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# Correlation of Plasma Concentration and Effects of Succinylcholine in Dogs

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**ABSTRACT:** The study was undertaken to determine the pharmacokinetic values for half-life, volume of distribution, and clearance for succinylcholine (SCh) based on measurements of the drug in plasma. Three intravenous (i.v.) doses (0.5, 1.0, and 5.0 mg/kg) were compared to study the time course of paralysis and recovery and to describe the relationship of plasma concentration (Cp) and the pharmacologic effects of SCh in canines. The physiologic response to the neuromuscular blocking drug was monitored using train-of-four stimulation of the left sciatic nerve and recording the response of the corresponding gastrocnemius muscle. Time courses for paralysis and recovery were monitored, and the results were used to predict the kinetic values for the pharmacologic effects. Blood samples were taken following drug administration for direct pharmacokinetic estimations. SCh determinations were performed using ion-pair extraction, chemical demethylation, and gas chromatography with nitrogen phosphorous detection. Both kinetic analyses showed the beta half-life for SCh to be approximately 5 min for all doses. SCh has a distribution half-life of less than 1 min. There appears to be a threshold Cp below which neuromuscular function returns. Recovery following SCh induced paralysis had a rapid onset, but the duration of paralysis and the rate of recovery were especially prolonged for the 5.0-mg/kg treatment group.

**KEYWORDS:** toxicology, succinylcholine, pharmacokinetics, pharmacodynamics

In 1969, Dal Santo described the kinetics of the distribution of radioactively labeled succinylcholine (SCh) in dogs [*1*]. Levy has described the kinetics of the pharmacologic activity of SCh in man [*2,3*] and in newborns [*4*] based upon bioassay data. The plasma disappearance of toxic doses has not been adequately described, nor have correlations of drug effect with plasma concentrations (Cp) been measured.

A good understanding of the pharmacology of SCh has not been achieved to date. Pharmacokinetic parameters of SCh based on direct plasma measurements have not been reported. This has largely been due to the lack of an adequate assay. In addition, there is a lack of information on the inactivation, metabolism, or disposition of the drug and on the contribution of each to the overall effects. With the current availability of a method to detect and quantitate SCh in biological tissues and fluids, one objective of this study was to relate the degree of neuromuscular blockade to the actual plasma levels of the drug. By defining values for the volume of distribution, clearance, half-life, distribution constants, and possi-

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ble models explaining the drug's action and dynamics, we would make available the data to understand better the pharmacology and toxicology of this popular drug.

Three doses (0.5, 1.0, and 5.0 mg of SCh/kg) were selected for the studies. All doses effected complete paralysis. A 1.0-mg/kg dose is typically used in the clinical setting to relax muscles for intubation or surgery or both for humans. The 5.0-mg/kg dose was selected to evaluate toxic effects that might occur in cases of homicide or suicide. The lowest dose was included to determine threshold effects of SCh, a possible dose-response relationship, and the limits of the detectability of the drug in plasma.

## Experimental Procedure

Twelve mongrel dogs were anesthesized with sodium pentobarbital and placed on a mechanical ventilator. SCh was administered intravenously into the cephalic vein. Heart rate, electrocardiogram (EKG), mean arterial pressure, and response of the gastrocnemius muscle to electrical stimulation were recorded simultaneously. A Grass 48-nerve stimulator was set to deliver train-of-four square pulses (Fig. 1) with a frequency of 2 Hz and of 0.2-m/s duration that were repeated every 10 s. A stimulus isolation unit was interfaced with the system to reduce stimulus artifact. The stimuli were delivered to the left sciatic nerve which had been severed to prevent stimulation in the retrograde direction. Recordings were taken from the corresponding left gastrocnemius muscle via an F-10 force transducer connected to a Beckman dynograph recorder. One hundred grams of tension were applied to the muscle to calibrate the transducer. The stimulation voltage was adjusted 10 to 15% above that required to yield a maximal twitch response to assure adequate detection of muscle response throughout the experiment.

Blood gases, pH, and hematocrit were measured at regular intervals. Nine-millilitre blood samples were collected from a surgically implanted venous (femoral) catheter over a sixty-minute period following drug administration. The blood was placed into heparinized Venoject tubes containing physostigmine (0.01% final concentration). The blood samples were

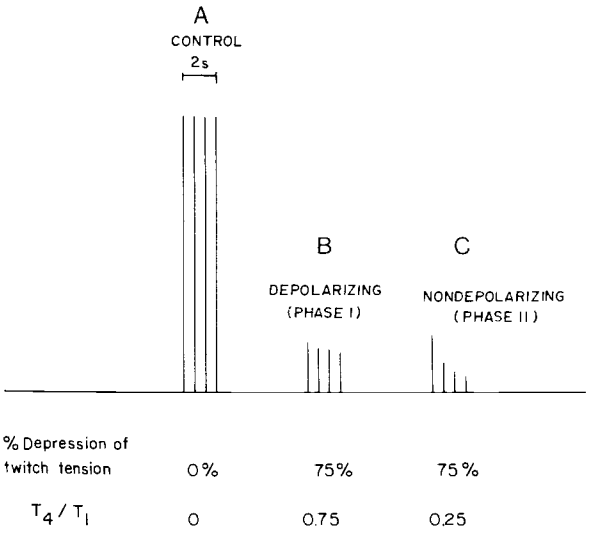


FIG. 1—*Diagramatic representation of evoked muscle twitch response to train-of-four stimuli.* (a) *Control response;* (b) *type of block produced by depolarizing muscle relaxant; and* (c) *nondepolarizing fade.*

centrifuged, and 1 mL of plasma was analyzed for SCh content. Blood was also sampled before and after drug administration to determine cholinesterase and pentobarbital concentrations. Physostigmine was not added to the latter samples.

## Results

Figure 2 shows a plasma concentration versus time curve following a typical 1-mg/kg intravenous (i.v.) dose of SCh. The initial phase is characterized as having a steep slope where the plasma concentration fell rapidly as the drug became distributed from the vascular compartment (central compartment). This is also referred to as the alpha phase.

When the distribution process is near completion, elimination plays a more dominant role and influences the shape of the terminal portion of the curve, also called the beta phase. The beta half-life of SCh in plasma was approximately 5 min. The kinetic processes of SCh were more complex than for a one-compartment model as proposed by Levy [*4*]. When the plasma disappearance data were fitted to model systems [*5*], the best correlation was attained using a two-compartment model (Fig. 3). SCh became distributed rapidly throughout the blood and was carried to tissues receiving the largest blood supplies, such as the kidney, liver, and highly vascularized muscle groups. The drug became distributed more slowly to other tissue sites (peripheral compartment). Equilibration of drug occurred with a net transfer of SCh to the peripheral compartment ($k_{12} = 0.673 \pm 0.14$ min-1 and $k_{21} = 0.33 \pm 0.06$ min-1, where $k_{12}$ was the distribution constant relating drug transfer to the peripheral compartment and $k_{21}$ was the distribution constant relating drug transfer to the central from the periph-

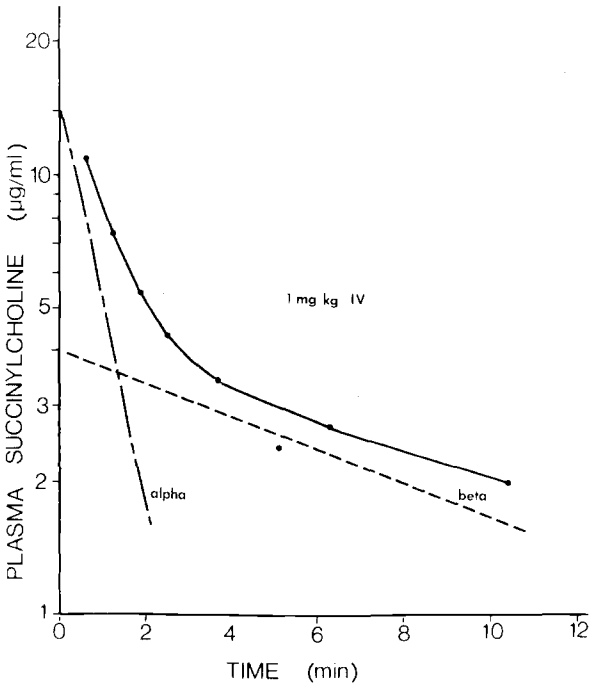


FIG. 2—*Concentration versus time plot showing actual data points with calculated distribution (alpha) and elimination (beta) lines.*

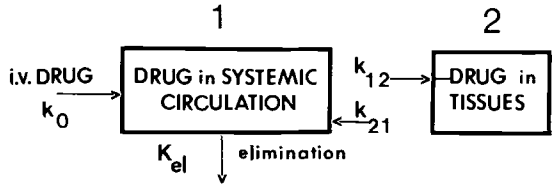


FIG. 3—*Schematic representation of two-compartment model.*

eral compartment. The central compartment also receives drug via $k_0$. The mean volume of the central compartment ($V_1$) was 0.487 ± 0.015 L (Table 1).

$V_d$ area reflects the total volume of distribution. The $V_{d\ area}$ for SCh was 2.184 ± 0.22 L. Clearance is a pharmacokinetic term defined as the volume of biological fluid cleared of a substance (drug) per unit time. SCh plasma clearance averaged 0.32 L/min and the half-life 4.69 min (arithmetic mean)/2.35 min (harmonic mean).

The pharmacologic values, such as half-life, that were determined based upon measurement of the pharmacologic effects (time course of paralysis) of SCh were similar to those determined from measurement of SCh disappearance from plasma as described previously.

Figure 4 depicts a 1-min portion of a physiograph printout during i.v. injection of a 5-mg/kg dose. Complete paralysis was produced by 25 s. Muscular block was not complete following muscular fasciculations (depicted on the bottom trace), and the $T_4/T_1$ ratio was 0.61, indicating a transition block that was neither depolarizing or nondepolarizing by definition. The ratio of the height of the fourth deflection $T_4$ to the height of the first deflection $T_1$ is used by anesthesiologists to monitor neuromuscular function since it distinguishes between depolarizing ($T_4/T_1 > 0.7$) and nondepolarizing ($T_4/T_1 < 0.3$) types of block. Total paralysis lasted 35 min, and recovery of $T_1$ was complete by 1 h 20 min. The SCh Cp at initial recovery was 1.06 μg/mL. At 1 h the SCh Cp was 0.43 μg/mL.

Two of the three dogs given 0.5 mg/kg remained paralyzed for 15.5 min and the third for 26 min. For the 1.0-mg/kg treatment group, duration of paralysis was 20 min, and the 5.0-mg/kg group exhibited no recovery until 40 min after injection.

Peak plasma concentrations for 0.5-, 1.0-, and 5.0-mg/kg doses were 22, 76, and 132 μg SCh/mL plasma, respectively. Recovery was plotted by measuring $T_1$ deflection amplitudes (in millimetres) (see Fig. 1) and comparing them with predose $T_1$ values. These were expressed as percent. The lowest initial Cp recorded at complete paralysis was 13 μg/mL, which was observed following a 0.5-mg/kg dose. This animal displayed a depolarizing-type of block. The lowest Cp (0.45 μg/mL) at initial recovery of $T_1$ was also observed following a dose of 0.5 mg/kg.

Figure 5 shows the relationship of i.v. dose (0.5 and 1.0 mg/kg) and duration of various degrees of neuromuscular inhibition in dogs. $T_{10}$, $T_{50}$, and $T_{90}$ represent the time required to recover 10, 50, and 90% of normal muscle contractile force as measured by $T_1$ amplitude. This figure suggests that a linear relationship may exist between the duration of a given degree of muscular paralysis and the dose of SCh administered, as reported by Levy [*3*].

From Fig. 5, minimum effective dose (Amin) values were determined for 10, 50, and 90% muscle paralysis. Figure 6 shows the results via a plot of Amin versus the intensity of pharmacologic effect as percent paralysis. The slope of the resulting line was used to predict the elimination rate constant for SCh.

Figure 7 shows the degree of muscle paralysis as a function of time after i.v. administration for the three treatment groups. At 0.5 and 1.0 mg/kg, recovery rates appeared to be independent of the dose administered; the slopes were parallel, and the relationship was

TABLE 1—*Pharmacokinetic parameters for succinylcholine following a single intravenous dose in twelve dogs.*

| Dog | Dose mg/kg | $k_{21}$, $min^{-1}$ | $k_{el}$, $min^{-1}$ | $k_{21}$, $min^{-1}$ | $V_1$, L/kg | $V_{d\ area}$, L/kg | $T^{-1}/2$, min | AUC, $\mu g$ min/L | Cl, L/min |
|---|---|---|---|---|---|---|---|---|---|
| 7888 | 0.5 | 0.222 | 0.113 | 0.814 | 0.432 | 3.036 | 6.12 | 36.06 | 0.344 |
| 7893 | 0.5 | 0.333 | 0.352 | 0.109 | 0.134 | 0.951 | 1.97 | 38.52 | 0.335 |
| 7910 | 0.5 | 0.534 | 0.196 | 1.681 | 0.327 | 2.065 | 3.52 | 33.27 | 0.406 |
| | | | | | | | | 35.95 ± 1.52 | |
| 7770 | 1.0 | 0.215 | 0.135 | 0.607 | 0.450 | 2.866 | 5.12 | 44.30 | 0.387 |
| 7774 | 1.0 | 0.188 | 0.129 | 1.124 | 0.151 | 2.021 | 5.35 | 73.37 | 0.262 |
| 8034 | 1.0 | 0.270 | 0.140 | 0.942 | 0.134 | 2.459 | 4.94 | 81.11 | 0.344 |
| 8071 | 1.0 | 0.323 | 0.139 | 0.428 | 0.743 | 2.645 | 4.99 | 71.89 | 0.367 |
| 8072 | 1.0 | 0.917 | 0.285 | 0.439 | 0.409 | 0.651 | 2.43 | 94.48 | 0.185 |
| 8126 | 1.0 | 0.198 | 0.112 | 0.576 | 0.575 | 2.664 | 6.18 | 84.78 | 0.296 |
| | | | | | | | | 74.99 ± 9.89 | |
| 7796 | 5.0 | 0.384 | 0.132 | 0.295 | 0.380 | 1.714 | 5.23 | 598.82 | 0.227 |
| 7811 | 5.0 | 0.171 | 0.123 | 0.032 | 1.968 | 3.078 | 5.63 | 351.20 | 0.379 |
| 7831 | 5.0 | 0.210 | 0.143 | 1.030 | 0.138 | 2.061 | 4.83 | 351.81 | 0.296 |
| | | | | | | | | 433.94 ± 82.44 | |
| Mean | | 0.330 | 0.167 | 0.673 | 0.487 | 2.184 | 4.69 | | 0.319 |
| S.E. | | 0.06 | 0.02 | 0.14 | 0.15 | 0.22 | 0.39 | | 0.02 |

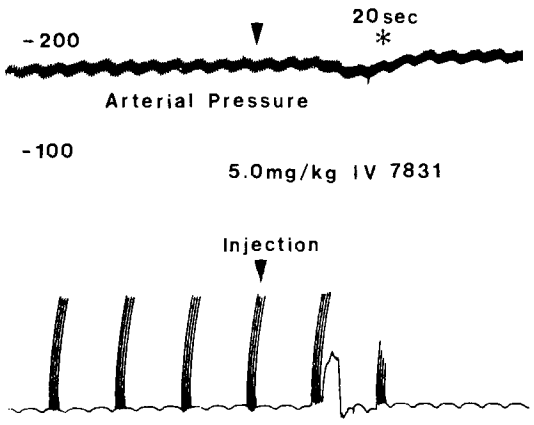


FIG. 4—*Physiologic trace depicting canine arterial pressure and evoked muscle twitch response resulting from injection of succinylcholine.*

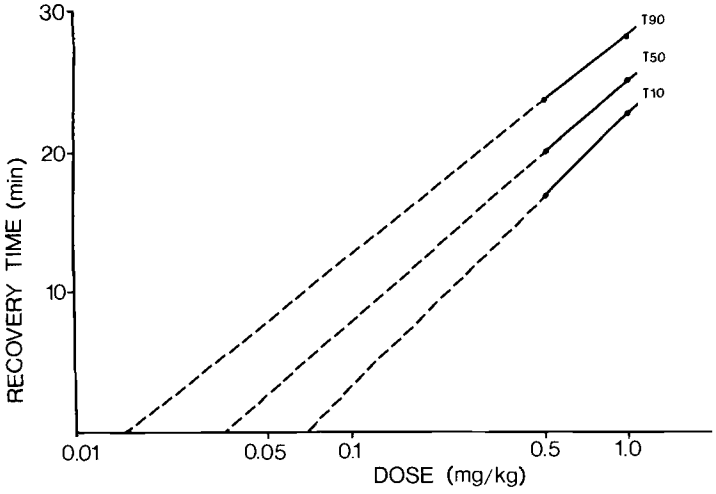


FIG. 5—*Relationship between dose of succinylcholine and time to recover 10, 50, and 90% of neuromuscular function.*

linear. This relationship was first described by Levy using data from human subjects [*3*]. The elimination rate constant was calculated by dividing the slope from the Amin plot into the slope of the lines in Fig. 7. The resultant value was 0.13 min, which was similar to the value calculated from direct plasma measurements.

At the 5.0-mg/kg dose, the prolonged duration of paralysis and slow recovery rate could be due to differences in Amin, intensity of effects, drug recirculation, receptor desensitization, enzyme inhibition, or induction of a nondepolarizing-type of block at this high dose. (SCh is a highly polar molecule. It does not readily cross cell membranes, the intestinal wall, the blood brain barrier, or the placenta. It is therefore confined to the aqueous portions of the body limited by nonporous membrane systems. SCh can therefore be redistributed [recirculated] throughout the vascular compartment.)

These results demonstrated a significantly decreased rate of recovery at a dose fivefold

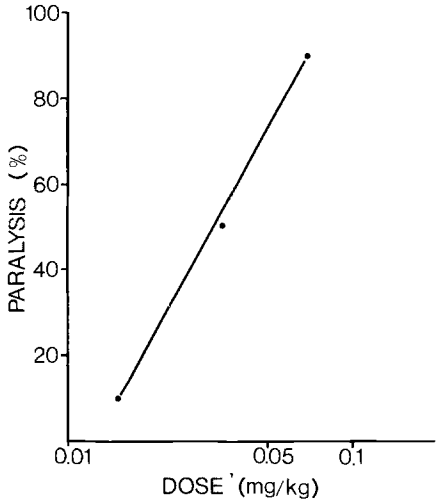


FIG. 6—*Dose-response plot for minimum effective dose to cause specific degree of paralysis.*

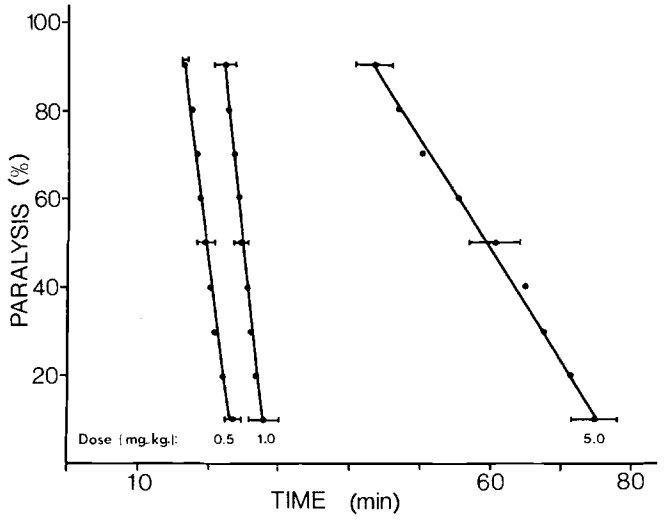


FIG. 7—*Degree of canine muscle paralysis as function of time for 0.5-, 1.0-, and 5.0-mg/kg i.v. doses of succinylcholine.*

greater than a clinical dose (5 versus 1 mg/kg). Figure 8 shows the times required for 10 and 90% recovery of $T_1$ for the three dosage groups. These results showed a tendency for recovery time to increase with increasing doses. To recover to 90% of predose $T_1$, nearly 20 min were required following a 0.5-mg/kg dose in canines and 75 min were required after 5.0-mg/kg i.v. Therefore, the time required for ventilatory support was dose-dependent. In homicidal cases, relatively small increases in the dose of SCh may result in respiratory paralysis lasting longer than 6 min, which would be fatal.

Figure 9 combines results from both pharmacokinetic and pharmacodynamic data to illustrate the relationship of muscular paralysis to SCh Cp. When comparing SCh Cps during

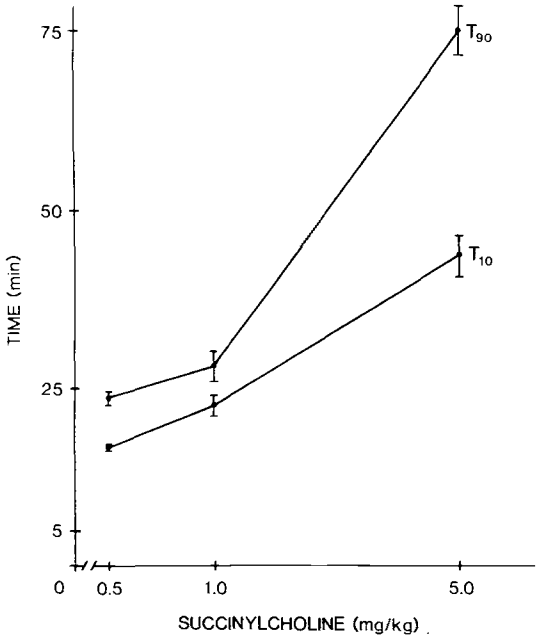


FIG. 8—*Times required to achieve 10 and 90% recovery of* $T_1$ *in relation to dose of succinylcholine administered.*

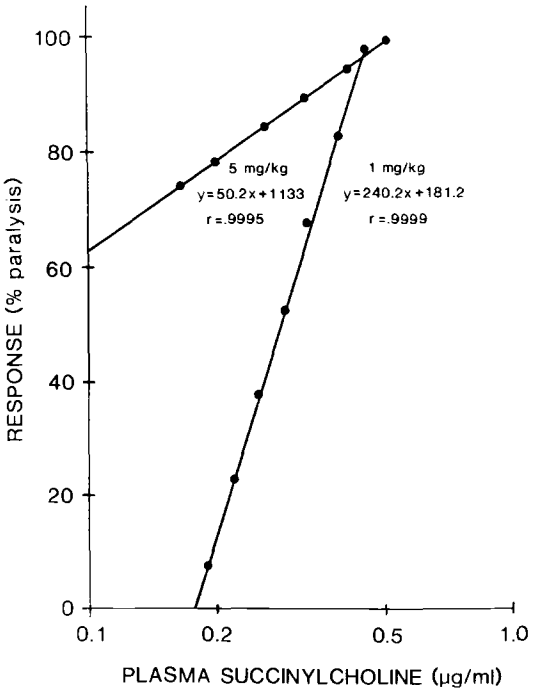


FIG. 9—*Plasma succinylcholine concentrations and percent inhibition of* $T_1$ *in canines for 1-mg/kg-dosed dogs and 5-mg/kg-dosed dogs. Equations for both lines are given along with correlation coefficients.*

recovery, there appeared to be a threshold concentration below which neuromuscular function returned (0.46 μg SCh/mL).

Following a 5.0-mg/kg dose, the degree of paralysis was greater than that observed with a low dose (1 mg/kg) despite similar Cps. Recovery was prolonged for high doses. One possible explanation for this is desensitization of the receptor. This could represent a protective biological role to allow sufficient time for saturated or inhibited enzyme systems to recover. Taussig et al. [*6*], reported that butyrocholinesterase (BuChE) was inhibited by SCh at high concentrations (31.32 μg/mL). This may also explain why Cps taken at 4 min (Table 2) increased exponentially with increasing doses. With the enzyme inhibited, less drug would be degraded. Schuh [*7*] also found the rate of recovery following i.v. doses of SCh to be dose-dependent rather than independent as suggested by Levy [*2,3*].

Muscle concentrations of SCh are influenced by blood supply. With longer exposure to drug (recirculation), tissue can accumulate the drug. Recirculation occurs with SCh as soon as 3 min after injection [*8*]. Those findings were based on bioassay of SCh, and since it is now known that SCh is present in tissue and blood long after recovery from paralysis, recirculation of SCh may play an important role in distribution and time-dependent tissue accumulation. In muscle groups receiving an abundant blood supply, the concentration of SCh will continue to increase as long as the drug remains in the blood in significant concentrations. In the study of triceps muscle, SCh levels in the tissue were higher at 70% recovery than at initial recovery (Table 2).

## Conclusions

Results from plasma disappearance studies of SCh have shown that clearance, half-life, and volume of distribution values were similar for all doses. Only the area under the curve (AUC) differed significantly, and that was significantly greater with high doses. This was to be expected with higher peak plasma concentrations and similar clearance and elimination rate values. The in vivo canine studies to evaluate SCh pharmacokinetics demonstrated the best model to be a two-compartment model with rapid distribution to a central compartment and slower distribution to a second compartment.

An evaluation of SCh pharmacodynamics indicated a dose-dependent relationship between the duration of paralysis as well as the rate of recovery at doses at or below 1.0 mg/kg. Recovery from paralysis following a dose of 5 mg SCh/kg was prolonged and not explained by difference in clearance or half-life.

New information was provided, such as SCh Cps at initial recovery and complete recovery, as well as the corresponding rates of recovery following three doses. For the first time, kinetic analyses based upon measurement of SCh in plasma showed that the values for clearance, half-life, volume of distribution, and distribution rate constants did not differ significantly among doses. The pharmacologic estimate for clearance and half-life based on nerve stimulation and recording recovery of $T_1$ were similar to the direct plasma measurements in the dog, thereby for the first time relating actual plasma concentrations to neuromuscular effect. Many factors play a role in the resultant action of this drug: blood flow, enzymatic hydrolysis, and, of course, the dose administered. The rate of recovery appears to be dose-

TABLE 2—*Succinylcholine concentrations in triceps (μg/g).*[a]

| Time | Mean ± S.E. | 10 Days | 20 Days |
|---|---|---|---|
| Initial | 0.14 ± 0.04 | 0.12 ± 0.05 | 0.10 ± 0.05 |
| 40% | 0.14 ± 0.02 | 0.15 ± 0.05 | 0.12 ± 0.05 |
| 70% | 0.24 ± 0.04 | 0.19 ± 0.05 | 0.16 ± 0.05 |

[a]Three dogs were given 1 mg/kg succinylcholine i.v.

dependent, and Schuh [7] described the dose-response curve as sigmoid in shape. This can be explained when considering that SCh is a potent cholinergic antagonist and therefore only a fraction of the receptors need to be occupied to achieve a full paralytic effect.

Therefore, SCh's pharmacologic action may not be directly proportional to dose. Van Rossum and Burgers [9] suggested that diffusion of the drug through the tissues may play a greater role than kinetics of receptor occupation. SCh also causes release of catecholamines [10], specifically norepinephrine. When considering the effects that changes in blood flow exert on the time course of action of SCh, as well as the cardiovascular effects as a result of norepinephrine release, the effects of small changes in cardiovascular function (ionotropic or chronotropic or both) may greatly influence resultant kinetics.

Studies can now be conducted to simulate homicidal situations to determine resultant drug concentrations in nonventilated animals. Urinary concentrations of SCh can provide valuable information in addition to the more commonly used kidney, liver, and muscle tissues.

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