Anesthesiology
61:534–539, 1984

Metabolites of Neostigmine and Pyridostigmine Do Not Contribute to Antagonism of Neuromuscular Blockade in the Dog

Pim J. Hennis, M.D., Roy Cronnelly, M.D., Ph.D.,† Manohar Sharma, Ph.D.,‡*
Dennis M. Fisher, M.D.,§ Ronald D. Miller, M.D.¶

The authors sought to determine whether the metabolites of neostigmine and pyridostigmine contribute to antagonism of neuromuscular blockade. Accordingly, the dose–response relationship, onset and duration of action (n = 60), and pharmacokinetics (n = 22) of neostigmine, pyridostigmine, their metabolites 3-hydroxyphenyltrimethylammonium (PTMA) and 3-hydroxy-N-methylpyridinium (MP), and edrophonium were determined in dogs anesthetized with sodium pentobarbital. The force of contraction of the anterior tibialis muscle was maintained at constant 90% depression by infusing pancuronium. Then, a single iv bolus dose of one of the drugs under study was injected while the pancuronium infusion was continued. Venous blood, urine, and bile were sampled for four hours. Concentrations were determined by liquid chromatographic techniques; a three-compartment pharmacokinetic model was fitted to the serum concentration data. The doses producing 50% antagonism were 6.5, 52, 69, and 40 μg/kg for neostigmine, pyridostigmine, edrophonium, and PTMA, respectively. MP was inactive as an antagonist. By comparing approximately equipotent doses, time to peak antagonism (onset) and until 30% of peak antagonism remained (duration) were shorter for both edrophonium and PTMA than for neostigmine and pyridostigmine. Slow distribution and elimination half-lives, volume of distribution at steady state (VD_{ss}), and total plasma clearance (Cl) were similar for the drugs except for a smaller Vd_{ss} and lower Cl for MP. More than 60% of the dose of each drug was recovered unchanged from urine; less than 1% was recovered from bile. Less than 10% of the dose of neostigmine was recovered as PTMA. Since PTMA was a weak antagonist and MP had no antagonist activity, the authors conclude that their contribution to antagonism of neuromuscular blockade is minimal. Therefore, the slower onset of neostigmine and pyridostigmine than of edrophonium cannot result from the time required for the formation of their metabolites. Also, differences in potency and duration between the drugs cannot be explained by pharmacokinetics. These results support the belief that there are pharmacodynamic differences between the drugs. (Key words: Antagonists, neuromuscular relaxants: edrophonium; neostigmine; pyridostigmine. Metabolites: neostigmine; pyridostigmine. Pharmacokinetics.)

WHEN NEOSTIGMINE AND PYRIDOSTIGMINE antagonize neuromuscular blockade induced by nondepolarizing muscle relaxants, their onset is slower than that of edrophonium.[1–3] When neostigmine and pyridostigmine react with acetylcholinesterase (AChE) at the neuromuscular junction, their primary metabolites, 3-hydroxyphenyltrimethylammonium (PTMA) and 3-hydroxy-N-methylpyridinium (MP), respectively, are formed (fig. 1). In contrast, edrophonium is not metabolized by AChE.[4,5]

We investigated the possibility that the slower onset of neostigmine and pyridostigmine than of edrophonium results from the time required for the formation of their metabolites. To explore this possibility, we assessed the contribution of the metabolites to antagonism of neuromuscular blockade. Thus, we determined the dose–response relationship, the onset and duration of action, and the extent of metabolism of neostigmine, pyridostigmine, PTMA, MP, and edrophonium in anesthetized dogs. To explain the results, we also determined the pharmacokinetics of these drugs.

Methods

We studied 60 mongrel dogs weighing 15–35 kg. After anesthesia was induced with sodium pentobarbital (20–30 mg/kg iv), the trachea was intubated, and the lungs were ventilated with air by a Harvard® pump, adjusted to maintain normocarbia. Anesthesia was maintained with additional doses of sodium pentobarbital (3–5 $mg \cdot kg^{-1} \cdot h^{-1}$). Esophageal temperature was maintained between 36 and 38° C with heating blankets. A femoral artery and vein and an external jugular vein were cannulated for measurement of blood pressure, injection of drugs, and sampling of blood, respectively. Following laparotomy, the common bile duct and urinary bladder were cannulated for collection of bile and urine. The incision then was closed to prevent excessive heat and fluid loss. Throughout the experiment, the dogs received 0.9% saline at a rate of 5–15 $ml \cdot kg^{-1} \cdot h^{-1}$.

The common sciatic nerve was stimulated with supramaximal square-wave stimuli of 0.15 ms duration at 0.15 Hz delivered by Grass S-44® stimulator. The resultant force of contraction (twitch tension) of the anterior tibialis muscle was quantitated with a Grass FT 10c® transducer and recorded on a polygraph. Pancuronium was injected as an iv bolus and then infused continuously at a rate sufficient to produce 90% depres-

* International Fellow in Clinical Pharmacology.
† Assistant Professor of Anesthesia and Pharmacology.
‡ Assistant Research Chemist.
§ Assistant Professor of Anesthesia and Pediatrics.
¶ Professor and Vice Chairman of Anesthesia, Professor of Pharmacology.

Received from The Department of Anesthesia, S-436, University of California, San Francisco, California 94143. Accepted for publication April 24, 1984. Supported in part by GM26403. Dr. Hennis is supported by a grant from the Merck Company Foundation.

Address reprint requests to Dr. Cronnelly.

sion of the twitch tension. When the pancuronium infusion rate and the twitch were unchanged for at least 20 min, a single bolus dose of one of the antagonists or their metabolites was injected. The pancuronium infusion was continued until the experiment was completed. The drugs were neostigmine methylsulfate [3.3 (n = 3), 5 (n = 3), 6.7 (n = 4), or 13.4 (n = 4) μg/kg] pyridostigmine bromide [35 (n = 3), 70 (n = 4), or 140 (n = 4) μg/kg], edrophonium chloride [40 (n = 5), 80 (n = 5), or 160 (n = 6) μg/kg]. PTMA iodide [25 (n = 5), 50 (n = 5), or 100 (n = 6), or 100 (n = 6) μg/kg], and MP iodide [50–10,000 μg/kg (n = 3)]. The doses represent the base rather than the salt of the drugs.

PTMA and MP were prepared from 3-dimethylaminophenol and 3-hydroxypyridinium (Aldrich) by quaternizing the latter compounds with methyliodide. The structure and purity of PTMA and MP were established by nuclear magnetic resonance, mass spectrometry, and the determination of melting points.

To determine the dose–response relationship, we calculated antagonism as:

$$\frac{\text{Peak twitch tension after injection} - \text{Twitch tension at time of injection}}{100 - \text{Twitch tension at time of injection}} \times 100\%$$

All values for twitch tension in this equation are expressed as percentage of the prepancuronium control twitch. For example, if twitch tension recovers from 10 to 50% of control twitch, this is expressed as 50–10/100–10, or 44% peak antagonism. For each drug, least-squares regression was used to analyze the percentage of antagonism *versus* the logarithm of the dose. From the regression line, the ED_{50} (dose producing 50% antagonism) was calculated. By comparing these ED_{50} values, the relative potencies of the drugs were determined. The slopes and positions of the regression lines were compared by analysis of covariance.[6]

To determine the onset and duration of action, the times from injection of the drug to peak antagonism and until 30% of peak antagonism remained were measured. Mean values of onset and duration after the various doses of each drug were compared within each drug group and between the drug groups by analysis of variance and the Student-Newman-Keuls test.[6]

The pharmacokinetic studies (n = 22) were performed in the same dogs used for the dose–response studies (*i.e.*, a drug was given, its response measured, and blood drawn for serum concentration measurements). The doses for pyridostigmine (n = 5), edrophonium (n = 5), and PTMA (n = 4) were 140, 160, and 100 μg/kg, respectively. For MP (n = 3), 1,000 μg/kg was injected and for neostigmine (n = 5) 260 μg/kg. The large dose of neostigmine was selected after preliminary experiments demonstrated a short detection period of neostigmine in serum. Venous blood was sampled before injection and at 1, 2, 4, 6, 8, 10, 15, 20, 25, 30, 45, 60, 90, 120, 150, 180, 210, and 240 min after injection. Blood samples were kept on ice and centrifuged immediately after the experiment; serum was stored at −70° C until it was analyzed. To determine the concentrations of neostigmine, pyridostigmine, edrophonium, and PTMA, an ion-pair extraction, reversed-phase high-performance liquid chromatographic technique was used. This technique separates parent compounds from their metabolites.[7] The lowest level of detection is 10 mg/ml; the coefficient of variation at that level is 8%. To determine the concentrations of MP an ion-pair extraction, ion-exchange liquid chromatographic technique was used. Its lowest level of detection is 50 ng/ml; the coefficient of variation at that level is 9.6% (see "Appendix").

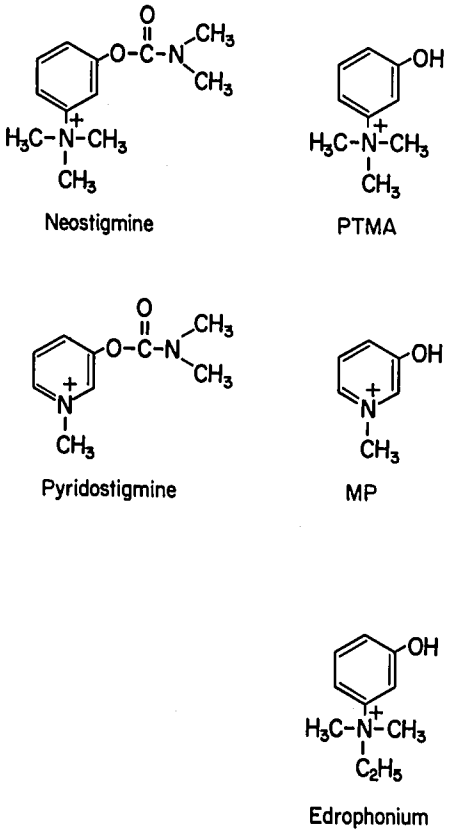

FIG. 1. Structures of neostigmine, pyridostigmine, edrophonium, 3-hydroxyphenyltrimethylammonium (PTMA), and 3-hydroxy-N-methylpyridinium (MP).

Two- and three-compartment pharmacokinetic models were fitted to the measured serum concentration data using a least-squares nonlinear regression[8]; residuals were weighted by the inverse square of the measured serum concentration. To select between the models, the residual sums of squares for each dog were compared.[9] Using standard formulas,[10] the following pharmacokinetic variables were determined: rapid and slow distri-

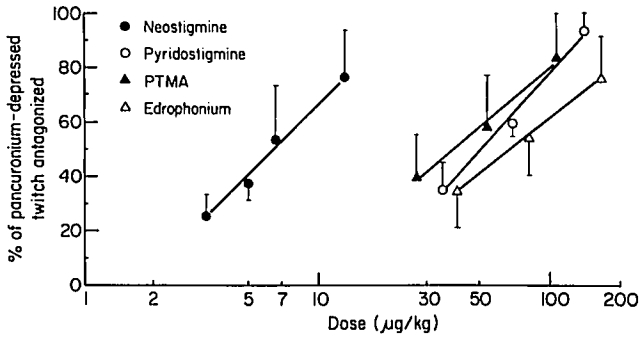

FIG. 2. Dose–response curves for neostigmine, pyridostigmine, PTMA, and edrophonium. Values plotted are means ± SD of the antagonism at each dose. Doses are expressed as quantity of the base.

bution half-lives ($t_{1/2}\pi$ and $t_{1/2}\alpha$); elimination half-life ($t_{1/2}\beta$); volume of the central compartment (Vl); volume of distribution at steady state (Vd_{ss}); and total plasma clearance (Cl). Mean values for each drug were compared by analysis of variance and the Student-Newman-Keuls test.[6]

To determine the extent of metabolism, urine and bile aliquots were collected every half hour for 4 h and their volumes were measured. The methods for determination of drug concentrations in urine and bile were similar to that used for serum. These techniques permit determination of the fraction of neostigmine and pyridostigmine eliminated as parent compound and as primary metabolite. The amounts recovered from urine and bile were expressed as percentage of the injected dose.

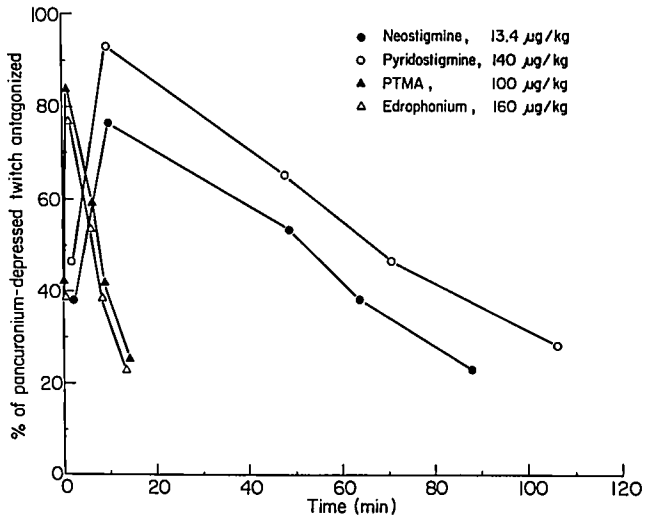

FIG. 3. Time course of antagonism of the pancuronium-depressed twitch tension by neostigmine (n = 4), pyridostigmine (n = 4), PTMA (n = 6), and edrophonium (n = 6). Values plotted are means. Onset is expressed as the time from injection to peak antagonism; duration as the time from injection until 30% of peak antagonism remains.

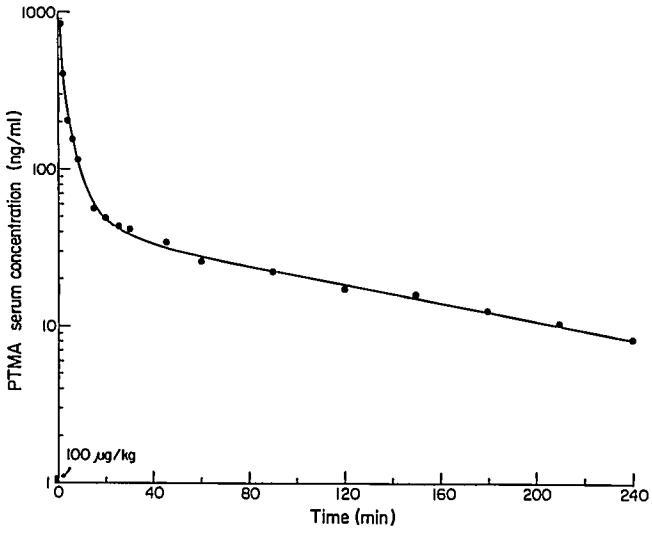

FIG. 4. Serum concentration *versus* time data for one dog that received 100 μg/kg PTMA. Circles represent measured concentrations of PTMA; the solid curve represents the fitted function as determined by nonlinear regression.

For all statistical comparisons, differences were considered significant at a $P < 0.05$.

Results

In the dose–response study, the regression lines for neostigmine, pyridostigmine, edrophonium, and PTMA did not deviate from parallelism. The regression line for neostigmine was positioned to the left of those for the other drugs (fig. 2). The ED_{50} values were 6.5, 52, 69, and 40 μg/kg for neostigmine, pyridostigmine, edrophonium, and PTMA, respectively. Thus, neostigmine was 8, 10.6, and 6.1 times more potent than pyridostigmine, edrophonium, and PTMA, respectively. MP, in doses up to 10,000 μg/kg, had no effect on twitch tension.

There was a dose-dependent increase in duration of antagonism for all drugs. However, the onset time was not changed. After approximately equipotent doses, the onset time for both edrophonium (1.3 ± 0.4 min, mean ± SD) and PTMA (1.0 ± 0.4 min) was shorter than that for neostigmine (10 ± 4 min) and pyridostigmine (10 ± 2 min) (fig. 3). Similarly, the duration for both edrophonium (13 ± 3 min) and PTMA (14 ± 6 min) was shorter than that for neostigmine (88 ± 30 min) and pyridostigmine (106 ± 45 min). There were no significant differences between edrophonium and PTMA or between neostigmine and pyridostigmine.

In the pharmacokinetic study, all drugs could be identified in serum for at least 4 h after injection. A three-compartment pharmacokinetic model was used to fit data from each dog. An example of the quality of data characterization by the model is shown for PTMA

TABLE 1. Pharmacokinetic Variables for Neostigmine, Pyridostigmine, Edrophonium, PTMA, and MP in Dogs

	n	$t_{1/2}\pi$ (min)	$t_{1/2}\alpha$ (min)	$t_{1/2}\beta$ (min)	V_1 (l/kg)	Vd_{ss} (l/kg)	Cl (ml·kg^{-1}·min^{-1})
Neostigmine	5	1.6 ± 0.6	11.1 ± 5.6	93 ± 33	0.14 ± 0.04	1.3 ± 0.4	24.5 ± 10.5
Pyridostigmine	5	1.3 ± 0.4	8.4 ± 5.4	122 ± 12	0.13 ± 0.07	2.0 ± 0.9	18.3 ± 7.0
Edrophonium	5	0.7 ± 0.4*	5.6 ± 3.2	85 ± 23	0.12 ± 0.03	1.6 ± 0.3	18.4 ± 1.1
PTMA	4	0.7 ± 0.2*	6.0 ± 3.0	98 ± 18	0.10 ± 0.07	1.5 ± 0.6	14.3 + 2.6
MP	3	1.0 ± 0.5	9.8 ± 6.2	107 ± 17	0.10 ± 0.06	0.5 ± 0.3†	4.6 ± 1.8†

Data are mean ± SD.
* Different from neostigmine and pyridostigmine ($P < 0.05$).
† Different from neostigmine, pyridostigmine, edrophonium, and PTMA ($P < 0.005$).

(fig. 4). A similar approximation of the data points by the fitted function was obtained in each case. The pharmacokinetic variables for the drugs differed in only two respects. First, the rapid distribution half-life for both edrophonium and PTMA was shorter than that of neostigmine and pyridostigmine (table 1). Second, volume of distribution at steady state was smaller and plasma clearance was lower for MP than for the other drugs.

Only 7 ± 3% of neostigmine was recovered from urine as PTMA. After pyridostigmine, no MP was recovered. For all drugs, more than 60% of the injected dose was recovered unchanged (table 2); more than 70% of the recovered amounts was excreted within 1–1.5 h. Less than 1% was recovered from bile.

Discussion

We conclude that the contribution of the metabolites of neostigmine and pyridostigmine to antagonism of neuromuscular blockade is minimal. Although PTMA has been reported to facilitate neuromuscular transmission,[11,12] we found marked differences in antagonist potencies between the metabolites, PTMA, and MP, and their parent compounds. PTMA was a weak antagonist (6.1 times less potent than neostigmine), and MP had a no antagonistic potency in doses up to 10,000 μg/kg. This also has been reported in the rat.[12]

Perhaps we underestimated the contribution of the metabolites to antagonism. The concentration of the metabolites at the neuromuscular junction might be higher when they are formed locally compared with their systemic administration. In addition, the metabolites may be cleared from the neuromuscular junction at a slower rate than their parent compounds. Although unlikely, both of these conditions may increase potency of the metabolites following *in vivo* production.

Since the contribution of the metabolites to antagonism is minimal, the slower onset of neostigmine and pyridostigmine than of edrophonium cannot be explained by the time required for the formation of their metabolites. The rapid onset of PTMA is not in disagreement with this explanation because the formation rather than the onset of PTMA would be the rate limiting step for the onset of neostigmine. However, as we discussed earlier, it is unlikely that sufficient PTMA is formed to offset its lesser potency.

The duration of action of PTMA was very similar to that of edrophonium. However, the short duration of edrophonium in the dog is in contrast to observations in humans that with appropriate doses its duration is similar to that of neostigmine.[3] Perhaps the duration of PTMA in humans is also longer than that in the dog and in adequate dosage this drug might be a clinically useful antagonist because of its rapid onset of action.

We determined the pharmacokinetics of the drugs to explain the results of the dose–response and onset and duration studies. However, the differences between potencies of neostigmine and or pyridostigmine, edrophonium, and PTMA cannot be explained by differences in volumes of distribution, since these were similar for the four drugs. MP had a smaller volume of distribution and a lower clearance than its parent compound. Thus inactivity cannot be explained by pharmacokinetics. The pharmacokinetics of PTMA have been determined in the rat and are similar to those for neostigmine.[12] Although our values are different from those previously determined, differences in species easily would account for the discrepancies.

Similarly, the shorter duration of edrophonium and PTMA than of neostigmine and pyridostigmine cannot

TABLE 2. Excretion of Neostigmine, Pyridostigmine, Edrophonium, PTMA, and MP into Urine and Bile in Dogs

	n		Urine*	Bile*
Neostigmine	4	(as (Neostigmine)	62 ± 9	<1
		(as PTMA)	7 ± 3	<1
Pyridostigmine	4	(as Pyridostigmine)	74 ± 2	<1
		(as MP)	<1	
Edrophonium	4		74 ± 24	<1
PTMA	4		71 ± 22	<1
MP	3		89 ± 7	<1

Data are mean ± SD.
*Per cent of injected dose recovered.

be explained by differences in pharmacokinetics, since their clearances and elimination half-lives were not different. Thus, although the blood concentrations of the drugs decrease at a similar rate, there are differences in the decline of their antagonistic effects. Although both the onset time and the rapid distribution half-life for edrophonium and PTMA were shorter than those for neostigmine and pyridostigmine, these findings probably are not associated. The error in estimating rapid distribution half-life is large, ranging from 25 to 50% for each pharmacokinetic fit. Therefore, comparing rapid distribution half-lives should be suspect. Also, slight differences in distribution half-lives cannot explain the vast differences in onset times. Lastly, in the isolated rat diaphragm preparation (in which distribution is not an issue), the onset of edrophonium is, as *in vivo*, much more rapid than that of neostigmine.[13]

Since neither differences in onset nor differences in duration of these drugs can be explained by their pharmacokinetics, they probably reflect differences in their pharmacodynamics. One explanation for the faster onset of edrophonium might be its faster rate of AChE inhibition. This explanation is supported by *in vitro* studies demonstrating that, in the absence of acetylcholine, edrophonium inhibits AChE at a faster rate than neostigmine and pyridostigmine.[14] A second explanation is that edrophonium has a presynaptic (*i.e.*, acetylcholine release) rather than a postsynaptic (*i.e.*, AChE inhibition) action, whereas the postsynaptic action is predominant for neostigmine and pyridostigmine.[15–18] Therefore, onset times may reflect differences in rate constants associated with the two mechanisms of action.

If AChE inhibition is the mechanism of action of these drugs, differences in dissociation of the AChE-inhibitor complex may account for the differences in their duration of action. Because edrophonium and PTMA do not contain a carbamate group (fig. 1), their binding to AChE is easily reversible and transient. Carbamylation of AChE by neostigmine and pyridostigmine produces a longer lasting inhibition.[19,20] Alternatively, differences in rates associated with mechanisms other than AChE inhibition may account for different durations. Although the parallelism in the dose–response curves of the drugs in our study suggests a common mechanism of action, our data do not permit us to decide on the nature of this mechanism of action.

Neostigmine, pyridostigmine, their primary metabolites, and edrophonium appear to be eliminated predominantly by renal excretion, since more than 60% of the dose was recovered unchanged from urine during 4 h and less than 1% was recovered from bile. Therefore, total plasma clearance is due largely to renal clearance. The plasma clearance values found in this study exceed known values of glomerular filtration rate, which suggests the importance of tubular secretion. Urinary excretion of neostigmine and pyridostigmine in rats can be blocked by Cyanine 863®, an inhibitor of tubular secretion of basic drugs,[21,22] which further suggests that tubular secretion is an active process.

In summary, we conclude that the primary metabolites of neostigmine and pyridostigmine, PTMA, and MP respectively, contribute minimally to antagonism of neuromuscular blockade. Therefore, we cannot explain the slower onset times of neostigmine and pyridostigmine than of edrophonium by the time required for the formation of their metabolites. Differences in dose–response relationships, onset, and duration of action between these drugs cannot be explained by pharmacokinetics. These results support the belief that there are pharmacodynamic differences between the drugs.

Appendix

Quantitation of 3-Hydroxy-N-methylpyridinium in Serum, Urine, and Bile

A series of standards, ranging from 50 to 400 ng of 3-hydroxy-N-methylpyridinium (MP) in 1 ml of serum, diluted urine, or bile, was prepared. To these standards, as well as to 1 ml of timed samples from the dog, a fixed amount of edrophonium chloride (internal standard) was added. The *p*H of each sample was adjusted to 4.5 with 4 N acetic acid, and both MP and edrophonium were extracted by methods described elsewhere.[7] The extracted samples were analyzed by an ion-exchange liquid chromatographic technique using an SCX cation exchange column (25 × 0.46 cm, Brownlee). The mobile phase was acetonitrile and 0.06 M sodium sulfate in sulphuric acid (*p*H 2.0) in a ratio of 16:84 (v/v); the flow rate was 2.5 ml/min. Both MP and edrophonium were monitored by a fixed wavelength ultraviolet detector (214 nm, LDC III 1203); ratios of the peak heights were used for quantitation. A calibration curve covering the standard concentrations was determined by weighted linear regression analysis. Concentrations of timed samples were calculated from the slope of the regression curve.

Absolute recoveries of MP and edrophonium were 50% and 80%, respectively. Linearity was demonstrated over the range of standards. The within-run precision was 9.6%, 8.1%, 5.0%, and 4.2% (coefficient of variation) at 50, 100, 200, and 400 ng/ml (six determinations per concentration), respectively.

References

1. Ferguson A, Egerszegi P, Bevan DR: Neostigmine, pyridostigmine, and edrophonium as antagonists of pancuronium. ANESTHESIOLOGY 53:390–394, 1980
2. Morris RB, Cronnelly R, Miller RD, Stanski DR, Fahey MR: Pharmacokinetics of edrophonium and neostigmine when antagonizing *d*-tubocurarine neuromuscular blockade in man. ANESTHESIOLOGY 54:399–402, 1981
3. Cronnelly R, Morris RB, Miller RD: Edrophonium: duration of action and atropine requirement in humans during halothane anesthesia. ANESTHESIOLOGY 57:261–266, 1982
4. Koelle GB: Anticholinesterase agents, The Pharmacological Basis

of Therapeutics. Edited by Goodman LS, Gilman A. New York, MacMillan, 1975, pp 445–466

5. Pantuck EJ, Pantuck CB: Cholinesterases and anticholinesterases, Muscle Relaxants. Edited by Katz RL. New York, American Elsevier Publishing, 1975, pp 143–163
6. Zar JH: Biostatistical Analysis. Englewood Cliffs, Prentice-Hall, 1974, pp 133–137, 151–155, 228–234
7. De Ruyter MGM, Cronnelly R: Reversed-phase, ion-pair liquid chromatography of quaternary ammonium compounds: Determination of pyridostigmine, neostigmine and edrophonium in biological fluids. J Chromatogr 183:193–201, 1980
8. Dixon WJ, Brown HB: Biomedical Computer Programs P-Series. Berkeley, University of California Press, 1979, pp 464–483
9. Boxenbaum HG, Riegelman S, Elashoff RM: Statistical estimation in pharmacokinetics. J Pharmacokinet Biopharm 2:123–148, 1974
10. Gibaldi M, Perrier D: Pharmacokinetics. New York, Marcel Dekker, 1975, pp 89–95, 175–180
11. Randall LO, Lehman G: Pharmacological properties of some neostigmine analogues. J Pharmacol Exp Ther 99:16–32, 1950
12. Barber HE, Calvey TN, Muir KT: The relationship between the pharmacolainestics, cholinesterase inhibition and facilitation of twitch tension of the quaternary drugs, neostigmine, anticholinesterase, pyridostigmine, edrophonium and 3-hydroxy-phenyltrimethylammonium. Br J Pharmacol 66:525–530, 1979
13. Hobbiger F: The mechanism of anticurare action of certain neostigmine analogues. Br J Pharmacol 7:223–236, 1952
14. Wilson IB: The interaction of tensilon and neostigmine with acetylcholinesterase. Arch Int Pharmacodyn 109:204–213, 1955
15. Blaber LC, Bowman WC: A comparison between the effects of edrophonium and choline in the skeletal muscle of the cat. Br J Pharmacol 14:456–466, 1959
16. Blaber LC, Christ DD: The action of facilitatory drugs on the isolated tenuissimus muscle of the cat. Int J Neuropharmacol 6:473–484, 1967
17. Blaber LC: The mechanism of the facilitatory action of edrophonium in cat skeletal muscle. Br J Pharmacol 46:498–507, 1972
18. Kitz RJ: The chemistry of anticholinesterase activity. Acta Anaesthesiol Scand 8:197–218, 1964
19. Wilson IB, Harrison MA, Ginsburg S: Carbamyl derivatives of acetylcholinesterase. J Biol Chem 236:1498–1500, 1961
20. Kitz RJ: Human tissue cholinesterases: Rates of recovery after inhibition by neostigmine; Michaelis-Menton constants. Biochem Pharmacol 13:1275–1282, 1964
21. Roberts JB, Thomas BH, Wilson A: Metabolism of [^{14}C]-neostigmine in the rat. Br J Pharmacol 25:763–770, 1965
22. Birtley RDN, Roberts JB, Thomas BH, Wilson A: Excretion and metabolism of [^{14}C]-pyridostigmine in the rat. Br J Pharmacol 26:393–402, 1966