Medicament Release from Suppository Bases I: Physicochemical Characteristics and Bioavailability of Indomethacin in Rabbits

NICHOLAS J. VIDRAS *, VINCENT E. REID *, N. R. BOHIDAR ‡, and FOTIOS M. PLAKOGIANNIS *x

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Abstract □ This investigation was designed to determine the *in vitro* release of indomethacin from suppository bases and the *in vivo* bioavailability in rabbits. Suppositories containing 25 mg of indomethacin were made by the fusion method with theobroma oil, esterified fatty acids (C_{10}–C_{18}), and polyethylene glycol 1000. To produce an exact dosage form, a formula for the determination of the displacement value was derived, and it was found that theobroma oil > esterified fatty acids (C_{10}–C_{18}) > polyethylene glycol 1000. The suppository hardness was determined by using appropriate apparatus and it was found that the esterified fatty acids (C_{10}–C_{18}) allowed the formation of more brittle suppositories. The release rates were determined with the USP dissolution apparatus, with or without cellophane membrane, and it was found that polyethylene glycol 1000 > esterified fatty acids (C_{10}–C_{18}) > theobroma oil. The bioavailability of indomethacin after rectal administration was greater with polyethylene glycol base. Significant correlation was obtained during the first 45 min between the *in vitro* release (dialyzing tubing) and the *in vivo* bioavailability.

Keyphrases □ Indomethacin—physicochemical characteristics, bioavailability, rabbits, suppository bases □ Bioavailability—indomethacin physicochemical characteristics, rabbits, suppository bases □ Suppositories—bases, indomethacin, physicochemical characteristics, bioavailability, rabbits

Indomethacin, a nonsteroid drug, was first synthesized in 1961 (1). It has been proven to have anti-inflammatory and analgesic effects in rheumatoid arthritis (2–6), gout (7), osteoarthritis (8–10), ankylosing spondylitis (11), glomerulonephritis (12), and acute musculoskeletal disorders (13). Unfortunately, like other analgesic and anti-inflammatory agents, it carries the risk of GI irritation. The tablets and capsules, which are presently used, have led to peptic ulceration and anorexia (14, 15), nausea, vomiting, dyspepsia, and diarrhea (16). Other side-effects have involved the central nervous system, including headache, giddiness, mental changes, faintness, drowsiness, and blurring of vision. An attractive route of administration for patients with peptic ulcers and for those who are unable to tolerate the drug could be the rectal route.

Previous studies on the rectal administration of indomethacin (17–20) have given little information regarding the suppository bases used, the influence of the suppository base on the release of the drug, or the effects of the physicochemical properties such as hardness or melting range on *in vitro* and *in vivo* drug release. In this study, indomethacin suppositories in three different bases, polyethylene glycol 1000, esterified fatty acids (C_{10}–C_{18}), and theobroma oil were used to evaluate their physicochemical properties, to examine the *in vitro* release of indomethacin, its *in vivo* release in rabbits, and to correlate if possible the *in vitro* with *in vivo* data.

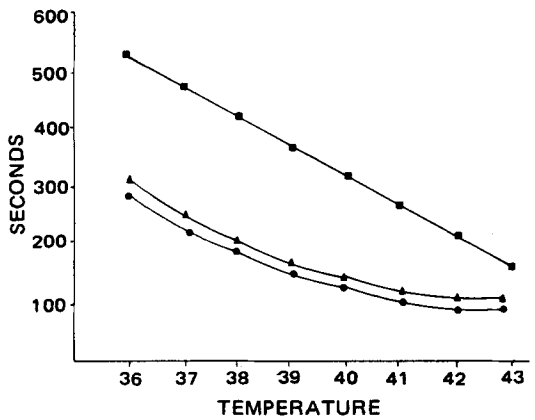

Figure 1—*Time interval for complete melting of indomethacin suppositories as a function of temperature using the USP disintegration apparatus. Key: (■) polyethylene glycol; (▲) esterified fatty acids (C_{10}–C_{18}); (●) theobroma oil.*

EXPERIMENTAL

Chemicals—Indomethacin[1], polyethelene glycol 1000[2], esterified fatty acids (C_{10}–C_{18})[3], theobroma oil[4], heptane[4], phosphate buffer[4] solutions, (pH 7–9), sodium hydroxide[4], and hydrochloric acid[4]. All reagents were analytical or reagent grade purity.

Preparation of Suppositories—All suppositories were prepared by the fusion method using a metal mold with 12 cavities (10). Drug displacement (3) in polyethylene glycol 1000, esterified fatty acids (C_{10}–C_{18}),

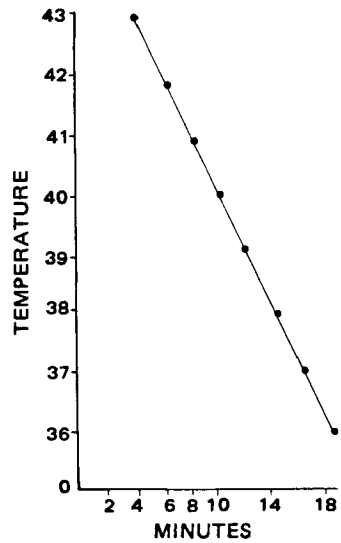

Figure 2—*Time interval for complete melting of indomethacin–polyethylene glycol base as a function of temperature using the commercial disintegration apparatus.*

[1] Merck, Sharp and Dohme, West Point, Pa.
[2] J. T. Baker Chemical Co., Philipsburg, N.J.
[3] Witepsol H-15, Kay-Fries Chemical Inc., Montvale, NJ 07645.
[4] Fisher Scientific Co., Springfield, N.J.

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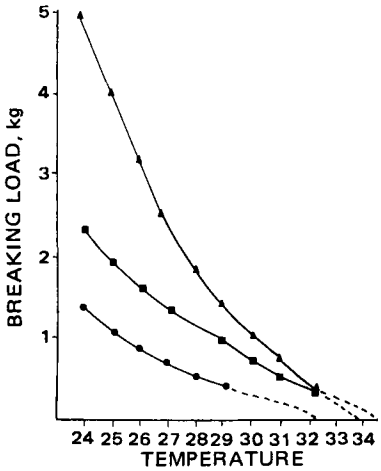

Figure 3—*Breaking load of the indomethacin suppositories as a function of temperature. Key: (■) polyethylene glycol; (▲) esterified fatty acids (C_{10}–C_{18}); (●) theobroma oil.*

and theobroma oil was first determined, and then the amount of indomethacin required was calculated.

Weight Variation—Twenty suppositories from each base were weighed, and the average weight and percent deviation for each suppository was calculated.

Content Uniformity Test—Although the USP does not specify a content uniformity for suppositories, the relative potency of the suppositories was determined. Thirty suppositories were randomly selected from each base, 10 of which were assayed individually. A preweighed suppository was melted and dissolved in 15 ml of phosphate buffer solution (pH 8). The buffer solution was added to a final yield of 1 liter. The adsorbance was measured on a spectrophotometer[5] at 318 nm. Blank suppositories were tested, and it was determined that the suppository vehicles had no effect on the UV absorbance at 318 nm. The concentration of indomethacin in the samples was determined from a standard curve.

Melting Range Test (Macro-Melting Range Test)—It has been shown (4, 5) that a narrow melting range is important in maintaining the shape of the suppository in ambient temperatures and in controlling the melting time of the suppository after insertion. The melting range test is a time measure for complete melting or dispersal of the suppository when immersed in a constant temperature water bath. Two apparatuses were used for the determination of the melting range. The USP tablet disintegration apparatus[6] was set as required in USP XIX, using water as the immersion fluid. A suppository, stored for at least 24 hr at room temperature, was placed in each tube of the basket, and each tube was covered with a disk. The time required for each suppository to completely melt at a specific temperature was determined. The temperature for this study ranged from 36 to 43°. The melting point testing apparatus for suppositories[7] was also used.

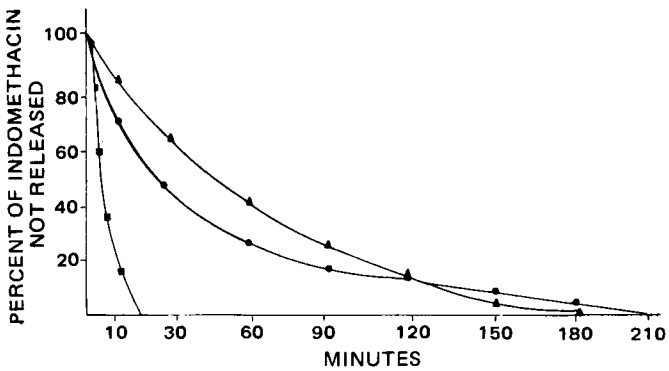

Figure 4—*Percentage of indomethacin not released from different suppository bases* versus *the time (min), using the USP dissolution apparatus. Key: (■) polyethylene glycol; (▲) esterified fatty acids (C_{10}–C_{18}); (●) theobroma oil.*

[5] Bausch-Lomb double beam spectronic 2000 UV by Shimadly.
[6] Erweaka, model ZT3, New York, N.Y.
[7] American Optical Co., New York, N.Y.

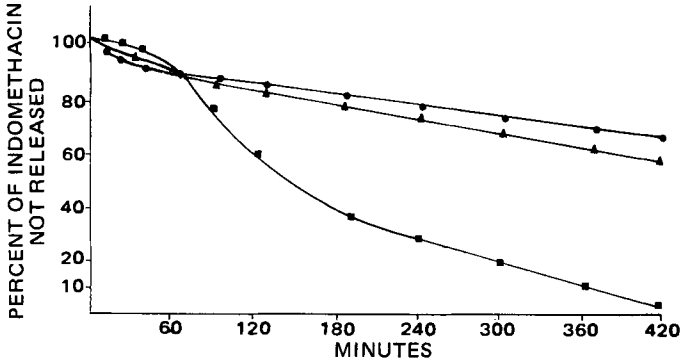

Figure 5—*Percentage of indomethacin not released from different suppository bases* versus *the time (min), using the USP dissolution apparatus and cellophane membrane. Key: (■) polyethylene glycol; (▲) esterified fatty acids (C_{10}–C_{18}); (●) theobroma oil.*

Breaking Test (Hardness)—The breaking test was designed to measure the brittleness and fragility of suppositories (1). This was measured by the weight in kilograms under which a suppository collapses at a specific temperature. The hardness of each suppository was determined by utilizing the fracture point testing apparatus for suppositories[7].

Release of Indomethacin from Suppository Bases—Three different methods were used for the determination of the release rates:

Method A—The USP rotating basket dissolution apparatus was used for the determination of release rates by this method. Each suppository was placed in the wire basket, which was lined from the inside with filter paper, and lowered into a flask containing 600 ml of phosphate buffer solution (pH 8). (The filter paper was used as a barrier for the diffusion of the suppository base.) The basket was rotated at 100 rpm at a constant temperature (37 ± 0.5°). Five-milliliter samples were withdrawn at appropriate time intervals and assayed to obtain a dissolution profile. Five milliliters of phosphate buffer was added to the dissolution medium to compensate for sampling. The absorbances of these solutions were measured at 318 nm on a spectrophotometer and the concentrations were determined from the standard curve.

Method B—The same apparatus and the conditions as previously described were employed. The modification was to place the suppository in a cellophane membrane[8] before exposure to the buffer solution.

Method C—This method employed dialysis. The dialyzing bags were prepared from dialysis tubing tied with plastic cord and soaked overnight in the phosphate buffer solution (pH 8). After rinsing the bags twice, 20 ml of phosphate buffer (pH 8) and one suppository were placed in each bag and suspended in a 500-ml wide-mouth bottle containing 400 ml of the phosphate buffer solution. The bottle was placed in a water bath at a constant temperature[9] of 39 ± 0.5° and agitated with a magnetic stirrer. At constant time intervals, a 5-ml sample was removed from the bottle and assayed to obtain a dissolution profile. Five milliliters of phosphate

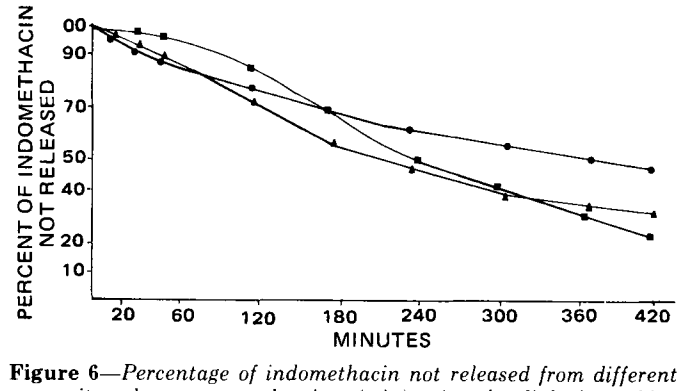

Figure 6—*Percentage of indomethacin not released from different suppository bases* versus *the time (min) using the dialyzing tubing method. Key: (■) polyethylene glycol; (▲) esterified fatty acids (C_{10}–C_{18}); (●) theobroma oil.*

[8] Dupont Co.
[9] The temperature, 39°, was chosen in order to initiate the *in vivo* conditions as closely as possible (the temperature of the rectum of the rabbits was 39°) and ensure complete melting of the suppositories.

Table I—Related Bioavailability Parameters from Serum Level Data

Parameter	Treatment A: Polyethylene Glycol 1000 Suppositories	Treatment B: Esterified Fatty Acids (C_{10}–C_{18}) Suppositories	Treatment C: Theobroma Oil Suppositories	Treatment D: Powder Suspension
Average of Peaks of Individual Serum Concentrations (Time Curve), μg/ml	1.35	1.12	1.17	1.64
Time of Peak Value of Individual Serum Concentration–Time Curves, min	60	90	90	90
Average of Area under Individual Serum Concentration–Time Curves, μg/ml min	178.53	182.42	177.92	215.10

buffer (pH 8) was added to the dissolution medium to compensate for sampling. The absorbances were measured at 318 nm in a spectrophotometer and the concentrations of indomethacin were obtained from the standard curve.

***In Vivo* Studies**—Twelve healthy white New Zealand male rabbits (3–4.5 kg) were randomly divided into four groups of three animals each. Four different dosage forms of indomethacin were administered, and each dosage form provided 25 mg of indomethacin.

At time zero, each rabbit received indomethacin as follows: Group A, one polyethylene glycol 1000 suppository; Group B, one esterified fatty acids (C_{10}–C_{18}) suppository; Group C, one theobroma oil suppository; and Group D, 25 mg of indomethacin suspended in ~100 ml of water administered orally. Retention of the suppositories by the rabbits was ensured by the use of an appropriately sized styrofoam disk taped to the rectum after insertion. Blood (2–5 ml) was taken by cardiac puncture from each rabbit at different time intervals up to 4 hr.

Assay of Serum—The serum samples were assayed by modifying a method described previously (17). One milliliter of serum was pipeted into a glass stoppered centrifuge tube containing 2 ml of a 1 *M* citrate buffer and 25 ml of heptane containing 5% isoamyl alcohol. The test tube was mechanically agitated for 30 min and then centrifuged. Twenty milliliters of the heptane phase was pipeted into a second centrifuge tube containing 5 ml of a 0.2 *M* citrate buffer. This was agitated for 25 min and then centrifuged. Ten milliliters of the organic phase was pipeted into a third centrifuge tube containing 3 ml of 0.1 *N* NaOH and shaken for 5 min. The organic phase was removed by aspiration and 2 ml of the aqueous phase transferred to a quartz cell for immediate spectrofluorophotometric determination (activation maximum, 305 nm; fluorescence maximum, 400 nm)[10,11].

RESULTS AND DISCUSSION

In the preparation of suppositories some difficulty is experienced in achieving the exact dosage. This is because the volume of suppositories from a particular mold is uniform, but its weight will vary because the density of the drug usually differs from the density of the base with which the mold is calibrated. Each mold should be calibrated before use by preparing suppositories using the base alone, weighing the blank suppositories, and taking the mean weight as the calibration factor. Therefore, to prepare accurate suppositories, it is essential to derive a general expression (Eq. 1) to account for the displacement value of a drug, which is defined as the number of parts by weight of the drug that displaces one part by weight of the base:

$$F = \frac{XB}{100\,(A - B) + XB} \qquad \text{(Eq. 1)}$$

where F is the displacement value of a drug; X is the percentage of drug used; B is the weight of n suppositories containing X% of a drug; and A is the calibration factor, *i.e.*, weight of unmedicated n suppositories.

According to the definition, the displacement value of a drug is:

$$F = \frac{\text{Amount of the drug in each suppository}}{\text{Amount of base displaced in each suppository}} \qquad \text{(Eq. 2)}$$

Since the amount of drug in each suppository is $X/100\,B$, and the amount of base displaced by the above amount of drug is $(A - (100 - X)/100\,B)$, Eq. 2 becomes:

$$F = \frac{\frac{X}{100}B}{A - \frac{100 - X}{100}B} = \frac{XB}{100\,(A - B) + XB} \qquad \text{(Eq. 3)}$$

Therefore, the quantity of base required is calculated using the following equation:

$$P = (N \times S) - \frac{D}{F} \qquad \text{(Eq. 4)}$$

where P is the amount of base required (in grams); N is the number of the prepared suppositories; S is the size of the mold used (in grams); and D is the amount of drug (in grams) that is required.

The displacement values of indomethacin were calculated in the polyethylene glycol 1000, esterified fatty acids (C_{10}–C_{18}), and theobroma oil suppository bases, and were found to be 1.72 ± 0.21, 2.06 ± 0.14, and 2.44 ± 0.39, respectively.

The weight variation of 20 randomly selected indomethacin suppositories containing 25 mg of the drug in the three bases was determined and found to be from 1.71 to 1.84 g for polyethylene glycol 1000, 1.38 to 1.48 g for esterified fatty acids (C_{10}–C_{18}), and 1.09 to 1.17 g for theobroma oil. All the suppositories prepared met the acceptable limits of the German, Russian, and Nordica pharmacopeias (21), which state that individual weight variations of rectal suppositories can vary by ±5, ±5, and ±10% of their average weight, respectively. The USP does not have any limits for acceptable weight variation of rectal suppositories. Although the USP does not specify content uniformity standards, the content uniformity of indomethacin suppositories in polyethylene glycol 1000, esterified fatty acids (C_{10}–C_{18}), and theobroma oil was determined and found to be within −5.2 to +12.2, −8.2 to +9.8, and −8.2 to +12.2 of the labeled amounts, respectively.

The definitions of rectal suppositories as reported in different pharmacopeias, specifically state the "suppositories should soften, melt, or dissolve at body temperature." However, these are of little value, as pointed out previously (22), unless the method of determining these characteristics is given. This is because different methods give different results and, consequently, suppositories may be accepted as satisfactory, although they do not melt, soften, or dissolve in the rectal environment. Furthermore, if a uniform method for determining the melting range could be developed, it would be quite useful in deciding if a suppository is sufficiently firm to be introduced into the rectum or handled and stored at ordinary room temperature or in warm environments. The melting range was determined by using the USP disintegration apparatus and a commercial disintegration apparatus[6]. Figure 1 shows the time for

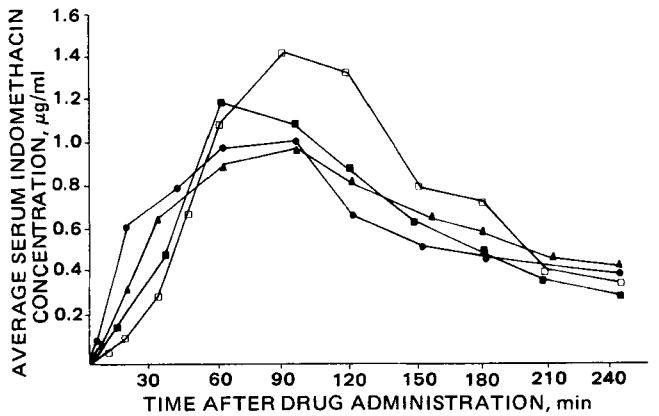

Figure 7—*Average serum indomethacin concentrations (μg/ml) obtained for three rabbits after receiving 25 mg of indomethacin given as: (■) polyethylene glycol; (▲) esterified fatty acids (C_{10}–C_{18}); (●) theobroma oil; (□) oral dose.*

[10] Aminco–Browman Spectrofluorophotometer, American Instrument Co.

[11] Prolonged exposure to daylight or delay in taking readings must be avoided because indomethacin is light sensitive.

Table II—Analysis of Variance of Plasma Concentration [a]

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-ratio
Treatments (T), (A, B, C, D)	3	0.1618	0.0539	
Rabbits/Treatment (R) (Error-T)	8	3.9365	0.4921	
Time Period, (P)	10	21.7512	2.1751	
$T \times P$ interaction	30	2.9795	0.0993	2.31[b]
$(T \times R)/D$ Error P	80	3.4433	0.0430	
Total	131	32.2723		

[a] Based on a split-strip-plot configuration (31, 32). [b] Implies that the interaction is significant ($p < 0.05$).

complete melting of indomethacin suppositories as a function of temperature. It is apparent from Fig. 1 that the polyethylene glycol 1000 indomethacin suppositories require a longer time for complete dissolution than that of esterified fatty acids (C_{10}–C_{18}) and theobroma oil in the USP disintegration apparatus. Figure 2 shows the time that is required to dissolve the polyethylene glycol 1000 indomethacin suppository as a function of temperature in the commercial disintegration apparatus[6]. This apparatus can be used for bases having densities >1 g/cm^{-3}. Polyethylene glycol 1000 can be measured in the apparatus but esterified fatty acids (C_{10}–C_{18}) (0.97 g/cm^{-3}) and theobroma oil (0.86 g/cm^{-3}) cannot.

The breaking load of the indomethacin suppositories in the three bases as a function of temperature is shown in Fig. 3. As can be seen from these curves, it is possible to determine the degree of deformation. A steep curve (esterified fatty acids, C_{10}–C_{18}) indicates a brittle base, whereas a flat curve (theobroma oil) indicates greater elasticity of the suppository base and a longer softening interval. A large number of methods are available for determination of the release of medicament from suppository bases (23–30); however, to our knowledge none have been proven to be totally satisfactory in correlating *in vitro* release with *in vivo* bioavailability. The release of indomethacin from the three suppository bases was determined by using three methods: two existing methods and a new, modified method.

Using the USP dissolution apparatus, the percent of indomethacin released is shown in Fig. 4. The data indicate that the time required to release the total amount of indomethacin from the polyethylene glycol 1000 suppository base was ~10 times less than that of the other two bases due to the water solubility of polyethylene glycol 1000 base. Therefore, the USP dissolution apparatus cannot be used for the determination of the medicament release from water soluble suppository bases without the use of membranes or filters. The inability of the USP dissolution method to be used with water soluble bases led to the use of membranes to hinder the erosion process.

The plot of indomethacin released using Method B is shown in Fig. 5. The data indicate that in the first hour the order of release was theobroma oil > esterified fatty acids (C_{10}–C_{18}) > polyethylene glycol 1000, but after the first hour, the order changed to polyethylene glycol 1000 > esterified fatty acids (C_{10}–C_{18}) > theobroma oil. The use of cellophane membranes and the dialysis technique, Methods B and C (Fig. 6), may represent an additional slow step in the overall release of indomethacin from suppositories, which may prevent a comparison of the methods. Therefore, an investigation is under way to determine and calculate the possible lag time in the methods.

***In Vivo* Studies**—The plasma indomethacin concentration–time relationshihps obtained for three rabbits after administration of single

Table III—Analysis of Variance for Each Time Period

Time Period, min	Levene Test[a]	ANOVA[b] Test
0	no test	no test
5	NS[c]	NS
12–15	NS	—[d]
30–40	NS	NS
60	NS	NS
90–95	NS	NS
120	NS	NS
145–155	NS	NS
180	NS	NS
205–210	NS	NS
240	NS	NS

[a] Levene Test is used for the comparison of variabilities. [b] ANOVA: Analysis of variance. [c] NS: Not significant at $p = 0.05$. [d] Highly significant ($p < 0.01$).

Table IV—Analysis of Variance For the 12–15-Min Time Period

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-Ratio
Treatments	3	0.6134	0.2045	8.127[a]
Within Treatments	8	0.2013	0.0252	
Total	11	0.8146		

[a] Highly significant ($p < 0.01$).

25-mg doses of indomethacin are shown in Table I and Fig. 7. As can be seen from the data, the peak levels obtained were 1.35 μg/ml for Treatment A, 1.12 μg/ml for Treatment B, 1.17 μg/ml for Treatment C, and 1.64 μg/ml for Treatment D. Furthermore, the average peak of indomethacin concentration obtained after rectal administration of 25 mg of indomethacin was 82.31, 68.29, and 71.34% for Treatments A, B, and C, respectively, of that when given orally.

The absorption of indomethacin from all suppository bases, as well as from those administered orally, was rapid, as reflected by the relatively short time to attain the peak plasma indomethacin level (Fig. 7 and Table I). The time of occurrence of the maximum peak level was considerably less after Treatment A (60 min) than that after Treatments B, C, and D (90 min).

The area under the plasma concentration–time curve (AUC) was calculated by the trapezoidal rule. The values of AUC measurements, as well as the average of peaks of individual serum concentration–time curves and the time of peak value of individual serum concentration–time curves, are given in Table I.

Statistical Analysis—The mean plasma concentration of indomethacin associated with polyethylene glycol 1000, esterified fatty acids (C_{10}–C_{18}), theobroma oil, and suspension was statistically compared, based on three rabbits per each treatment and 11 sampling time periods. The ANOVA for a split-strip-plot (31–32) design suitable for time-dependent responses was conducted and the results presented in Table II.

The ANOVA indicated that there is an interaction between treatment and time periods. The interpretation of the significant interaction indicates that there exists one or more time periods in which the mean plasma concentration of the treatments are significantly different. This information led to the second set of analyses in which an ANOVA was conducted for each time period to identify the time period in which the treatments were significantly different. These results are provided in Table III. Some of the collection times in Table III show a range of time periods and not a specific collection time, because the ANOVA is used to identify the time period in which the treatments are significantly different. Therefore, the exact collection time should be used. The 12–15 min time period indicates that some blood samples at the third collection time were collected at 12 min, while others at 15 min. Specifically, the collection time for the indomethacin–polyethylene glycol 1000 suppositories was 12 min, while for the other treatments it was 15 min.

It can be seen that there is a significant difference among the treatments in the 12–15-min time period. This is the most critical information of the experiment. The ANOVA for this period is given in Table IV. A Duncan's Multiple Range Test was conducted, and the results (Table V) revealed that Treatment C ($\bar{C} = 0.70$) was significantly higher) ($p > 0.05$) than that of Treatment A ($A = 0.18$) and Treatment D ($\bar{D} = 0.12$). There was no significant difference between Treatments B and C. As time increased, the variability of the plasma concentration increased, and thus, no significant differences among the treatments were detected (31, 32).

The present results indicate that the indomethacin is well absorbed from the rectum, although the peak serum concentration and AUC were less than after an equivalent oral dose, which is in accordance with previous experiments (17).

Table V—Analysis of Variance According To Duncan's Multiple Range Test

	Significance Triangle			
	D	A	B	C
0.6967 C	—[a]	—[a]	0[b]	
0.400 B	0			
0.1800 A	0			
0.1200 D				

[a] Implies significant difference between C and D and between C and A ($p < 0.05$). [b] 0 implies no significant difference at $p = 0.05$.

An attempt to correlate the *in vivo* with the *in vitro* release of the indomethacin proved to be unsatisfactory because of the wide variance in the results from three *in vivo* treatments and three dissolution methods. However, the dialyzing tubing method gave the best correlation with the *in vivo* data in three suppository bases, whereas the USP dissolution method gave the best correlation only with the use of fatty suppository bases (esterified fatty acids (C_{10}–C_{18}) and theobroma oil). Attempts to use a sequential order correlation between the three dissolution methods and the *in vivo* results show that the best correlation was found during the first 45 min of the experiment.

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NOTES

High-Performance Liquid Chromatographic Determination of Proglumide in Plasma

TERUYOSHI MARUNAKA ˣ, YOSHINORI MINAMI, YUKIHIKO UMENO, and EIJI MATSUSHIMA

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Abstract □ A rapid and sensitive method for determining the anticholinergic agent, proglumide, in plasma by high-performance liquid chromatography is described. Samples were acidified with hydrochloric acid and extracted with chloroform. The dried extract was resolved in chloroform and chromatographed on an adsorption chromatographic column using a mobile phase of chloroform–methanol (24:1) on a high-performance liquid chromatograph equipped with a UV absorbance detector (240 nm). The detection limit for proglumide was 0.05 μg/ml.

Keyphrases □ Proglumide—high-performance liquid chromatographic determination in plasma □ High-performance liquid chromatography—analysis, proglumide in plasma □ Anticholinergic agent—proglumide, high-performance liquid chromatographic determination in plasma

Proglumide (xylamide, DL-4-benzamido-*N*,*N*-dipropylglutaramic acid) is a derivative of the amino acid that was developed as an anticholinergic agent (1–7). The GLC methods have been reported for the assay of proglumide in plasma (8–10). Proglumide has been assayed for its methyl derivative (8, 9), and its trimethylsilyl derivative determined (10).

The present report describes a rapid, precise, and sen-

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