Thiamine Whole Blood and Urinary Pharmacokinetics in Rats: Urethan-Induced Dose-Dependent Pharmacokinetics

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Abstract □ The whole blood pharmacokinetics of thiamine after intravenous administration of thiamine hydrochloride (4, 12, and 36 mg/kg) to rats anesthetized continuously with ether (inhalation) or urethan (1 g/kg ip) were studied. Urinary excretion of thiamine after intravenous administration of thiamine hydrochloride to rats lightly anesthetized with ether was also investigated. At any particular dose, thiamine displayed apparent classical two-compartment model behavior in the time range studied. Under urethan anesthesia, thiamine displayed apparent dose-dependent kinetics as measured by the changes in the pharmacokinetic parameters, AUC, $V_{d(area)}$, $t_{0.5\beta}$, and total body clearance, Cl_{TB}, with dose. However, when ether anesthesia was used, thiamine displayed dose-independent pharmacokinetic behavior. These results suggest that care should be taken in the interpretation of pharmacokinetic data obtained in anesthetized animals, particularly when urethan anesthesia is used.

Keyphrases □ Thiamine—whole blood and urinary pharmacokinetics after urethan and ether administration, rats □ Urethan—effect on whole blood and urinary pharmacokinetics of thiamine □ Anesthetic agents—urethan and ether, effect on whole blood and urinary pharmacokinetics of thiamine, rats □ Pharmacokinetics—of thiamine, effect of urethan and ether administration to rats

Obtaining reliable and accurate blood concentration *versus* time data is crucial in determining a drug's true disposition in any animal species. To minimize pain and discomfort to laboratory animals and provide convenience to the researcher, experiments are often carried out under anesthesia. Reported are some observations on the apparent effects of urethan anesthesia on the pharmacokinetics of thiamine in rats.

Urethan is used as an anesthetic agent for laboratory and agricultural animals. Administered intraperitoneally, it produces a long-lasting narcosis and therefore is used primarily for prolonged, terminal experiments. As opposed to barbituates, urethan has a wide margin of safety and gives a more stable, longer anesthesia (1). Thiamine pharmacokinetics at a dose of 4 mg/kg iv in rats under urethan anesthesia were reported previously (2–4). The present study examined the dose-independent kinetics in unchanged thiamine urinary excretion and the pharmacokinetics of thiamine in whole blood in lightly ether-anesthetized rats. The apparent dose dependency of thiamine pharmacokinetics under urethan anesthesia was also studied.

EXPERIMENTAL

Materials and Methods—Male Sprague-Dawley rats[1], 230–400 g, were used. Prior to intravenous thiamine hydrochloride[2] administration *via* the dorsal penis vein (5) at three dose levels (4, 12, and 36 mg/kg), the rats received either urethan or ether as the anesthetic agent. Urethan[2] was administered intraperitoneally as a 500-mg/ml solution at a dose of 1.0 g/kg; however, some rats required additional urethan, the total not exceeding 2.0 g/kg, to induce continuous anesthesia during the experiment. Ether[3] was administered by inhalation using two different methods. The first method, called light ether anesthesia, involved initially anesthetizing the animal sufficiently to permit the injection and removing the animal to a restraining cage for the remainder of the blood sampling phase. Ether was not administered at any other point in this experiment. The second method used, the "open technique" (6), involved continuous ether; *i.e.*, after initially being anesthetized, the animal was kept unconscious by a constant supply of ether vapors. Forced air was conducted over liquid ether to an open funnel maintained over the nose and mouth of the rat for the remainder of the experiment. Since hypothermia may accompany general anesthesia, each rat's body temperature was monitored by a rectal thermistor probe and the animal was maintained at 37 ± 1° by means of a thermostatically-controlled, heated surgical table. After dosing with thiamine hydrochloride, a total of 10 100-μl blood samples were collected from the tail vein. Free thiamine (nonphosphorylated) in whole blood was determined as previously described (3).

In rats where urinary thiamine was determined, light ether anesthesia was used. Subsequently, the rats were placed in metabolism cages. Urine was collected from the rats at set time intervals after dosing and the free thiamine in urine was determined by standard methods (7–10). The turnover of endogenous thiamine in urine, *i.e.*, urinary excretion of thiamine under conditions of normal dietary intake, was determined in rats over the 24-hr interval prior to intravenous administration of exogenous thiamine hydrochloride. In other words, the rats were placed in individual metabolism cages 24 hr prior to exogenous thiamine administration and urine collected during this period was assayed for thiamine. The amount of thiamine excreted in this 24-hr period for each animal was assumed to be the turnover of endogenous thiamine.

Calculations—The time course of thiamine in blood displayed apparent two-compartment model behavior (Figs. 1–3). Individual rat blood concentration–time profiles were fit to Eq. 1 using two programs, a simplex optimization of the parameters[4] (11, 12), and the NONLIN program (13).

$$C_B = Ae^{-\alpha t} + Be^{-\beta t} \qquad \text{(Eq. 1)}$$

where C_B is the blood levels in μg/ml and A, B, α, and β are standard symbols used to describe classical two-compartment behavior.

The significance of differences between mean pharmacokinetic parameters for each dose was evaluated by a one-tailed t-test, single clas-

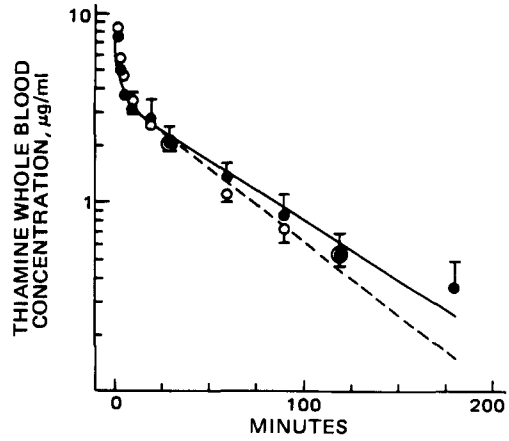

Figure 1—*Thiamine blood levels after treatment with thiamine hydrochloride (4 mg/kg iv) in rats under urethan (○) and light ether anesthesia (●). Concentration of thiamine is expressed as thiamine hydrochloride in micrograms per milliliter.*

[1] ARS/Sprague-Dawley, Madison, Wis.
[2] Sigma Chemical Co., St. Louis, Mo.
[3] Mallinckrodt Chemical Works, St. Louis, Mo.

[4] The program was developed by Dr. W. White, M. Mintun, and various students in the Department of Pharmaceutical Chemistry of the University of Kansas.

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Table I—Effect of Anesthesia on the Pharmacokinetic Parameters[a] for Thiamine Disposition in Rats after Administration of Thiamine Hydrochloride, 4 mg/kg iv

Parameter	Anesthetic Agent		Statistical Significance of Difference
	Light Ether[b]	Urethan[c]	
A, μg/ml	9.6 ± 2.3	10.3 ± 1.5	
B, μg/ml	3.38 ± 0.60	3.70 ± 0.41	
α, min^{-1}	0.64 ± 0.16	0.469 ± 0.094	
β, min^{-1}	0.0168 ± 0.0016	0.0190 ± 0.0021	
$t_{0.5\beta}$, min	42.2 ± 3.5	41.1 ± 4.4	NS[d]
AUC, μg min/ml	271 ± 48	228 ± 17	NS
AUC/dose, min/ml/kg	0.068 ± 0.012	0.057 ± 0.004	NS
$V_{d(area)}$, ml/kg	1220 ± 180	1090 ± 130	NS
Cl_{TB}, ml/min/kg	17.5 ± 4.1	18.9 ± 1.6	NS

[a] Biexponential parameters determined by fitting blood concentration *versus* time data to Eq. 1 using simplex and NONLIN programs. [b] Mean ± *SE* from five animals. [c] Mean ± *SE* from 11 animals. [d] Not significantly different at $p = 0.05$.

sification Model I ANOVA, and the Student–Newman–Keuls multiple comparison test (14). The exact test applied will be discussed. The parameters calculated (Eqs. 2–5) and compared were area under the curve (AUC), volume of distribution [$V_{d(area)}$], half-life of elimination from blood ($t_{0.5\beta}$), and total body clearance (Cl_{TB}), which were calculated from individual rat blood concentration-time profiles derived by fitting Eq. 1 to the data and employing Eqs. 2–5 (15):

$$AUC_{0\to\infty} = A/\alpha + B/\beta \quad \text{(Eq. 2)}$$

$$V_{d(area)} = \frac{\text{dose}}{\beta(AUC_{0\to\infty})} \quad \text{(Eq. 3)}$$

$$t_{0.5\beta} = 0.693/\beta \quad \text{(Eq. 4)}$$

$$Cl_{TB} = \text{dose}/AUC_{0\to\infty} \quad \text{(Eq. 5)}$$

where $AUC_{0\to\infty}$ is the area under the curve, zero time to infinity.

For urine studies, the doses of thiamine on a milligram per kilogram basis were converted to absolute amounts by using the rat weight. The fraction of the exogenous thiamine dose excreted in the urine was determined by plotting the total amount of thiamine excreted in the 24-hr postdosing minus the amount of thiamine excreted from normal thiamine turnover, determined from the 24-hr predosing, *versus* the dose administered. A linear plot suggests apparent dose-independent elimination kinetics with the slope indicative of the fraction of dose excreted in the urine. Similarly, the fraction of dose excreted in the urine in any time interval may be determined. The terminal half-life for thiamine elimination was determined from a plot of the logarithm of the amount of unchanged drug remaining to be excreted *versus* time. Renal clearance was estimated by dividing the urinary excretion rate by the whole blood concentration at the midpoint of the urine collection period.

RESULTS

Figures 1, 2, and 3 compare the mean blood thiamine concentrations after urethan and light ether anesthesia where thiamine hydrochloride was administered intravenously at 4, 12, and 36 mg/kg, respectively. The curves drawn in Figs. 1–3 were generated by Eq. 1 using the mean pharmacokinetic parameters (Tables I–III) obtained by averaging the parameters determined by computer fitting individual animal blood thiamine concentration *versus* time data.

Tables I–III compare the effects of various anesthetic agents on the pharmacokinetic behavior of thiamine. In addition to the use of light ether anesthesia as a control, continuous ether administration sustaining the rats' unconsciousness throughout the entire sampling period (as was the case with the urethan anesthesia) was used for further comparison at the 36-mg/kg dose. Included in Tables I–III are the parameters A, α, B, β, and the calculated model-dependent parameters, $V_{d(area)}$ and $t_{0.5\beta}$, as well as the model-independent parameters, $AUC_{0\to\infty}$ and Cl_{TB}.

Analyses by an unpaired Student t test of these pharmacokinetic parameters indicated no significant differences between the mean parameters for light ether and urethan anesthesia after intravenous treatment with 4 mg of thiamine hydrochloride/kg. At a dose of 12 mg of thiamine hydrochloride/kg, analyses of pharmacokinetic parameters for light ether and urethan anesthesia by a Student t test indicated highly significant differences between mean $AUC_{0\to\infty}$ ($p < 0.005$), $t_{0.5\beta}$ ($p < 0.1$), and Cl_{TB} ($p < 0.005$), but no significant difference between the mean volumes of distribution.

At a dose of 36 mg of thiamine hydrochloride/kg, the pharmacokinetic parameters for urethan anesthesia were compared with both modes of ether anesthesia, light ether (ether administered only initially), and ether administered continuously. Analyses of variance indicated highly significant differences between mean $AUC_{0\to\infty}$ ($p \ll 0.001$), $t_{0.5\beta}$ ($p \ll 0.001$), and Cl_{TB} ($p \ll 0.001$). No significant difference between the mean volumes of distribution for urethan *versus* light ether anesthesia was seen. When continuous ether-treated animals were compared to those treated with light ether, a significant increase in volume of distribution and Cl_{TB} was noted, but the half-lives were unaffected by the different mode of ether administration.

As shown, urethan markedly affected all of the thiamine pharmaco-

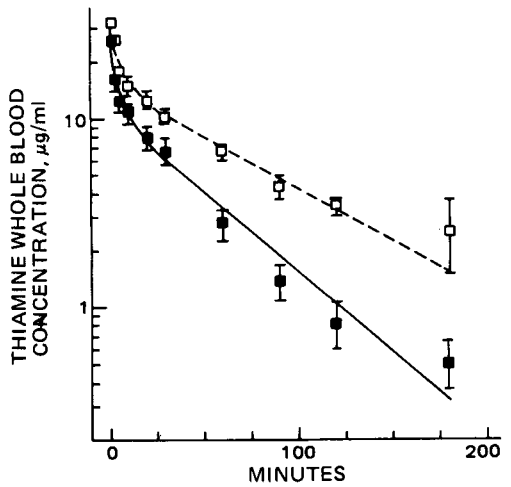

Figure 2—*Thiamine blood levels after treatment with thiamine hydrochloride (12 mg/kg iv) in rats under urethan (□) and light ether anesthesia (■). Concentration of thiamine is expressed as thiamine hydrochloride in micrograms per milliliter.*

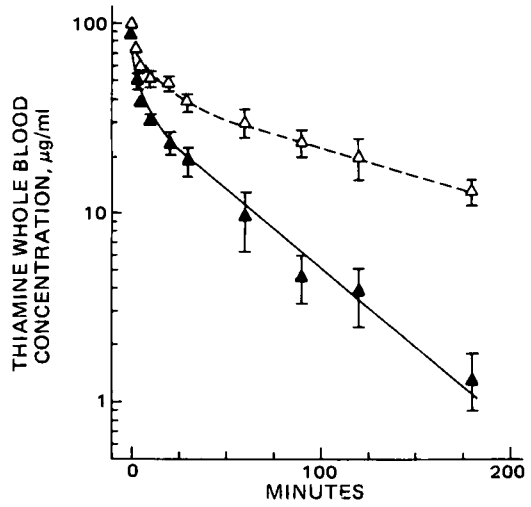

Figure 3—*Thiamine blood levels after treatment with thiamine hydrochloride (36 mg/kg iv) in rats under urethan (△) and light ether anesthesia (▲). Concentration of thiamine is expressed as thiamine hydrochloride in micrograms per milliliter.*

Table II—Effect of Anesthesia on the Pharmacokinetic Parameters[a] for Thiamine Disposition in Rats after Administration of Thiamine Hydrochloride, 12 mg/kg iv

Parameter	Anesthetic Agent		Statistical Significance of Difference
	Light Ether[b]	Urethan[c]	
A, μg/ml	33.0 ± 6.2	36.1 ± 6.1	
B, μg/ml	10.9 ± 1.3	15.1 ± 1.6	
α, min^{-1}	0.57 ± 0.13	0.57 ± 0.10	
β, min^{-1}	0.0201 ± 0.0014	0.0141 ± 0.0014	
$t_{0.5\beta}$, min	35.2 ± 2.2	51.4 ± 4.5	$p < 0.01$[d]
AUC, μg min/ml	627 ± 91	1320 ± 120	$p < 0.005$[d]
AUC/dose, min/ml/kg	0.052 ± 0.008	0.110 ± 0.010	$p < 0.05$[e]
$V_{d(area)}$, ml/kg	1020 ± 110	790 ± 82	NS[f]
Cl_{TB}, ml/min/kg	20.6 ± 2.8	9.6 ± 0.8	$p < 0.005$[d]

[a] Biexponential parameter determined by fitting blood concentration *versus* time data to Eq. 1. [b] Mean ± *SE* for five animals. [c] Mean ± *SE* for seven animals. [d] Parameters under urethan anesthesia significantly different from parameters under light ether anesthesia using one-tailed t test (14) at the level indicated. [e] Parameter under urethan anesthesia significantly different from each value for this parameter in Tables I–III using the Student–Newman–Keuls test (15). [f] Not significantly different at $p = 0.05$.

kinetic parameters (with the exception of the volume of distribution) after intravenous administration of thiamine hydrochloride at 12 and 36 mg/kg. Dose-independent pharmacokinetic parameter changes were observed under light ether anesthesia as demonstrated by comparing the $AUC_{0\to\infty}$/dose. The Student–Newman–Keuls multiple comparison test (15) applied to the mean $AUC_{0\to\infty}$/dose parameter for each anesthetic agent used and at each dose of thiamine hydrochloride, indicated no significant difference among means from light ether-anesthetized animals. This was consistent with results from the urinary excretion studies. The pattern for excretion of thiamine by the renal route is shown in Fig. 4. After taking into account the turnover of endogenous thiamine, ~75% of the exogenous thiamine dose administered was excreted unchanged in the first 2 hr and the amount of thiamine excreted unchanged in 48 hr accounted for 96% of the administered dose as shown in Fig. 5. The urinary excretion rate of thiamine in light ether-anesthetized rats closely parallels the time course of whole blood concentrations, with approximately the same terminal half-life for all the doses studied. In addition, the renal clearance closely correlated with Cl_{TB} for rats anesthetized with ether, since the primary elimination route at these doses is the renal route.

As opposed to the results for thiamine pharmacokinetics after ether anesthesia, the Student–Newman–Keuls test indicated significant differences between mean $AUC_{0\to\infty}$/dose values for urethan and ether-anesthetized animals, and also suggested that differences between the mean $AUC_{0\to\infty}$/dose values at each dose of thiamine hydrochloride for urethan-anesthetized animals were significant.

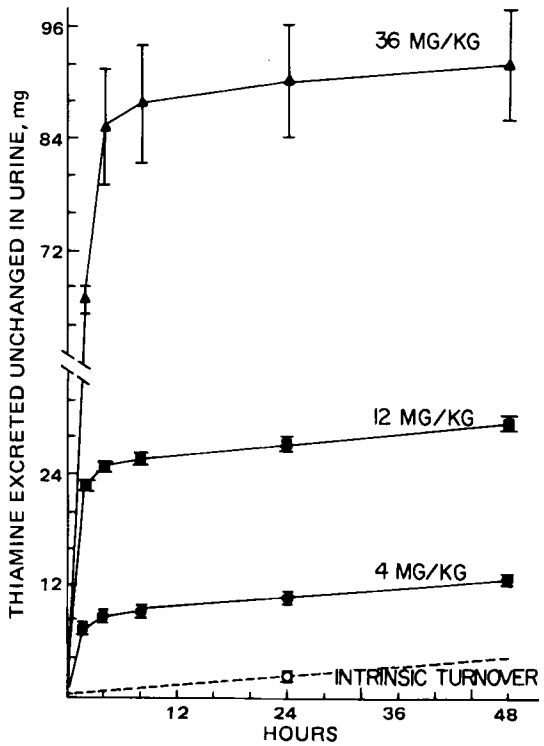

Figure 4—*Cumulative urinary excretion patterns of thiamine after administration of thiamine hydrochloride in rats at 4, 12, and 36 mg/kg iv and the intrinsic turnover of thiamine from the diet.*

DISCUSSION

In the present investigation, urethan anesthesia significantly prolonged the half-life of thiamine in rats after administration of thiamine hydrochloride at 12 and 36 mg/kg relative to the 4-mg/kg dose. Total body clearance significantly decreased, primarily due to the increased half-life (decreased β) and not due to the small changes, if any, in volumes of distribution.

Urinary excretion of unchanged thiamine is the principal elimination route of thiamine in the dose range studied. Since thiamine appears to be exclusively eliminated by the renal route, total body clearance and renal clearance are approximately equal. The total body clearance of thiamine in rats anesthetized with ether (~19 ml/min/kg) is greater than the glomerular filtration rate (10 ml/min/kg) in rats (16). This result is consistent with the known tubular secretion of thiamine and other quaternary ammonium drugs (17–19). Thiamine is also known to be reabsorbed from the renal tubules by a saturable process (19).

The mechanism of urethan alteration of thiamine elimination after thiamine hydrochloride administration is most likely very complex, since urethan changes a number of physiological parameters. It suppresses hematopoiesis and is hepatotoxic (20). Urethan also causes acute changes in blood and blood vessels, hemolysis, and red blood cell breakdown (1). Hemodynamic alterations caused by urethan are slow to occur, and other factors may alter or accelerate these hemodynamic effects (21). It was shown (22) that urethan in anesthetic doses increases the hematocrit,

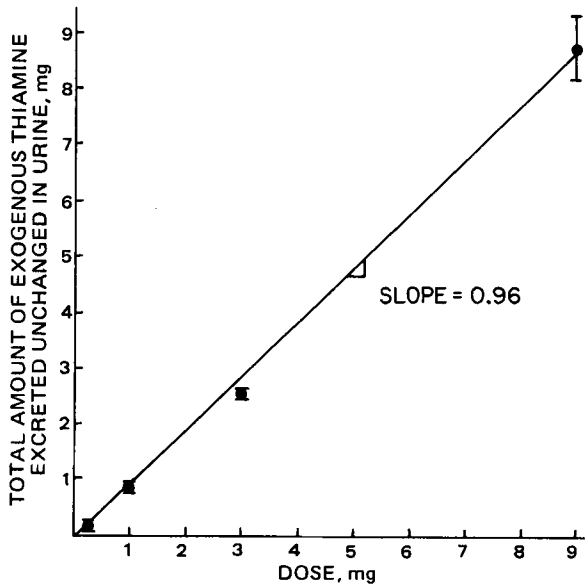

Figure 5—*Plot of the total amount of thiamine excreted unchanged in urine in 48 hr (corrected for the intrinsic turnover of thiamine in 48 hr)* versus *the dose of thiamine hydrochloride administered. The line drawn was generated by least-squares analysis.*

Table III—Effect of Anesthesia on the Pharmacokinetic Parameters [a] for Thiamine Disposition in Rats after Administration of Thiamine Hydrochloride, 36 mg/kg iv

Parameter	Anesthetic Agent			Statistical Significance of Difference
	Light Ether [b]	Continuous Ether [b]	Urethan [c]	
A, μg/ml	142.5 ± 7.6	70.2 ± 5.1	70 ± 10	
B, μg/ml	35.2 ± 5.2	17.9 ± 5.1	43.4 ± 4.1	
α, min^{-1}	0.76 ± 0.20	0.239 ± 0.069	0.232 ± 0.052	
β, min^{-1}	0.019 ± 0.002	0.020 ± 0.004	0.0066 ± 0.0008	
$t_{0.5\beta}$, min	38.1 ± 4.2	37.9 ± 5.6	97.9 ± 7.4	$p \ll 0.001$ [d]
AUC, μg min/ml	2090 ± 290	1304 ± 95	6230 ± 880	$p \ll 0.001$ [d]
AUC/dose, min/ml/kg	0.058 ± 0.008	0.036 ± 0.003	0.173 ± 0.024	$p < 0.05$ [e]
$V_{d(area)}$, ml/kg	830 ± 44	1530 ± 260 ($p < 0.05$) [d]	841 ± 92	NS [f]
Cl_{TB}, ml/min/kg	18.4 ± 2.3	28.0 ± 1.8 ($p < 0.01$) [d]	5.7 ± 1.0	$p \ll 0.001$ [d]

[a] Biexponential parameters determined by fitting blood concentration *versus* time data to Eq. 1. [b] Mean ± SE for five animals. [c] Mean ± SE for six animals. [d] Significantly different from light ether and/or continuous ether using single classification Model I ANOVA (15). [e] Parameter under urethan anesthesia significantly different from each value for this parameter in Tables I–III using the Student–Newman–Keuls test (15). [f] Not significantly different at $p = 0.05$.

decreases blood pressure, increases plasma glucose, and causes local tissue damage to intra-abdominal organs and loss of blood plasma from circulation.

Ether is also known to alter many hemodynamic functions (23). To dramatize these effects, ether was administered continuously in the 36-mg/kg base study. The use of continuous ether anesthesia compared to light ether anesthesia significantly affected only the volume of distribution, and in an increasing direction. As noted, mean pharmacokinetic parameters for thiamine disposition in rats under urethan were significantly different from those in rats under light ether and continuous ether anesthesia.

Hypothermia may accompany general anesthesia due to hemodynamic alterations and general depression of the temperature regulating centers in the hypothalamus (24). Hypothermia was recently shown to alter the pharmacokinetics of pancuronium in the cat (25) and propranolol in the dog and humans (26). It was found (25) that pancuronium was eliminated more slowly and the volume of distribution and total plasma clearance was less in the cat when body temperature was lowered by 10°. Another investigation (26) reported that cooling to 26° markedly reduced the apparent volume of distribution and the total body clearance of propranolol in dogs. Although hypothermia may alter a drug's disposition kinetics, the temperature decreases examined previously were large (≥10°) and in the present investigation the animals under continuous anesthesia were maintained under thermal control.

Most striking and perhaps most consequential to thiamine disposition and elimination may be the pronounced hypocalcemic effect of urethan in rats. This effect was studied (27) with ether and halothane anesthesia. Urethan administered intraperitoneally at anesthetic doses significantly lowered serum calcium within 15 min after injection and this effect lasted for more than 20 hr.

The relationship between calcium and thiamine is well documented. Calcium and magnesium deficiencies alter thiamine distribution in the rat brain and liver. Calcium also plays a significant role in binding thiamine to nerve membrane structure (28–30). Although it is suspected that the hypocalcemic effect of urethan may bear primary consideration in the alteration of thiamine elimination, hemodynamic changes may also contribute.

Since it is not usually necessary to maintain animals under continuous anesthesia during many simple pharmacokinetic studies, the use of urethan or continuous ether anesthesia is no longer common practice. However, for many experiments where significant surgery is required or the animals are exposed to painful techniques, continuous anesthesia is desirable. The results of the present study demonstrate the care which must be taken in the interpretation of pharmacokinetic data otained in anesthetized animals, particularly when urethan anesthesia is used.

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