Stable Nonaqueous Pentobarbital Sodium Solutions for Use in Laboratory Animals

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Abstract □ The degradation kinetics of pentobarbital sodium in propylene glycol-based solutions were studied along with the *in vivo* effects in laboratory animals. The degradation rate constant was directly proportional to the water concentration in propylene glycol–water solvent systems. An activation energy of 23.4 kcal/mole was obtained in propylene glycol–water (1:1). Pentobarbital sodium solutions in anhydrous propylene glycol and 9:1 mixtures of propylene glycol with ethanol, glycerin, or dimethylacetamide gave relatively slow degradation rates at 100°, with all projected 25° $t_{99\%}$ values greater than 4.5 years. Intravenous administration of pentobarbital sodium in various anhydrous propylene glycol-based vehicles to rats produced no hemolysis or gross organ damage that would interfere with pathological evaluations. Results of an intraperitoneal sleeptime study indicated that pentobarbital sodium produced consistent hypnotic effects when administered as an aqueous solution or in anhydrous propylene glycol-based vehicles.

Keyphrases □ Pentobarbital sodium—degradation kinetics in propylene glycol-based solutions, *in vivo* effects in laboratory animals □ Degradation kinetics—pentobarbital sodium in propylene glycol-based solutions, *in vivo* effects in laboratory animals □ Stability—pentobarbital sodium in propylene glycol-based solutions, *in vivo* effects in laboratory animals □ Hypnotics—pentobarbital sodium, degradation kinetics in propylene glycol-based solutions, *in vivo* effects in laboratory animals

Pentobarbital has been recommended frequently as a drug of choice for producing euthanasia in laboratory animals whenever its use will not interfere with experimental results (1). In the dog, the maximum lethal dose is generally considered to be about 50 mg/kg. It is also widely used intraperitoneally to induce anesthesia in small laboratory animals.

A major concern in the use of injectable pentobarbital sodium products is the chemical instability in aqueous solutions (2, 3). At the high pH level necessary to maintain pentobarbital sodium in solution, the degradation rate is fast enough to cause crystallization of the water-insoluble degradation product within several months at room temperature. The use of vehicles containing 20–60% propylene glycol in water to overcome the stability problem was reported (4, 5), but detailed kinetic and toxicological data were not included. However, the utility of propylene glycol in parenteral products is well established (6).

This paper describes studies on propylene glycol-based solutions of pentobarbital sodium intended for parenteral use in producing euthanasia or anesthesia in laboratory animals. High temperature kinetic studies established the degradation rate reduction in the propylene glycol-based vehicles. The effects of water concentration and other solvents on the degradation rate also were evaluated. Additionally, acute intravenous toxicity and intraperitoneal sleeptime studies were conducted in rats to evaluate the drug availability and pathological changes associated with propylene glycol-based formulations.

EXPERIMENTAL

Kinetic Procedure—Solutions were prepared by dissolving sufficient pentobarbital sodium in the desired solvent to give a concentration of 50 mg/ml. The solution was then divided into several 10-ml ampuls, which were sealed and placed in a mechanical convection oven[1] maintained at a constant temperature ±0.2°. Periodically, ampuls were removed and assayed for the remaining pentobarbital sodium concentration.

Approximately 15 assays were obtained in each kinetic study, with each assay obtained from a separate ampul. All data were treated statistically, using linear regression analyses to establish rate constants and confidence intervals (7).

Assay—A differential UV method, similar to that reported by Tishler *et al.* (8) for phenobarbital degradation, was used to assay the remaining pentobarbital sodium. The sample was diluted in 1.0 *N* NH_4OH to a concentration of 4–20 μg/ml of pentobarbital sodium, and a UV spectrum from 220 to 300 nm was obtained[2]. An additional sample was diluted similarly in 0.1 *N* HCl, and a UV spectrum was obtained.

The difference in acidic and basic absorbances at 240 nm was used to determine the drug concentration. A standard curve obtained with this method, using concentrations of 4, 8, 12, 16, and 20 μg/ml, showed a slope of 0.03598 with an intercept of 0.0089 and a correlation coefficient of 0.9999.

Acute Intravenous Toxicity Studies—Forty-four male Sprague–Dawley rats[3], 160–180 g (7–8 weeks old), were divided into 11 treatment groups of four each. All animals were housed individually in stainless steel hanging cages with ambient temperature maintained at 22–24°. Food[4] and water were supplied *ad libitum*.

Single 100-mg/kg doses of five pentobarbital sodium formulations (200

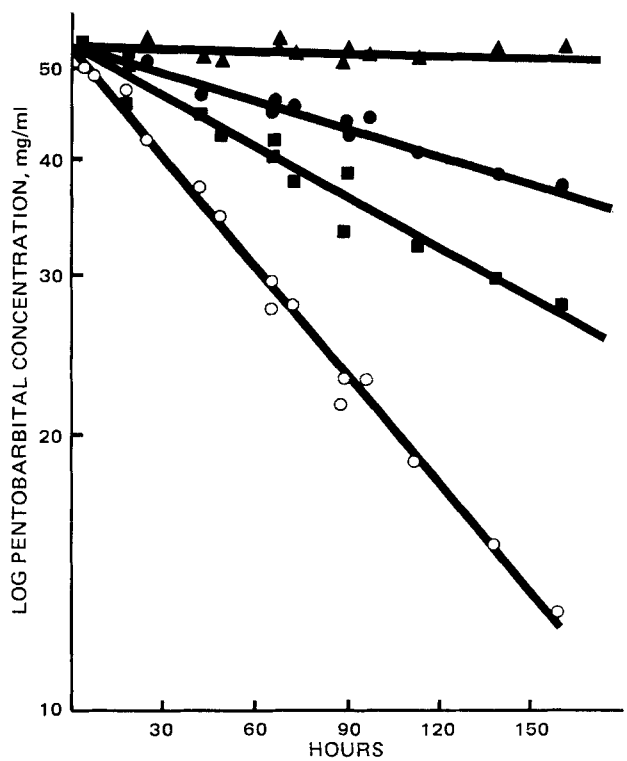

Figure 1—*Hydrolysis of pentobarbital sodium at 100° in solvents composed of varying propylene glycol–water ratios and an initial concentration of 50 mg/ml. Key: ○, 0:4; ■, 2:2; ●, 3:1; and ▲, 4:0.*

[1] Stabil-Therm, Blue M Electric Co., Blue Island, Ill.
[2] Coleman model 124, Perkin-Elmer Co., Norwalk, Conn.
[3] Sprague-Dawley Co., Madison, Wis.
[4] Purina rat chow, Ralston-Purina Co., St. Louis, Mo.

Table I—Effect of Propylene Glycol–Water Ratio on Hydrolysis Rate of Pentobarbital Sodium at 100°

Propylene Glycol–Water Ratio[a]	$k_1 \times 10^3$, hr^{-1} ± 95% Confidence Interval	$t_{1/2}$, hr
0:4	8.92 ± 0.45	78
2:2	4.07 ± 0.63	170
3:1	2.10 ± 0.54	330
4:0	0.04 ± 0.33	16,100

[a] Relationship between k_1 and percent water yields a correlation coefficient of 0.999 by linear regression analysis.

Table II—Effect of Temperature on Rate Constants Obtained from a Pentobarbital Sodium Solution in Propylene Glycol–Water (1:1)

Temperature	$k_1 \times 10^3$, hr^{-1} ± 95% Confidence Interval	$t_{1/2}$, hr
100°	4.07 ± 0.63	170
90°	1.61 ± 0.16	430
80°	0.683 ± 0.064	1016
70°	0.252 ± 0.049	2750

mg/ml), the five corresponding vehicles (0.5 ml/kg), or 100 mg/kg of a pentobarbital sodium aqueous solution (positive control) were administered to each group intravenously by the tail vein. In the drug-treated animals, euthanasia was produced by the intravenous administration of the pentobarbital sodium; these rats were evaluated for gross anatomical changes. Vehicle-treated rats were placed in a carbon dioxide chamber immediately after injection as a means of inducing euthanasia for evaluation of pathological changes associated with vehicle treatment. Immediately after death, all animals were necropsied; observations were made for gross pathological changes in the heart, liver, lungs, kidneys, spleen, and brain.

Intraperitoneal Sleeptime Studies—Eighty-five male Sprague–Dawley rats[3], 160–180 g (7–8 weeks old), were divided into six groups of 10 rats and five groups of five rats each. Pentobarbital sodium doses of 25 mg/kg in five different formulations and an aqueous solution (200 mg/ml) were administered to each group of 10 rats by the intraperitoneal route. The corresponding formulation vehicles without drug were administered to the five remaining groups of five rats using volumes equivalent to those used for the drug-treated rats (0.125 ml/kg). Sleeptime was defined as the time interval from loss to restoration of the righting reflex. The righting reflex was considered recovered when the animal erected itself three times within 30 sec.

RESULTS AND DISCUSSION

Degradation Kinetics—In all cases, the degradation of pentobarbital sodium followed first-order kinetics with the logarithm of drug concentration remaining linear with time. Garrett *et al.* (3) demonstrated first-order kinetics in hydrolysis studies with barbiturates, including pentobarbital, under neutral and alkaline conditions. Since the rate constant is independent of the initial concentration in first-order kinetics, the values obtained using 50 mg/ml (in most experiments) would be applicable to any other pentobarbital sodium concentration.

Table III—Degradation Rate Constants for Pentobarbital Sodium at 100° in Nonaqueous Solvent Systems

Solvent[a]	$k_1 \times 10^4$, hr^{-1} ± 95% Confidence Interval	Maximum $k_1 \times 10^4$ hr^{-1}	Minimum Projected $t_{99\%}$ at 25°, years[b]
100% Propylene glycol	0.43 ± 3.29	3.72	8.84
10% Ethanol in propylene glycol	3.69 ± 1.14	4.83	6.81
10% Glycerin in propylene glycol	5.67 ± 1.36	7.03	4.68
10% Dimethylacetamide in propylene glycol	3.40 ± 0.55	3.95	8.32

[a] Percentage refers to grams per 100 ml of solution. [b] Time for 1% degradation calculated from maximum rate constant at 100° and energy of activation of 23.4 kcal/mole.

Table IV—Stability of Nonaqueous Pentobarbital Sodium Solutions in 100-ml Injectable Vials[a] Maintained at 40°

Solvent System	Pentobarbital Sodium Concentration[b], mg/ml at Initial	3 Months	6 Months	9 Months	19 Months
Propylene glycol	206.1	205.1	203.9	204.7	201.4
10% Dimethylacetamide in propylene glycol	207.4	205.9	210.5	205.3	205.2
20% Dimethylacetamide in propylene glycol	207.0	199.8	200.6	201.1	209.0
10% Ethanol in propylene glycol	211.2	208.2	210.1	204.9	205.1
10% Glycerin in propylene glycol	205.0	206.9	207.0	202.4	201.4

[a] Type 2 glass and butyl rubber closures. [b] All assay values for stability samples are means from two vials, one maintained in an upright position and the other inverted. Initial values are from a single assay.

Propylene Glycol–Water Ratio—Kinetic experiments were run at 100° using various ratios of propylene glycol–water as the solvent to assess the magnitude of the degradation rate reduction with increasing proportions of propylene glycol (Fig. 1 and Table I). The data clearly show the decrease in the hydrolysis rate constant with an increased percentage of propylene glycol in the solvent. In fact, the rate constant appeared to be approximately proportional to the water concentration in the solvent, a condition consistent with a consideration of water as a first-order reactant in the hydrolysis. In anhydrous propylene glycol, the calculated rate constant was extremely low, with the statistical maximum still less than one-fifth of the rate constant in 75% propylene glycol.

Temperature Effect—Table II summarizes the kinetic results obtained at four temperatures with a 50-mg/ml pentobarbital sodium solution in propylene glycol–water (1:1). When the data were fitted to the Arrhenius relationship by plotting the logarithm of the rate constant *versus* the reciprocal of absolute temperature, excellent linearity was obtained, with a calculated activation energy of 23.4 kcal/mole (Fig. 2).

Table V—Acute Intravenous Toxicity[a] and Intraperitoneal Sleeptime in Rats

Pentobarbital Sodium Concentration, mg/ml	Solvent System[b]	Sleeptime Duration, min[c]
200	Water	45.7 ± 4.5
200	Propylene glycol	49.5 ± 6.1
0	Propylene glycol	0
200	10% Dimethylacetamide in propylene glycol	51.6 ± 8.9
0	10% Dimethylacetamide in propylene glycol	0
200	20% Dimethylacetamide in propylene glycol	51.2 ± 8.6
0	20% Dimethylacetamide in propylene glycol	0
200	10% Glycerin in propylene glycol	46.6 ± 7.2
0	10% Glycerin in propylene glycol	0
200	10% Ethanol in propylene glycol	44.0 ± 6.2
0	10% Ethanol in propylene glycol	0

[a] No gross pathology was found based on observations of four rats using 0.5 ml/kg of solution, equivalent to a pentobarbital sodium dose of 100 mg/kg. [b] Percentage refers to grams per 100 ml of solution. [c] Values represent mean ± 1 *SD* from 10 rats treated with drug-containing solutions and five rats treated with vehicles. All doses were 0.125 ml/kg, equivalent to 25 mg/kg of pentobarbital sodium, except with propylene glycol vehicle where 0.5 ml/kg was used.

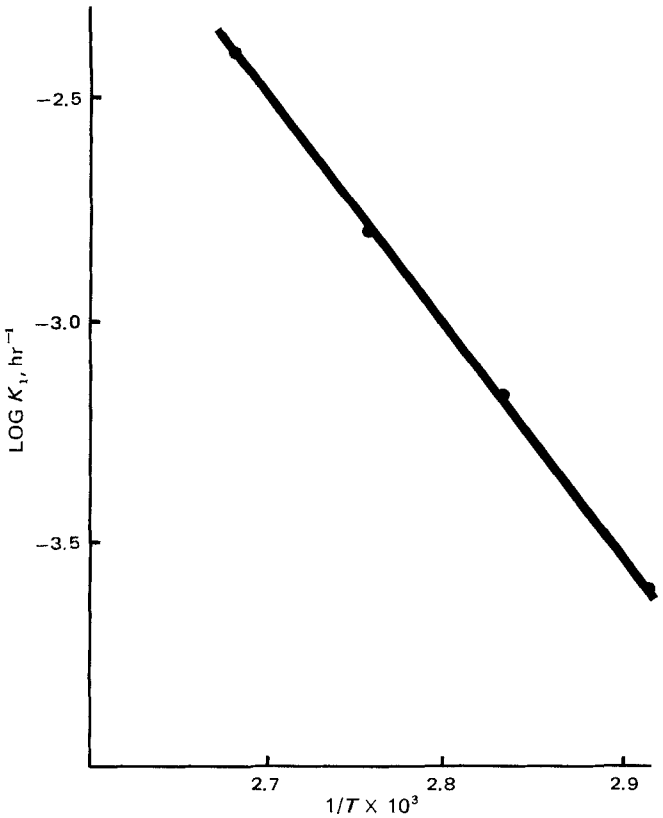

Figure 2—*Linear relationship between log* k_1 *and the reciprocal of absolute temperature in the hydrolysis of pentobarbital sodium in propylene glycol–water (1:1) using initial concentrations of 50 mg/ml. Energy of activation = 23,400 ± 1700 (p = 0.05) cal/mole; correlation coefficient = 0.9997.*

Extrapolation of this relationship to 25° yielded a rate constant of 1.42×10^{-6} hr^{-1}, a value 3.49×10^{-4} times the magnitude of the rate constant at 100°. When using this extrapolated constant, the calculated time for 1% pentobarbital sodium degradation in propylene glycol–water (1:1) at 25° would be 295 days; the time for 10% degradation would be 8.49 years.

Other Solvents—Degradation rates were determined at 100° for solutions containing 50 mg/ml of pentobarbital sodium and 10% ethanol, glycerin, or dimethylacetamide in propylene glycol (Table III). The glycerin-containing solvent gave the fastest degradation rate, while the pure propylene glycol system appeared to be the most stable. However, all anhydrous formulations showed substantially more stability than the propylene glycol–water solvent systems studied.

Based on the energy of activation determined with propylene glycol–water (1:1), the listed anhydrous solvents have projected minimum $t_{99\%}$ values (time for 1% degradation) at 25° of at least 4.68 years. As a comparison, the projected 25° $t_{99\%}$ values in water and propylene glycol–water (1:1) are 0.37 and 0.81 year, respectively. Actual stability assays on formulations based on the anhydrous systems showed no significant degradation, even after 19 months at 40° (Table IV).

Acute Intravenous Toxicity—Five anhydrous propylene glycol-based formulations containing 200 mg/ml of pentobarbital sodium were tested for acute intravenous toxicity in rats along with the corresponding vehicles (Table V). The pentobarbital sodium dose was 100 mg/kg, equivalent to 0.5 ml of formulation/kg. No drug-related pathological changes occurred in the organs of rats treated intravenously with the various pentobarbital sodium solutions or their corresponding vehicles. In all cases, organs were grossly normal. Thus, it may be concluded that the use of these solutions to produce euthanasia should not interfere with pathological evaluations or interpretations in rats.

Intraperitoneal Sleeptime—Sleeptimes induced by the various pentobarbital sodium solutions varied from 44.0 to 51.6 min (Table V). The values were not statistically different. The lack of statistically significant differences in the mean sleeptime durations indicated that the use of anhydrous propylene glycol-based vehicles did *not* affect pentobarbital sodium bioavailability. None of the vehicles (without drug) produced hypnotic effects in rats.

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