pressure were statistically significant but physiologically inconsequential.

Oxybutynin produced apparently dose-related tachycardia. A significant peak tachycardic response (21 beats/min) appeared after the 0.3-mg/kg dose, and a significant peak (52 beats/min) occurred after the 3.0-mg/kg iv dose. Although there was a consistent tachypnea after each oxybutynin dose, none of the respiratory changes was significant.

The effects of the cumulative 4.4-mg/kg iv oxybutynin dose on the autonomic responses in conscious dogs are summarized in Table II. Oxybutynin produced several significant autonomic responses. The tyramine-induced pressor response was significantly enhanced. The tyramine-evoked tachycardic response was significantly depressed to +3.2 beats/min. The acetylcholine-induced depressor and associated tachycardic responses were significantly inhibited. The epinephrine pressor response amplitude was significantly enhanced by oxybutynin treatment, although the associated heart rate response was inhibited by from +11 to −1 beats/min. Other significant changes included enhanced histamine tachycardia, increased norepinephrine brachycardia, and increased diastolic depressor response to isoproterenol.

When oxybutynin was administered to anesthetized dogs, the systolic and diastolic pressure responses were opposite to those observed in the conscious state (Table III). Oxybutynin (1.0, 3.0, and 10.0 mg/kg iv) produced systolic/diastolic depressor responses of −8/−5, −30/−26, and −31/−37 mm Hg, respectively. The associated significant tachycardic responses occurred only after the 1.0- and 3.0-mg/kg doses. The 10.0-mg/kg oxybutynin dose produced profound and significant depressor responses but insignificantly increased heart rate. Perhaps at this dose in the anesthetized dog the heart rate response, which appears to be compensatory to the systolic/diastolic depression, is pharmacologically antagonized by oxybutynin. The possibility of a toxicologic response to the 10.0-mg/kg dose is supported by the appearance of a marked and statistically significant tachypnea (+17 breaths/min).

Table IV describes the effects of a total cumulative 14-mg/kg iv oxybutynin dose of the autonomic treatment responses of anesthetized dogs. The acetylcholine-induced arterial pressure depressor response was inhibited. Similarly, the acetylcholine-induced tachycardia was markedly inhibited following oxybutynin. The epinephrine pressor response was enhanced, as was the heart rate response. The systolic/diastolic pressures and heart rate response to norepinephrine were also significantly altered. The respiratory rate response to norepinephrine was slightly, but significantly, increased. Only the diastolic pressure response to isoproterenol was significantly increased following oxybutynin.

Divergent responses due to the "state-of-the-animal" were reported previously (1–3). The influence of differential state on response must be considered when interpreting the experimental data, particularly from the anesthetized preparation.

The differences in systolic/diastolic arterial pressures, heart rates, and respiration rates are summarized in Table V. Basal heart rate was considerably lower in the conscious state, while basal respiratory rate was significantly greater. The amplitude of the changes indicated that the conscious animal exhibits greater oxybutynin sensitivity and response intensity. Greater oxybutynin tachycardic response in conscious animals can be explained on the basis of the Law of Initial Values (8).

In the conscious dog, the oxybutynin response was pressor; following pentobarbital anesthesia, the same oxybutynin doses evoked a systolic/diastolic arterial depressor response. These findings are inexplicable.

The consistent and significant tachycardia produced by oxybutynin in conscious and anesthetized dogs and the selective oxybutynin inhibition of the acetylcholine-induced cardiovascular responses in both conditions support the earlier pharmacological report. Oxybutynin has mild to moderate cardiovascular effects, probably through an anticholinergic mechanism of action.

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Amino Acid Effect on Aspirin Stability in Propylene Glycol

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Received July 6, 1978, from the *School of Pharmacy, West Virginia University, Morgantown, WV 26506.* Accepted for publication November 6, 1978. *Present address: School of Pharmacy, University of Maryland, Baltimore, MD 21201.

Abstract □ Temperature stability studies were conducted on 0.36 *M* (6.5% w/v) aspirin solutions including either 0.02 *M* L-methionine or 0.02 *M* histidine in propylene glycol. Aspirin was determined spectrophotofluorometrically as salicylic acid content at 412 nm. A 0.36 *M* aspirin in polyethylene glycol 400 solution was studied concurrently. Aspirin degradation rate constants, *k*, obtained from semilogarithmic plots of percent drug remaining *versus* time at 30–70 ± 0.5° were used for preparing Arrhenius plots. Good correlation was seen between predicted aspirin stability and experimental $k_{25°}$ values. L-Methionine and histidine markedly reduced aspirin stability.

Keyphrases □ Aspirin—stability in propylene glycol, effect of L-methionine and histidine, temperature □ Stability—aspirin in propylene glycol, effect of L-methionine and histidine, temperature □ Propylene glycol—stability of aspirin solutions, effect of L-methionine and histidine, temperature

Aspirin remains the most sought-after, nonprescription analgesic. It possesses a combination of anti-inflammatory, antipyretic, and analgesic properties unparalleled by other "aspirin-like" compounds. Aspirin is, however, a notoriously unstable drug and degrades to the less potent salicylic acid in the presence of moisture (1). Liquid aspirin dosage forms present a challenging pharmaceutical problem because of this instability in water and in vehicles containing traces of water.

Numerous attempts to stabilize aspirin solutions, especially in nonaqueous solvents such as polyethylene glycol 400 (2), glycerol and propylene glycol (3, 4), ethanol, (5), and esterified polyethylene glycols (6) have been reported. The objective of the present study was to measure

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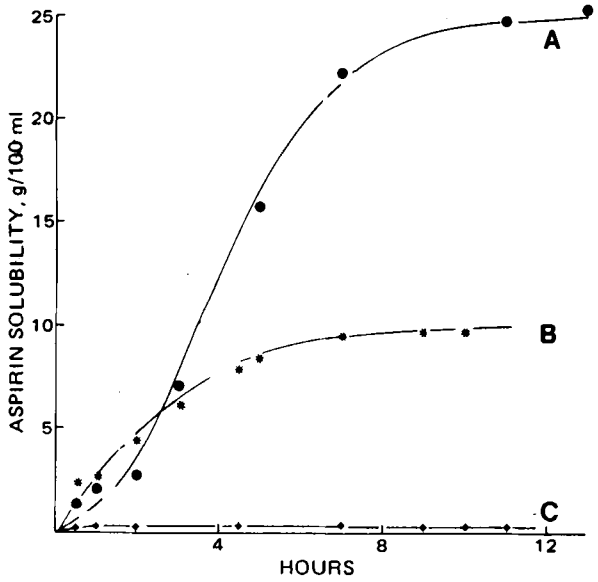

Figure 1—*Aspirin dissolution rates in polyethylene glycol 400 (A), propylene glycol (B), and water (C) by mechanical rotary agitation at 25 ± 0.1°.*

Table I—Aspirin Formulations I–IV

Formulation	Aspirin, 6.5% w/v (0.36 *M*)	Histidine, 0.32% w/v (0.02 *M*)	L-Methionine, 0.30% w/v (0.02 *M*)	Propylene Glycol	Polyethylene Glycol 400
I	+			+	
II	+		+	+	
III	+	+		+	
IV	+				+

aspirin stability in propylene glycol and in the presence of several amino acids. L-Methionine and histidine were chosen since they were slightly soluble in this solvent; furthermore, past reports indicated that histidine pro-

Table II—Predicted Stability of Aspirin Formulations I–IV for $t_{10\%}$ at 25 and 5°

Formulation[a]	$t_{10\%}$ 25°	$t_{10\%}$ 5°
I	151.2 days	55.8 months
II	75.6 days	20.4 months
III	55 days	13.3 months
IV	18.8 days	2.88 months

[a] See Table I for formulation contents.

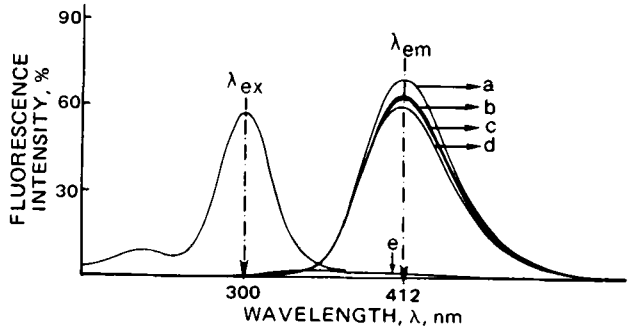

Figure 2—*Effect of 3.6 × 10^{-4} M aspirin solution and 3.6 × 10^{-4} M salicylic acid solution in pH 7 phosphate buffer on fluorescence intensity. Key: a, 11 μl of salicylic acid solution plus 40 μl of aspirin solution; b, 10 μl of salicylic acid solution; c, 10 μl of salicylic acid solution plus 10 μl of aspirin solution; d, 10 μl of salicylic acid solution plus 20 μl of aspirin solution; and e, 20 μl of aspirin solution.*

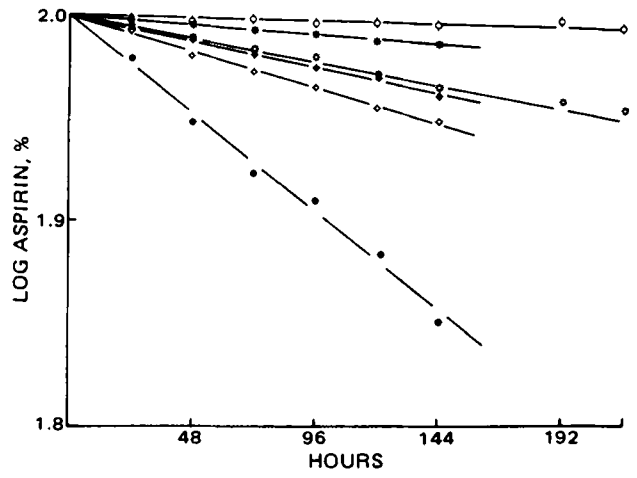

Figure 3—*Percent aspirin remaining as a function of time and temperature for I. Key: ◇, 30°; ✱, 40°; ☼, 50°; ◆, 55°; ◈, 60°; and ●, 70°.*

duced remedial effects in the treatment of peptic ulcers (7) and that methionine effected favorable histological changes in parenchymal cells of the liver (8).

EXPERIMENTAL

Materials—Aspirin[1], histidine[2], L-methionine[3], propylene glycol[2], polyethylene glycol 400[4], and salicylic acid[2] were used as received. Methanol[2] was spectroscopic grade.

Aspirin Formulations—Aspirin and the amino acids were dissolved in the glycol solvents by mechanical agitation for 4–6 hr on a rotary shaker[5] at ambient room temperatures. All aspirin formulations con-

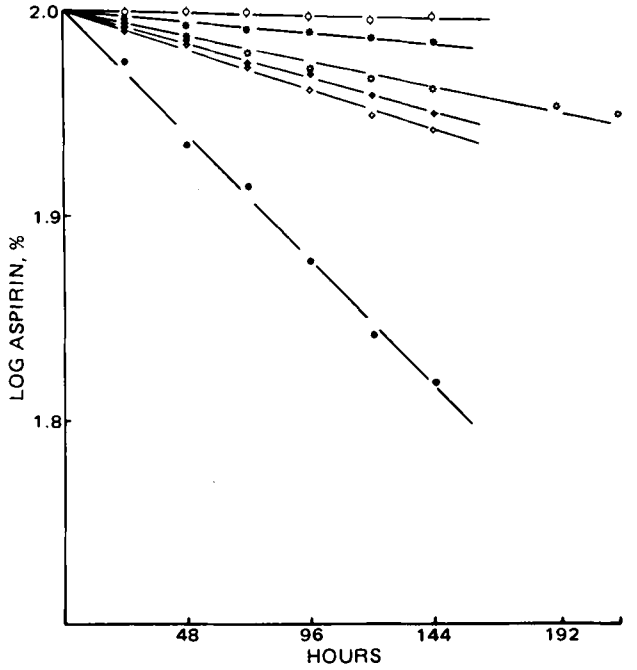

Figure 4—*Percent aspirin remaining as a function of time and temperature for II. Key: ◇, 30°; ✱, 40°; ☼, 50°; ◆, 55°; ◈, 60°; and ●, 70°.*

[1] Amend Drugs and Chemicals, Irvington, N.J.
[2] Fisher Scientific Co., Fair Lawn, N.J.
[3] Mann Research Laboratories, New York, N.Y.
[4] Ruger Chemical Co., Irvington, N.J.
[5] Gyrotory shaker, model S3, New Brunswick Scientific Co., New Brunswick, N.J.

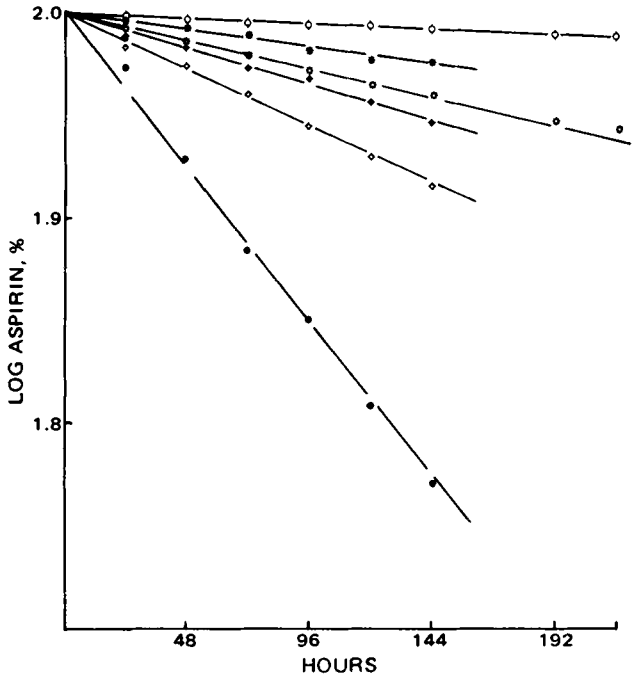

Figure 5—*Percent aspirin remaining as a function of time and temperature for III. Key: ○, 30°; ✱, 40°; ☼, 50°; ◆, 55°; ⊖, 60°; and ●, 70°.*

tained 0.36 *M* (6.5% w/v) aspirin and 0.02 *M* of either amino acid (II or III). A 0.36 *M* aspirin in polyethylene glycol 400 (IV) solution was included for comparison (Table I).

Aspirin Content Analysis—Aspirin was assayed as salicylic acid in pH 7 phosphate buffer at 25 ± 0.1°, using a spectrophotofluorometer[6] equipped with a 150-w xenon lamp/LP 21 photomultiplier tube, at the excitation and emission wavelengths of 300 and 412 nm, respectively. A 1.3 conversion factor, representing the aspirin to salicylic acid molecular weight ratio, was used to compute aspirin concentrations from salicylic acid concentrations. Salicylic acid concentrations were determined from

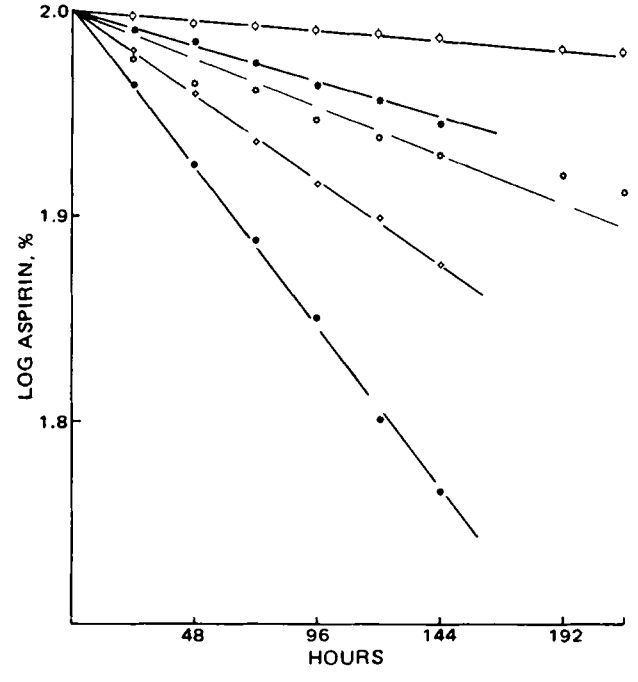

Figure 6—*Percent aspirin remaining as a function of time and temperature for IV. Key: ○, 30°; ✱, 40°; ☼, 50°; ⊖, 60°; and ●, 70°.*

[6] Aminco-Bowman, American Instrument Co., Silver Spring, Md.

Table III—Effect of Water Dilution on pH of Formulations I–III

Formulation	Percent Water (v/v) 0	10	25	50	70
I	3.1	3.1	3.1	3.0	3.0
II	3.8	3.8	3.7	3.3	3.0
III	3.9	3.9	3.8	3.5	3.1

a standard plot of salicylic acid, prepared from a fresh 0.36 *M* salicylic acid stock solution in glycol or methanol and appropriately diluted with pH 7 phosphate buffer for the fluorescence measurements.

Stability Studies—The aspirin formulations were placed in screw-capped, 30-ml, amber-colored prescription bottles and were immersed in controlled temperature water baths at 30–70 ± 0.5°. At 24-hr intervals, analytical samples were pipetted into ice-cold tubes, sealed, and stored at refrigeration temperatures until analysis. Semilogarithmic plots of percent aspirin remaining as a function of time and temperature were used to obtain the degradation rate constants, *k*. Arrhenius plots based on these rate constants were derived to predict aspirin stability at 25 and 5°. These predictions were compared with the experimental values from samples left at ambient room temperatures (25 ± 2°).

RESULTS AND DISCUSSION

Complete solubility of 6.5% (w/v) aspirin in propylene glycol or polyethylene glycol 400 was attainable within 6–8 hr of continuous agitation at 40 oscillations/min on a shaker bath maintained at 25 ± 0.1° (Fig. 1). This concentration represents the average adult dose (~325 mg/teaspoonful). Figure 2 shows the relative salicylic acid fluorescence intensity as a function of wavelength. Aspirin exerted negligible fluorescence at 412 nm.

Figures 3–6 show the semilogarithmic plots of percent aspirin remaining as a function of time and temperature for I–IV. The degradation rate constants, *k*, derived from these plots show that IV was the least stable (Fig. 7). This result may be attributed to transesterification (6) between aspirin and the polyethylene glycols. Formulation II ($k = 0.54 \times 10^{-4}$ hr^{-1}) exhibited almost twice the I degradation rate, indicating that amino acids decrease aspirin stability in propylene glycol. Table II summarizes the predicted shelflife stability at 25 and 5° for I–IV in terms of a 10% aspirin breakdown. As anticipated, the shortened shelflife of aspirin in propylene glycol due to the presence of the amino acids cor-

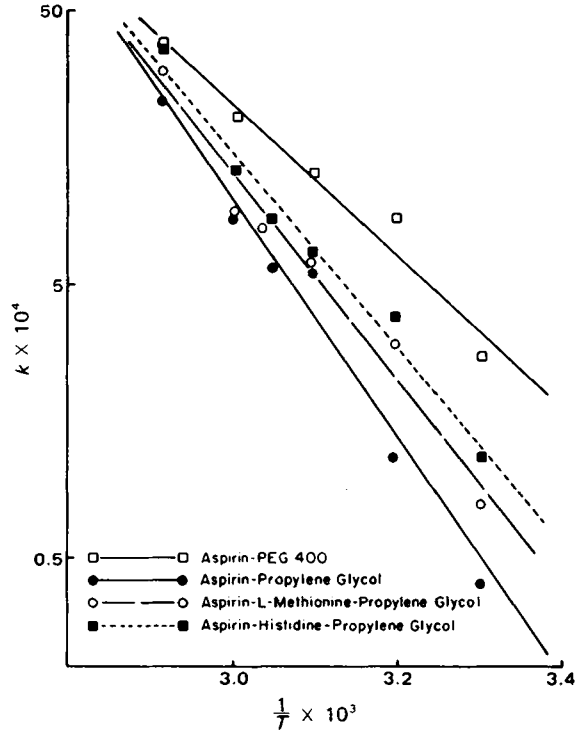

Figure 7—*Arrhenius plot showing aspirin degradation rate constants, k, in I–IV exposed to 30–70 ± 0.5°.*

Table IV—Predicted (Extrapolated) and Experimental (Actual) Degradation Rate Constants at 25° for Formulations I–IV

Formulation	Degradation Rate Constants, $k \times 10^{-4}$	
	Experimental[a]	Predicted
I	0.28	0.29
II	0.54	0.58
III	0.72	0.79
IV	3.49	2.33

[a] Ambient room temperature, 25 ± 2°.

responded with the slight increases in pH on dilution with water (Table III).

A fair correlation was evident between the predicted and experimental degradation rate constants, $k_{25°}$ (Table IV), thus supporting the predicted stability values (Table II). Preliminary IR studies showed a carbonyl band shift from 1595 cm^{-1} of L-methionine[7], where no bonding takes place, to 1640 cm^{-1} when the amino acid was dissolved in propylene glycol, indicating weak hydrogen bonding. Similarly, weak hydrogen bonding was indicated between aspirin and propylene glycol because of a slight carbonyl band shift to a higher frequency.

[7] Kaydol, Ruger Chemical Co., Irvington, N.J.

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Radiochemical Plasma Salicylamide Assay Using Ring-Labeled Tritiated Salicylamide

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Abstract □ A rat plasma salicylamide assay was developed using ring-labeled tritiated salicylamide, synthesized by reacting salicylamide with tritium oxide in the presence of heptafluorobutyric acid. The reaction yielded ^3H-salicylamide of specific activity up to 8.41 mCi/mmole, 60% yield. Plasma containing ^3H-salicylamide and its metabolites was extracted with a toluene-based scintillation fluid, which was subsequently counted. Specificity for free salicylamide was demonstrated by radiochemical and standard fluorescence plasma salicylamide level–time curves. Specificity resulted from nonextraction of the salicylamide sulfate and glucuronide metabolites. Sulfatase and β-glucuronidase treatment allowed the analysis of plasma sulfate and glucuronide conjugates as free salicylamide. This procedure should be effective for the analysis of salicylamide and its metabolites in the presence of similar phenolic compounds.

Keyphrases □ Salicylamide—radiochemical analysis, from plasma, compared to fluorescence assay, rats □ Radiochemistry—analysis, salicylamide in plasma, compared to fluorescence assay, rats □ Fluorometry—analysis, salicylamide in plasma, compared to radiochemical assay, rats

In a study of the effects of alternate substrates on their ability to block temporarily the first-pass metabolism (1, 2) of phenolic drugs, the oral administration of salicylamide to rats was chosen as a suitable animal model because of the similarity of salicylamide's metabolic behavior in rats (3–7) and humans (8, 9). Numerous assays for salicylamide have been proposed (9–11). The fluorometric assay of Barr and Riegelman (9) is the most extensively used procedure for the analysis of salicylamide and its metabolites in biological fluids. This sensitive and specific fluorometric assay, along with a few modifications, has been the assay of choice in numerous studies (3, 9, 12).

Since the long-term objective of this project was to screen the effects of various alternate substrates on their ability to block temporarily the sulfation–glucuronidation first-pass effect of a model phenolic compound, salicylamide, many of the alternate substrates would most likely be other phenolic compounds. Some of these phenolic alternate substrates have physical, chemical, and fluorescense properties so similar to salicylamide that they interfere with the fluorometric procedure (9). For this reason, an assay for salicylamide and its metabolites was developed using ring-labeled tritiated salicylamide. The assay selectivity was due to the separation of salicylamide from its sulfate and glucuronide metabolites by a relatively nonpolar organic solvent.

EXPERIMENTAL

Reagents and Materials—All reagents were analytical grade, and all aqueous solutions were prepared using glass-distilled, deionized water. The scintillation fluid was prepared by stirring overnight 1000 ml of toluene[1] with 0.5 g of 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene[2] and 4 g of 2,5-diphenyloxazole[2].

[1] Scintillation grade, Research Products International Corp., Grove Village, Ill.

[2] Amersham/Searle Corp., Arlington Heights, Ill.

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