratories, Elkhart, IN 46514.
[2] Unpublished study.
[3] Obtained from Laboratory Research Enterprise, Kalamazoo, Mich. The dogs were maintained in an animal care facility fully accredited by the American Association for Laboratory Animal Care.
[4] Wayne Dog Krumettes, Allied Mills, Chicago, Ill.
[5] Lot 9601, New England Nuclear, Boston, Mass.

Table II—Summary of Average Daily Fecal Blood Loss Values

Dog	Average Daily Fecal Blood Loss, ml/Period in which Treatment was Administered							
	Control Period 1	A2	Control Period 2	B2	Control Period 3	C2	Control Period 4	D2
13	0.85	0.56/3	0.76	0.76/4	0.62	1.45/2	0.61	2.51/1
14	0.58	0.61/3	0.49	0.90/2	0.41	0.98/1	0.31	2.00/4
15	1.06	0.56/4	0.66	1.16/3	0.52	1.81/1	0.40	4.27/2
16	0.60	0.47/4	0.59	1.02/2	0.33	1.59/3	0.54	3.17/1
17	0.69	0.91/2	0.63	2.97/1	0.52	1.73/3	0.75	6.33/4
18	0.65	0.61/1	0.46	0.76/3	0.44	1.08/2	0.33	1.74/4
19	0.40	0.63/1	0.40	1.00/4	0.42	0.93/3	0.46	0.90/2
20	0.49	0.84/3	0.74	1.16/1	0.65	1.23/4	0.42	1.68/2
21	0.66	0.62/2	0.70	1.29/4	0.46	1.33/1	0.48	3.04/3
22	0.79	1.18/2	0.74	2.16/3	0.50	1.89/4	1.16	3.59/1
23	0.97	1.17/4	0.66	2.46/1	0.50	1.12/2	0.55	2.89/3
24	0.78	0.85/1	0.67	1.49/2	0.54	1.45/4	0.52	2.56/3
Mean	0.70	0.75[a]	0.62	1.43[b]	0.50	1.37[b]	0.54	2.89[c]
±*SE*	0.05	0.07	0.03	0.21	0.02	0.09	0.07	0.41

[a] Significantly ($p < 0.05$) different from B2, C2, and D2. [b] Significantly ($p < 0.05$) different from A2 and D2. [c] Significantly ($p < 0.05$) different from A2, B2, and C2.

of body weight, (*b*) no significant differences among periods, and (*c*) no significant residual effects of the treatments. The finding that there were significant differences between squares is consistent with the dose-dependent nature of aspirin-induced GI microbleeding in dogs (7), since all dogs received a fixed aspirin-equivalent dose regardless of body weight. Similarly, the finding of significant differences between dogs within squares is consistent with numerous reports indicating that the response of individuals to aspirin varies significantly.

DISCUSSION

These findings support the hypothesis that the differential effect of C2 and D2 tablets on the gastric mucosa is attributable to the sodium acetylsalicylate component of D2 being contained in a dissolving core. Of the four quantitatively similar, but physically different, formulations, only with D2, the dissolving core tablet, would significant reconversion of sodium acetylsalicylate to the unionized acid be expected to occur (if the dissolving sodium acetylsalicylate core became adherent to the gastric mucosa during tablet disintegration); D2 caused significantly more GI microbleeding than the other formulations. Therefore, the physical form of a buffered acetylsalicylate formulation is critical in the effect of such formulations on GI microbleeding.

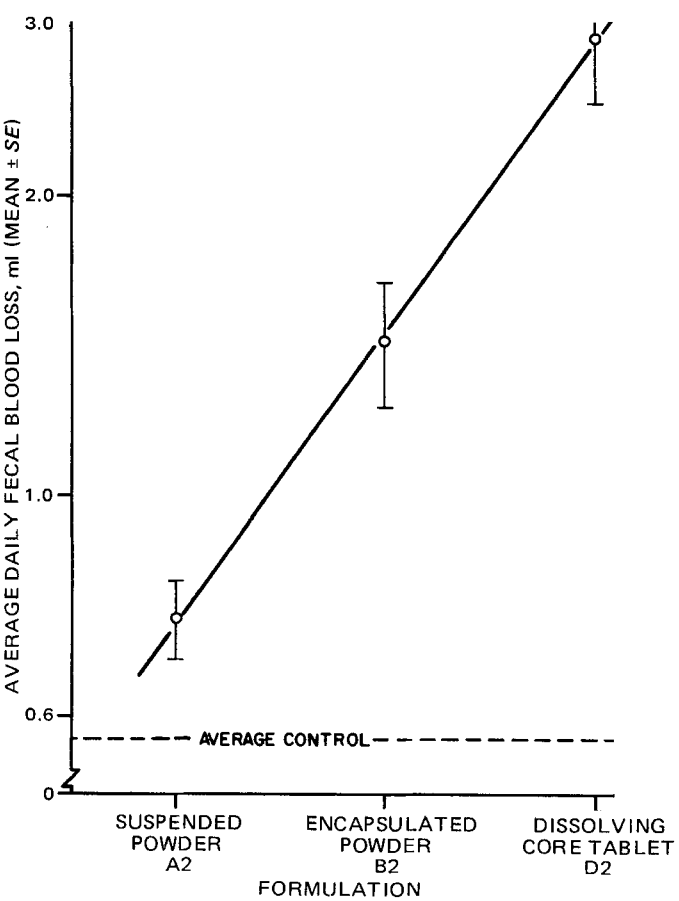

Figure 1—*Relationship of physical form to GI microbleeding.*

Aspirin, in the unionized form, must enter into the gastric mucosal cell to initiate gastric mucosal erosion. Therefore, the relationship of the physical form of a buffered sodium acetylsalicylate formulation to the GI microbleeding produced by the formulation is most likely attributable to the dissolution rate. In the case of a formulation with a rapid dissolution rate, there is little chance for reconversion of sodium acetylsalicylate to the acid; the converse is true for a formulation with a slow dissolution rate.

If it is assumed that the suspended (A2) and encapsulated (B2) powder formulations represent D2 tablet formulations with infinitely short dissolution and disintegration times, respectively (the fact that the encapsulated powder is not presented to the gastric mucosa until the capsule dissolves should be considered a form of delayed drug administration rather than delayed disintegration, since the material is completely disintegrated once presented to the mucosa), these results indicate clearly that a correlation exists between the rate of tablet dissolution and the effect on the gastric mucosa that is independent of total buffer capacity. Thus, a product with finite disintegration and solution times (D2) elicits significant GI microbleeding, while a product with an infinitely short disintegration time but a finite solution time (B2) causes significantly less microbleeding; a product with an infinitely short dissolution time (A2) causes even less microbleeding, despite the fact that all three formulations have similar total buffer capacities (Fig. 1). Almost certainly the fecal blood loss observed during treatment with A2 is not significantly greater than the control, although this possibility could not be tested statistically since no placebo was employed.

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Quick Estimation of Kinetic Parameters for a Compartment with Exponential Absorption Rate and First-Order Elimination Rate

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Abstract □ Two methods are presented for the quick estimation of kinetic parameters for a compartment with an exponential absorption rate and a first-order elimination rate. The first method is by direct computation from the observed levels of substance in the compartment at times t, $2t$, and $3t$, where t is arbitrary. The second method uses a numerical table to estimate the parameters from the observed peak level, the time of the peak level (or the time when the level rises to half of the peak level), and the time when the level has declined to half of its peak value. Some approximation equations also are given.

Keyphrases □ Kinetic parameters—compartment with exponential absorption rate and first-order elimination rate, two methods for quick estimation □ Pharmacokinetics—compartment with exponential absorption rate and first-order elimination rate, two methods for quick estimation of kinetic parameters

If the rate of absorption of a substance into an initially empty, well-stirred compartment declines exponentially with time and the rate of elimination is first order, then the quantity or concentration, y, of the substance in the compartment is a function of time, t, of the general form:

$$y = \frac{C(e^{-k_1 t} - e^{-k_2 t})}{k_2 - k_1} \qquad \text{(Eq. 1)}$$

where C, k_1, and k_2 are constants. The method usually recommended for estimating these constants from experimental data is a graphical procedure known as "peeling," "feathering," or the "method of residuals" (1, pp. 281–292), complemented by least-squares adjustment by computer (1, 2). The graphical procedure can be computerized, and these methods are entirely satisfactory if suitable computer programs and services are available. Without a computer, however, these methods are time consuming and are quite unwieldy for preliminary evaluation of data, rough comparison of published reports, and double-checking calculations.

This report describes methods of rapid, direct computation of C, k_1, and k_2 from experimental data; these methods may be useful for applications not requiring high accuracy or careful statistical weighting.

THEORY AND DISCUSSION

Estimation from y Values at t, $2t$, and $3t$—In principle, any three data points will determine the three parameters. In practice, however, the resulting three simultaneous equations cannot always be solved for the parameters. Therefore, direct computation of the parameters requires a suitable selection of data points.

Let t be any convenient time and let y_1, y_2, and y_3 be the observed levels at t, $2t$, and $3t$, respectively. Equation 1 gives:

$$y_j = \frac{C(e^{-k_1 jt} - e^{-k_2 jt})}{k_2 - k_1} \qquad \text{(Eq. 2)}$$

where $j = 1$, 2, or 3. For the solution of these three simultaneous equations, let:

$$r = +\sqrt{\frac{y_1 y_3}{y_2^2} - \frac{3}{4}} \qquad \text{(Eq. 3)}$$

Then:

$$k_1 = -\frac{1}{t}\log_e\left[\frac{y_2}{y_1}\left(\frac{1}{2} + r\right)\right] \qquad \text{(Eq. 4)}$$

$$k_2 = -\frac{1}{t}\log_e\left[\frac{y_2}{y_1}\left(\frac{1}{2} - r\right)\right] \qquad \text{(Eq. 5)}$$

and:

$$C = \frac{y_1^2(k_2 - k_1)}{2y_2 r} \qquad \text{(Eq. 6)}$$

The labeling of the constants k_1 and k_2 is entirely arbitrary. The conventions adopted here regarding the algebraic sign of r (Eqs. 3–5) assign the label k_2 to the larger of the two. If $r = 0$, then $k_1 = k_2 = k$ and Eq. 1 takes the limiting form:

$$y = Cte^{-kt} \qquad \text{(Eq. 7)}$$

If the quantity under the radical in Eq. 3 is negative, the data are inconsistent with the model underlying Eq. 1.

Equations 3–6 become simpler if t is selected on the rising limb of the y curve (Fig. 1) in such a way that $2t$ intercepts the falling limb at just the same level; *i.e.*, $y_2 = y_1$. Then the limiting case ($k_1 = k_2 = k$, Eq. 7) will have $y_3 = (\frac{3}{4})y_1$ (*cf.*, Eq. 3).

The use of Eqs. 3–6 may be illustrated by the example of Fig. 1. The steps for estimating the parameters are:

1. From the curve of Fig. 1, read the values of y at $t = 2$, 4, and 6: $y_1 = 1.85$, $y_2 = 1.43$, and $y_3 = 1.02$, respectively.
2. Compute $r = \sqrt{(1.85)(1.02)/(1.43)^2 - 0.75} = 0.416$ and $y_2/y_1 = 1.43/1.85 = 0.773$.
3. Compute $k_1 = -(\frac{1}{2})\log_e[0.773(0.5 + 0.416)] = 0.17$, $k_2 = -(\frac{1}{2})\log_e[0.773(0.5 - 0.416)] = 1.4$, and $C = fDk_a/V = (1.85)(1.4 - 0.17)/[2(0.773)(0.416)] = 3.5$.

Like any method of fitting the curve of Fig. 1, this analysis does not tell which of the two constants, k_1 (the smaller) or k_2 (the larger), is identified with k_a or k_e, nor does it evaluate the several factors of the coefficient C.

Estimation from Peak Level, y_m, Time of Peak Level, t_m, and Time of Decline to Half of Peak Level, t_{h_2}—Let y_m be the peak level, t_m be the time of the peak level, t_{h_1} be the time when the rising level first reaches $y_m/2$, and t_{h_2} be the time (after t_m) when the declining level reaches $y_m/2$ (Fig. 1). The theoretical equations (Eq. 1) for these ob-