
5 Pharmacokinetics and Pharmacodynamics of Carbamates under Physical Stress

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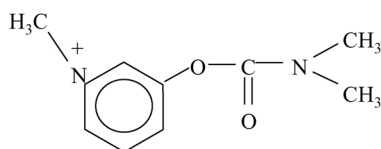
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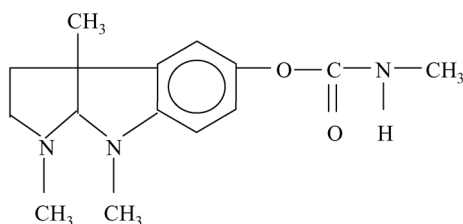
I. INTRODUCTION

Carbamates (reversible cholinesterase inhibitors) are potential pretreatment agents against nerve gas poisoning. This chapter discusses the pharmacokinetics and pharmacodynamics of pyridostigmine, physostigmine, and neostigmine in human beings and various animal species, under normal, disease states, and stressful conditions. The chemical structures of these carbamates, pyridostigmine, physostigmine, and neostigmine, are given in Figure 5.1.

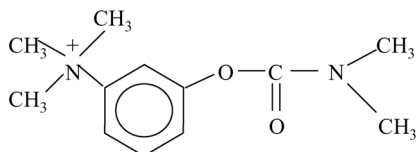
Pyridostigmine (Mestinon), the N,N-dimethyl-carbamate of 3-hydroxy-N-Methyl pyridinium, was first synthesized in 1946 by R. Urban.¹ It is a quaternary ammonium compound and dispensed as a bromide salt, pyridostigmine bromide (PB). PB is a reversible anticholinesterase drug most frequently used in the treatment of patients with myasthenia gravis. It has been proposed as a pretreatment drug against nerve agent poisoning.² The hypothesis is well established that short-lasting AChE inhibitor drugs (PB and physostigmine) protect the ChE enzyme against inactivation by nerve agents.²⁻⁴



PYRIDOSTIGMINE



PHYSOSTIGMINE



NEOSTIGMINE

FIGURE 5.1 Chemical structures of carbamates: pyridostigmine, physostigmine, and neostigmine.

The carbamate anticholinesterases such as PB bind reversibly with ChE enzyme, yet spontaneously reactivate relatively rapidly. However, nerve agents (organophosphate compounds) bind with the ChE irreversibly and form a much more stable phosphorylated enzyme (ChE-OP) complex. PB binds to peripheral ChE at anionic and esteratic sites and thus carbamylates the enzyme. The carbamylated enzyme sites cannot bind with nerve agents. In the meantime, some of the nerve agents are hydrolyzed to inactive metabolites by nonspecific hydrolases. The decarbamylation of the ChE takes place at the alcohol moiety on the esteratic site, regenerating the ChE enzyme to sustain life.

Physostigmine (also called eserine) is an alkaloid obtained from the leguminous plant Calabar or ordeal bean—the dried, ripe seed of *Physostigma Venenosum* Balfour, a perennial plant in tropical West Africa. The main alkaloid was first isolated from the seeds of the Calabar bean in a pure form in 1864 by Jobst and Hesse, who called it physostigmine.⁵ One year later, it was obtained in a crystalline form by Vee and LeVen, who called it eserine.⁶ Physostigmine (PHY) is the first anticholinesterase agent known to man and is used in the treatment of atropine-induced intoxication.

Neostigmine (also called prostigmine) was first synthesized by Aeschlimann and Reinert in 1931.⁷ Among the quaternary nitrogen compounds synthesized, they found that the dimethyl carbamate ester of 3-oxy-phenyl-trimethyl ammonium (neostigmine) was one of the most active compounds, and had actions similar to physostigmine. This agent has been employed in the treatment of myasthenia gravis. It is also used to reverse the neuromuscular blockade caused by anesthetic agents.

II. PYRIDOSTIGMINE BROMIDE

A. GENERAL ASPECTS

Pyridostigmine is a close congener of neostigmine, with a longer duration of action and fewer muscarinic effects. Pyridostigmine acts by competing with ACh for its binding site on the enzyme acetylcholinesterase (AChE). Thus, PB interferes with the enzymatic destruction of ACh, potentiating the action of ACh on both the skeletal muscle (nicotinic effect) and gastrointestinal tract (muscarinic effect). The symptoms associated with PB intoxication are tremors, diarrhea, hypersalivation, abdominal cramps, muscle weakness, fatigue, blurred vision, fasciculations, and urinary incontinence.^{8–10} PB was used as a pretreatment drug to protect soldiers in the event of nerve gas exposure during the Persian Gulf War. Many war veterans complained of various side effects ascribed to PB use and about half of the total veterans during the Gulf War complained of PB side effects.^{11–13} These veterans received a 30 mg oral dose of PB three times a day for 2 weeks. This dose was used because it was suggested to be a symptom-free dose from experimental studies and is intended to produce 30–40% AChE inhibition. The most common symptoms reported by the veterans included effects on the gastrointestinal and genitourinary systems.¹¹ These symptoms are likely to be enhanced by other stress factors including physical stress. Subchronic oral doses of PB (0.5–60 mg/kg/day) administered for 13 weeks in rats showed toxicity (tremors and ChE inhibition) above the 5 mg/kg/day dose.¹⁴ Pyridostigmine administered i.v. to dogs showed initial cardiopulmonary toxicity and

ChE inhibition at 2 and 5 mg/kg/day doses.¹⁵ These and other side effects are related to dose, route of administration, and the disposition of drug from the body.

B. ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

ABSORPTION: Absorption of PB, a quaternary ammonium compound, appears to be poor and erratic from the gastrointestinal tract after oral administration. This is because, being a polar compound, PB passes poorly across biological membranes. Hence much larger doses are required for the pharmacological effect by the oral route as compared to the parenteral routes. A study reported that the oral dose required to produce an effect with PB is 30 times that of the i.v. dose producing the same effect.¹⁶ After oral administration of PB, the onset of action occurs after 30 to 45 min, and the duration of action is approximately 3 to 6 h. Oral bioavailability of PB is affected by inter-individual differences in gastrointestinal absorption, peristalsis, and possible differences in metabolism in the gastrointestinal tract and liver. These factors are likely to be of importance in relation to the symptoms experienced by the Gulf War veterans who received PB.

The absorption and bioavailability of PB have been evaluated in studies, both in healthy subjects and in patients of myasthenia gravis.^{16–19} In healthy subjects, who received 60 mg oral PB, the oral bioavailability ranged from 11.5 to 18.9%, and maximum plasma levels were attained between 1.5 and 5 h after dosing.¹⁷ The results suggested that PB absorption occurs at a slower rate than its elimination, and this may be affected further by prior food consumption. Although ingestion of food with PB delays the time to reach peak plasma concentration by about 90 min, the extent of absorption of PB though is not affected.²⁰ It has been suggested that the considerable variations in daily dosage requirements in myasthenic patients may be due to inter-individual differences in disease severity and in absorption or metabolism of PB.¹⁶ In patients, oral PB (dose range of 30 to 240 mg) administered over a period of 7 to 22 h, resulted in an oral bioavailability of 3.6%. In another study, the bioavailability of PB was found to be 7%.²¹ Malabsorption with orally administered PB was also found to occur in patients with myasthenia gravis.¹⁹ This may be responsible for inadequate disease control in these patients. The authors suggest that this malabsorption may be the result of PB-induced alterations in the gastrointestinal epithelium. The pharmacokinetics of absorption are given in the following section and also in [Table 5.1](#).

DISTRIBUTION: The distribution of ¹⁴C-pyridostigmine was studied by Birtley et al.²² Ten per cent of the i.m. dose was present in the alimentary tract within 1 h after injection and 0.3% of the dose is secreted in the bile. A high concentration of radioactivity occurred in the kidney when excretion in the urine was at its maximum level. Lower concentrations were present in the liver, intestinal contents, heart, blood, and muscle. Radioactivity was also detected in the lungs, spleen, and skin, but not in the brain, thymus gland, intestinal wall, or body fat. The detection of radioactive respiratory CO₂ suggested that to a small extent pyridostigmine may be metabolized by another route. The serum concentration was dose-dependent and was correlated with the clinical response.²³ A radio-immunoassay (RIA) method was developed to determine the plasma concentration time profiles and tissue distribution of PB in rat following its i.m. administration.²⁴ This study found that PB had a half-life (t_{1/2}) of 25

min and was not detectable in plasma 6 h following its administration. Studies with ^{14}C -pyridostigmine have shown that PB gets trapped in various tissue compartments.^{20, 25} It is suggested that the uptake of PB into the liver and kidney is concentration dependent and is responsible for its metabolism and elimination. The steady-state volume of distribution (V_d) of PB is relatively small (0.3–0.7 l/kg), suggesting its limited distribution to the muscle and other organs/tissues.²⁵ The mean plasma $t_{1/2}$ after oral PB was 200 min, while after i.v. infusion of 4 mg, the $t_{1/2}$ was 97 min.¹⁷ It has been reported that neither PB nor its metabolite 3-hydroxy-N-methyl-pyridinium is bound to plasma proteins.^{20, 26} In order to find the distribution and retention of radioactivity of PB in the body, ^{14}C -pyridostigmine (463 μg , 1.78 $\mu\text{mol}/2.2 \mu\text{Ci/kg}$) was administered s.c. twice a day for up to 16 days. Four rats were sacrificed on days 1, 4, 8, 12, and 16. Tissues such as ear, eye, heart, kidney, liver, lung, muscle, skin, sternum, and tail were analyzed. The results indicated the consequent increase in radioactivity per g of tissues from day 1 to day 16; cartilaginous tissues particularly accumulated, increasing concentrations with subsequent doses of pyridostigmine. This increase in radioactivity in the body tissues after chronic dosage was indicative of its binding to macromolecules, such as negatively charged chondroitin sulfate (unpublished data, Somani 1983). However, the study did not determine the radioactivity in brain tissue. Recently, ^{11}C labeled PB was administered to mice and its accumulation was measured in brain tissues.²⁷ The study documented no difference in the brain radioactivity between swim exercise-stressed mice and controls. Obviously, there was no change in blood brain barrier permeability and this may be related to variables such as age, strain, or dose of PB.²⁸ It is quite possible that stress may alter the expression of CNS AChE. However, it would be important to re-examine the effect of physical stress (exercise training/swim exercise) on permeability of quaternary ammonium compound through blood/brain compartments. It would also be necessary to study the possible distribution of radioactivity in brain regions after single and chronic dosages of ^{14}C -labeled PB under normal and stressful conditions.

METABOLISM: Metabolism of ^{14}C -pyridostigmine in myasthenic patients was studied by Kornfeld et al. and Somani et al.^{29, 30} Liver seems to be the main site of metabolism of pyridostigmine.²² We have shown that PB is metabolized to 3-hydroxy-N-methyl pyridinium (3-HNMP) in man, (Figure 5.2),³⁰ and both the parent drug and metabolite seem to accumulate in the muscles after chronic administration of the drug to rats.³¹ The pharmacological properties of 3-HNMP are largely unknown other than that it is less toxic than 3-hydroxy-phenyl-trimethyl ammonium, a metabolite of neostigmine.³² The authors reported that the LD_{50} of these metabolites in mice after s.c. injection were 1350 mg/kg and 100 mg/kg, respectively.

Metabolism and excretion of PB after multiple dosing was studied in albino Sprague-Dawley rats by Somani.³¹ ^{14}C -pyridostigmine (463 μg , 1.78 $\mu\text{mol}/2.2 \mu\text{Ci/kg}$) was administered s.c. twice a day for 16 days. At the end of the sixteenth day, rats were found to have gained weight from 30 to 45%. Urine and feces were collected every 24 h and the radioactivity was measured in the excreta. The proportion of unchanged PB, 3-HNMP, and other metabolites were determined by paper chromatography in urine samples. The daily excretion of PB in urine ranged from 75–81% as an unchanged drug and that of 3-HNMP from 15–20% and unidentified

TABLE 5.1
Pharmacokinetics of Pyridostigmine in Humans and Animals

| Species | Dose | Route of Admin. | Peak Plasma Conc. or AUC | $t_{1/2}$ | Vd | Cl | F | Ref |
|------------------------|-----------------|-----------------|--|--------------------|----------------------|---------------------------------|----------------------|-----|
| Healthy Human | 4 mg | i.v. | 6.7 ± 2.1 mg/ml \times min (AUC) | 97 min | 1.03 ± 0.35 l/kg | 0.63 ± 0.20 l/kg | | 17 |
| Healthy Human | 60 mg | Oral | 20–100 mg/l | 200 min | | | 14.3% (11.5–18.9) | 17 |
| Myasthenic Patients | 60 mg | Oral | 40–60 mg/l | 30–90 min | 0.5–1.7 | | 10% | 20 |
| Myasthenic Patients | 180–1440 mg/day | Oral | 180 mg/l | 1.5 h | 0.65 l/kg | 0.29 ± 0.30 l/h/kg | 3.6% (2.9–4.2) | 16 |
| Myasthenic Patients | 60–540 mg/day | Oral | 12.4–64.5 mg/ml | | | | | |
| Myasthenic Patients | 240–1080 mg/day | | 15.3–144.0 mg/ml | | | | | |
| Healthy Male | 2.5 mg | i.v. | | 1.52 h | 1.43 l/kg | 0.65 l/kg \times h | | 21 |
| | 120 mg | Oral | 40–60 mg/l | 1.78 ± 0.24 hr | $1.64 \pm .29$ l/kg | 0.66 ± 0.22 l/kg \times h | $7.6 \pm 2.4\%$ | |
| Human (normal patient) | 0.35 mg/kg | i.v. | 700–1800 mg/ml and after 3–4 h 40–60 mg/ml | 112 ± 12 min | 1.1 ± 0.3 l/kg | 9 ± 2 ml/kg/min | | 43 |
| Anephric patient | 0.35 mg/kg | i.v. | | 379 ± 162 min | | 2 ± 0.6 ml/kg/min | | 43 |
| Myasthenic Patients | | | | | | 349–481 ml/min | | 160 |
| Elderly Patient | 0.25 mg/kg | i.v. | | 157 ± 56 min | 1.4 ± 0.4 l/kg | 6.7 ± 2.2 ml/kg/min | | 105 |

| Species | Dose | Route of Admin. | Peak Plasma Conc. or AUC | $t_{1/2}$ | Vd | Cl | F | Ref. |
|----------------------|-----------------------------------|-----------------|--|-------------------------------|--|----------------------------|----------|------|
| Young Patient | 0.25 mg/kg | i.v. | | 140±60 min | 1.8±0.7 l/kg | 9.5±2.7 ml/kg/min | | 105 |
| Human Volunteers | 60 mg | Oral | 0.28±0.18 mg/h/mo Absorption rate constant 0.23/h | Elimination rate constant 2/h | | 6.65±2.4 ml/min/kg (renal) | | 26 |
| Myasthenic Patient | 60–120 mg | Oral | 588–4560 mg/ml | | | | | 39 |
| Healthy Human Male | 30 mg/daily every 8 h for 21 days | Oral | 311±120 l/h | | | 221±13.4 l/hr | 12% | 44 |
| Healthy Human Female | | Oral | Absorption rate constant 0.32±0.02/h | | | 172±11.4 l/h | 12% | 44 |
| Human Male | 3.65 mg/70 kg | i.v. | | 22.79 min | 247–834 ml/kg | 9.3–26.5 ml/min/kg | | 40 |
| Myasthenic Patient | 420–2100 mg daily | Oral | 15–60 mg/l | Time to peak conc. 2 h | | | | 19 |
| Myasthenic Patient | 5–6 mg | i.v. | 61–80 h.ng/ml | 1.05±0.32 h | 1.76±.54 l/kg | 1.0±0.01 l/h | | 18 |
| Dog | 0.6 mg/kg | i.v. | | 8.3 hr±2.1 h | Lambda 2 8.7±1.9 l/kg Steady state 3.9±0.9 l/kg | 13.0±1.0 ml/min/kg | | 42 |
| Dog | | Oral | | | | | 33.6±9.5 | |
| Rat | 0.056 mg/kg | i.m. | 1010 mg × min per ml | 24.8 min | 1.97 l/kg | | | 41 |
| Rat | 0.5–2.0 μmol/kg | i.v. | | 24.2±4.2 min | 0.35±0.05 l/kg | 15.0±0.2 ml/min/kg | | 25 |

Note: Bioavailability (F) value is the fraction of the dose that reaches the systemic circulation. $t_{1/2}$ is the plasma half-life; Vd is the steady-state volume of distribution. AUC refers to the area under the plasma concentration vs. time curve, and Cl refers to the clearance.

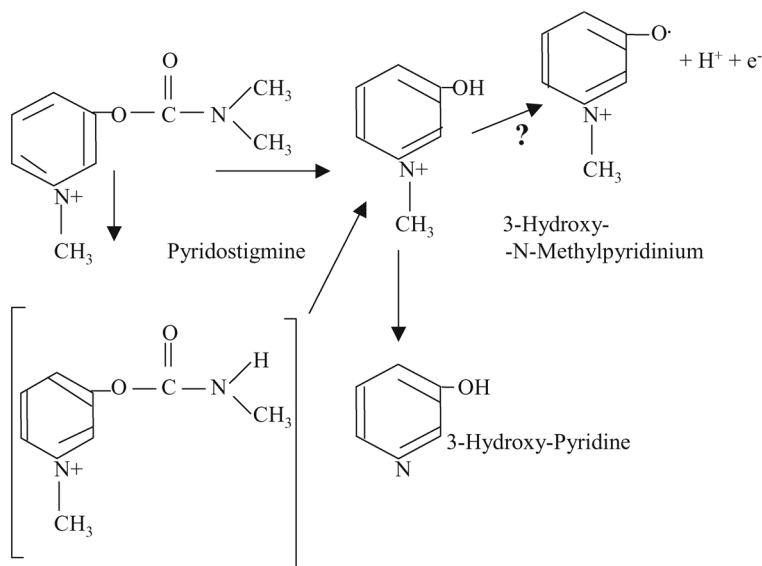


FIGURE 5.2 Metabolic pathway of pyridostigmine showing possible formation of reactive metabolite under physical stress.

metabolite (1–4%) as percent of daily dose. There was no consistent increase or decrease in the excretion of PB and its metabolites during the whole study time, suggesting that there was no stimulation or inhibition of metabolism. The elimination of radioactivity in feces ranged from 3–10% as a percent of daily dose. The average amount of PB in the body at steady state ranged from 4.83–8.77:g, corresponding to 7–12% of the administered dose. These studies suggest that PB may accumulate in the body after multiple dosing. The metabolite 3-HNMP did not form glucuronide or sulfate conjugates *in vivo*, in isolated liver perfusion, and in isolated liver microsomal studies.³¹ A recent report on pyridostigmine by Dr. Beatrice A. Golomb of the Rand Corporation incorrectly mentioned the formation of glucuronide of 3-hydroxy-N-methyl pyridinium.³³ However, 3-hydroxy-N-trimethyl-phenyl ammonium, a metabolite of neostigmine formed the glucuronide conjugate in *in vivo* and *in vitro* studies.^{34,35} This is the first study to show that the quaternary ammonium compound, despite being highly polar, formed glucuronide conjugate.

EXCRETION: Earlier work on disposition of PB reported that 50% of the dose rapidly appeared in the urine after a s.c. injection of pyridostigmine (3 mg/kg) in the dog, and a total of 67% of the dose in 24 h.³⁶ There was no further urinary excretion of the drug by this route of administration. However, after oral administration of pyridostigmine (7.5 mg/kg) to the dog, the unchanged drug was excreted in the urine up to 36 h, at which time 57% of the dose had been recovered. The rapid excretion of ¹⁴C-pyridostigmine radioactivity in hen was blocked by prior administration of a dye, Cyanine 863, indicating PB was secreted by the renal tubules in the hen and probably also in the rat and man.²² The maximum excretion of radioactivity occurred

between 1 and 3 h after oral administration of single doses of ^{14}C -pyridostigmine to the rat.³⁷ In 24 h, 42% of the dose was excreted in the urine, and 38.4% was present in the feces and intestinal contents. The peak concentration of radioactivity in liver and blood occurred about 2 h after administration and about 75% of the radioactivity in the urine was present as unchanged pyridostigmine, the remainder as metabolites. The main route of excretion of PB is via the kidneys; renal clearance occurs both by glomerular filtration and tubular secretion.²⁰

C. PHARMACOKINETICS OF PYRIDOSTIGMINE

The pharmacokinetics of pyridostigmine in healthy human volunteers, myasthenic patients, and in animal species such as dogs and rats are enumerated in Table 5.1. The numerical data on pharmacokinetic parameters, such as $t_{1/2}$ bioavailability (F), area under the plasma concentration vs. time curve (AUC), volume of distribution (Vd), and clearance (Cl) after i.v. and oral administration to humans and animals, are summarized in this table.

Pyridostigmine bromide is eliminated from plasma in a biexponential manner.³⁸ There is a direct linear relationship between the area under plasma concentration time curve and total daily dose of pyridostigmine in myasthenic patients which indicates linear pharmacokinetic modeling.³⁹ Calvey et al.⁴⁰ studied the pharmacokinetics of ^{14}C -pyridostigmine based on a two-compartment model after i.v. administration to myasthenic patients. The fast disposition half-life of pyridostigmine ranged from 0.61–1.78 min and the terminal half-life from 14.81–37.01 min (mean half-life = 22.79 min).⁴⁰ Pyridostigmine clearance (9.3–26.5 ml/min/kg) was invariably greater than the presumptive value for glomerular filtration rate, and the volume of distribution of the drug ranged from 0.25–0.83 l/kg. The steady state kinetics of PB in myasthenic patients indicated that the routine measurement of plasma pyridostigmine concentration had little to offer in the management of myasthenic patients.¹⁶ These investigators reported a relatively stable kinetic parameter of PB in and between individual patients. The maximal effect of pyridostigmine with a “bell shaped” dose response curve occurred at a concentration of 30–60 ng/ml in plasma of 20 myasthenic patients (both male and female).¹⁸ The pharmacokinetics of PB were determined in rats after intramuscular administration in the dose of 0.056 $\mu\text{g/kg}$.⁴¹ The maximum plasma concentration (C_{max}) was found to be 21.3 $\mu\text{g/ml}$ and the time to reach C_{max} was approximately 9 min. PB pharmacokinetics were determined in beagle dogs after i.v. infusion and oral doses of syrup and tablet, all in the dose of 0.6 $\mu\text{g/kg}$.⁴² PB had a relatively long terminal $t_{1/2}$ of 8.3 h and a high Vd of 8.7 l/kg. The drug showed affinity for the peripheral tissues, as evidenced by a 4-fold higher residence time in tissues, as compared to plasma. The renal clearance of PB in volunteers under the influence of ranidine and pirenzepine was studied.²⁶ Its renal clearance average was about 74% due to tubular secretion. In an earlier similar study, it was shown that the renal route accounts for 75% of the pyridostigmine clearance in anesthetized patients.⁴³ PB has a short $t_{1/2}$, 1.0 h after i.v. and 3.7 h after oral administration.¹⁷ Recently Marino et al.⁴⁴ carried out the studies to determine the time course of plasma PB concentration and RBC AChE activity after administration of 30 mg PB three times a day in healthy human subjects. They correlated the plasma

concentration of pyridostigmine with RBC AChE activity and determined pharmacokinetic parameters using NONMEN-IV version 2.1. This study was population-based and carried out in 90 male and female volunteers. This study showed that pharmacokinetics of PB are both gender and weight dependent and the pharmacodynamic effects did not lag significantly from plasma concentration and returned to near normal within 8 h.

Table 5.2 depicts the differences in the pharmacokinetics of PB within and among different species. Some of the human data indicated that the Vd of pyridostigmine being a quaternary amine is higher. Although these pharmacokinetic data are under normal conditions, there is no literature on pharmacokinetic data under stressful

conditions such as under heavy military duty, mild or strenuous exercise, or after swim exercise. It is imperative to assume that exercise or stressful conditions might alter pharmacokinetics of pyridostigmine which in turn will alter the pharmacodynamics of this drug.⁴⁵ This is an area which needs further exploration especially in light of the use of PB as a pretreatment drug against possible threat from exposure to nerve gases.

D. PHARMACODYNAMICS OF PYRIDOSTIGMINE BROMIDE: USE AS A PRETREATMENT DRUG

Pharmacodynamic actions of PB were studied as early as 1946 by Koster³ and Koelle.² Anticholinesterase activity of PB was about one-fifth of the activity of neostigmine;⁴⁶ whereas, the comparative activity reported by Blaschko et al.⁴⁷ was about one-tenth. One of the most noticeable differences between neostigmine and pyridostigmine is the inability of the latter compound to produce a direct stimulant action on smooth muscle either *in vitro*,⁴⁶ or *in vivo*.⁴⁸ It has been suggested that this may account for the occurrence of fewer unpleasant side effects when pyridostigmine is used clinically.^{49–51} Foldes and Smith⁵² reported maximum inhibition of butylcholinesterase with 7×10^{-9} M PB at 1 h.

Pyridostigmine bromide was employed as a pretreatment drug against possible threat of the nerve agent sarin by soldiers during the Persian Gulf War. It has been suggested that the effectiveness of pyridostigmine pretreatment is due to the carbamylation of a portion of tissue AChE that protects it against irreversible inhibition by sarin. Spontaneous decarbamylation of PB produces sufficient free AChE to restore normal function.⁵³ The pharmacodynamics (cholinesterase inhibition) in RBC and plasma of humans and animal species following pyridostigmine administration are depicted in Table 5.2. Pyridostigmine pretreatment reversed the neuromuscular blockade produced by sarin. The rate of recovery was similar in rhesus monkey, cats, and rabbits, suggesting a common mechanism of action. The effectiveness of pyridostigmine pretreatment in nonhuman primates (marmosets and rhesus monkeys) on sarin poisoning was assessed.⁵⁴ PB produced a dose-related blood AChE inhibition in these animal species. The time to peak carbamylation occurred within 10–20 min of i.v. dosing of PB. PB pretreatment supported by therapy with atropine protected the primates against sarin poisoning. Hence, these authors suggested that pyridostigmine pretreatment could be effective in humans. The relationship between reversible

TABLE 5.2

Time Course of Pyridostigmine Bromide (PB) Action (Cholinesterase [ChE] Activity: Percent of Control) in Plasma and RBC in Various Animal Species and Humans, under Normal Conditions

| Species | Dose (mg/kg) | ChE Activity—% of Control | | | Ref. |
|---------------|--|---------------------------|---------|------------------|------|
| | | Plasma | RBC | Time after PB | |
| Mouse | 0.20 (p.o.) | | 70 | 60 min | 64 |
| | 0.82 (p.o.) | | 40 | 60 min | |
| Rat | 39.2 mg/ml | | 72 | Day 1 | 69 |
| | 0.50:l/h | | 74 | Day 4 | |
| | (s.c.) | | 70 | Day 8 | |
| | for 14 days | | 80 | Day 14 | |
| | | | (blood) | | |
| Rat | 5 | | 102 | 18 h | 14 |
| Male | 15 | | 57 | | |
| | 30 | | 66 | | |
| | 60 | | 51 | | |
| | (p.o.) for 13 weeks | | | | |
| Rat Female | 5 | | 95 | 18 h | 14 |
| | 15 | | 81 | | |
| | 30 | | 58 | | |
| | 60 | | 71 | | |
| | (p.o.) for 13 weeks | | | | |
| Rat | 0.075 | | 94 | 5 min | 63 |
| | (i.m.) | | 54 | 20 min | |
| | | | 70 | 35 min | |
| | | | 88 | 50 min | |
| | | | (blood) | | |
| Rat | 20 | 25 | 20 | 3 h | 57 |
| | 40 | 50 | 30 | 3 h | |
| | 80 | 20 | 20 | 2 h | |
| | (p.o.) | 50 | 30 | 4 h | |
| Rat | 90/day | | 13 | Day 1 | 58 |
| | (p.o.) | | 17 | Day 2 | |
| | for 15 days | | 25 | Day 4 | |
| | | | 26 | Day 7 | |
| | | | 10 | Day 15 | |
| Rat | 12 ml/h (low dose) (s.c. infusion) for 14 days | | 68 | Day 6 | 161 |
| | | | 52 | Day 7 | |
| | | | 60 | Day 14 | |
| | | | 95 | 7 days post-A | |
| | 60 ml/h (high dose) (s.c. infusion) for 14 days | | 25 | Day 6 | |
| | | | 30 | Day 8 | |
| | | | 32 | Day 14 | |
| | | | 100 | 7 days post-A | |

continued

TABLE 5.2 (continued)

| Species | Dose (mg/kg) | ChE Activity—% of Control | | Ref. |
|------------|------------------------------|---------------------------|------------------|------|
| | | Plasma | Time after PB | |
| Rat | 2.0 (i.p.) | | 95 | 56 |
| | | | 115 | |
| | | | 125 | |
| | | | 100 | |
| | 5.0 (infusion) | | 68 | |
| | | | 72 | |
| | | | 100 | |
| | | | 90 | |
| | 25.0 (infusion) | | 67 | |
| | | | 50 | |
| | | | 57 | |
| | | | 47 | |
| Rat | 0.2 | | 70 | 55 |
| | 0.025 | | 80 | |
| | 0.010 | | 90 | |
| | (i.m.) | | | |
| Guinea Pig | 0.10 | | 51 | 53 |
| | (i.v.) | | (blood) | |
| Guinea Pig | 0.47 | | 70 | 64 |
| | 1.9 | | 40 | |
| | (p.o.) | | | |
| Guinea Pig | 0.94 | | 92 | 162 |
| | | | 74 | |
| | | | 53 | |
| | | | 45 | |
| | | | 50 | |
| | | | 57 | |
| Guinea Pig | 0.2 | | 30 | 55 |
| | 0.025 | | 55 | |
| | 0.010 | | 70 | |
| | (i.m.) | | | |
| Guinea Pig | 0.05 μmol/kg (i.v.) | | 28 | 66 |
| | | | 25 | |
| | | | 29 | |
| Marmoset | 0.20 | | 39 | 54 |
| | (i.v.) | | (blood) | |
| Dog | 0.5 | | 80 | 15 |
| | | | 80 | |
| | | | 80 | |
| | 2.0 | | 55 | |
| | | | 58 | |
| | | | 58 | |
| | 5.0 | | 50 | |
| | | | 50 | |
| | | | 56 | |
| | i.v. infusion over 15 min | | (blood) | |

TABLE 5.2 (continued)

| Species | Dose (mg/kg) | ChE Activity—% of Control | | | Ref. |
|-----------------------------|--|---------------------------|----------------------------|--|------|
| | | Plasma | RBC | Time after PB | |
| Monkey | 0.2 (i.v.) | | 46 (blood) | 15 min | 54 |
| Monkey | 0.25 | 80 | | Mean readings over 5 days | 62 |
| | 0.50 | 70 | | | |
| | 1.0 | 40 | | | |
| | s.c. infusion over 5 days | (serum) | | | |
| Monkey | 0.12 | 66.6 | | 30 min | 59 |
| | 0.24 | 46.5 | | | |
| | 0.48 | 29.7 | | | |
| | 0.96 | 16.9 | | | |
| | (s.c.) | (serum) | | | |
| Monkey | 40 mg/day, 5 days of wk for 10 wk | | 54 49 69 80 92 | 2 h 4 h 8 h 16 h 24 h | 61 |
| Hen | 5/day for 2 months | | 17 | | 103 |
| Human | 30 mg every 8 h (p.o.) for 3 weeks | | 90 77 78 91 | 1 h 2 h 4 h 8 h | 44 |
| Human | 30 mg every 8 h (p.o.) for 10 days | | 77 77 101 (blood) | 6th day 9th day 5th day post Rx | 60 |
| Human | 630 mg (p.o.) | 46 (serum) | | 50 min | 8 |
| Human | 30 mg every 8 hours (p.o.) for 7 days | | 64 59 61 59 | Day 1 Day 3 Day 5 Day 7 | 88 |
| Human (Healthy Males) | 90 mg/day Wk. 3 & 4 on M, T, W only (p.o.) | | 72 69 68 | Day 1 Day 2 Day 3 | 86 |

ChE inhibition by pyridostigmine at different doses and its efficacy against soman toxicity was studied in rats and guinea pigs. The study concluded that ChE inhibition may provide some protection against soman poisoning by as low as 10%.⁵⁵

The effects of pyridostigmine (2, 5, and 25 mg/kg, i.p.) on RBC cholinesterase and skeletal muscle contraction were studied in rats.⁵⁶ The high dose (25 mg/kg)

significantly inhibited ChE activity at different times; however, the study did not show a correlation between the ChE inhibition and decrease in muscle contraction. The acute toxicity of pyridostigmine at three oral doses (20, 40, and 80 mg/kg) produced acute focal necrosis, leukocytic infiltration, and marked changes in the motor end-plates of skeletal muscle of rat. The changes were more prominent in the diaphragm than the quadriceps muscle. The whole blood and RBC cholinesterase (ChE) activity was reduced to considerably less than one-half the normal value.⁵⁷

Oral administration of pyridostigmine for 13 weeks in male and female Sprague-Dawley rats caused tremors and significant RBC ChE inhibition (51 to 81% of control) at 15 mg/kg dosage and higher, indicating toxicity.¹⁴ However, no toxic effects were observed with 5 mg/kg pyridostigmine dose. This study also noted differences in ChE inhibition between male and female rats. At different doses of PB, the effects of PB (90 mg/kg/day) in diet for 15 days on blood ChE activity and myofiber morphology in diaphragm of rats was evaluated.⁵⁸ Electron microscopy showed maximal changes in the post-synaptic areas of the neuro-muscular junction of diaphragm. It was observed that subchronic PB induced primarily myopathic changes in rat diaphragm; however, some mechanism of adaptation seems to be activated that minimizes skeletal muscle injury 1 week after stoppage of pyridostigmine.

Different s.c. doses of pyridostigmine in primates indicated that serum cholinesterase activity dose-dependently decreased 30 min after pyridostigmine administration but did not significantly alter performance.⁵⁹ These investigators concluded that pyridostigmine is a very safe pretreatment drug for nerve agent poisoning. The increasing doses of pyridostigmine promptly and progressively lowered the AChE activity of blood to a minimum of 40% of control at a 5 mg/kg dose in beagle dogs.¹⁵ With higher doses (5 mg/kg), the cardiac output was unchanged; however, airway resistance increased significantly. The lowest dose (0.5 mg/kg) produced minimal effects on the cardiovascular and respiratory systems. These authors hypothesized that PB in low doses would cause little or no adverse effects in normal humans when used as a protective agent. Acute oral pyridostigmine overdose (390–900 mg) in nine patients during the Persian Gulf War showed serum cholinesterase activity inhibition 21 to 75% of control, 20–90 min after pyridostigmine ingestion. The data indicated that serum ChE inhibition was a reliable diagnostic tool in pyridostigmine poisoning; however, no correlation between the extent of ChE inhibition and the incidence or severity of the cholinergic signs and symptoms was found.⁸ The possible detrimental effects of oral pyridostigmine 30 mg three times a day on human neuromuscular function was assessed.⁶⁰ Muscle strength and endurance were tested before and after treatment; electro-diagnostic tests such as EMS nerve conduction and response to repetitive stimulation were also carried out before and after treatment (8th day). It was shown that pyridostigmine causing 20–30% ChE inhibition in healthy young men produced no significant neuromuscular adverse effects in humans.

The efficacy of orally administered pyridostigmine syrup, when used as a pretreatment for rhesus monkeys exposed to sarin and treated with antidotes such as atropine sulfate, the oxime TMB-4 and the anticholinergic agent benactyzine hydrochloride, was evaluated.⁶¹ The authors showed that oral PB treatment followed

by antidotal therapy was effective in protecting rhesus monkeys against repeated exposure to lethal concentrations of sarin. The protective period of oral pyridostigmine supported by the antidotal therapy was between $\frac{1}{2}$ and 8 h. In another study, the continuous infusion of PB producing 30 and 60% of normal serum ChE inhibition provided protection against the behavioral toxicity induced by five daily repeated low doses of soman in monkeys.⁶²

The efficacy of pyridostigmine pretreatment at symptom-free doses was studied at various times (5, 20, 35, and 50 min) prior to exposure to sarin in rats. Significant inhibition of whole blood and lung cholinesterase activity occurred 20 min after pyridostigmine administration, suggesting this to be the optimal time for protection against sarin inhalation toxicity.⁶³ The effects of PB pretreatment on antidotal efficacy of atropine and 2-PAM in sarin, tabun, and VX poisoning in mice and guinea pigs has been evaluated. Further the oxime-induced reactivation of VX-inhibited whole blood AChE of guinea pigs was studied.⁶⁴ This study showed that 1 h prior to organophosphate exposure, pyridostigmine induced 30 and 60% inhibition of RBC cholinesterase activity at 0.47 and 1.9 mg/kg oral doses in guinea pigs and 0.20 and 0.82 mg/kg in mice. The data also showed that PB significantly enhanced the efficacy of antidotes atropine and 2-PAM against tabun in both animal species, whereas it reduced or did not increase the efficacy of these antidotes against sarin or VX in both species. Pretreatment with PB also reduced significantly the recovery of VX-inhibited AChE activity by 2-PAM. In a previous report in male rhesus monkeys, it was found that the combination of PB pretreatment and prompt post-treatment with atropine and 2-PAM chloride resulted in greatly improved protection (protective ratio >40, compared to control) against soman intoxication.⁶⁵ The effect of pyridostigmine pretreatment on cardiorespiratory function in tabun poisoning was evaluated in guinea pigs.⁶⁶ The study found significant inhibition of RBC cholinesterase at different times after intravenous administration of pyridostigmine. The investigators concluded that pyridostigmine enhanced circulatory depression, decreased survival time and rate in tabun poisoned animals. Lintern et al.⁶⁷ have demonstrated that repeated administration of pyridostigmine (0.4 μ moles/kg, s.c.) twice a day for 3 weeks altered the molecular forms of AChE activity in the soleus, extensor digitorum longus (EDL), and diaphragm muscle of mice.

Pyridostigmine pretreatment was used by the Gulf War veterans to obtain 20–30% cholinesterase inhibition in order to enhance the efficacy of the standard therapeutic regimen for possible nerve gas intoxication.^{11,12} Pyridostigmine reversibly inhibits cholinesterase (60–70% of control) in the peripheral tissues and blood when given in symptom-free doses in primates and rodents 15–30 min after dosing.^{62,68} A symptom-free dose of pyridostigmine for 14 days caused inhibition of whole blood cholinesterase (70–80% of control) in rats.⁶⁹ Somani et al.⁷⁰ have demonstrated that, under resting conditions, pyridostigmine in a symptom-free dose (1.2 mg/kg, p.o.) for 14 days inhibited plasma BChE activity (87% of control) in mice 4 weeks after the last dose of pyridostigmine indicating the delayed effect of the drug. Thus, subchronic exposure to pyridostigmine might have influenced the *de novo* synthesis of enzyme. BChE is a nonspecific choline-ester hydrolase which is synthesized in the liver.⁷¹ Moreover, data of Somani et al. further show that

pyridostigmine did not significantly alter AChE activity in blood cells (RBC and platelets) indicating that most of the pyridostigmine or its metabolites interacted with plasma cholinesterase which is the scavenger of the anticholinesterase agents.⁷⁰ Pyridostigmine 30 mg every 8 h produced RBC cholinesterase inhibition greater than 10% at the time of trough in approximately 70% of individuals.⁴⁴ These investigators based their estimations using the pharmacodynamic model that best fit RBC AChE activity using an E_{\max} value compared to baseline. Pharmacodynamic effects and the toxicity of pyridostigmine have clearly shown the importance of dose, time of administration, and the time course of disposition (pharmacokinetics), which could determine the protective efficacy of PB against nerve gas poisoning.

E. FACTORS INFLUENCING PHARMACOKINETICS AND PHARMACODYNAMICS OF PYRIDOSTIGMINE BROMIDE

1. Stress

The effects of physical stress on the disposition and pharmacokinetics of drug is not well recognized. Recently, Somani and Kamemori⁴⁵ have reviewed the effect of exercise on absorption, distribution, metabolism, and excretion of drugs and chemicals. During exercise, cardiac output increases with the intensity of workload and concomitant changes in regional blood flow distribution occurs. Thus, blood flow to skeletal muscles and skin is greatly increased, while, on the other hand, hepatic blood flow decreases during exercise.⁷² Therefore, the clearance of drugs that are primarily eliminated by liver metabolism may be impaired due to decrease in hepatic blood flow.^{73,74} Hepatic clearance is the product of hepatic blood flow and the hepatic extraction ratio. The decrease in hepatic blood flow could theoretically result in a diminished clearance of drug or chemical, thereby resulting in the body's accumulation of the drug and metabolites during chronic administration. This increased concentration could cause potentially detrimental effects of the drug or chemical. Several studies with PB under stressful conditions have been carried out by various investigators and are summarized in [Table 5.3](#).

The effect of ChE inhibition induced by pyridostigmine pretreatment on endurance, thermoregulation, and pathophysiology during exercise in a hot environment was studied.⁷⁵ As a result of exercise, after PB administration, no change in ChE inhibition was observed due to the hot environment and exercise, after PB administration. However, the endurance time for pyridostigmine treated animals was only 23 min compared to 35 min for the control animals. It was concluded that intense ChE inhibition induced by pyridostigmine administration severely limited the endurance capacity of rats working in the heat.⁷⁵ The effects of chronic oral PB treatment and a single i.m. atropine injection on thermoregulatory effector responses of patas monkeys was evaluated.⁷⁶ The study reported a 20–25% decrease in serum ChE activity with daily oral PB treatment; however, thermoregulatory or cardiovascular functions were not affected. The potential muscle damage produced by pyridostigmine pretreatment (14 days) when given alone or when combined with physical exercise was investigated in a mouse model.⁶⁹ It was found that only the combination of pyridostigmine plus physical exercise contributed to a loss of integrity in skeletal muscle,

TABLE 5.3
Time Course of Pyridostigmine Bromide (PB) Action (Cholinesterase [ChE] Activity: Percent of Control) in Plasma and RBC in Various Animal Species and Humans, under Stressful Conditions

| Species | Dose (mg/kg) | ChE Activity—% of Control | | | | Ref. |
|-----------------------------|---|---|---------------|-----------------|------------------|------|
| | | Stress as a Factor | Plasma | RBC | Time after PB | |
| Mouse | 1.2 (p.o.) 2 weeks | None | 87 | 96 | 4 weeks after | 70 |
| | | Exercise training | 79 | 103 | PYR treatment | |
| Rat | 0.6 (i.p.) | None | 36 | | 105 min | 75 |
| | | Heat (35°C) + Exercise | 38 | | 120 min | |
| Guinea Pig Male | 0.20 (s.c.) | Low Stress | | 35 | 2 h | 80 |
| | | Median Stress | | 35 | | |
| | | High Stress | | 31 | | |
| Monkey | 0.4 every 8 h (p.o.) for 7 days | None | 70 | | Day 1 | 76 |
| | | | 75 | | Day 7 | |
| | | Heat Stress | NC (serum) | | | |
| Human | 30 mg (p.o.) | None | | 61 | 150 min | 82 |
| | | Exercise (58% VO ₂ max) for 30 min | | NC | 150 min | |
| Human | 30 mg (p.o.) | None | | 33.4 | 110 min | 87 |
| | | Cold Stress | | 30.2 | 127 min | |
| Human | 60 mg 30 mg (p.o.) | Healthy | | 28.4 | 150 min | 83 |
| | | Mildly Asthmatic | | 76.7 (blood) | 150 min | |
| Human | 30 mg every 8 h (p.o.) | Healthy | | 80.7 | 9.6 h | 89 |
| | | Symptomatic | | 81.2 (blood) | 7.1 h | |
| Human | 30 mg (p.o.) | Heat (35° C) and Hypohydration | | 73.1 | 90 min | 84 |
| | | | | 66.9 | 160 min | |
| | | | | 69 | 220 min | |
| Human (Male Soldiers) | 30 mg every 8 h (p.o.) for 7 days | Heat Stress plus Exercise | | 76.3 | Day 1 | 85 |
| | | | | 72.9 | Day 3 | |
| | | | | 58.8 | Day 5 | |
| | | | | 65.7 | Day 7 | |
| | | | | | (2 h after PB) | |
| Human Volunteers | 30 mg × 4 at 8 h intervals (p.o.) | Normal | | 75.8 | 60 min | 81 |
| | | | | 64.4 | 120 min | |
| | | | | 67.2 | 240 min | |
| | | Heat-Exercise Stress | | | NC | |

as evidenced by increased CPK activity in serum and enhanced urinary creatinine excretion rate. A recent study that used an acute, much higher PB dose has demonstrated that forced swim exercise enhances brain AChE inhibition, AChE mRNA, and neuronal c-fos oncogene by pyridostigmine in mice.⁷⁷

The delayed and interactive effects of PB and exercise training on BChE, AChE, creatine phosphokinase (CPK), and malondialdehyde (MDA) in peripheral and cerebral tissues of mice were reported.⁷⁰ The mice were sacrificed 4 weeks after the last dose of PB or saline and 24 h after the last exercise. Blood, muscle, and nerve tissues were isolated and analyzed. BChE and AChE activity significantly decreased to 79% of control in plasma and 78% of control in triceps muscle of mice, respectively, in PB + exercise group. Creatine phosphokinase activity increased 122% of control in plasma in PB + exercise group indicating enhanced neuromuscular effect of combination. Malondialdehyde concentration (lipid peroxidation end product) significantly increased to 124% of control in triceps muscle in PB + exercise group indicating the oxidative stress of the combination. This study showed that the interactive and delayed effects of PB (even after 4 weeks of stoppage of dosage) and exercise training occurred primarily in peripheral tissues.

The delayed effects of PB and exercise training on muscle tension in the mouse lower extremity were studied.⁷⁸ This study reported the interactive effects of PB and exercise training on muscle tension elicited in mouse lower extremity anterior muscular compartment by dorsiflexion of the foot with stimulation of the peroneal nerve. Experiments on muscle tension were conducted 4 weeks after the last dose of PB or saline and 24 h after exercise training. The muscle tension was measured in right and left legs using a tension transduction device connected to a polygraph. There was a significant increase in the muscle tension of combined legs ($p < 0.05$) in the group treated with PB plus exercise as compared to control and exercise groups (Figure 5.3). A significant reduction in acetylcholinesterase activity ($p < 0.01$) was also observed in the triceps muscle in mice treated with PB plus exercise when compared to control and exercise groups. These results suggest that delayed effects of PB (even after 4 weeks of stoppage of dosage) and interaction with exercise training leads to a reduction in muscle AChE activity. This may be due to an increase in acetylcholine leading to increased muscle tension. Exercise in combination with pyridostigmine may lead to a loss of some muscle fibers, which may be compensated for by hypertrophy of the remaining fibers involving contractile proteins. These changes in triceps muscle proteins and muscle fiber profile can alter the muscle tension in mice. A recent study examined the relationship between intake of PB and handgrip strength in Gulf War veterans, in comparison to control humans.⁷⁹ The report showed that handgrip strength was negatively associated with age and female gender. Further, the data suggested no relationship between PB intake and postwar handgrip strength.

The AChE activity in different brain areas of heat-stressed guinea pigs was measured after administration of pyridostigmine for 2 h at temperatures of 41.5°C, 42.6°C, and 44.3°C. The study found no entry of pyridostigmine into cortex, striatum, and hippocampus following tritiated pyridostigmine administration and autoradiographic evaluation. The results indicated that heat stress did not induce pyridostigmine penetration into brain areas of guinea pigs.⁸⁰ Pyridostigmine inges-

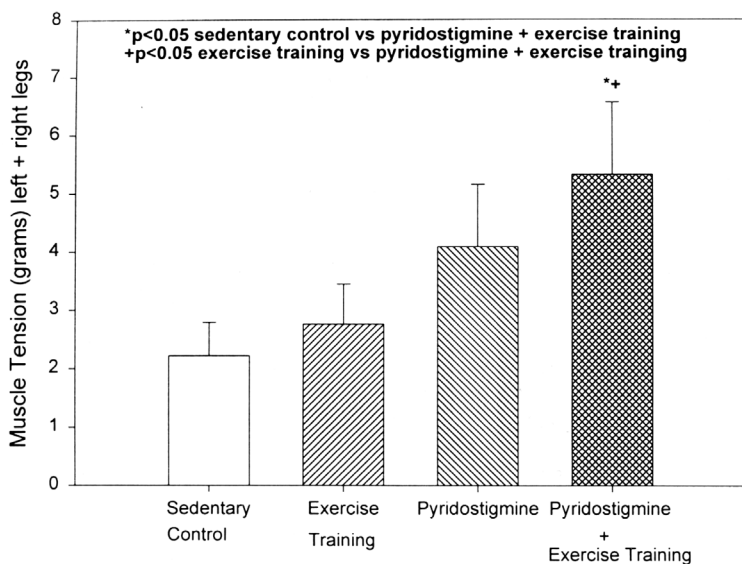


FIGURE 5.3 Muscle tension (gm) in mice ($n = 5$) in lower extremity anterior muscular compartment by dorsiflexion of left plus right legs with stimulation of peroneal nerve.

tion at a dose of 30 mg 8 hourly for 4 days resulted in significant inhibition of whole blood ChE activity; however, no significant differences were found between treatments on physiological responses and heat-balance parameters.⁸¹ The study concluded that pyridostigmine ingestion did not increase the physiological strain resulting from exercise stress in hot conditions.

Oral PB ingestion in human subjects who underwent exercise at high environmental temperature (36°C), reduced skin blood flow, which may limit exercise thermoregulation.⁸² The respiratory function in healthy and asthmatic volunteers following a single oral dose of pyridostigmine was evaluated.⁸³ The study found a correlation between ChE inhibition and forced expiratory volume in 1 s (FEV_1) at 60 mg oral dose in healthy subjects. Based on this result, 30 mg PB was suggested to be an appropriate dose for mild asthma patients producing similar level of ChE inhibition. The authors concluded that a certain threshold of ChE inhibition must be reached before the effect of pyridostigmine on respiratory function could be observed.⁸³ A few measurable physiological effects (responses to heat, exercise, and hypohydration) were studied after 30 mg oral pyridostigmine ingestion. Pyridostigmine had little significant and practical effect on human physical responses to moderate exercise-heat stress (35% VO_2 maximum at 35°C temperature and up to 75% relative humidity).⁸⁴

A double-blind study investigated the effects of multiple-dose oral PB on physiological responses to heat stress tests in a hot, dry environment (42°C and 20% relative humidity) in seven male soldiers.⁸⁵ The authors found that a 7 day oral PB course caused very mild effects on physiological responses in men undergoing moderate treadmill exercise in a hot environment.

The deleterious effects, if any, of pyridostigmine on acceleration tolerance or performance was evaluated in healthy male human volunteers.⁸⁶ These authors found that any significant decline in the optimum performance of normal and healthy aircrew receiving prophylactic doses of pyridostigmine during aerial combat was unlikely. The effects of oral pyridostigmine bromide (PB) on human thermoregulation during cold water immersions (20°C) was also investigated.⁸⁷ These authors concluded that a 30 mg oral dose of pyridostigmine did not increase individual susceptibility to hypothermia during cold water immersion. However, in combination with cold stress, pyridostigmine may result in marked abdominal cramping and limit cold tolerance. Acute and chronic oral ingestion of pyridostigmine (30 mg three times a day) by healthy human volunteers did not alter thermoregulatory or metabolic effects during moderate activity in cold climates.⁸⁸ The levels of cholinesterase inhibition did not correlate with the type or severity of symptoms in Gulf War veterans after oral ingestion of pyridostigmine.⁸⁹

Since Gulf War veterans underwent physical stress (exercise) and were exposed to pyridostigmine, it is possible that exercise could cause significant effects on pharmacokinetics and pharmacodynamics of PB under conditions that reasonably simulate heavy military duty. Based on the data from previous studies, we believe that physical exercise will increase the inhibition of ChE activity by PB after its administration. The stressful demands of modern military duty include a broad range of activities, especially during war time. The demanding physical tasks of a combat infantry soldier can be expected to result in significant physical and chemical changes within the body.⁹⁰ Notwithstanding this, physiological stress is still to be expected, because of the redistribution of blood flow to serve the demands of active muscle cells⁹¹ as well as to meet the needs of temperature regulation in the body. In addition, a considerable production of metabolic acids from substrate catabolism will lead to a marked reduction of the intracellular pH.^{92, 93} The time course of a drug in the body may be influenced by exercise dynamics.^{94, 95} Hence, it is important to investigate further how physical activity interacts with a drug that may potentially be administered under combat field conditions.

Physical stress increases the oxygen consumption in the body, generates reactive oxygen species in tissues, and exerts oxidative stress response.^{96, 97} The interaction of exercise and ethanol resulted in an inhibition of AChE in certain brain regions of rats and the enzyme inhibition was well correlated with enhanced lipid peroxidation in corresponding brain regions.^{98, 99} AChE is a membrane-bound enzyme with lipid dependence.¹⁰⁰ The present data show that interaction of pyridostigmine and exercise enhanced AChE inhibition and lipid peroxidation in triceps muscle of mice indicating an enhanced oxidative stress response of the combination. Earlier studies from our laboratory have also reported that physical stress enhanced the inhibition of cholinergic enzymes elicited by a centrally acting reversible cholinesterase inhibitor (physostigmine) in skeletal muscle of rats.¹⁰¹ The accumulation of pyridostigmine and metabolite 3-HNMP may likely be due to its existence in zwitter ionic form at the acidic pH of the muscle of mice that have undergone physical stress which can cause detrimental effects on skeletal muscle. Further studies are needed to confirm this phenomenon. A recent preliminary report on the neuro-endocrine-immune

effects of either PB and/or exercise training on treadmill for 14 days showed a significant decrease in plaque-forming cell response and altered splenic and thymic CD4/CD8 sub-populations following 40 and 60 min exercise and/or PB treatment to adult female mice. However, no effect was observed in lymphoproliferation or natural killer cell activity after either treatment. Administration of PB did not show any effect on thymic and splenic weights, but the physical stress resulted in a significant decrease in both spleen and thymus weight at 40 and 60 min exercise training protocols on treadmill.¹⁰²

Stress is an important factor that can alter the pharmacokinetic and pharmacodynamic effects of PB resulting in increased toxicity, and this area of research needs further investigation.

2. Environmental Exposures

Single and combined effects of pyridostigmine, DEET, and permethrin on plasma butyrylcholinesterase activity and lethality in hens was assessed.¹⁰³ It was found that pyridostigmine (5 mg/kg/day) for 2 months resulted in significant inhibition of plasma ChE activity in hens. The authors concluded that carbamylation of peripheral esterases by pyridostigmine reduces the hydrolysis of DEET and permethrin and increases their availability to the nervous system. The doses of pyridostigmine and chlorpyrifos and the duration of exposure used by Abou-Donia et al.¹⁰³ were approximately four times higher than what veterans used during the Persian Gulf War. These investigators had used pyridostigmine (5 mg/kg, p.o.) for 2 months, along with other chemicals, to show neurotoxic effects in hens.

3. Gender and Age

The effect of oral doses of pyridostigmine in different dosages (0.5, 15, 30, and 60 mg/kg/day) on RBC cholinesterase inhibition was evaluated in male and female Sprague-Dawley rats.¹⁴ The study showed differences in RBC cholinesterase inhibition between male and female rats at different dosages. The study suggested that male rats may be more sensitive to RBC cholinesterase inhibition, especially at doses of 15 mg/kg/day and higher.

We have recently studied the influence of physical stress (acute exercise) and PB on ChE activity in blood and brain regions of male (NIH Swiss) and female (C₃H-He/N-ve) mice.¹⁰⁴ This study examined the interaction of acute exercise and a single PB dose (2 mg/kg, p.o.) on ChE activity in blood and brain regions of these two different strains of male and female mice. PB significantly decreased BChE activity (61% of control) in male and (31% of control) in female mice. PB significantly decreased AChE activity in RBC (72% of control) in male and (61% of control) in female mice. The interaction of PB and exercise resulted in a significant inhibition of plasma BChE activity (58% of control), RBC AChE activity (72% of control), and cortical AChE activity (84% of control) in male mice. However, there was a significant inhibition of plasma BChE activity (37% of control), RBC AChE activity (51% of control) and cortical AChE activity (80% of control) in female mice. These results showed the differences in ChE activity in male and female, which are

differentially influenced by exercise. Physical stress seems to increase the permeability of PB in brain, thereby inhibiting cerebral AChE activity in both species.

The influence of age on the pharmacokinetics of PB has been evaluated in subjects under anesthesia and paralyzed with neuromuscular blockers.¹⁰⁵ The plasma concentrations of PB were determined by radioimmuno-assay and after 1 h were found to be greater in the elderly (71–85 years of age) compared to the younger patients (21–51 years of age). It was found that the plasma clearance of PB was significantly reduced in the elderly (6.7 ml/kg/min). The study concluded that the prolonged duration of action of pyridostigmine in the elderly is due to the reduced plasma clearance of the drug. The relationship between the pharmacokinetic parameters and variables such as gender and weight in response to PB was evaluated in healthy subjects.⁴⁴ The relation between oral clearance and gender was found to be significant. The clearance in men was found to be 221 l/h while that in women was found to be 172 l/h. On the other hand, the relationship between the pharmacodynamic parameters elimination rate constant from the effect compartment (K_{eo}) and concentration at steady state giving half maximal effect (EC_{50}) was not significant. There have been very few studies of pyridostigmine evaluating its pharmacokinetics and pharmacodynamics with respect to gender and age. These could be very significant especially with the use of pyridostigmine as a pretreatment under stressful conditions.

III. PHYSOSTIGMINE

A. GENERAL ASPECTS

Physostigmine (PHY) is one of the oldest drugs, isolated from Calabar beans and successfully used for the treatment of glaucoma in 1864. It gained further prominence due to its use in the clinical trials of Alzheimer's disease. Physostigmine is also a potent prophylactic antidote for organophosphate poisoning. It is a reversible cholinesterase inhibitor and has a short duration of action. Being a tertiary amine structurally, it is lipid soluble and hence crosses the blood-brain barrier readily to produce central actions.

B. PHARMACOKINETICS OF PHYSOSTIGMINE

The pharmacokinetics of PHY in rat showed a biexponential disappearance after i.v. dosage, suggesting a two-compartment model.¹⁰⁶ The half-life of distribution phase ($t_{1/2\beta} = 1.31$ min) of PHY suggests rapid equilibration of the drug with tissues. The half-life of elimination phase was found to be 16 min following i.v. administration and 17 min after i.m. administration in rat.¹⁰⁷ These half-lives are different from the half-lives of PHY in dog (30.7 ± 17.1 min),¹⁰⁸ in man (21.7 min),¹⁰⁹ and in guinea pig (40–50 min).¹¹⁰ The Vd of 1.36 l/kg is higher than the total body volume which is indicative of a sequestration of this drug in tissues. The Vd in the dog and man was also higher than body water. Studies with radioactive 3H-PHY have shown that about

30–40% of the radioactivity (RA) was irreversibly bound to liver after i.v. or i.m. administration.¹¹¹ The clearance of $62 \text{ ml min}^{-1} \text{ kg}^{-1}$ in rat was higher than the dog ($41 \text{ ml min}^{-1} \text{ kg}^{-1}$) and man ($22 \text{ ml min}^{-1} \text{ kg}^{-1}$). The high clearance in rat may be related to increased metabolism of PHY in this species. Hepatic clearance plays a major role in the elimination of PHY. Physostigmine excretion in urine is less than 4% in 24 h after a single dose of i.m. administration.¹¹² In comparison to renal excretion, biliary excretion plays a greater role in that about 27% of the dose is excreted in bile of the rat.¹¹² The pharmacokinetics of physostigmine in Alzheimer patients showed an elimination half-life of 20–30 min.¹¹³ Clearance and volume of distribution were 7.7 ± 0.9 (SE) l/min and 2.4 ± 0.6 (SE) l/kg, respectively. Butyrylcholinesterase inhibition half-life was 83.7 ± 5.2 (SE) minutes. During sustained steady-state infusion, plasma physostigmine concentration ($r = 0.95$) and butyrylcholinesterase inhibition ($r = 0.99$) were linearly correlated with the dose.¹¹⁴

The absorption rate constant (K_a) and the elimination rate constant (K_e) of PHY after oral administration are 0.1 ± 0.07 and 0.036 ± 0.024 , respectively. $C_{p_{\max}}$ and t_{\max} are 3.3 ng/ml and 16 min. The clearance of PHY is $80.95 \text{ ml min}^{-1} \text{ kg}^{-1}$. The bioavailability (F) value, the fraction of the dose that reaches the systemic circulation, is 0.02. The lack of parent drug in systemic circulation and low AUC ($160.57 \text{ ng min}^{-1} \text{ kg}^{-1}$) and high extraction ratio (0.98) after oral administration strongly suggest “first pass” effect.¹¹⁵

The half-life of PHY in the liver and the muscle was found to be 24 and 20 min, respectively, after i.v. administration, whereas the studies after i.m. administration gave a half-life of 26 min in the liver. The elimination rate constant for liver (K_l) and muscle (K_m) after i.v. administration was found to be 0.0288 and 0.0351 min^{-1} , respectively, and after i.m., K_l was 0.027 min^{-1} for the liver.¹¹¹

The half-life of PHY in rat brain was 11 min. PHY is rapidly concentrated in the rat's brain. The drug, or metabolite, appears to be concentrated in mitochondria in greater amounts by a mechanism other than simple diffusion. The effect of the drug on mitochondrial function are not known, nor is it known how these effects are related to the toxicity of PHY in humans.¹¹⁶

Plasma protein binding studies are important in determining drug distribution and excretion. The Scatchard plot for the binding of PHY to rat plasma and rat serum albumin resulted in negative slope.¹¹⁷ Somani et al.¹¹⁸ reported that the binding of PHY to plasma proteins decreases in the presence of quinidine, furosemide, acetaminophen, theophylline, and verapamil. The binding of $[3H]$ physostigmine to crystallized human serum albumin was investigated using equilibrium dialysis. The percentage bound to 1% (w/v) human serum albumin decreased from 18 to 4% as the total concentration of physostigmine increased from 3.3 nM to 2.7 μM (0.9 to 750 ng ml⁻¹). A single class of specific binding sites with a large affinity constant, $K = 8 \times 10(7) \text{ l mol}^{-1}$, was identified.¹¹⁹

The distribution and metabolism of PHY in the body determines its duration of action. After i.m. administration, the time course of PHY indicates that PHY is metabolized rapidly in plasma and comparatively slowly in the brain. PHY is distributed in all tissues and sequestered in the liver. Distribution studies showed that the

radioactivity per gram of tissue was highest in kidney and liver, whereas the percentage of the administered dose in terms of radioactivity was maximum in muscle, followed by liver.^{106,107} Lukey et al.¹¹⁰ studied the pharmacokinetics of PHY in guinea pigs following i.m. doses of 5, 27, and 146 $\mu\text{g}/\text{kg}$. Plasma PHY concentrations were analyzed by HPLC and it was found that the peak concentration of the drug was reached at 30 min for all doses. There was a linear relationship between the PHY dose and AUC and C_{max} . Therefore, i.m. PHY administration to guinea pigs resulted in rapid absorption, distribution, and elimination and showed linear pharmacokinetics.

A physiological model for physostigmine disposition was developed in the rat which incorporated anatomical, physiological, and biochemical parameters, i.e., tissue volume, plasma flow rates, drug metabolism, and tissue-to-plasma partition coefficients.¹²⁰ Predicted concentrations of physostigmine in different tissue compartments were consistent with the experimental observations in the rat following an i.v. dose. Part of this study also compared the time course changes in measured effect, as percentage change in cholinesterase activity in brain and related these changes to the plasma or brain drug level in either a combined pharmacokinetic-pharmacodynamic (plasma physostigmine-effect relationship) or a dynamic model (brain physostigmine-effect relationship).

One of the major factors that determines the duration and intensity of the pharmacological activity of a drug is the rate and pattern of its metabolism. Little is known about the fate of PHY in the body. Although PHY has been in use for more than a century, to date there is still no data available on its metabolism and mechanism of toxicity other than from excessive cholinergic activity. PHY is metabolized to eseroline and three other metabolites (M_1 , M_2 , and M_3) that have not been identified.¹¹¹ This reference showed that about 90% of the drug reaching the liver is metabolized within 5 min indicating the importance of hepatic elimination of this drug. The nature of the metabolites and their possible toxic effects have not been studied. The liver sequestered radioactivity after i.m. or i.v. administration of ^3H -PHY.^{106,107} When PHY is administered to humans by the oral route, it is anticipated that most of the drug will be metabolized as soon as it reaches the liver. PHY is hydrolyzed to eseroline, which has a phenyl hydroxyl group and is capable of forming conjugates with endogenous substrates. However, eseroline could be further oxidized to catechol and then to quinone (rubreserine type).¹²¹ Such metabolites are highly reactive and can act as strong electrophiles (Figure 5.4). These electrophiles can form covalent conjugates with nucleophilic amine and sulfhydryl groups of cysteine and glutathione. The metabolism of physostigmine was studied by its incubation with the microsomal fraction of mouse liver. The metabolites formed were separated by reversed-phase ion-pair liquid chromatography and detected amperometrically by dual electrodes. Two major and six minor metabolites were found. Retention times and electrochemical characteristics were studied for these and compared with the hydrolyzed products of physostigmine: eseroline and rubreserine. None of the major metabolites was identical with these standards.¹²²

The time course of subcellular distribution of radioactivity in rat brain after i.v. administration of ^3H -PHY was studied.¹¹⁶ The concentration of radioactivity was

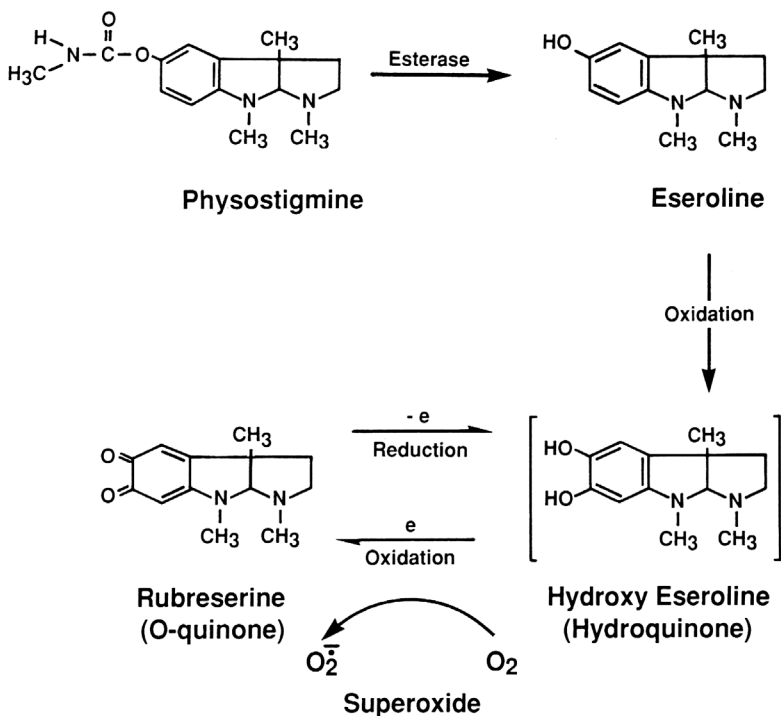


FIGURE 5.4 Possible metabolic pathway of physostigmine. (Adapted from Somani et al.¹²³)

higher in mitochondrial fraction and continuously increased from 5–60 min. The amount of radioactivity in synaptosome and microsomes increased up to 30 min and then declined at 60 min. Irreversible binding of xenobiotics to proteins can result in toxicity. The “covalent binding” is an experimental parameter that serves as an index of the formation of highly reactive metabolites that are difficult to measure by other means. “Reactive” metabolites can possibly reduce molecular oxygen to superoxide anion, which can in turn produce highly reactive singlet oxygen, and then to hydrogen peroxide and hydroxyl radical.

PHY is metabolized to eseroline which is further hydroxylated to form catechol and its oxidative product rubreserine (o-quinone). Eseroline causes damage to neuronal cells.¹²³ In another investigation, the changes in antioxidant enzymes were studied in brain regions in response to chronic infusion of PHY (34.5 $\mu\text{g/kg/hr}$) in rats that were sacrificed at the end of days 1, 7, and 12 of infusion. PHY infusion increased superoxide dismutase (SOD) activity in brain stem (122 and 123% of control) and in striatum (119 and 117% of control) on days 7 and 12, respectively. PHY infusion depressed catalase activity in the brain stem, while glutathione peroxidase activity increased in the brain stem (153 and 151% of control) and in cortex (114 and 138% of control) on days 7 and 12 of PHY infusion, respectively. This study suggests

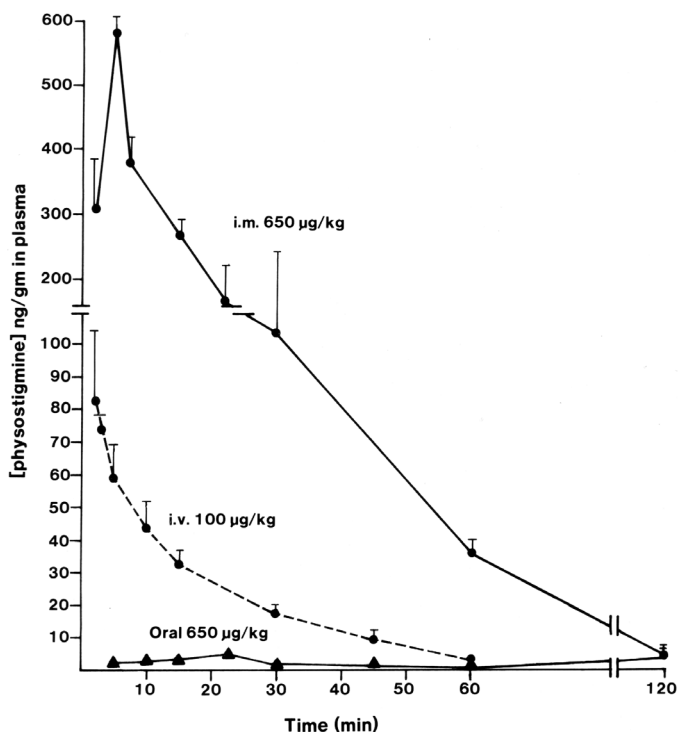


FIGURE 5.5A Time course of plasma and brain physostigmine concentration and corresponding plasma BChE and brain AChE inhibition (% of control) after different routes of administration: (A) time course plasma physostigmine concentration.

that PHY and its metabolites influenced the antioxidant enzyme activity selectively in different brain regions possibly as a compensatory mechanism of electrophilic stress of PHY metabolites.

Time course of PHY concentration in plasma and brain was compared after 650 µg/kg dose i.m. and oral and 100 µg/kg after i.v. administration as shown in Figure 5.5. BuChE activity in plasma and AChE activity in brain was also compared after these doses. The figure shows the pharmacokinetic and pharmacodynamic effects of PHY. PHY does not reach an effective concentration in the brain after oral administration because of its first-pass effect. However, it is an effective pretreatment drug after i.v. and i.m. routes of administration.

C. PHARMACODYNAMICS OF PHYSOSTIGMINE

PHY is a short-acting anticholinesterase agent and could potentially be utilized as a prophylactic agent against OP intoxication as it reversibly inhibits a portion of tissue cholinesterase, thereby preventing phosphorylation and aging of this enzyme by organophosphates.^{2, 124–126} ChE is an important parameter to monitor the efficacy of PHY in OP intoxication.

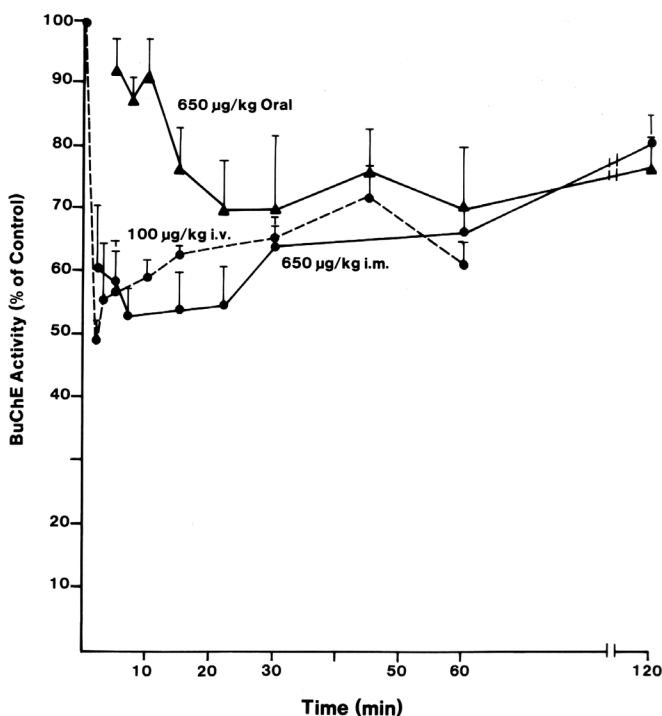


FIGURE 5.5B (B) time course of plasma BChE activity (% of control).

Earlier studies showed that PHY afforded protection against several lethal doses of DFP. The increased toxicity of PHY following the previous administration of DFP is to be expected due to the fact that a large portion of the tissue cholinesterase would be inactivated at the time when PHY was given. As a result, only a small amount of cholinesterase would have to be inactivated by PHY to produce death.¹²⁷ It is conceivable that the protective action exerted by PHY when injected prior to DFP probably results from the reversible combination of PHY with the active groups of the cholinesterase molecules, thereby blocking access to DFP and the subsequent formation of an irreversible ChE-OP complex. During the time necessary for the dissociation of the PHY-cholinesterase complex, part of the uncombined DFP would be excreted or hydrolyzed and the decarbamylated cholinesterase would then resume its physiological function. Therefore, the hypothesis was well established that short-lasting anticholinesterase drugs (carbamates) may protect ChE enzymes against inactivation by irreversible anticholinesterase (organophosphates).^{2,4} This type of protective action is of interest in many respects, but particularly with a view to developing drugs which are potentially effective against poisoning by nerve agents.^{3, 54, 125, 128–130}

Physostigmine has been used as a prophylactic treatment regimen to antagonize the toxic effects of soman in animals.^{126, 131–136} Studies done on the protection afforded by the carbamate AChE inhibitors generally used survival as an end point. Solana et al.¹³⁷

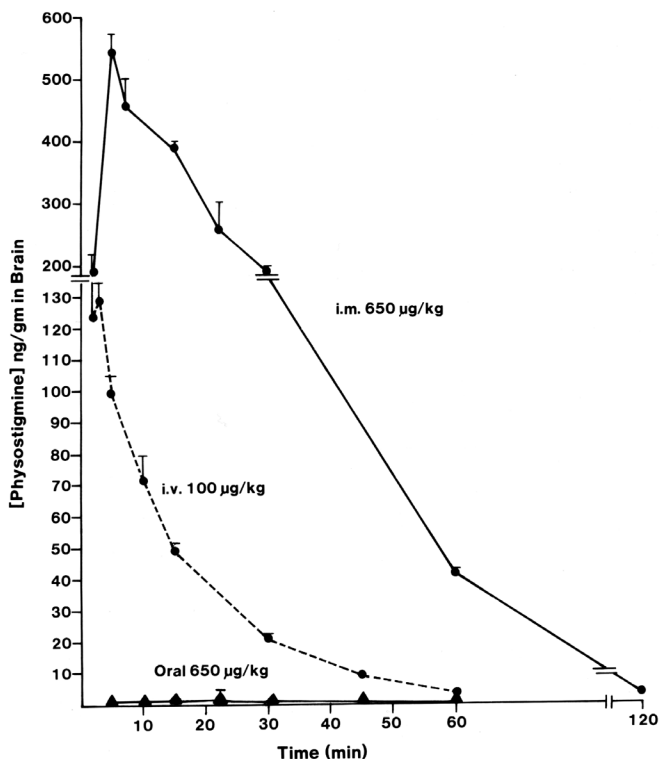


FIGURE 5.5C (C) Time course of brain physostigmine concentration.

evaluated the combination pretreatments PHY/pyridostigmine in guinea pigs challenged with 2 LD₅₀ of soman. Both carbamates contributed to blood AChE inhibition. However, PHY alone seems to protect the ChE inhibition by soman as much as the protection by optimal dose of combination. The pretreatment regimen against soman, sarin, and VX intoxication in guinea pigs was studied by Lennox et al.¹³⁸ These regimens include PHY and an adjunct: apophen, atropine, azapophen, benactyzine, benterpine, scopolamine, or trihexyphenidyl. These investigators reported that several regimens were effective against several organophosphates. PHY subacute in conjunction with acute adjunct (scopolamine or trihexyphenidyl) is effective as pretreatment against 5LD₅₀ of soman and 2 LD₅₀ of VX in guinea pigs.¹³⁹

Harris et al.¹⁴⁰ administered sustained-release PHY (0.4, 10, or 50 mg/ml) to rat at a rate of 2.5 µl/h for 28 days. The blood AChE was inhibited about 11, 42, and 66% corresponding to the above rates, respectively. These PHY dosages did not decrease the performance of rat on an accelerating rotarod. Harris et al.¹⁴⁰ suggested that “in a pretreatment mode, 42–66% inhibition of AChE by sustained exposure to PHY, with an acute dose of cholinolytic, would suffice to protect against lethality and motor performance decrement by a toxic level of soman.

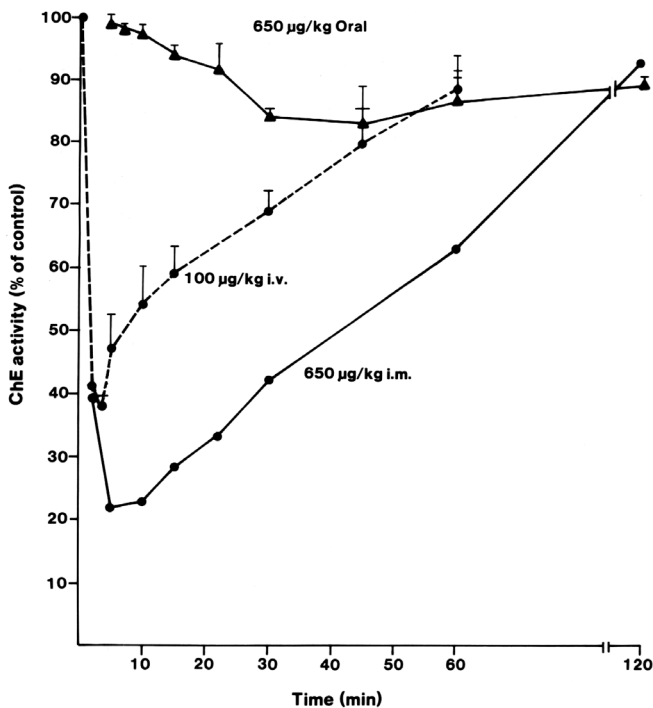


FIGURE 5.5D (D) time course of brain AChE activity (% of control).

D. INFLUENCE OF PHYSICAL STRESS ON PHARMACOKINETICS AND PHARMACODYNAMICS

This section discusses the observation that exercise alters the pharmacokinetic and pharmacodynamic parameters of PHY, a flow-limited drug. Exercise increases cardiac output but diverts blood flow away from the liver^{73,141} and could decrease the clearance of drugs, particularly those flow-limited drugs which are hepatic extractable, such as propranolol.^{142,143} PHY is highly extracted by liver and its clearance may be dependent on hepatic blood flow.¹⁴⁴ A decrease in liver blood flow due to exercise will decrease the amount of PHY reaching the parenchymal cells, which in turn reduces the metabolism of PHY, thereby decreasing the clearance of PHY and increasing the area under the curve and $t_{1/2}$. The pharmacokinetics and disposition of flow-limited drugs are more likely to be affected by exercise, whereas the pharmacokinetics and disposition of capacity-limited drugs which are strongly bound and poorly extracted are less likely to be influenced by exercise.¹⁴⁴ Exercise also causes a plasma shift¹⁴⁵ which results in a decrease in plasma volume and a change in the volume of distribution.

The time course of PHY distribution is different in tissues in trained exercise rats compared to control rats.¹⁰⁶ Training exercise altered the time taken by different tissues to reach peak concentration of the drug plus metabolites. These results showed

that the radioactivity of PHY + metabolites was higher as percent of control in brain (133%), liver (126%), heart (191%), kidney (385%), lung (106%), and muscle (180%) at 2 min post exercise in endurance-trained rats. However, radioactivity in trained rats declined below control at 5 min post exercise in kidney, muscle, brain, heart, and lung; whereas, in the liver, radioactivity declined below control at 15 min post exercise. The amount of total radioactivity in the different tissues reveals the distribution of PHY and its metabolite's affinity to different tissues. The highest amount of radioactivity accumulates in liver when compared to other tissues. Peak concentrations of RA were observed in 2 min time in heart and lung—the organs of very high blood flow. It seems the distribution of RA was dependent on blood flow. Peak concentration of RA was observed in brain at the 5-min time point and decreased within 30 min. Muscle showed peak concentration of RA only after 15 min. The blood flow to different organs changes with intensity of exercise. During exercise the arterioles in muscle will inflate, and during cessation of exercise these arterioles will return to normal condition, which will not allow higher blood flow from arterioles, thereby helping to sequester the drug in muscle mass. In plasma, RA showed a decreasing trend from the beginning. The half lives of RA in trained vs. control rats were for brain, 18 vs. 20 min; liver, 25 vs. 35 min; heart, 31 vs. 26 min; kidney, 30 vs. 28 min; lung, 26.5 vs. 30 min; and muscle, 45 vs. 31 min. There was no significant difference in $t_{1/2}$ except muscle and liver. Exercise influences the profile of distribution of RA in all tissues and pharmacokinetics of PHY. It appears that these influences may be due to the flux of blood flow after the cessation of exercise, severity of exercise, pH changes due to lactic acid production, ionization of the drug, lipid solubility, and other undetermined factors.

Acute exercise increases behavioral sensitivity of PHY.¹⁴⁶ Carbamate-induced decrease in performance has been shown to be restored with diazepam and atropine.¹⁴⁷ The combined effect of physical exercise and physostigmine on AChE activity in different tissues of rat has been extensively studied by Somani and co-workers.¹⁴⁸ Matthew and co-workers¹⁴⁹ have studied the acute and chronic administration of physostigmine on ChE inhibition and performance (endurance) of exercising rats. Acute physostigmine administration in exercising rats resulted in an inhibition of blood ChE and a reduction in endurance (performance); whereas, chronic administration attenuated the decrease in ChE activity and the endurance of exercising rats. These studies suggest that decreases in performance, caused by acute drug administration, may be attenuated through accommodation with chronic administration. Dube et al.¹⁵⁰ reported the interactive effects of physostigmine and exercise on cholinesterase activity in RBC and tissues of rat. The results indicate that in control rats not given physostigmine, different intensities of acute exercise affect the cholinesterase enzyme to a moderate degree in red blood cells and heart without affecting brain, diaphragm, and thigh muscles. Acute exercise modifies the effect of physostigmine by increasing the cholinesterase inhibition in red blood cells and brain without affecting other tissues.

The central and peripheral responses of rats were altered due to the interactive effect of acute exercise and endurance training in the presence of physostigmine. The data indicated that endurance training delayed ChE recovery; however, there was almost complete recovery in rats given acute exercise plus physostigmine and slower

recovery in endurance training plus physostigmine as compared to physostigmine alone. Physostigmine's rate of decarbamylation of cholinesterase enzyme (K_d) due to acute and/or trained exercise in brain, heart, diaphragm, and muscle of rat have been studied.¹⁵¹ Acute exercise + PHY increased, whereas endurance training + PHY decreased ChE activity in brain, red blood cells, and various tissues as compared to PHY alone. The results shown in Table 5.4 suggested that acute exercise and endurance training have opposite effects on K_d after PHY administration.

PHY and exercise have significant effects on the synthetic (ChAT) and degradative (AChE) enzymes of acetylcholine in active EDL muscle. Exercise has prolonged the inhibitory effect of PHY on ChAT and AChE activities both in active EDL and passive soleus muscles.¹⁰¹ The interactive effect of PHY and concurrent acute exercise resulted in a slight decrease in ChE activity in the brain.¹⁵⁰ The interaction of exercise and subacute PHY decreased AChE activity in both corpus striatum and hippocampus after PHY, as well as PHY plus acute or trained exercise. AChE activity in cerebral cortex was inhibited by PHY plus exercise, (acute or trained). AChE activity decreased in the brain stem in all groups except in PHY plus acute or trained exercise rats.¹⁴⁸ The study indicated that PHY, exercise, or the combination of both decreased AChE activity in a regionally selective pattern. The data are consistent with the hypothesis that elevation in ACh levels down-regulates the ongoing cholinergic neurotransmission through a negative feedback mechanism.

TABLE 5.4
Effect of Acute or Trained Exercise on Rate of Decarbamylation (K_d)—in min^{-1} of ChE in RBC and Tissues of Rat

| GROUP Treatment | | IV PHY | V AE + PHY | VI ET + PHY |
|--------------------|------------------------|-----------|---------------|----------------|
| RBC | $K_d \text{ min}^{-1}$ | 0.021 | 0.024 | — |
| | r | 0.93 | 0.95 | — |
| | $t_{1/2} \text{ min}$ | 33.5 | 29.0 | — |
| Brain | $K_d \text{ min}^{-1}$ | 0.014 | 0.0252 | 0.009 |
| | r | 0.97 | 0.90 | 0.91 |
| | $t_{1/2} \text{ min}$ | 50.0 | 27.5 | 75.0 |
| Heart | $K_d \text{ min}^{-1}$ | 0.019 | — | 0.008 |
| | r | 0.95 | — | 0.98 |
| | $t_{1/2} \text{ min}$ | 37.5 | — | 85.0 |
| Diaphragm | $K_d \text{ min}^{-1}$ | 0.01 | 0.039 | .008 |
| | r | 0.97 | 0.99 | 0.98 |
| | $t_{1/2} \text{ min}$ | 67.5 | 17.5 | 84.0 |
| Muscle | $K_d \text{ min}^{-1}$ | 0.012 | 0.008 | 0.012 |
| | r | 0.91 | 0.89 | 0.79 |
| | $t_{1/2} \text{ min}$ | 55.0 | 83.5 | 60.0 |

Note: r is the correlation coefficient for % ChE inhibition vs. time for the declining curve; $t_{1/2}$ is the half-life in min for recovery of ChE enzyme.

E. EFFECT OF SOMAN ON PHARMACOKINETICS AND PHARMACODYNAMICS

Soman is a potential irreversible cholinesterase (ChE) inhibitor. Its extreme toxicity and rapid irreversible inhibition of ChE have been studied by several authors. Poisoning by soman does not respond to treatment with a combination of atropine and an oxime. Pretreatment with a carbamate offered the possibility of devising a drug treatment that would be effective against poisoning by an organophosphate (OP) anticholinesterase, including soman. Recently, emphasis has been placed on two carbamates, physostigmine and pyridostigmine, because they are effective as pretreatment compounds in mammals for treatment of the reversal of central anticholinergic syndrome.

Pharmacokinetics of physostigmine (PHY) was compared after pretreatment with different routes of administration and then soman challenge.¹⁵² Rats were dosed with ³H-PHY (i.v., 100; i.m., 500; oral, 650 µg/kg), 5 or 15 min prior to soman (105 µg/kg s.c.; 1.5 LD₅₀ or 35 µg/kg s.c. 0.5 LD₅₀) treatment and were sacrificed at various times. Pharmacokinetic parameters were determined for PHY using JANA and PC-NONLIN programs. AUC decreased from 1372 to 603 and from 4502 to 1610, indicating 56% and 64% reduction in systemic availability of PHY after i.v. and i.m. dose, respectively, in the presence of soman. CI increased from 73 to 165 and from 111 to 310 ml min⁻¹ kg⁻¹ in the pretreated rats with i.v. and i.m. PHY, respectively. On the other hand, systemic availability of PHY increased by about 100% (an increase in AUC from 152 to 312), and total CI decreased from 4254 to 2080 ml min⁻¹ kg⁻¹ after oral pretreatment with PHY. In the presence of soman, hepatic CI decreased from 31.85 to 29.9 ml min⁻¹ kg⁻¹ and intrinsic CI from 1592.5 to 373.7 ml min⁻¹ kg⁻¹. PHY was slightly less metabolized in soman-challenged rats.

Time course of ³H-PHY concentration and ChE activity in plasma, muscle, and brain were studied in rats pretreated with PHY and then soman challenge.¹⁵² BuChE activity in plasma was 5% of control from 7–30 min after PHY (100 µg/kg, i.v.) pretreatment and then soman challenge (105 µg/kg, s.c.), or treatment with soman alone. Plasma PHY concentration steadily declined from 32.6 ng/ml at 7 min to 15.0 ng/ml at 30 min. ChE activity in muscle was 60–50% of control for PHY pretreatment, but soman alone gave 85–72% of control activity from 2–30 min. Brain ChE activity was about 5% of control within 2 min after soman challenge; however, with PHY pretreatment and soman challenge, the activity was about 40% at 10 min, 28% at 15 min, which recovered to 45% of control at 30 min, indicating that PHY protected brain ChE. Brain PHY concentration steadily declined from 58.6 ng/gm at 7 min to 11.7 ng/gm at 30 min. However, pretreatment of rat with a higher dose of PHY (500 µg/kg, i.m.) and then soman (105 µg/kg) challenge showed BChE in plasma and ChE activity in brain and muscle to be about 25, 30, and 62% of control in comparison to about 5% of control in plasma and brain with soman alone, indicating the protection of ChE enzyme with higher PHY pretreatment dose.¹⁵² The protective role of PHY seen in total brain was not consistent for all brain regions. Soman alone produced a 95% ChE inhibition and there were no differences in its effect between total

brain or brain areas.¹⁵² Pretreatment of the rat with PHY produced a protective effect upon ChE activity up to 30 min.

However, after pretreatment with oral administration of PHY (650 $\mu\text{g/kg}$), the BChE activity in plasma was lowest (12.4% of control) at 20 min whereas PHY concentration was maximum (5.5 ng/ml) at 15 min. BuChE activity remained the same up to 90 min and recovered to 30% at 120 min. Brain ChE did not show any protection after oral administration.

ChE activity in total brain was 12, 30, and 24% at 5, 15, and 30 min after PHY (100 $\mu\text{g/kg}$) pretreatment with a higher dose than soman challenge (105 $\mu\text{g/kg}$, s.c.). After pretreatment with a higher dose of PHY (500 $\mu\text{g/kg}$), ChE activity was found to be 4, 13, and 19% at 5, 15, and 30 minutes. The non-significant difference in ChE activity from 100–500 $\mu\text{g/kg}$ PHY/kg might indicate that higher doses of PHY do not necessarily provide more protection of the enzyme from soman than lower doses. However, protective role of PHY seen in total brain was not consistent for all brain regions. Soman alone produced a 95% ChE inhibition and there were no differences in its effect between total brain or brain areas. Pretreatment of the rat with PHY produced a protective effect upon ChE activity up to 30 min. However, no protective effect on survival was observed.

The effects of soman challenge on ChE activity in diaphragms of rats pretreated with PHY were studied by Somani et al.¹⁵² After an i.m. PHY dose (500 $\mu\text{g/kg}$), ChE activity was 49, 76, and 74% of control in diaphragm at 5, 20, and 45 min, respectively, which rapidly recovered to 88% at 60 min. A semilog plot of % ChE inhibition vs. time gave a 0.02 min^{-1} rate of recovery of the enzyme. ChE activity in soman (105 $\mu\text{g/kg}$, s.c.) challenged rats was about 16–22% of control from 2 to 30 min; however, with i.m. PHY (500 $\mu\text{g/kg}$) pretreatment, the activity recovered to about 35% of control at 20 min and 43% of control at 30 and 45 min. ChE activity after oral administration of PHY (650 $\mu\text{g/kg}$) was found to be 57% of control at 5 min, which rapidly recovered to about 83% at 22 min, then slowly recovered to 98% of control in 120 min. The rate of recovery of fast phase was 0.053 min^{-1} and slow phase was 0.017 min^{-1} . ChE activity in the soman (105 $\mu\text{g/kg}$, s.c.) challenged rat was found to be 16–22% of control from 2 to 30 min. However, with oral pretreatment and then soman challenge, the ChE activity was found to be 67, 76, and 87% of control at 15, 45, and 120 min, respectively. These results indicate that PHY pretreatment by oral route of administration gave some protection to diaphragm ChE.

In conclusion, pretreatment of PHY and then soman challenge decreased systemic availability of PHY after i.v. and i.m. administration. However, systemic availability of PHY after oral administration was increased in the presence of soman. Pretreatment with PHY produced protective effect upon ChE enzyme in CNS and peripheral tissues.

IV. NEOSTIGMINE

Neostigmine (NEO) is a reversible cholinesterase inhibitor that was introduced into therapeutics in 1931 due to its stimulant action on the intestinal tract. This quaternary ammonium compound has greater stability and potency compared to physostigmine

and pyridostigmine. Neostigmine has been used in the treatment of myasthenia gravis for more than 60 years.¹⁵³ This drug is widely used in anesthesia to antagonize the effects of muscle relaxants after operative surgery. Early work had shown that the anticholinesterase activity of neostigmine was five times greater than that of pyridostigmine.⁴⁶ Further, an important difference between neostigmine and pyridostigmine is the inability of the latter compound to produce a direct action on smooth muscle either *in vitro*⁴⁶ or *in vivo*.⁴⁸ This may account for the occurrence of fewer unpleasant side-effects when pyridostigmine is used clinically.^{49–51}

The pharmacokinetics and metabolism of neostigmine has been studied in rats after intramuscular and oral administration.^{153–156} The metabolic pathway of neostigmine was elucidated as shown in Figure 5.6. 3-Hydroxyphenyltrimethylammonium (3-HPTMA) and the glucuronide of 3-HPTMA were isolated and characterized. Small amounts of 3-hydroxyphenyldimethylamine (3-HPDMA) and two unidentified metabolites were also detected. Liver is the organ of metabolism for neostigmine. This drug is rapidly taken up by the hepatic cells as shown by the liver perfusion technique.¹⁵⁷ The drug and its charged metabolites were retained for prolonged time within the rat liver. The formation of a glucuronide of a quaternary ammonium compound (3-HPTMA) was shown *in vitro* in rat liver microsomes supplemented with UDP-glucuronic acid.³⁵ However, 3HNMP did not form the glucuronide in *in vivo* as well as *in vitro* studies utilizing rat liver microsomes.

The distribution and metabolism of neostigmine in different tissues of rat were studied after acute and chronic s.c. administration of ¹⁴C-neostigmine.³⁴ The $t_{1/2}$ of

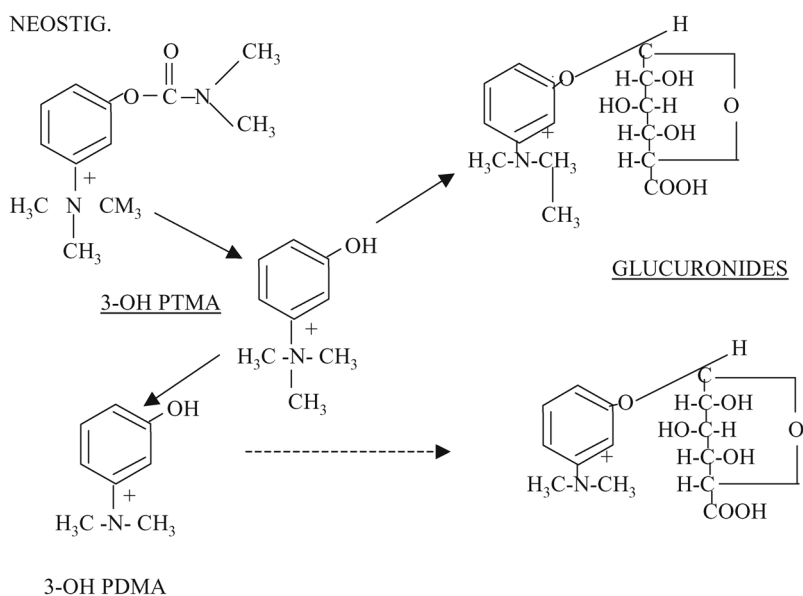


FIGURE 5.6 Probable metabolic pathways of neostigmine. (Adapted from Somani et al.¹⁵³)

neostigmine and its metabolites on average is 10 min in plasma, 33 min in liver, and 1.5 h in muscle. Neostigmine is metabolized in the liver at the rate of $2.24 \text{ H}10^{-2} \mu\text{mol/min/g}$ and 3-HPTMA is metabolized at the rate of $2.89 \text{ H}10^{-2} \mu\text{mol/min/g}$. Metabolic degradation of neostigmine proceeds in muscle at the rate of $2.1 \text{ H}10^{-2} \mu\text{mol/g}$. During chronic administration, the concentration of neostigmine and its metabolites rose in the liver between days 1 to 8 from 0.63 to $5.89 \mu\text{mol/g}$ of tissue and in muscle from 0.085 to $0.39 \mu\text{mol/g}$ of tissue. Liver contained the highest concentration of glucuronide of 3-HPTMA (G-3-HPTMA) followed by neostigmine and 3-HPTMA. G-3-HPTMA concentration was $0.321 \mu\text{mol}$ on day 1 and increased to $2.89 \mu\text{mol/g}$ liver on day 8. The neostigmine concentration increased from 0.14 to $1.62 \mu\text{mol/g}$ of liver during the same period. Similar increases in the concentration of neostigmine and its metabolites were also observed in the muscle under the same conditions. 3-HPDMA, a probable metabolite and an unknown metabolite, also consistently increased in the liver and muscle.³⁴ Thus, the liver is a significant reservoir for neostigmine and G-3-HPTMA. Neostigmine and its major metabolites also accumulate in the muscle. Muscle and cartilage tissues contain chondroitin sulfate, a negatively charged macromolecule, and a constituent of mucopolysaccharide. The binding of quaternary amines to chondroitin sulfate was carried out *in vitro* by ultracentrifugation techniques using varying concentrations of ^{14}C -neostigmine from $1.79 \text{ H} 10^{-8}$ to $1.43 \text{ H} 10^{-7} \text{ mol}$; ^{14}C -HPTMA, $3.59 \text{ H} 10^{-8} \text{ mol}$; and ^{14}C -pyridostigmine from $7.66 \text{ H} 10^{-9}$ to $3.8 \text{ H} 10^{-7} \text{ mol}$. Increasing concentrations of NEO, HPTMA, and pyridostigmine bind in an increasing amount to chondroitin sulfate (Somani, unpublished data).

Neostigmine kinetics and metabolism were studied after i.m. administration in 8 patients with myasthenia gravis.¹⁵⁸ The plasma neostigmine level declined mono-exponentially from 21 ± 2 to $9 \pm 1 \text{ ng/ml}$ between 30 and 120 min. Estimates of plasma half-life ($t_{1/2}$) ranged from 51.1 to 90.5 min; apparent volume of distribution varied from 32.0 to 60.6 l/kg ; and total body clearance from 434 to 549 ml/min . Approximately 80% of the drug was eliminated in urine within 24 h either unchanged or as metabolites. Approximately 50% of the dose was eliminated as the unchanged drug, 15% as 3-hydroxyphenyltrimethylammonium, and 15% as other unidentified metabolites. The neostigmine $t_{1/2}$, based on the urinary excretion of the unchanged drug, ranged from 90.2 to 118.7 min. Therefore, neostigmine is eliminated by renal and extrarenal mechanisms. Calvey et al.¹⁵⁹ have shown that the elimination of neostigmine and its metabolites also occurs via bile, this being the secondary route after urine.

The clinical pharmacology and kinetic interaction of neostigmine and pyridostigmine has been evaluated in patients with myasthenia gravis.^{18,39} These two drugs showed similar pharmacokinetic profiles with plasma $t_{1/2}$ of 0.9 and 1.4 h for neostigmine and pyridostigmine, respectively. The oral bioavailability was however higher for pyridostigmine (7.6%) compared to neostigmine (2%). Aquilonius et al.¹⁸ observed no pharmacokinetic interaction between neostigmine and pyridostigmine in five myasthenic patients, when these drugs were given in combination by the oral route. On the contrary, another study suggested that neostigmine might interfere with the bioavailability of pyridostigmine when both drugs are administered orally at the

same time.³⁹ However, a combination of i.m. and oral routes of these quaternary amines may be more advantageous in the treatment of myasthenia gravis.

Neostigmine and PB have similar chemical structure and pharmacological effects. However, neostigmine is more potent and is metabolized extensively to an unusual glucuronide metabolite. Pyridostigmine does not form the glucuronide conjugate and is metabolized in the liver to its major metabolite 3-hydroxy-N-methyl pyridinium. Further, NEO sequesters in the liver, whereas, PB does not. It is thought that the differences in the distribution and metabolism of these two drugs play a role in their duration of action and influence the pharmacodynamic effects after single and multiple dosages.

V. SUMMARY

Pyridostigmine bromide, a peripheral anticholinesterase drug, was used for the first time on a mass scale by military personnel during the Persian Gulf War as a protective measure against possible nerve gas exposure. The soldiers took PB 30 mg tablets three times a day for 2 weeks. Gulf War veterans reported various illnesses, months and years after the war. PB may be implicated in the etiology of Gulf War illnesses. This has led to an increase in research with PB and its interaction with other factors such as the environment, chemicals, and possible low-level exposure to nerve gas. This chapter deals with the pharmacokinetics and pharmacodynamics of carbamates such as PB, physostigmine, and neostigmine. The absorption, distribution, metabolism, and excretion of PB has been reported extensively in various animal species and human beings. The pharmacokinetics of PB plays an important role in determining the pharmacodynamic effects in normal, disease, or stressful conditions, and in the presence of chemicals and low-level nerve gas exposure. The pharmacodynamic effects and toxicity of PB have clearly shown the importance of dose, time of administration, and disposition of this drug, which could determine its protective efficacy against nerve gases. However, there is little information on pharmacokinetics and pharmacodynamics of PB under stressful conditions and also with respect to gender and age. This area of research needs further investigation. Although PB was prescribed during the Persian Gulf War, this chapter also discusses PHY, a centrally acting carbamate that also has potential as a pretreatment drug against nerve agents. PHY does not attain an effective concentration in the brain after oral administration due to its first-pass effect. However, this drug was found to be efficacious centrally and peripherally after i.v. and i.m. administration. Physical stress influences the pharmacokinetics and pharmacodynamics of this drug. The pharmacokinetics and metabolism of neostigmine (a close congener of PB) has also been compared with PB. Neostigmine is more potent pharmacodynamically, and its metabolite forms a unique glucuronide of quaternary amine and is sequestered in the liver. The metabolite of PB does not form the glucuronide. Environmental factors play a role in altering pharmacokinetics and pharmacodynamics of PB and PHY. Physical stress seems to enhance the pharmacodynamic effects of PB. Therefore, it may be necessary to titrate the dosage of PB under stressful conditions to enable its safe and effective use.

ACKNOWLEDGMENTS

The authors thank Judith M. Bryan for technical support in preparation of this manuscript. The authors sincerely acknowledge their gratefulness to Drs. James A. Romano, Brian J. Lukey, and Benedict Capacio of the U.S. Army Medical Research Institute of Chemical Defense, 3100 Ricketts Point Road, Aberdeen Proving Ground, MD 21010-5400, and Dr. David A. Gelber, Associate Professor of Neurology, Department of Neurology, Southern Illinois University School of Medicine, Springfield, IL 62702, for their thorough review of this chapter.

REFERENCES

1. Urban, R., Pyridostigmine, U.S. Patent 2, 1951. *Pharmacol. Ther.*, 13(3), 393, 1972.
2. Koelle, G.B., Protection of cholinesterase against irreversible inactivation by DFP *in vitro*, *J. Pharmacol. Exp. Ther.*, 88, 323, 1946.
3. Koster, R., Synergism and antagonisms between physostigmine and ci-isopropylfluorophosphate in cats, *J. Pharmacol. Exp. Ther.*, 88, 39, 1946.
4. Bergman, F. and Shimona, A., Quaternary ammonium salts as inhibitors of acetylcholine release, *Biochem. Biophys. Acta.*, 8, 520, 1952.
5. Jobst, J. and Hesse, O., Über die bohne von calabar, *Ann. Chem. Pharm.*, 129, 115, 1864.
6. Vee, M. and LeVen, M., De L'alcaloide de la feve de calabar et experiences physiologiques avec ce meme alcaloide, *J. Pharm Chimie*, 1, 70, 1865.
7. Aeschlimann, J.A. and Reinert, M., Pharmacological action of some analogs of physostigmine, *J. Pharmacol. Exp. Ther.*, 43, 413, 1931.
8. Almog, S., Winkler, E., Amitai, Y., Dani, S., Shefi, M., Tirosch, M., and Shemer, J., Acute pyridostigmine overdose: A report of nine cases, *Isr. J. Med. Sci.*, 27, 659, 1991.
9. Park, K.H., Kim, D.E., Arnold, T.W., Oh, S.J., and Bradley, R., Pyridostigmine toxicity electrophysiological study, *Electromyogr. Clin. Neurophysiol.*, 33, 323, 1993.
10. Kluwe, W.M., Page, J.G., Toft, J.D., Ridder, W.E., and Chung, H., Pharmacological and toxicological evaluation of orally administered pyridostigmine in dogs, *Fundam. Appl. Toxicol.*, 14, 40, 1990.
11. Keeler, J.R., Hurst, C.G., and Dunn, M.A., Pyridostigmine used as a nerve agent pretreatment under wartime conditions, *JAMA*, 266, 693, 1991.
12. Institute of Medicine, Health consequences of service during the Persian Gulf War: Initial findings and recommendations for immediate action, National Academy Press, Washington, D.C., 1992.
13. Cook, J. and Kolka, M., Chronic pyridostigmine bromide administration: Side effects among soldiers working in a desert environment, *Mil. Med.*, 157, 250, 1992.
14. Levine, B.S., Long, R., and Chung, H., Subchronic oral toxicity of pyridostigmine bromide in rats, *Biomed. Environ. Sci.*, 4, 283, 1991.
15. Caldwell, R.W., Lowensohn, H.S., Chryssanthi, M.A., and Nash, C.B., Interactions of pyridostigmine with cardiopulmonary systems and their relationships to plasma cholinesterase activity, *Fundam. Appl. Toxicol.*, 12, 432, 1989.
16. Sorensen, P.S., Flachs, H., Friis, M.L., Hvidberg, E.F., and Paulson, O.B., Steady state kinetics of pyridostigmine in myasthenia gravis, *Neurology*, 34, 1020, 1984.
17. Breyer-Pfaff, U., Maier U., Brinkmann, A.M., and Schumm, F., Pyridostigmine kinetics in healthy subjects and patients with myasthenia gravis, *Clin. Pharmacol. Ther.*, 37, 495, 1985.

18. Aquilonius, S.M., Eckernas, S.A., Hartvig, P., Lindstrom, B., and Osterman, P.O., Clinical pharmacology of neostigmine and pyridostigmine in patients with myasthenia gravis, *J. Neurol. Neurosurg. Psychiat.*, 46, 929, 1983.
19. Cohan, S.L., Dretchen, K.L., and Neal, A., Malabsorption of pyridostigmine in patients with myasthenia gravis, *Neurology*, 27, 299, 1977.
20. Aquilonius, S.M. and Hartvig, P., Clinical pharmacokinetics of cholinesterase inhibitors, *Clin. Pharmacokinetics*, 11, 236, 1986.
21. Aquilonius, S.M., Eckernas, S.A., Hartvig, P., Lindstrom, B., and Osterman, P.O., Pharmacokinetics and oral bioavailability of pyridostigmine in man, *Eur. J. Clin. Pharmacol.*, 18, 423, 1980.
22. Birtley, R.D., Roberts, J.B., Thomas, B.H., and Wilson, A., Excretion and metabolism of ^{14}C -pyridostigmine in the rat, *Br. J. Pharmacol.*, 26(2), 393, 1966.
23. Cohan, S.L., Pohlman, J.L.W., Mikszewski, J., and O'Doherty, D.S., The pharmacokinetics of pyridostigmine, *Neurology*, 26, 536, 1976.
24. Miller, R.L. and Verma, P., Radio immuno assay of pyridostigmine in plasma and tissues, *Pharmacol. Res.*, 21(4), 359, 1989.
25. Yamamoto, K., Sawada, Y., and Iga, T., Comparative pharmacokinetics of four cholinesterase inhibitors in rats, *Biol. Pharmaceut. Bull.*, 18, 1292, 1995.
26. Eiermann, B., Sommer, N., Winne, D., Schumm, F., Maier, U., and Breyer-Pfaff, U., Renal clearance of pyridostigmine in myasthenic patients and volunteers under the influence of ranitidine and pirenzepine, *Xenobiotica*, 23, 1263, 1993.
27. Telang, F.W., Ding, Y.-S., Volkow, N.D., Molina, P.E., and Gatley, S.J., Letter to the Editor, *Nucl. Med. Biol.*, 26, 249, 1999.
28. Sharma, H.S., Navarro, J.C., and Dey, P.K., Increased blood brain barrier permeability following acute short-term swimming exercise in conscious normotensive rats, *Neurosci. Res.*, 10, 211, 1991.
29. Kornfeld, P., Samuels, A.J., Wolf, R.L., and Osserman, K.E., Metabolism of ^{14}C -labelled pyridostigmine in myasthenia gravis. Evidence for multiple metabolites, *Neurology*, 26, 634, 1970.
30. Somani, S.M., Roberts, J.B., and Wilson, A., Pyridostigmine metabolism in man, *Clin. Pharmacol. Therap.*, 393, 13, 1972.
31. Somani, S.M., Metabolism and pharmacokinetics of pyridostigmine in rat after multiple dosing, *Pharmacologist*, 25, 97, 1983.
32. Howrath, R.D., Lamberton, A.H., and Woodcock, D., Investigations on the influence of chemical constitution upon toxicity. Part II. Compounds related to "prostigmine," *J. Chem. Soc.*, Part 1, 182, 1947.
33. Golomb, B.A., *Pyridostigmine Bromide*, Vol. 2, Rand Corporation, 1999, 1.
34. Somani, S.M., Distribution of neostigmine and its metabolites in rat tissues after acute and chronic administration, *Eur. J. Pharmacol.*, 30, 336, 1975.
35. Somani, S.M. and Anderson, J.H., *In vitro* glucuronization of 3-hydroxy-phenyl-trimethyl ammonium by rat liver microsomes, *Drug Metabol. Dispos.*, 5, 15, 1977.
36. Fromberz, K. and Pellmont, B., Pharmakologische wirkung des mestinon, "*Roche*" *Schweiz. Med. Wschr.*, 49, 1187, 1953.
37. Husain, M.A., Roberts, J.B., Thomas, B.H., and Wilson, A., The excretion and metabolism of oral ^{14}C -pyridostigmine in the rat, *Br. J. Pharmacol.*, 34(2), 445, 1968.
38. Burdfield, P.A., Calvery, T.N., and Roberts, T.B., *In vitro* metabolism of neostigmine and pyridostigmine, *J. Pharm. Pharmacol.*, 25(4), 428, 1973.
39. Chan, K., Davison, S.C., Dehghan, A., and Hyman, N., The effect of neostigmine on pyridostigmine bioavailability in myasthenic patients after oral administration, *Meth. Find. Exper. Clin. Pharmacol.*, 3, 291, 1981.

40. Calvey, T.N., Chan, K., and Dehghan, A., Kinetics of intravenous pyridostigmine in man, *Br. J. Clin. Pharmacol.*, 11, 406, 1981.
41. Meyer, H.G., Lukey, B.J., Gepp, R.T., Corpuz, R.P., and Lieske, C.N., A radioimmunoassay for pyridostigmine, *J. Pharmacol. Exper. Therapeu.*, 247, 432, 1988.
42. Taylor, T., Hawkins, D.R., Forest, T.J., and Chung, H., Pharmacokinetics of pyridostigmine in dogs. *J. Pharmacol. Sci.*, 80, 353, 1991.
43. Cronnelly, R., Stanski, D.R., Miller, R.D., and Sheiner, L.B., Pyridostigmine kinetics with and without renal function, *Clin. Pharmacol. Therapeu.*, 28, 78, 1980.
44. Marino, M.T., Schuster, B.G., Brueckner, R.P., Lin, E., Kaminskis, A., and Lasseter, K.C., Population pharmacokinetics and pharmacodynamics of pyridostigmine bromide for prophylaxis against nerve agents in humans, *J. Clin. Pharmacol.*, 38, 227, 1998.
45. Somani, S.M. and Kamemori, G.H., Exercise and absorption, distribution, metabolism, excretion and pharmacokinetics of drugs and chemicals, in *Pharmacology in Exercise and Sports*, Somani, S.M., ed., CRC Press, Inc., Boca Raton, FL, 1996, 1.
46. Hobbiger, F., The action of carbamic esters and tetraethylpyrophosphate on normal and curarized frog rectus muscle, *Br. J. Pharmacol. Chemother.*, 5, 37, 1950.
47. Blaschko, H., Bulbring, E., and Chou, T.C., Tubocurarine antagonism and inhibition of cholinesterase, *Br. J. Pharmacol. Chemother.*, 4, 29, 1949.
48. Desmedt, J.E. and LaGrutta, G., Sur le mode d'action de l'ester dimethyl carbamique de la 3-hydroxy-methyl pyridine (Mestinon), *Rev. Neurol.*, 91, 457, 1954.
49. Tether, J.E., Mestinon in myasthenia gravis (Preliminary report), *Dis. Nervous Syst.*, 15, 227, 1954.
50. Osserman, K.E., Teng, P., and Kaplan, L.I., Studies in myasthenia gravis, Preliminary report on therapy with mestinon, *JAMA*, 155, 961, 1954.
51. Schwab, R.S. and Timberlake, W.H., Pyridostigmine (mestinon) in the treatment of myasthenia gravis, *NEJM*, 251, 271, 1954.
52. Foldes, F.F. and Smith, J.C., The interaction of human cholinesterases with anticholinesterases used in the therapy of myasthenia gravis, *Ann. N.Y. Acad. Sci.*, 135, 287, 1964.
53. Dirnhuber, P. and Green, D.M., Effectiveness of pyridostigmine in reversing neuromuscular blockade produced by soman, *J. Pharm. Pharmacol.*, 30, 419, 1978.
54. Dirnhuber, P., French, M.C., Green, D.M., Leadbeater, L., and Stratton, J.A., The protection of primates against soman poisoning by pretreatment with pyridostigmine, *Pharm. Pharmacol.*, 31, 295, 1979.
55. Lennox, W.J., Harris, L.W., Talbot, B.G., and Anderson, D.R., Relationship between reversible acetylcholinesterase inhibition and efficacy against soman lethality, *Life Sci.*, 37, 793, 1985.
56. Anderson, R.J., Chamberlain, W.L., Roesner, M., Dacko, C., and Robertson, D.G., Decreased tetanic contracture of rat skeletal muscle induced by pyridostigmine, *J. Toxicol. Environ. Health*, 18, 221, 1986.
57. Gebbers, J.-O., Lotscher, M., Kobel, W., Portmann, R., and Laissue, J.-A., Acute toxicity of pyridostigmine in rats: Histological findings. *Arch. Toxicol.*, 58, 271, 1986.
58. Bowman, P.D., Schuschereba, S.T., Johnson, T.W., Woo, F.J., McKinney, L., Wheeler, C.R., Frost, D., and Korte, D.W., Myopathic changes in diaphragm of rats fed pyridostigmine bromide subchronically, *Fundam. Appl. Toxicol.*, 13, 110, 1989.
59. Blick, D.W., Murphy, M.R., Brown, G.C., Yochmowitz, M.G., Fanton, J.W., and Hartgraves, S.L. Acute behavioral toxicity of pyridostigmine or soman in primates, *Toxicol. Appl. Pharmacol.*, 126, 311, 1994.

60. Glikson, M., Achiron, A., Ram, Z., Ayalon, A., Karni, A., Sarova-Pinchas, I., Glovinski, J., and Revah, M., The influence of pyridostigmine administration on human neuromuscular functions—Studies in healthy human subjects. *Fund. Appl. Toxicol.*, 16, 288, 1991.
61. Von Bredow, J.D., Adams, N.L., Groff, W.A., and Vick, J.A., Effectiveness of oral pyridostigmine pretreatment and cholinolytic-oxime therapy against soman intoxication in nonhuman primates, *Fund. Appl. Toxicol.*, 17, 761, 1991.
62. Blick, D.W., Kerenyi, S.Z., Miller, S., Murphy, M.R., Brown, G.C., and Hartgraves, S.L., Behavioral toxicity of anticholinesterases in primates: Chronic pyridostigmine and soman interactions, *Pharmacol. Biochem. Behav.*, 38, 526, 1991.
63. Vijayaraghavan, R., Husain, K., Kumar, P., Pandey, K.S., and Das Gupta, S., Time dependent protection by carbamates against inhaled sarin aerosols in rats. *Asia Pac. J. Pharmacol.*, 7, 257, 1992.
64. Koplovitz, I., Harris, L.W., Anderson, D.R., Lennox, W.J., and Stewart, J.R., Reduction by pyridostigmine pretreatment of the efficacy of atropine and 2-PAM treatment of sarin and VX poisoning in rodents, *Fund. Appl. Toxicol.*, 18, 102, 1992.
65. Kluwe, W.M., Efficacy of pyridostigmine against soman intoxication in a private mode, in *Proceedings of the 6th Medical Chemical Defense Bioscience Review*, Aberdeen Proving Ground, MD, U.S. Army Medical Research Institute of Chemical Defense, 1987, 227.
66. Worek, F., Kleine, A., and Szinicz, L., Effect of pyridostigmine pretreatment on cardiorespiratory function in tabun poisoning, *Human Exper. Toxicol.*, 14, 634, 1995.
67. Lintern, M.C., Smith, M.E., and Ferry, C.B., Effects of repeated treatment with pyridostigmine on acetylcholinesterase in mouse muscles, *Human Exp. Toxicol.*, 16, 158, 1997.
68. Husain, K., Vijayaraghavan, R., and Marjit, D.N., Effect of pyridostigmine and physostigmine against acute toxicity of inhaled DFP in rats, *Arch. Ind. Hyg. Toxicol.*, 41, 19, 1990.
69. Hubert, M. and Lison, D., Study of muscular effects of short-term pyridostigmine treatment in resting and exercising rats, *Human Exp. Toxicol.*, 14, 49, 1995.
70. Somani, S.M., Husain, K., Asha, T., and Helfert, R., Interactive and delayed effects of pyridostigmine and physical stress on biochemical and histopathological changes in peripheral tissues of mice, *J. Appl. Toxicol.*, 20, 327, 2000.
71. Augustinsson, K.B. and Nachmansohn, D., Distinction between acetylcholinesterase and other choline ester splitting enzymes, *Science*, 110, 98, 1949.
72. Wade, O.L. and Bishop, J.M., *Cardiac Output and Regional Blood Flow*, Blackwell, Oxford, 1962.
73. Rowell, L.B., Blackmon, J.R., and Bruce, R.A., Indocyanine green clearance and estimated hepatic blood flow during mild to maximal exercise in upright man, *J. Clin. Invest.*, 43, 1677, 1964.
74. Ballard, B.E., Pharmacokinetics and temperature, *J. Pharm. Sci.*, 63, 1345–1357, 1974.
75. Francesconi, R., Hubbard, R., and Mager, M., Effects of pyridostigmine on ability of rats to work in the heat. *J. Appl. Physiol.: Respirat. Environ. Exercise Physiol.*, 56, 891, 1984.
76. Avlonitou, E. and Elizondo, R., Effects of atropine and pyridostigmine in heat-stressed patas monkeys, *Aviat. Space Environ. Med.*, 59, 544, 1988.
77. Friedman, A., Kaufer, D., Shemer, J., Hendler, I., Soreq, H., and Tur-Kaspa, I., Pyridostigmine brain penetration under stress enhances neuronal excitability and induces early immediate transcriptional response, *Nat. Medicine*, 2, 1382, 1996.
78. Verma-Ahuja, S., Husain, K., Verhulst, S., Espinosa, J.A., and Somani, S.M., Delayed effects of pyridostigmine and exercise training on muscle tension in mouse lower extremity, *FASEB J.*, 818, 5, 1999.

79. Kaiser, K.S., Hawksworth, A.W., and Gray, G.G., Pyridostigmine bromide intake during the Persian Gulf War is not associated with postwar handgrip strength, *Mil. Med.*, 165, 165, 2000.
80. Lallement, G., Foquin, A., Baubichon, D., Burckhart, M.-F., Carpentier, P., and Canini, F., Heat stress, even extreme, does not induce penetration of pyridostigmine into the brain of guinea pigs, *Neurotoxicology*, 19, 759, 1998.
81. Epstein, Y., Seidman, D.S., Moran, D., Arnon, R., Arad, M., and Varssano, D., Heat-exercise performance of pyridostigmine-treated subjects wearing chemical protective clothing, *Aviat. Space Environ. Med.*, 61, 310, 1990.
82. Kolka, M.A. and Stephenson, M.S., Human temperature regulation during exercise and after oral pyridostigmine administration, *Aviat. Space Environ. Med.*, 61, 220, 1990.
83. Ram, Z., Molcho, M., Danon, Y.L., Almog, S., Baniel, A.K., and Shemer, J., The effect of pyridostigmine on respiratory function in healthy and asthmatic volunteers, *Isr. J. Med. Sci.*, 27, 664, 1991.
84. Wenger, C.B. and Latzka, W.A., Effects of pyridostigmine bromide on physiological responses to heat, exercise and hypohydration, *Aviat. Space Environ. Med.*, 63, 37, 1992.
85. Wenger, B., Quigley, M.S., and Kolka, M.A., Seven-day pyridostigmine administration and thermoregulation during rest and exercise in dry heat, *Aviat. Space Environ. Med.*, 64, 905, 1993.
86. Forster, E.M., Forster, J.S., Barber, B.A., Parker, Jr., F.R., Whinnery, J.E., Burton, R.R., and Boll, P., Effect of pyridostigmine bromide on acceleration tolerance and performance, *Aviat. Space Environ. Med.*, 65, 110, 1994.
87. Prusaczyk, W.K. and Sawka, M.N., Effects of pyridostigmine bromide on human thermoregulation during cold water immersion, *J. Appl. Physiol.*, 71, 432, 1991.
88. Roberts, D.E., Sawka, M.N., Young, A.J., and Freund, B.J., Pyridostigmine bromide does not alter thermoregulation during exercise in cold air, *Can. J. Physiol. Pharmacol.*, 72, 788, 1994.
89. Sharabi, Y., Danon, Y.L., Berkenstadt, H., Almog, S., Mimouni-Bloch, A., Zisman, A., Dani, S., and Atsmon, Survey of symptoms following intake of pyridostigmine during the Persian Gulf War, *Isr. J. Med. Sci.*, 27, 656, 1991.
90. Brooks, G.A. and Fahey, T.N., *Exercise Physiology*, John Wiley & Sons, New York, 1984, 726.
91. Connolly, R.J., Flow patterns in the capillary bed of rat skeletal muscle at rest and after repetitive tetanic contraction, in *Microcirculation*, Grayson, J. and Zingg, W., Eds., Plenum Press, New York, 1976.
92. Sahlin, K., Intracellular pH and energy metabolism in skeletal muscle of man with special reference to exercise, *Acta Physiol. Scand. Suppl.*, 455, 1, 1978.
93. Hughson, R.L. and Green, H.J., Blood acid-base and lactate relationships studies by ramp work tests, *Med. Sci. Sports Exerc.*, 14, 297, 1982.
94. Day, R.E., Effects of exercise performance on drugs used in musculoskeletal disorders, *Med. Sci. Sports Exerc.*, 13, 272, 1981.
95. Schwartz, G., Estimating the dimension of a model, *Ann. Stat.*, 6, 461, 1978.
96. Somani, S.M., ed., *Pharmacology in Exercise and Sports*, CRC Press, Inc., Boca Raton, FL, 1996, 1.
97. Powers, S.K., Criswell, D., Lawler, J., Martin, D., Lieu, F., Ji, L.L., and Herb, R.A., Rigorous exercise training increases superoxide dismutase activity in ventricular myocardium, *Am. J. Physiol.*, 34, 2094, 1993.
98. Husain, K. and Somani, S.M., Influence of exercise and ethanol on cholinesterase activity and lipid peroxidation in blood and brain regions of rat, *Prog. Neuro-Psychopharmacol. Biol. Psychiat.*, 21, 659, 1997.

99. Husain K. and Somani, S.M., Effect of exercise training and chronic ethanol ingestion on cholinesterase activity and lipid peroxidation in blood and brain regions of rat, *Prog. Neuro-Psychopharmacol. Biol. Psychiat.*, 22, 411, 1998.
100. Ott, P., Membrane acetylcholinesterases: Purification, molecular properties and interactions with amphiphilic environments, *Biochem. Biophys. Acta*, 822, 375, 1985.
101. Babu, S.R., Somani, S.M., and Dube, S.N., Effect of physostigmine and exercise on choline acetyltransferase and acetylcholinesterase activities in fast and slow muscles of rat, *Pharmacol. Biochem. Behav.*, 45, 713, 1993.
102. Peden-Adams, M.M., Dudley, A.C., EuDaly, J.G., Gilkeson, G.S., and Keil, D.F., Effects of exercise stress on pyridostigmine bromide on immune function parameters in mice, *Toxicologist*, 54, 162, 2000.
103. Abou-Donia, M.B., Wilmarth, K.R., Jensen, K.F., Oehme, F.W., and Kurt, T.L., Neurotoxicity resulting from coexposure to pyridostigmine bromide, DEET, and permethrin: Implications of Gulf War chemical exposures, *J. Toxicol. Environ. Health*, 48, 35, 1996.
104. Husain, K. and Somani, S.M., Influence of physical stress and pyridostigmine on cholinesterase activity in blood and brain regions of male and female mice, *FASEB J.*, 818, 1999.
105. Stone, J.G., Matteo, R.S., Ornstein, E., Schwartz, A.E., Ostapkovich, N., Jamdar, S.C., and Diaz, J., Aging alters the pharmacokinetics of pyridostigmine, *Anes. Analgesia*, 81, 773, 1995.
106. Somani, S.M. and Khalique, A., Pharmacokinetics and pharmacodynamics of physostigmine in the rat after intravenous administration, *Drug Metab. Dispos.*, 15, 627, 1987.
107. Somani, S.M. and Khalique, A., Distribution and pharmacokinetics of physostigmine in rat after intramuscular administration, *Fundam. Appl. Toxicol.*, 6, 327, 1986.
108. Giacobini, E., Somani, S.M., McIlhany, M., Downen, A., and Hallak, M., Pharmacokinetics and pharmacodynamics of physostigmine after i.v. administration in beagle dogs, *Neuropharmacology*, 26, 831, 1987.
109. Hartvig, L., Wiklund, and Lindstrom, B., Pharmacokinetics of physostigmine after intravenous, intramuscular and subcutaneous administration in surgical patients, *Acta Anaesthesiol. Scand.*, 30, 177, 1986.
110. Lukey, B.J., Parrish, J.H., Marlow, D.D., Clark, C.R., and Sidell, F.R., Pharmacokinetics of physostigmine intramuscularly administered to guinea pigs, *J. Pharm. Sci.*, 79, 796, 1990.
111. Unni, L.K. and Somani, S.M., Hepatic and muscle clearance of physostigmine in the rat, *Drug Metab. Dispos.*, 14, 183, 1986.
112. Somani, S.M. and Boyer, A., *Eur. J. Drug Metab. Pharmacokin.*, 10, 343, 1985.
113. Johansson, M. and Nordberg, A., Pharmacokinetic studies of cholinesterase inhibitors, *Acta Neuro. Scand.*, S149, 22, 1993.
114. Asthana, S., Greig, N.H., Hegedus, L., Holloway, H.H., Raffaele, K.C., Schapiro, M.B., and Soncrant, T.T., Clinical pharmacokinetics of physostigmine in patients with Alzheimer's disease, *Clin. Pharmacol. Thera.*, 58, 299, 1995.
115. Somani, S.M., Pharmacokinetics and pharmacodynamics of physostigmine in the rat after oral administration, *Biopharm. Drug Dispos.*, 10, 187, 1989.
116. King, B.F. and Somani, S.M., Distribution of physostigmine and metabolites in brain subcellular fractions of the rat, *Life Sci.*, 41, 2007, 1987.
117. Unni, L.K. and Somani, S.M., Binding of physostigmine to rat and human plasma and crystalline serum albumins, *Life Sci.*, 36, 1389, 1985.
118. Somani, S.M., Unni, L.K., and McFadden, D.L., Drug interaction for plasma protein binding: Physostigmine and other drugs, *Int. J. Clin. Pharmacol. Ther. Toxicol.*, 25, 412, 1987.

119. Whelpton, R. and Hurst, P. R., The binding of physostigmine to human serum albumin, *J. Pharm. Pharmacol.*, 42, 804, 1990.
120. Somani, S.M., Gupta, S.K., Khalique, A., and Unni, L.K., Physiological pharmacokinetic and pharmacodynamic model of physostigmine in the rat, *Drug Metab. Disp.*, 19, 655, 1991.
121. Hemsworth, B.A. and West, G.B., Anticholinesterase activity of some degradation products of physostigmine, *J. Pharm. Sci.*, 59, 118, 1970.
122. Isaksson, K. and Kissinger, P.T., Metabolism of physostigmine in mouse liver microsomal incubations studied by liquid chromatography with dual-electrode amperometric detection, *J. Chromatog.*, 419, 165, 1987.
123. Somani, S.M., Kutty, R.K., and Krishna, G., Eseroline, a metabolite of physostigmine, induces neuronal cell death, *Toxicol. Appl. Pharmacol.*, 106, 28, 1990.
124. Fleisher, J.H. and Harris, L.W., Dealkylation as a mechanism for aging of cholinesterase after poisoning with pinacolyl methylphosphonofluoridate, *Biochem. Pharmacol.*, 14, 641, 1965.
125. Berry, W.K. and Davies, D.R., The use of carbamates and atropine in the protection of animals against poisoning by 1,2,2-trimethylpropylmethyl phosphonofluoridate, *Biochem. Pharmacol.*, 19, 927, 1970.
126. Heyl, W.C., Harris, L.W., and Stitcher, D.L., Effects of carbamates on whole blood cholinesterase activity: Chemical protection against soman, *Drug Chem. Toxicol.*, 3, 319, 1980.
127. McNamara, B.P., Koelle, G.B., and Gilman, A., The treatment of diisopropyl fluorophosphate (DFP) poisoning in rabbits, *J. Pharmacol. Exp. Ther.*, 88, 27, 1946.
128. Schoene, K., Steinhanses, J., and Oldiges, M., Protective activity of pyridinium salts against soman poisoning *in vivo* and *in vitro*, *Biochem. Pharmacol.*, 25, 1955, 1976.
129. Gordon, J.J., Leadbeater, L., and Maidment, M.P., The protection of animals against organophosphate poisoning by pretreatment with a carbamate, *Toxicol. Appl. Pharmacol.*, 43, 207, 1976.
130. Ashani, Y., Leader, H., Raveh, L., Bruckstein, R., and Spiegelstein, M., *In vitro* and *in vivo* protection of acetylcholinesterase against organophosphate poisoning by pretreatment with a novel derivative of 1,3,1-diolaphosphorinane 2-oxide, *J. Med. Chem.*, 26, 145, 1983.
131. Harris, L.W., Heyl, W.C., Stitcher, D.L., and Moore, R.D., The effect of atropine and/or physostigmine on cerebral acetylcholine in rats poisoned with soman, *Life Sci.*, 22, 907, 1978.
132. Harris, L.W., Stitcher, D.W., and Heyl, W.C., The effects of pretreatments with carbamates, atropine and mecamylamine on survival and on soman induced alterations in rat and brain acetylcholine, *Life Sci.*, 26, 1885, 1980.
133. Harris, L.W., Lennox, W.J., and Talbot, B.G., Toxicity of anticholinesterase: Interactions of pyridostigmine and physostigmine with soman, *Drug Chem. Toxicol.*, 7, 507, 1984.
134. Inns, R.H. and Leadbeater, L., The efficacy of bispiridinium derivatives in the treatment of organophosphate poisoning in the guinea pig, *J. Pharm. Pharmacol.*, 35, 427, 1983.
135. Karlsson, N., Larsson, R., and Puu, G., Ferrocene-carbamate as prophylaxis against soman poisoning, *Fund. Appl. Toxicol.*, 4, S184, 1984.
136. Leadbeater, L., Inns, R.H., and Pylands, J.M., Treatment of poisoning by soman, *Fund. Appl. Toxicol.*, 5, 225, 1985.
137. Solana, R., Gennings, C., Anderson, D., Lennox, W., and Carter, W., Jr., Absence of effect by pyridostigmine against organophosphate induced lethality and physical incapacitation, *FASEB J.*, 3, 3664A, 1989.
138. Lennox, W.J., Harris, L.W., Anderson, D., and Solana, R., Successful pretreatment/therapy of soman, sarin and VX intoxication, *FASEB J.*, 3, 3683A, 1989.

139. Anderson, D., Harris, L., and Lennox, W., Subacute carbamate plus acute adjunct pre-treatment against nerve agent intoxication, *FASEB J.*, 3, 3867A, 1989.
140. Harris, L.W., Anderson, D.A., Lennox, W.J., and Solana, R.P., Effects of subacute administration of physostigmine on blood cholinesterase activity, motor performance and soman intoxication, *Toxicol. Appl. Pharmacol.*, 97, 267, 1989.
141. McDonald, R.B., Hamilton, J.S., Stern, J.S., and Horwitz, B.A., Regional blood flow of exercise-trained younger and older cold-exposed rats, *Am. J. Physiol.*, 256, 41069, 1989.
142. Shand, D.G., Kornhauser, D.M., and Wilkinson, G.R., Effects of route of administration and blood flow on hepatic elimination, *J. Pharmacol. Exp. Ther.*, 195, 424, 1975.
143. Frank, S., Somani, S.M., and Kohnle, M., Effect of exercise on propranolol pharmacokinetics, *Eur. J. Clin. Pharmacol.*, 39, 391, 1990.
144. Somani, S.M., Gupta, S.K., Frank, S., and Corder, N., Effect of exercise on disposition and pharmacokinetics of drugs, *Drug Develop. Res.*, 20, 251, 1990.
145. Dill, D.B. and Costill, D.L., Calculation of percentage changes volumes of blood, plasma and red cells in dehydration, *J. Appl. Physiol.*, 37, 247, 1974.
146. McMaster, S.B. and Foster, R.E., Behavioral and morphological studies of the interaction between exercise and physostigmine, U.S. Army Medical Research and Development Command, *Sixth Ann. Chem. Def. Biosci. Rev.*, August, 629, 1987.
147. Matthew, C.B., Hubbard, R.W., Francesconi, R.P., and Thomas, G.J., Carbamate-induced performance and thermoregulatory decrements restored with diazepam and atropine, *Aviat. Space Environ. Med.*, 58, 1183, 1987.
148. Somani, S.M., Babu, S.R., Arneric, S.P., and Dube, S.N., Effect of cholinesterase inhibitor and exercise on choline acetyltransferase and acetylcholinesterase activities in rat brain regions, *Pharmacol. Biochem. Behav.* 39, 337, 1991.
149. Matthew, C.B., Bowers, W.D., Francesconi, R.P., and Hubbard, R.W., Chronic physostigmine administration in the exercising rat, Report U.S. Army Medical Research and Development Command, Natick, MA, March, 1990.
150. Dube, S.N., Somani, S.M., and Babu, S.R., Concurrent acute exercise alters central and peripheral responses to physostigmine, *Pharmacol. Biochem. Behav.*, 41, 773, 1993.
151. Somani, S.M. and Dube, S.N., Endurance training changes central and peripheral responses to physostigmine, *Pharmacol. Biochem. Behav.*, 41, 773, 1992.
152. Somani, S.M., Giacobini, E., Boyer, A., Hallak, M., Khalique, A., Unni, L., Hannant, M., and Hurley, E., Mechanisms of action and pharmacokinetics of physostigmine in relation to acute intoxication by organofluorophosphates, Reports submitted to U.S. Army Medical Research and Development Command, Fort Detrick, MD, 1988.
153. Somani, S.M., Roberts, J.B., Thomas, B.H., and Wilson, A., Isolation and characterization of metabolites of neostigmine from rat urine, *Eur. J. Pharmacol.*, 12, 114, 1970.
154. Roberts, J.B., Thomas, B.H., and Wilson, A., Distribution and excretion of ^{14}C -neostigmine in the rat and hen, *Br. J. Pharmacol. Chemotherap.*, 25, 234, 1965.
155. Roberts, J.B., Thomas, B.H., and Wilson, A., Metabolism of ^{14}C -neostigmine in the rat, *Br. J. Pharmacol. Chemotherap.*, 25, 763, 1965.
156. Roberts, J.B., Thomas, B.H., and Wilson, A., Excretion and metabolism of oral ^{14}C -neostigmine in the rat, *Biochem. Pharmacol.*, 15, 71, 1966.
157. Somani, S.M. and Anderson, J.H., Sequestration of neostigmine and metabolites by perfused rat liver, *Drug Metabol. Dispos.*, 3, 275, 1975.
158. Somani, S.M., Chan, K., Dehghan, A., and Calvey, T.N., Kinetics and metabolism of intramuscular neostigmine in myasthenia gravis, *Clin. Pharmacol. Ther.*, 28, 64, 1980.
159. Calvey, T.N., Somani, S.M., and Wright, A., Differences between the biliary excretion of tri[^{14}C]methyl-(3-hydroxy-phenyl)ammonium iodide in Wistar and Gunn rats, *Biochem. J.*, 119, 659, 1970.

160. Chan, K. and Calvey, T.N., Renal clearance of pyridostigmine in patients with myasthenia gravis, *E. Neurol.*, 16, 69, 1977.
161. Adler, M., Maxwell, D., Foster, R.E., Deshpande, S.S., and Albuquerque, E.X., *In vivo* and *in vitro* pathophysiology of mammalian skeletal muscle following acute and subacute exposure to pyridostigmine. Studies on muscle contractility and cellular mechanisms. *Proceedings of the Fourth Annual Chemical Defense Bioscience Review*, 1984, 173.
162. Capacio, B.R., Byers, C.E., Anderson, D.R., Matthews, R.L., and Brown, D.E., The effect of ondansetron on pyridostigmine-induced blood acetylcholinesterase inhibition in the guinea pig, *Drug Chem. Toxicol.*, 19, 1, 1996.