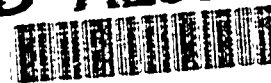


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# TRAINING PROGRAM FOR INSTRUMENTATION, TELEMETRY, AND EXERCISE ERGOMETRY

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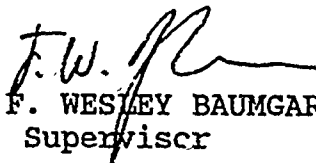
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CONTENTS

	<u>Page</u>
BACKGROUND . . . . .	1
Estimating the Threat . . . . .	1
Protecting Against the Threat . . . . .	2
Medical Care. . . . .	3
Pharmacological Countermeasures . . . . .	4
Scientific Issues . . . . .	4
Chemical Protection . . . . .	6
References . . . . .	9
 TRAINING PROTOCOL: Training Program for Instrumenta- tion, Telemetry, and Exercise Ergometry . . . . .	 15
Protocol Outcomes . . . . .	22
Primate Exercise Wheel System Modifications/ Debugging . . . . .	22
Specific Training Procedures/Primate Exercise Wheel/Primate Restraint Chair . . . . .	24
Typical Training Day . . . . .	24
Results. . . . .	25
Primate Exercise Wheel/Primate Restraint Chair. . . . .	25
Preliminary Draft of Paper Describing the Primate Exercise Wheel . . . . .	25
Primate Exercise Wheel . . . . .	26
Primate Exercise Wheel Controller/ Manual . . . . .	27
Primate Exercise Wheel Controller/ Automatic . . . . .	27
Shaping and Maintaining Exercise Behavior . . . . .	30
Metabolic and Physiological Data Collection . . . . .	32
References . . . . .	33
 EXPERIMENTAL PROTOCOL: Ergogenic Aids, Environmental And Physical Evaluation Model: Rest and Prior Exercise . . . . .	 34
 PROTOCOL ADDENDUM: Ergogenic Aids, Environmental And Physical Evaluation Model: Rest and Prior Exercise . . . . .	 44
Results. . . . .	48
Resting Experiments . . . . .	48
Pyridostigmine Dose Response Curve . . . . .	49
 EXPERIMENTAL PROTOCOL: Ergogenic Aids, Environmental and Physical Evaluation Model: Exercise Capacity . . . . .	 51

List of Figures

<u>Fig.</u> <u>No.</u>		<u>Page</u>
1.	Monkey seated in primate restraint chair . . . . .	19
2.	Primate Exercise Wheel . . . . .	26
3.	Block diagram of LabView program . . . . .	28
4.	Control panel for Primate Exercise Wheel . . . . .	29
5.	Effect of pyridostigmine infusion on cholinesterase inhibition at two ambient temperatures . . . . .	49
6.	Effects of pyridostigmine infusion on skin temperature at two ambient temperatures . . . . .	49
7.	Effects of cholinesterase inhibition on blood glucose and insulin . . . . .	50

List of Tables

<u>Table</u> <u>No.</u>		
1.	Subject Assignments . . . . .	45
2.	Results of Resting Experiments . . . . .	48
3.	Results of Pyridostigmine Experiments . . . . .	50



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TRAINING PROGRAM FOR INSTRUMENTATION,  
TELEMETRY, AND EXERCISE ERGOMETRY

BACKGROUND

Estimating the Threat

The overriding military problem is the threat that personnel may be exposed to organophosphate nerve agents during a conflict. Planning for such a possibility first requires estimates of the effects of agent exposure. Since human experimental subjects cannot be used to obtain such information, alternative approaches have been developed. These include: (a) analysis of clinical reports of accidental exposure to similar agents in industrial or agricultural settings, (b) use of mathematical models, and (c) use of animal models. Part of the research Systems Research Laboratories (SRL) has been conducting at the USAF School of Aerospace Medicine Radiation Biology Branch (USAFSAM/RZB) has been directed at the use of animal models for estimating the nature of nerve agent bioeffects (Blick et al., 1986a, 1987a-d, 1988, 1989; Murphy et al., 1984, 1985, 1988, 1990).

Three main types of bioeffects have been identified: The most obvious effect of nerve agent exposure is the production of casualties through death or severe incapacitation. SRL has studied this aspect with both rats and monkeys (Murphy et al., 1988, 1990; Blick et al., 1989).

A second main bioeffect of nerve agent is performance degradation, caused by the agent itself or interactions of the agent with physiological and environmental perturbations, such as the increase in body temperature that occurs when working while wearing the protective chemical defense ensemble. Exposure to the agent or its interactions with physiological and environmental burdens may not produce an obvious casualty, but it may impair job performance and thus compromise the mission. Examples include decreased time to feelings of fatigue or to exhaustion, muscle weakness, as well as reduced vision and impaired equilibrium. SRL has extensive experience in studying the effects of the nerve agent soman on performance deficits in monkeys and rats (Blick et al., 1986a, 1987a-d, 1988, 1989; Murphy et al., 1988, 1989, 1990).

A third bioeffect of nerve agent is possible delayed/residual damage, which may appear as impaired health or nervous disorders (behavioral, motor, or sensory). Exposure to nerve agents, with or without prophylaxis or treatment, may cause long-term injuries of delayed onset, the most insidious of which is brain damage (Churchill et al., 1985a; McDonough et al., 1986). Even before such effects could be diagnosed, affected individuals would likely be more susceptible to becoming a casualty during subsequent nerve agent exposures. The onset of delayed debilitation during military service would reduce the effectiveness of affected personnel, and after military service would reduce their

quality of life and place a burden on medical care facilities. SRL, in collaboration with USAFSAM, has studied the effects of nerve agent on chronic behavioral deficits and brain damage in rats (Murphy et al., 1988; Armstrong et al., 1989) and monkeys (Blick et al., 1989; Campbell et al., 1989; Murphy et al., 1990).

The threat of nerve agent exposure provides the enemy with a unique opportunity -- inflicting heat-related casualties by forcing our troops into wearing the chemical defense protective ensemble. The chemical defense protective equipment, like all types of protective garments, increases heat loading. Since the age range and general physiological condition of USAF personnel is similar, it is likely that heat casualties will occur in "epidemics", rather than as isolated cases, as might be expected with standard combat clothing. For example, in one Marine field exercise, 232 men in a single battalion were admitted to a field hospital in a 4-h period (Augerson et al., 1986). Besides overloading medical facilities, this situation causes two significant problems. First, the loss of operational capability and combat strength (the "cutting edge") can be critical and often decisive. Second, the early signs and symptoms of heat disorder: face flushed, nasal mucosa injected, sweating, throbbing headache, dyspnea, nausea, vomiting, irritability, etc., can easily be confused with nerve agent poisoning. This confusion increases the alarm and causes additional problems for both command and medical channels. For example, confusing a heat casualty with a nerve agent casualty could lead to treatment with atropine. This would be life saving if nerve agent exposure had occurred, but life threatening if the symptoms were caused by a heat disorder (Augerson et al., 1986).

#### Protecting Against the Threat

In addition to estimating the threat, planning for possible chemical conflict requires the development of measures to protect against exposure to organophosphate nerve agents. Protection schemes need to be addressed to the reduction or prevention of casualties, performance degradation, and delayed/residual damage. How is such protection to be accomplished?

The most effective method of ensuring U.S. Air Force personnel can continue to operate in a chemical agent environment is to provide protective systems which isolate them from the hazard. Such protection is usually in the form of personal or individual protection including mask, overgarments, boots, undergarments; and in the form of collective protection, which may be mobile (tents, International Standards Organization Shelters, chemical/biological/radiological protected vehicles, etc.) and hardened fixed shelters.

Individual protection allows the person to operate as a mobile entity to perform necessary duties related to operational requirements (i.e., generate sorties, rapid runway repair, explosive ordnance disposal, medical, etc.). The level of protection

afforded by fielded individual equipment and equipment under development generally provides the necessary level of protection against the agent. But protection from the agent extracts a price: operational burden (weight of the garment, decreased ability to use some types of tools, decreased communications capacity, etc.) and heat stress. Even at mild environmental temperatures and light work schedules, prolonged wearing of the current chemical defense ensemble creates a moist, warm, tropical microclimate next to the wearer's skin. When the environmental temperature exceeds 75°F, the effective temperature inside a chemical defense ensemble can reach 92° to 96°F and higher. The increased heat burden comes from two sources: 1) absorption of heat from the environment; and 2) increased heat storage of body heat. Heat is normally lost by radiation, conduction, convection, and evaporation, but each of these heat elimination paths is compromised by the chemical defense ensemble. In the case of evaporation, for example, an individual inside the chemical defense ensemble sweats, but the air inside the ensemble rapidly becomes saturated. Therefore the sweat cannot evaporate and this does not contribute to the elimination of heat stored in the body. The unevaporated sweat can increase discomfort and the risk of skin disorders, such as tropical immersion foot. Increasing work load increases the amount of heat stored in the body and thus the risk of heat disorders. In its mildest form, heat stress affects judgment, coordination, and mood (Critchley, 1947). The symptoms of heat fatigue include increased rate of sweating, lacrimation, nasal stuffiness, throbbing headache, dizziness, nausea, vomiting, anorexia, and mood changes ranging from severe irritability to apathy and severe depression. Assuming that the individual is not misdiagnosed as a nerve agent casualty (atropine, an appropriate treatment for a nerve agent casualty, would be contraindicated in a heat casualty), heat fatigue is usually self-limiting, especially if the individual can be allowed to rest in a cool environment and have free access to water. Untreated, it can lead to more serious and potentially life-threatening forms of heat injury, such as heat stroke.

#### Medical Care

A second means of protection is minimizing the effects of chemical exposure through efficient evacuation and medical treatment of chemical casualties. SRL has considerable experience in this area.

The goal of USAF medical support units is to provide an organized, well-rehearsed system of casualty management; a system using medical personnel at the highest level of capability and efficiency, maximizing return to duty of the minimally injured to ensure the highest possible sortie generation rate, providing rapid and effective medical stabilization of those casualties who are no longer mission capable to permit their timely evacuation to higher echelons of medical treatment, and protecting medical personnel and casualties from further injury. SRL has been the prime contractor for a number of programs that address the impact



of chemical warfare on casualty care procedures for an entire spectrum of cases: hardened fixed air base facilities, Air Force and Army mobile medical facilities, and aeromedical evacuation facilities. In each of these cases, SRL addressed the design of chemically protected medical facilities.

### Pharmacological Countermeasures

The third means of protection is pharmacological countermeasures (usually self-administered) that consist of a prophylaxis regimen and postexposure treatment. In addition to estimating the bioeffects of nerve agents, characterizing the physiological responses to thermal stress and exercise (as might be encountered when ground crews are working while wearing the protective chemical defense ensemble) and the interactions of these stressors with nerve agent prophylactic antidote drugs and combinations is an area in which USAFSAM Chemical Defense Branch (VNC) personnel and the on-site SRL team have contributed to the U.S. Air Force chemical defense effort, including:

Identifying Air Force needs and surveying existing literature to assess areas where specific new research is most needed to meet Air Force requirements.

Examining and calibrating the Primate Exercise Wheel (PEW).

Assisting in examining and calibrating the instrumentation for metabolic and physiological measurements, their interface to the Macintosh computer, and debugging the computer program used to acquire metabolic and physiological data.

Developing and examining the complete thermal profile of chaired rhesus (and ultimately patas) monkeys at rest at ambient temperatures of 25° and 35°C.

### Scientific Issues

These issues include examining the interactions of pharmacological countermeasures (such as pyridostigmine) with physiological and environmental stressors (as in a protective chemical defense ensemble exercise) and determining the effect of these interactions on potential ground crew performance and airbase operability, using a primate model of human responses.

The chemical warfare agents under consideration are organophosphate anticholinesterases such as tabun, sarin, and soman. The chemical defense program at USAFSAM has dealt exclusively with soman. These agents are believed to produce their major effect by inhibiting (i.e., blocking the action of) acetylcholinesterase (AChE), an enzyme needed to deactivate the critically important neurotransmitter, acetylcholine (ACh). In the normal function of the cholinergic part of the central and peripheral nervous system, nerve impulses cause ACh to be released at the

nerve ending into the synaptic cleft. The released ACh then combines with postsynaptic receptor sites on other nerves, muscle end plates, or glands to continue nervous integration/transmission, cause muscle contraction, or glandular secretion, respectively. Released ACh also combines with presynaptic receptors to regulate ACh synthesis, so that the communication provided by ACh can be discrete and postsynaptic processes have time to recover between activations. AChE is required to inactivate the ACh in the synaptic cleft by hydrolyzing it into its acid and alcohol components. If there is insufficient AChE, ACh accumulates in the synaptic cleft. At first the communication becomes less and less discrete and overstimulation of the cholinergic system results. Eventually, complete blockage of the cholinergic system occurs.

Anticholinesterase agents and drugs bind to and inhibit cholinesterases, thereby preventing them from inactivating ACh. Although there appears to be a surplus of ChE in the normal system, acute inhibition of more than about 70% causes unpleasant symptoms and debilitation (e.g., salivation, diarrhea, fasciculations); acute inhibition above 90% leads to convulsions, paralysis, respiratory failure, anoxia, and death.

Chronic ChE inhibition produces tolerance by a variety of mechanisms (Russell and Overstreet, 1987) so that higher levels of inhibition may be reached before toxic signs and collapse are produced (Blick et al., 1987d; Kerenyi et al., 1987; Murphy et al., 1988). Although the exact causal relationships are not known, it is clear that the complex of severe symptoms of anoxia and convulsion, resulting from organophosphate poisoning, can result in permanent brain damage (Churchill et al., 1985a; McDonough et al., 1986; Murphy et al., 1988). Organophosphates may cause hypoglycemia, indirectly, by increasing acetylcholine at vagal synapses on the beta cells of the Islets of Langerhans in the pancreas, which causes them to secrete excess insulin. When blood glucose levels approach 50 mg%, there is an increased likelihood of a seizure. If blood glucose levels remain low for a prolonged time period, it will likely lead to permanent brain damage, especially in the cerebral cortex, diencephalon, and medulla. Death can occur as a result of respiratory failure.

The neuropharmacology of the sites involved with exercise and stressors like heat are not completely known, but it is clear that cholinergic mechanisms that are sensitive to chemical defense compounds are an important part of the control mechanisms. In the central nervous system (CNS), acetylcholine is an important neurotransmitter, especially in the cerebral and cerebellar cortex, various sites in the thalamus, and in the hippocampus. In the preoptic/anterior hypothalamic area, which is involved in the control of body temperature, substantial cholinergic inputs have been documented, but their precise function is clouded by significant species-specific differences and complex interactions with noncholinergic systems (Crenshaw, 1979). Other CNS sites involved with temperature sensitivity and temperature control in the caudal hypothalamus, thalamus, septal nucleus, brainstem, and

spinal cord also have significant cholinergic inputs (Reaves and Hayward, 1979), as do brainstem sites involved with the reticular system and those sites involved in the control of heart rate, blood pressure, and respiratory variables. But, chemical defense compounds have many of their most profound and potentially life-threatening effects in the peripheral nervous system. For example, the neuromuscular junction between nerves and skeletal muscles, including the diaphragm, is a cholinergic synapse. The pre- and postganglionic parasympathetic fibers, (including those that innervate the heart and blood vessels), the preganglionic sympathetic fibers, and the postganglionic sympathetic fibers innervating the sweat glands, are cholinergic. Unfortunately, cholinergic receptors are not homogeneous. There are at least 2 types: nicotinic and muscarinic. They respond to nicotine (an alkaloid from *Nicotiana tabacum* and closely related plants) and muscarine (an alkaloid from *Amanita muscaria*, a mushroom), respectively, as if they were acetylcholine. Generally, receptors are either nicotinic or muscarinic; that is, they respond to nicotine or muscarine, but not to both. In the central nervous system, most of the nicotinic receptors are found in the thalamus and the cerebellar cortex; most of the other central receptors are muscarinic. In the peripheral nervous system, the neuromuscular receptor is nicotinic, while most of the synapses in the autonomic nervous system are muscarinic. In general, excitatory nicotinic receptors demonstrate rapid onset and short duration, while muscarinic receptors show slow onset and long duration. These receptors respond differently to drugs; i.e., atropine affects muscarinic synapses, but has little or no effect on nicotinic synapses.

#### Chemical Protection

Three main approaches to pharmacological protection against the effects of nerve agent have been examined:

1. Protective Pretreatment. A seemingly ideal approach to protecting against chemical warfare nerve agents would be to "chemically harden" personnel so that the nerve agent could not inactivate the ChE. The first step toward this ideal has been taken with the fielding of the drug pyridostigmine as prophylaxis to nerve agent poisoning. Pyridostigmine is a carbamate anticholinesterase that reversibly binds to ChE. Pyridostigmine is a quaternary amine and does not cross the blood-brain barrier. It affects only peripheral sites (Matthew et al., 1988). Acutely, pyridostigmine causes trembling due to stimulation of nicotinic receptors in skeletal muscle. This can be controlled by administration of diazepam (Matthew et al., 1987), but diazepam does cross the blood-brain barrier and can depress central cholinergic neurons by decreasing acetylcholine release. While temporarily bound to pyridostigmine, ChE molecules cannot be irreversibly bound to nerve agent and are, therefore, protected. The currently fielded regimen is three 30 mg tablets of pyridostigmine a day for 10 days, which is designed to chronically inhibit ChE by 30 to 40%. Although there is considerable individual variability in the actual level of inhibition, SRL research on primate and

rodent performance (Blick et al., 1986a, 1986b, 1987a, 1987b; Brown et al., 1988; Campbell et al., 1984) is in agreement with other animal research and recent human studies (Gawron et al., 1990) that prophylactic treatment with pyridostigmine at the proposed ChE inhibition levels is safe. Our animal work also indicates that there is a wide margin of safety for this drug.

There is no question that prophylaxis with pyridostigmine will help save lives. Pretreatment with pyridostigmine, in combination with atropine and/or oxime therapy, has been shown to provide protection against the lethal effects of the nerve agent soman in a number of species (Gordon et al., 1978; Lipp and Dola, 1980; Harris, 1981; Xia et al., 1981). In one study (Dirnhuber et al., 1979), pretreatment with pyridostigmine in combination with high dose atropine therapy was reported to increase the LD<sub>50</sub> of soman in rhesus monkeys by 28 times. Pyridostigmine pretreatment has also been shown to be effective in reversing the otherwise irreversible neuromuscular blockade produced by soman in rats (Dirnhuber and Green, 1978; French et al., 1979).

Pyridostigmine, when administered alone, causes an increase in sweating, with a decrease in skin and core temperatures (Avlonitou and Elizondo, 1988). The increase in sweating, with the concomitant water loss, may force the cardiovascular system to work at its limits. In patas monkeys, this water loss does not seem to affect ability to exercise (Elizondo, 1990), at least at modest (25-30%) cholinesterase inhibition. In rats, high levels (60%) of cholinesterase inhibition may compromise the ability to engage in moderate exercise, especially in a warm to hot environment (Francesconi et al., 1984), but this may be due to the way exercising rats dissipate heat (via their tail). But, even in rats, modest (20-40%) cholinesterase inhibition does not affect ability to exercise (Francesconi et al., 1986).

Another issue in the safe use of pyridostigmine is its interaction with low doses of nerve agent on a long-term basis. It is assumed that long-term exposure to soman, above a certain level, would cause cholinesterase to become more and more inhibited until symptoms and decrements appeared. It is possible that the continued use of pyridostigmine during chronic soman exposure might accelerate the inhibition of cholinesterase and the appearance of deficits. In collaboration with USAFSAM personnel, SRL recently investigated this question (Kerenyi, 1987), using a rodent model and found the LD<sub>50</sub> of repeated soman to be unaffected by the chronic administration of pyridostigmine that inhibited ChE by up to an average of 72%. However, the large component of aliesterase in the rat may weaken the applicability of this model. Chronic pyridostigmine did not increase the behavioral toxicity of long-term, low-dose soman exposure. It did provide a small, but variable, protection from soman-induced behavioral decrements (Blick et al., 1988).

2. Reactivation of Cholinesterase. Another approach to counteracting the effects of nerve agents is to pharmacologically

reactivate the inhibited cholinesterase. The oxime 2-PAM chloride has been fielded for this purpose. It and the anticholinergic drug atropine are the two parts of the standard therapeutic "combo-pen" that are to be administered as soon as possible after suspected nerve agent exposure. The theory behind the use of reactivation is that by disengaging the soman, the ChE can be restored to function. Therapeutic doses of 2-PAM chloride do not cause any change in core temperature, skin temperature, heart rate, or whole body sweating in resting man (Robinson and Michael, 1970), but do cause a small decrease in whole body sweating with moderate exercise in humans (Cummings et al., 1964) and monkeys (Kolka et al., 1987). Combining atropine and 2-PAM chloride may augment the atropine-induced increase in body temperature (Kolka et al., 1987; Cummings et al., 1964). Since 2-PAM chloride does not cross the blood-brain barrier, it affords relatively little reactivation of central cholinesterase. Unfortunately, proPAM, which does cross the blood-brain barrier, also provides relatively little reactivation and caused some transient, but pronounced behavioral toxicity (Kenley et al., 1982). While this approach works well for some nerve agents, it is almost completely ineffective against soman because the soman-ChE bond quickly becomes irreversible (a process called aging).

3. Anticholinergic Therapy. A third approach to protecting against nerve agents is to protect against the excess of ACh and associated convulsions and anoxia. Atropine, which is generally accepted as an effective treatment for anticholinesterase (carbamate or organophosphate pesticides, chemical warfare nerve agents) exposure, is one such treatment. It competes with the neurotransmitter acetylcholine for binding sites at muscarinic receptors in smooth muscle, heart, and sweat glands, while having little, if any, effect on nicotinic receptors, such as those at the neuromuscular junction. One effect of this competition is to reduce sweating in humans and other primates by 40-60% (Avlonitou and Elizondo, 1988; Kolka et al., 1987; Sato and Sato, 1981; Craig, 1952). This suppression of thermoregulatory sweating and evaporative heat loss results in a net heat storage (Avlonitou and Elizondo, 1988) with a reduced heat tolerance and reduced physical exercise performance (Craig, 1952; Cullumbine and Miles, 1956; Davies et al., 1978). Excessive and/or prolonged heat storage might be expected if personnel are exposed to a "hot" environment, moderate to severe exercise, or the combination of exercise in a "hot" environment, especially if these conditions are compounded by wearing a protective chemical defense ensemble. If such excessive and/or prolonged heat storage occurs, it can lead to heat-related disorders, which range from heat fatigue to heat exhaustion to heat stroke. Generally, heat fatigue and heat exhaustion are self limiting if the individual can be allowed to rest in a cool environment and is provided with fluids. Unfortunately, the symptoms of heat disorders resemble those of low-dose nerve agent exposure. Careful differential diagnosis is critical because treatment with nerve agent antidotes (atropine) is contraindicated and could be life-threatening. If such conditions are untreated, they may lead to heat stroke. This is characterized by headache, dizziness, numbness or drowsiness, purposeless

or uncoordinated movements, mental confusion (with mania), as well as muscle rigidity, generalized convulsions, tachypnea, tachycardia, high core and skin temperatures, with pupils constricted and pinpointed. Life-threatening emergency can occur within minutes to hours.

On the other hand, atropine also causes an "atropine flush", an increase in cutaneous blood flow (Davies et al., 1978). It is not known if this represents a mode of heat exchange. Atropine also causes an increase in heart rate, especially during exercise (Kolka et al., 1987; Avlonitou and Elizondo, 1988). It is not clear if this is caused by a decrease in venous blood pressure due by blood being shunted to the skin (the "atropine flush"), to some direct effect on the heart (such as decreased parasympathetic inputs), or by some other central or peripheral nervous system effect.

When administering atropine, it is important to consider what other anticholinergics might be present in the system. These include a wide variety of over-the-counter and prescription drugs, such as antihistamines, cold medications, antidiarrheal medications, as well as minor (antidepressant) and major (anti-psychotic) tranquilizers, as these agents may act additively or synergistically with atropine (Matthew et al., 1986). The route of administration is also important. Oral administration requires approximately twice the dose and twice the time to reach peak effectiveness (Mirakhur, 1978).

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## TRAINING PROTOCOL

1. TITLE: Training Program for Instrumentation, Telemetry, and Exercise Ergometry
2. PROJECT/TASK/WORK UNIT: 2729-03-30
3. PRINCIPAL CO-INVESTIGATORS/USERS:  
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5. USAF RELEVANCY AND INTERAGENCY DEPENDENCY: Military planners in all of the uniformed services require estimates of the effects of nerve agent exposure on personnel and their ability to carry out their missions. The effects on performance of proposed prophylactic and/or antidote drugs for nerve agent are also of great interest. USAFSAM/VNC and its in-house contractor group will engage in a research program that includes assessments of the effects of nerve agents, prophylactics, antidotes, and their combinations in a primate model that characterizes the human physiological responses to thermal stress and physical work. The tasking includes specific protocols to evaluate the impact of proposed chemical defense drugs, prophylactic drugs, ergogenic aids, and physical stress on potential ground crew performance and airbase operability. The primate model to be established at USAFSAM will provide a safe and ethical means to investigate physiological stress functions. The research will provide information to the Surgeon General and, ultimately, to commanders that will help them make crucial decisions concerning the potential impact of chemical attacks on airbase operability.
6. SCIENTIFIC OBJECTIVES: The purpose of the current protocol is to train a pool of research primates to accept thermal regulatory instrumentation and to exercise on a primate exercise wheel (PEW), (Curran et al., 1972). Subsequent testing protocols will address the addition of a thermal burden for the animal, along with the testing of drug effects.
7. TECHNICAL BACKGROUND: In general, the testing of the physiological effects of experimental drugs involves procedures which, for technical, legal, or ethical reasons, cannot be carried out in humans or with computer models. Nonhuman primate models have been successfully incorporated in these types of studies. Of general interest in the present effort are the potential applied effects of selected pharmacological drugs/agents associated with chemical warfare/defense (CWD) scenarios. Limited studies in man

(Kolka et al., 1984) and USAFSAM sponsored research (Avlonitou, 1987; Avlonitou and Elizondo, 1988) in primates have documented the potential deleterious effects of CWD drugs on thermoregulation and physical endurance. The suitability and broad applicability of this primate model for expanded USAF studies have been demonstrated. Therefore, the purpose of this effort is to establish this model in-house at USAFSAM.

Elizondo (1988) has described the appropriateness of the primate model for studying thermoregulation. Thermoregulatory function is an especially important concern in CWD scenarios because of the inhibitory effects of CWD garments on heat dissipation. The eventual increase in cumulative body heat storage results in early physical fatigue and collapse. A related parameter is the level of physical exertion, since vigorous activity increases metabolic heat production, perturbs physiological function, and promotes fatigue in itself (Rowell, 1974; Astrand and Rodahl, 1977). It is therefore necessary to evaluate the additional physical stressors of both environmental thermal burdens and exercise to more realistically represent the possible airbase operational environments for USAF ground crews in USAF CWD research.

In the laboratory, variations in the ambient environment can be easily accomplished using the thermal chambers at USAFSAM. Promoting an exercise perturbation, which is appropriate for the primate, is a more difficult task but not entirely novel. Curran (1972) at the Armed Forces Radiology Research Institute (AFRRI) first developed a primate exercise wheel (PEW) for use in studying the regression of atherosclerotic lesions with exercise. Robertshaw et al. (1973) and Gisolfi et al. (1978) have also incorporated physical work into their research with primates. More recently, Avlonitou (1987) and a USAFSAM contractor, Dr. R. Elizondo, have successfully modified the PEW for CWD research. We therefore plan to incorporate the experimental model established through previous USAFSAM-sponsored research into VNC's in-house research program.

The purpose of this protocol is to provide and maintain a pool of trained monkeys for studies incorporating this thermoregulatory/exercising primate model. This capability will then be utilized in specific CWD and related experiments after approval by the USAFSAM Animal Care and Use Committee and the Human Systems Division (HSD) Primate Use Committee.

#### 8. EXPERIMENTAL METHOD:

(a) Primate Exercise Wheel Training Procedures: The PEW can be described as a simple locomotor task. The method for training monkeys to operate the PEW is as follows:

Animals are individually selected from the available pool by the trainer. Aggressive animals are chosen whenever possible because experience has shown that they learn more quickly. A

normal, aggressive young monkey, placed in a new situation, is active and curious, which greatly facilitates the initial phases of training. Routine adaptation to the training environment is specifically avoided as it reduces the level of exploratory behavior, thus retarding the initial phase of training.

Primate Exercise Wheel Training: Animals will be moved into a mobile transfer cage and transported to the PEW. The subjects will be released from the transfer cage directly into the PEW. No direct human contact will be necessary. Training will include the following steps:

1. Approximately 1 min after release into the PEW, a red light mounted in front of the animal will be illuminated; 5 s following onset of the red light, shock will be administered to the bars of the PEW. Shock will be sufficient only to cause the animal to move about the wheel. The shock parameters are (a) approximately 0.1 mA, (b) 0.3 s duration, (c) repeat once per second until movement of the wheel occurs. One of the PI's, Dr. Constable, has manually tested this shock level and found it to be unpleasant, but not painful. Initially, the wheel will be manually moved by the experimenter coincident with the offset of the shock. Any movement of the wheel (by the animal or experimenter) will cause the shock to cease. The computer will be programmed to discontinue all shock, halt the entire program, and signal the operator if the animal fails to cause the wheel to move for any 15 s period.

2. Training, as per step 1, will continue until the animal initiates movement of the wheel and keeps the wheel in motion for at least 10 s. If the animal continues to move the wheel, the light will be extinguished and the wheel manually stopped until the next trial. The inter-trial interval (ITI) will be 30-60 s, depending on the animal's progress. Desirable (appropriate) animal responses will be given longer ITI times.

3. When the animal reliably starts on the red light cue, the length of the trial will gradually be increased to 2 min, with a 1 min ITI (rest period).

4. Prior to step 4, any wheel movement (per 5 s) delays any shock onset. In step 4, the white light will flash if the speed of the wheel falls below a preset speed, 0.3 miles per hour (mph). This step will be complete when the subject is able to work 2 min, with 1 min rest for 1 h with no more than 5 shocks for the hour.

5. The minimum speed required to postpone any shock initiation will be gradually increased in 0.2 mph increments up to 2.5 mph for 1 h.

6. The minimum speed will be set at 1.0 mph and the 1 min rest interval will be shortened by 10 s every third day. When the subject can continuously walk at least 1.0 mph for 1 full hour, with no more than 6 shocks, the minimum speed will be

increased in 0.2 mph increments until the subject can maintain 2.5 mph for the hour.

We know that these speed parameters are reasonable since they have been used previously for work that USAFSAM/VNC contracted to Dr. Elizondo (1988) and that they are much lower than speeds that have been used by others. (Curran (1972) set the lower limit at 3.0 and the upper limit at 6.0 mph. The animals operated at an average of 3.2 mph for 1 h nonstop.)

(b) Telemetry Instrumentation Procedures: In order to monitor body (core) temperature and heart rate, a Mini Mitter (Mini-Mitter Company, Inc., P.O. Box 3386, Sun River, OR 97707) Physiotel amplifier-transmitter (less than 2" in diameter, 1/4-in. thick convex shape) will be implanted subcutaneously. After a surgical level of anesthesia is achieved (induction ketamine 10 mg/kg, i.m.; maintenance isoflurane) a small midline incision will be made under aseptic conditions near the ventral midline, over the abdominal cavity. Blunt dissection will be used to open a subcutaneous pocket to accommodate the amplifier-transmitter package. The two electrocardiographic leads will be tunneled under the skin, from the implantation site to sites, one on each side of the chest, which provide the best electrocardiographic signal (monitored during implantation). The temperature probe (13 cm long, 3 mm in diameter) will be inserted into the abdominal cavity, via a small incision in the linea alba. Post-operative analgesia will be by buprenorphine 0.025 mg/kg). All surgical procedures will be performed in the operating room of the Research Support Section (RSS) under the supervision of the attending veterinarian.

The implanted amplifier-transmitter package is battery operated. The battery has a rated life of approximately 6 months (constant on). But this can be extended to 20+ months, by turning the amplifier-transmitter off, utilizing a built-in, three position, magnetically activated switch (on, off, test), when the animal is not to be involved in an experiment for an extended time.

Subcutaneous implantation of the amplifier-transmitter and all leads will minimize the amount of human contact required---limited to chairing the monkey and turning the amplifier-transmitter package on or off with a magnet.

The monkeys will be chaired (Fig. 1) 3-4 times for 1 h per session to adapt to the procedure. They will then be chaired periodically during training to obtain resting physiological (heart rate, temperature) readings. They will be placed in the chair by two technicians wearing standard protective clothing. An intermediate thoracic plate will be installed in the restraint chair to restrict the animal's ability to reach the leads on the chest area.

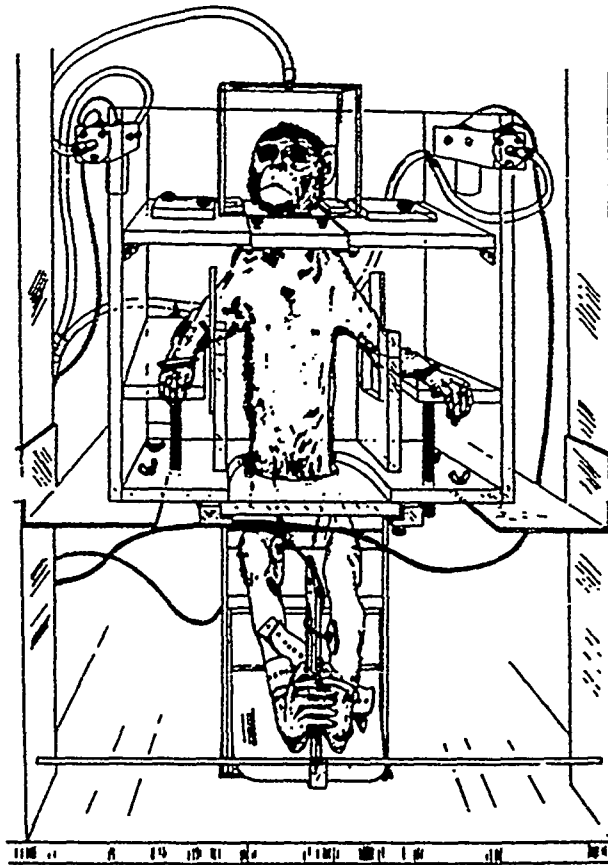


Figure 1. Monkey seated in primate restraint chair.

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10. DTIC LITERATURE SEARCH: Not applicable, as this protocol only prepares or maintains animals for use in specific experiments detailed in other protocols.
11. RESOURCE REQUIREMENTS:
  - a. Facilities: Initial housing for 6 rhesus monkeys in Building 185, USAFSAM, Brooks AFB, TX. Laboratory space for training PEW systems in Building 185. Future housing for 12-16 patas monkeys in Building 185 at any one time.
  - b. Equipment: Components for the 2 PEW systems, 2 primate chairs, and telemetry equipment required for this work are being ordered and assembled.
  - c. Animals:
    - (1) a. Rhesus: Six rhesus (2.5-4.5 kg) will be used initially. No additional rhesus are anticipated except as possible replacement animals due to health or difficulty of training.
    - b. Patas: Up to 16 Patas females at any one time, not less than 2.5 nor more than 4.5 kg, to be selected from the available commercial supplies. Dates of use will vary with the needs of the research program supported by this protocol. On average, we expect to use approximately 16 patas animals and replace animals if unexpected loss is sustained.
  - (2) Postexperimental Disposition: Successfully trained animals will always be transferred from this protocol to an experimental protocol. Experimental protocols will specify the disposition of the animals. Animals not amenable to training will be returned to the Veterinary Sciences Division (VS).

d. Support Personnel: All animal training will be conducted by SRL (Contractor) personnel.

e. General: Maintenance of the animals and their quarters will be the responsibility of USAFSAM/VSR. This protocol requires no special care or animal support.

f. Nutrition: The animals will be maintained on normal rations of monkey chow. Animals will not be trained in the PEW within 2 hr of normal ration feeding. A small amount of fruit will be given when the animal is placed in the PEW to facilitate handling.

12. HAZARDS:

The hazards associated with handling animals are minimized by adherence to standard laboratory procedures, with which all applicable personnel have been familiarized.

All personnel will be briefed on the hazards of direct contact with the animals. Standard protective clothing, gloves (rubber and/or leather), and face masks will be worn during the handling of any animal. Personnel will have read USAFSAM Regulation 161-1 covering the control management for herpes virus simiae (B-Virus). Every possible attempt will be made to prevent scratches and bites.

13. ANIMAL USE:

a. Alternative Species: The rhesus monkey is the immediately available species of choice because of the large data base on the species, including the area of exercise physiology, and the comparability to man. In some species, like the squirrel monkey, heat can be dissipated by sweating, but primarily through the palms and soles. The squirrel monkey adapts by making behavioral adjustments and vasomotor control changes to regulate body temperature (Kolka and Elizondo, 1983). The rhesus and patas monkey are qualitatively similar and, like humans, sweat over the entire body surface. However, the evaporative heat loss that is due to sweating is 40% higher in the patas (Elizondo, 1988). Because of the higher sweating capacity and other similarities with the human eccrine system, the patas is the more appropriate animal model for thermoregulatory studies. The patas can tolerate higher temperatures than the rhesus, this capacity being related to their adaptation to the savanna and subdesert areas that they inhabit (Kolka and Elizondo, 1983).

b. The care and use of animals in this study will be in accordance with USAFSAM Regulation 169-2.

c. Veterinary Consultant: Dr. Roger C. Harvey/VSR/43477

14. SIGNATURES OF PRINCIPAL INVESTIGATORS:

## Protocol Outcomes

### 1. Primate Exercise Wheel System Modification/Debugging

SRL employees suggested the following modifications in the PEW system that was delivered to USAFSAM/VNC: 1) setting the maximum number of sequential shocks that the monkey can receive and automatically turning off the program so that the monkey cannot receive any additional shocks unless the experimenter manually turns the program back on; 2) controlling the number and duration of work/rest cycles; 3) enlarging the light bar; 4) changing the "meaning" of the light signals: a) a green light indicates a work period (no tone); b) a yellow light plus tone indicates an underspeed condition, where the animal must increase speed to avoid a mild shock; c) light bar, lights off, house lights on indicates a rest period; and 5) monitoring the total distance traveled by the monkey, the number of grace periods (yellow light on) initiated, the cumulative grace period durations, and the number of shocks received in each work period and for the total session.

SRL employees worked with Rothe Development and USAFSAM Technical Services (TS) personnel to revise, debug, and modify the metabolic and physiological data acquisition program as written for the PEW system by Rothe Development.

Specifically, SRL employees suggested modifications in the way heart rate data was collected and confirmed; that all equations, especially those involving  $VO_2$ ,  $VCO_2$ , respiratory evaporation, surface area conductance (K), and MET, were correctly implemented. This was accomplished by modifying a copy of the program to allow data to be entered via the keyboard, then comparing the results with those obtained using the same data and equations with the manipulations made by hand calculators.

The correction factor for STPD is calculated as follows, (Weast, 1964; Hall and Brouillard, 1985).

$CF = (1 - PH_{20}/BAROPRESS) * (273.15/760) * (BAROPRESS/TEMPKDB)$ , where:

$PH_{20} = PH_{20}STADB - ((0.00066 * BAROPRESS * (TEMPDB - TEMPNB)) * (1 + .0011 * (TEMPDS - TEMPNB)))$ , and where:

$PH_{20}STADB = (10^{((-7.90298) * (373.16/TEMPKDB - 1))} + (5.02808 * (\text{LOG}(373.16/TEMPKDB)/\text{LOG}(10)))) - (1.3816 * 10^{-7}) * (10^{(11.334 * (1 - TEMPKDB/373.16) - 1)}) + (8.328 * 10^{-3}) * 10^{(-3.49149 * (373.16/TEMPKDB - 1)) - 1} * 760$ .

Oxygen Volume is calculated as follows, (Brown et al., 1984).

$VO_2 = |(((FLOWN_2 + DERVOLN_2) * DELTAO_2) + (VOLN_2 BOX * DERO_2))|$ , where:

$FLOWN_2 = FLOWSTPD * FEN_2$ ;

$DERVOLN_2 = BOXVOL * ((FEN_2 * CF) - (pFEN_2 * pCF)) / DELTATIME$ ;

$$\text{DELTAO}_2 = (\text{FEO}_2/\text{FEN}_2) - (\text{FIO}_2/\text{FIN}_2);$$

$$\text{VOLN}_2\text{BOX} = \text{BOXVOL} * \text{CF} * \text{FEN}_2;$$

$$\text{DERO}_2 = ((\text{FEO}_2/\text{FEN}_2) - (\text{pFEO}_2/\text{pFEN}_2)) / \text{DELTATIME}.$$

Carbon Dioxide Volume is calculated as follows (Brown et al., 1984):

$$\text{VCO}_2 = |(((\text{FLOWN}_2 + \text{DERVOLN}_2) * \text{DELTACO}_2) \div (\text{VOLN}_2\text{BOX} * \text{DERCO}_2))|,$$

where:

$$\text{FLOWN}_2 = \text{FLOWSTPD} * \text{FEN}_2;$$

$$\text{DERVOLN}_2 = \text{BOXVOL} * ((\text{FEN}_2 * \text{CF}) - (\text{pFEN}_2 * \text{pCF})) / \text{DELTATIME};$$

$$\text{DELTACO}_2 = (\text{FECO}_2/\text{FEN}_2) - (\text{FICO}_2/\text{FIN}_2);$$

$$\text{VOLN}_2\text{BOX} = \text{BOXVOL} * \text{CF} * \text{FEN}_2;$$

$$\text{DERCO}_2 = ((\text{FECO}_2/\text{FEN}_2) - (\text{pFECO}_2/\text{pFEN}_2)) / \text{DELTATIME}.$$

K is calculated as (Johnson and Elizondo, 1979; Kolka and Elizondo, 1983; Chen, 1989):

$$K = \frac{(\text{MET} - \text{EVAPBOX})}{(\text{CORETEMP} - \text{MEAN SKIN TEMP})}, \text{ where}$$

$$\text{MET} = \frac{[\text{VO}_2 * (3.83 + (1.2) * R)] * 69.77}{\text{S.A.}}, \text{ and}$$

$$\text{EVAPBOX} = ((\text{HUMIDRATIO}_{\text{EXP}} - \text{HUMIDRATIO}_{\text{ENV}}) * (\text{FLOW} * 0.1589)) * 453,$$

where:

$$\text{HUMIDRATIO}_{\text{EXP}} = (0.62198 * (\text{P}_{\text{W}_{\text{EXP}}} ((30.08/29.92) * 14.384))); \text{ and}$$

$$\text{HUMIDRATIO}_{\text{ENV}} = (0.62198 * (\text{P}_{\text{W}_{\text{ENV}}} ((30.08/29.92) * 14.384))); \text{ and}$$

$$\text{P}_{\text{W}_{\text{EXP}}} = \text{E}^{-\text{LN SAT PRESSURE}_{\text{EXP}}} \text{ and } \text{P}_{\text{W}_{\text{ENV}}} = \text{E}^{-\text{LN SAT PRESSURE}_{\text{ENV}}};$$

$$\text{and } \text{P}_{\text{W}_{\text{EXP}}} = \text{E}^{-\text{LN STAT PRESSURE}_{\text{EXP}}}.$$

$$\text{LN SAT PRESSURE}_{\text{EXP}} = (-10440.39708 / \text{TEMPWB}_{\text{EXP}}) + (-11.2946496) +$$

$$(-0.027022355 * \text{TEMPWB}_{\text{EXP}}) + (1.289036 * 10^{-5} * \text{TEMPWB}_{\text{EXP}}^2) +$$

$$(-2.478068 * 10^{-9} * \text{TEMPWB}_{\text{EXP}}^3) + (6.5459673 * \text{LN TEMPWB}_{\text{EXP}})$$

LN SAT PRESSURE<sub>ENV</sub> is calculated using the same equation, but substituting TEMPWB<sub>ENV</sub> in the equation above.

SURFACE AREA is calculated as:

$$SA=(0.107233\div(0.0013803*WT)-(0.0000873171*WT^2))*(WT^{2/3}).$$

MEAN SKIN TEMPERATURE is calculated as (Kolka and Elizondo, 1983):

$$\text{MEAN SKIN TEMPERATURE}=0.266*(T_{\text{CHEST}})+0.375*(T_{\text{BACK}})+.310*(T_{\text{THIGH}})+0.077*(T_{\text{CALF}})+0.152*(T_{\text{TAIL}}).$$

## 2. Specific Procedures/Primate Exercise Wheel (PEW)/Primate Restraint Chair

For training procedures for PEW, see Section 4b, on Shaping and Maintaining Exercise Behavior.

The monkeys were placed in a primate restraint chair by two experimenters for 1-2 h/day for 7-14 days, until they became acclimated to the procedure and stopped struggling. Once they became acclimated to the chairing procedure, they were instrumented as described in the protocol. Each time a monkey was removed from the primate restraint chair, it was examined to determine if the chairing or instrumental procedures caused any pathological processes, such as decubital ulcers, contusions, or dependent edema. It was unlikely that any pathological process would occur, but if it had, chairing procedures would have been temporarily suspended until the monkey had been examined by the veterinary consultant.

## 3. Typical Training Day

From early July, 1989 (when the PEW was initially delivered, modified, and debugged) until late August, SRL employees (Sherry and McGlothan) trained monkeys to run in the PEW under stimulus control and to sit quietly in a primate restraint chair. By the end of this time period, the first 6 rhesus monkeys were running 3 mph for 6-10 min work periods, with 1 min rest periods, under stimulus control, and sitting quietly in a primate restraint chair for 2-4 h.

Beginning in late August, monkeys were transported to the chamber where the experiments were to be conducted to allow them to adapt to this new setting. During this period, the two SRL employees transported a monkey to the chamber and placed it in the primate restraint chair. McGlothan would then return to the vivarium and run each monkey in the PEW, while Sherry remained with the monkey in the chamber to conduct the experiment.

On Labor Day weekend, McGlothan was injured in a non-work-related accident and was placed on restricted duty. SRL was able to reassign SRL employees (DeLaPena and Muraira) from another contractual effort so the monkeys could continue to be run in the PEW and the experiments could continue in the chamber. McGlothan was able to return to full duty in mid-December.

In mid-December, when the pyridostigmine experiments started, an SRL employee (Noonan) was able to interact with a Rothe Development employee (Parker) to facilitate setting up the cholinesterase assay.

#### 4. Results

##### a. Primate Exercise Wheel (PEW)/Primate Restraint Chair

Monkeys 521Z, 531Z, 567Z, 615Z, 619Z, 641Z, currently run in the PEW under stimulus control at 3 mph for 6 - 10 min exercise sessions separated by 1 min rest periods. These monkeys have also been trained to sit quietly in the Primate Restraint Chair for the duration of an experiment. Monkeys 603Z, 607Z, 611Z, and 623Z are currently being trained to run in the PEW.

##### b. Preliminary Draft of Paper Describing The Primate Exercise Wheel (PEW)

The level of exercise of humans is typically quantified by using stationary bicycles or treadmills. There are a variety of methods available to measure the exercise level of small animals, such as common laboratory rodents, and to evaluate the effects of drugs, central nervous system lesions, and other treatments on this activity. But it is often relatively difficult to develop a model of human physiological responses in small animals, such as rodents, because of differences in metabolic rate, basic physiological responses, etc. Primates physiological responses are similar to humans, so results obtained with a primate model can be more readily extrapolated to humans.

Unfortunately, adult and even juvenile nonhuman primates are unpredictable, difficult to handle, and potentially dangerous. So, they must be confined, either in a primate restraint chair or in a cage. Therefore, it is relatively more difficult to evaluate their exercise level. Smith described a bicycle ergometer for chaired primates (Smith et al. 1962), while Gisolfi described a primate "rowing machine" (Gisolfi et al., 1978). Both of these authors used positive reinforcement (preferred fluid and pellets, respectively) to motivate exercise behavior. Using positive reinforcers causes two potential problems: 1) eating or drinking and swallowing may interfere with breathing and may limit the duration of exercise because of satiation, and 2) it may not be possible to obtain or maintain the desired level of physical exertion. But both of these methods could probably be modified for use with aversive stimuli to motivate behavior. Robertshaw described a motor-driven treadmill for primates, but it took as long as 8 months to train monkeys to use this device and it could only be used with relatively tame monkeys (Robertshaw et al., 1973). Curran described an exercise wheel for primates (Curran et al, 1972) which has been modified and used by Avlontou (1987) and Elizondo (1990). We have made additional modifications and propose to describe the basic exercise wheel and our modifications here.

### Primate Exercise Wheel (PEW)

The wheel is constructed of 2 sheets of 3/4" plastic (Plexiglas ) and 148 round aluminum bars (6061 T6 aluminum bar stock) which form a cylindrical cage, 48" in diameter and 17" wide, as shown in Figure 2. The wheel is suspended via R-16 roller bearings on stub axles attached to the frame which is constructed of 14 gauge 1" square steel tubing. One of the Plexiglas walls contains two spring-loaded guillotine doors, each 10" by 16". The middle of the inner edge of each door is located 9" from the center of the wheel. Because of variations in the thickness of the plastic walls, the placement of the doors, etc., the wheel may not be perfectly balanced, so small external weights can be added to obtain satisfactory balance.

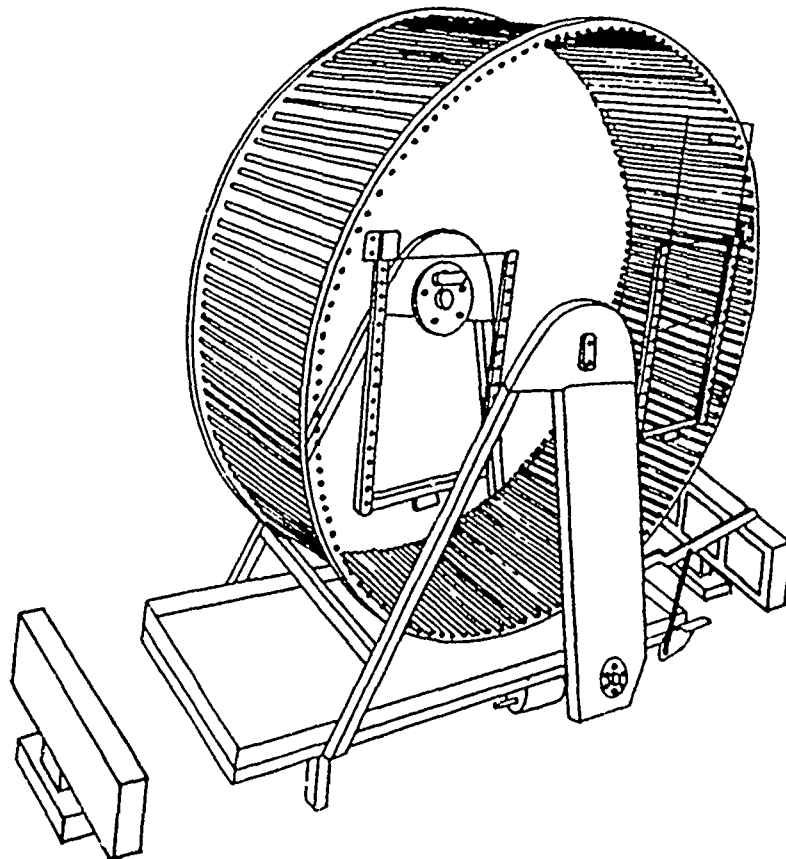


Figure 2. Primate Exercise Wheel

The aluminum bars act as a circular treadmill and provide the surface on which the monkey runs. They also act as a shocking grid to provide a negative reinforcement during the initial shaping and subsequent maintenance of the behavior (walking or running), using a free-operant (Sidman) avoidance paradigm with exteroceptive cues. Brass slip rings with electric motor brushes allow energizing of alternate bars when the wheel is in motion to provide the shock.

The wheel is supplied with a mechanical friction brake (Fig. 2), which can be applied manually or it can be controlled automatically by the Wheel Controller. The brake prevents the monkey from operating the wheel faster than an upper preset speed and stabilizes the wheel during rest periods.

Wheel rotation is detected by a Data Technology Optical Encoder (SM23-850-20/12). The output of this device is connected to the Wheel Controller, which has two modes of operation: manual and automatic.

#### PEW-Wheel Controller/Manual

When the Wheel Controller is switched from Automatic to Manual (by a toggle switch on the Controller), the overhead light is turned off and the green (middle) light on the light bar turns on. The experimenter has direct control of the yellow under speed (right) light on the light bar, shock initiation, and brake initiation via separate switches on a hand-held controller that is connected to the Wheel Controller. Normally the Manual mode would be used during the shaping of the behavior.

#### PEW--Wheel Controller/Automatic

In the automatic mode, the wheel is controlled by an Apple Macintosh II computer with National Instruments NB-MIO-16L and NI-MIO-16H boards, in computer slots 1 and 2 respectively, and using a program written in the National Instruments LabVIEW graphical programming language, version 1.2. A block diagram of the program is shown in Figure 3. The control panel as it appears on the Macintosh monitor is shown in Figure 4. The set variables are located in the lower right corner of the control screen and are altered by placing the cursor on the appropriate box with the Mouse and making the changes on the keyboard. The switches are toggled with the Mouse.

When all telemetry systems are functioning satisfactorily, the subjects will then be similarly instrumented and placed in the PEW. The animals will be tested for 1 h per session until the subjects satisfactorily adjust to the telemetry devices. Techniques will be patterned after the work by Avlonitou and Elizondo (1988).

Instantaneous speed, in miles and fractions of a mile per hour and total distance covered in miles and fractions of a mile, as well as the number of "grace" period initiations, the number of shocks since reset, the total number of shocks, the number of the current work cycle, and the elapsed time since the beginning of the current work or rest cycle are shown on readouts located on the left half of the controller screen.

Exteroceptive cues are provided by a pair of light bars mounted at each end of the wheel, a tone generator, and by an



overhead "house" light. Each light bar is divided into three light panels (each 4 in. square), from left to right, colored red, green, and yellow.

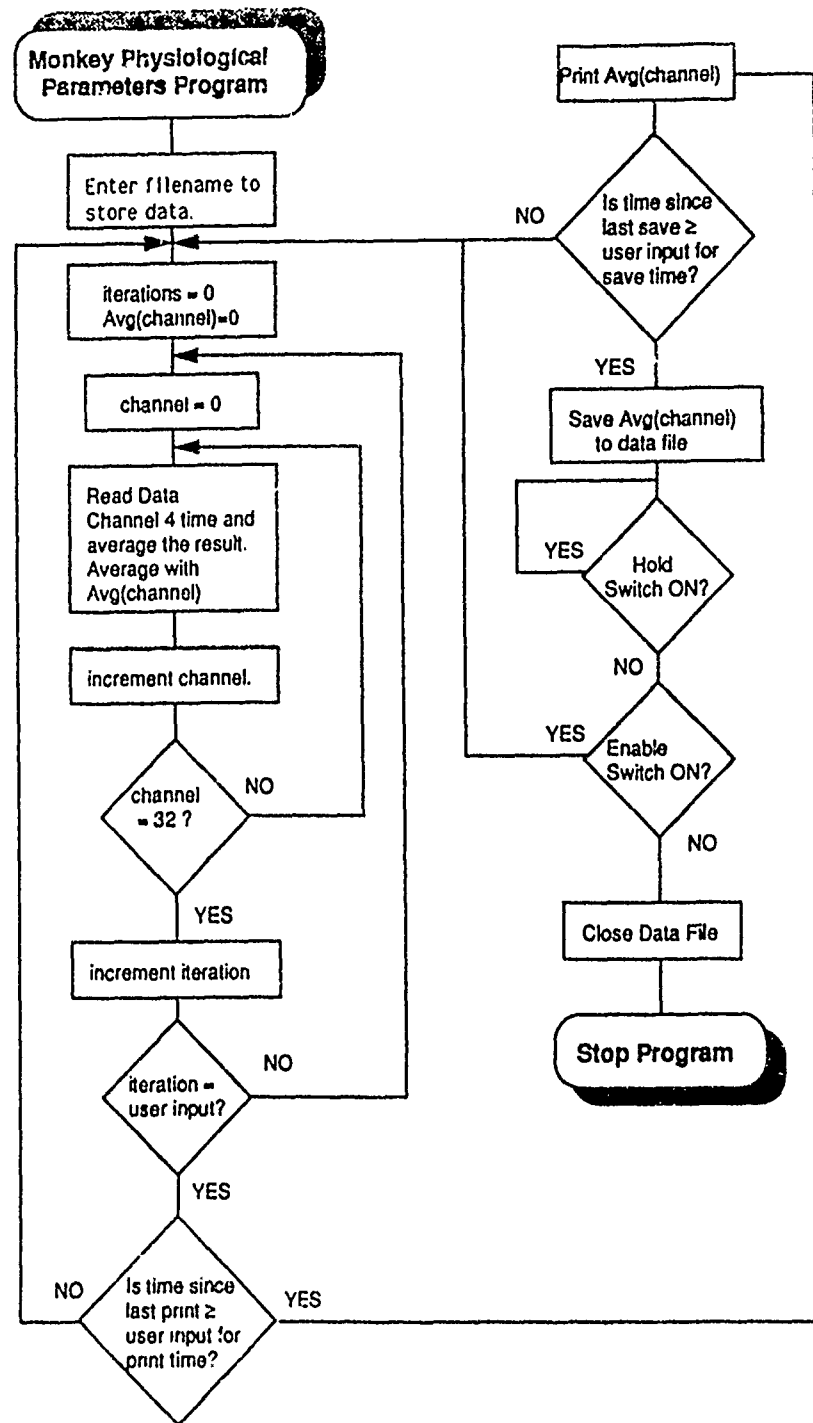


Figure 3. Block Diagram of LabView Program.



the controller. The yellow light can be accompanied by a 1900 Hertz (60-75 dB) tone generated by a Mallory SC-110-D tone generator. It is possible to turn the tone off when it is not needed. If the monkey has not increased the speed of the wheel above the minimum preset level during this "grace" period (duration set on controller), it will receive a shock (duration of shock, inter-shock interval, and number of sequential shocks set at controller). The monkeys rarely receive more than one or two sequential shocks (a trained monkey may not receive any shocks or at most 1 to 2 individual shocks during a 1-h training session). In the unlikely event that the monkey receives the number of sequential shocks set on the controller, the computer automatically turns the program (and shocking capacity) off and turns the lights (and tones) on the light bar off and the house light on (thus indicating a rest period to the monkey). The computer beeps to signal the operator to manually turn the program back on. The monkey cannot receive any shocks when the program is off. The shock intensity is controlled by a BRS Electronics Shock Generator (SG-002).

The left panel is red and lights whenever the monkey's speed exceeds the maximum speed that was set on the controller. At the end of the "grace" period, the brake automatically engages for 100 msec (set at controller) and continues to be applied at 1-s intervals (set at controller) until the wheel speed is brought below the preset maximum speed. Activation of the brake is accompanied by the "clicking" sound of the solenoid that works the brake, providing an additional exteroceptive cue.

At the beginning of a rest cycle (duration set at controller), the lights on the light bar turn off (as well as any tones). The overhead "house" light turns on and the brake is applied to stabilize the wheel.

At the end of the rest cycle, the house light turns off, the green light and the yellow light on the light bar turn on, indicating that a work cycle has begun and the wheel is currently below speed. At the end of the "grace" period, if the monkey has not brought the wheel up to speed, it receives a shock.

#### Shaping and Maintaining Exercise Behavior

Control of exercise behavior is based on a free-operant avoidance paradigm with visual and auditory exteroceptive cues. Although the animal could learn to exercise at a specific rate (as determined by the experimenter) using only proprioceptive cues, the exteroceptive cues are used to increase the speed with which the animal acquires the task and minimizes the probability of shock once a steady-state level of behavior is achieved.

Shaping the behavior is relatively straightforward. The monkey is placed in the PEW with the Wheel Controller in Manual. The brake and the overhead house light are turned off and the green light on the light bar turned on (indicating that a work

cycle is in effect). Most monkeys do not appear to like to sit on the "floor" of the wheel, but try to move up the side of the wheel. In a properly balanced wheel, even a small movement on the part of the monkey will "rock" the wheel. When this occurs, turn on the yellow light-tone, if the monkey increases its speed, turn off the yellow light-tone. If the monkey stops or does not increase its speed, wait 5 s (grace period) and then give the monkey a single shock. Generally, this will cause the animal to move forward. If this occurs, turn off the yellow light-tone and note the monkey's ambient speed.

Do not allow the monkey to "ride" the wheel; that is, do not allow it to grab onto the bars, hold on, and ride the wheel around in a complete or partial circle without moving. One simple way to prevent this behavior is to divide the wheel into two halves, an upper and a lower half. Whenever the monkey is in the upper half of the wheel, it receives a shock.

As with most operant tasks, it is best if the animal develops a stereotyped method of response, so it is best to allow the monkey to run in only one direction. The direction, either clockwise or counterclockwise, can be chosen by the experimenter or the monkey (that is, watch which direction the monkey chooses to run on its initial work in the wheel and then reinforce movement in that direction). The monkey quickly learns the relationship between the yellow light-tone and the shock.

Depending on the monkey's age and apparent physical condition, set up an appropriate number of work-rest cycles of an appropriate duration. Initially, determine a minimum acceptable speed by increasing the monkey's spontaneous speed by 0.2 to 0.5 mph. This can be increased over sessions by the same increment until the monkey reaches the desired speed. It is best to start relatively slowly and observe the animal closely to determine if the level of exercise causes physiological stress (panting, labored breathing, etc.). If these physiological signs do not occur, then increase the duration of work cycles to the desired duration over several sessions. This is the training protocol used in this laboratory, as well as by Curran and his colleagues (1972) and Elizondo and his colleagues (Avlonitou, 1987; Elizondo, 1990).

Curran, working with Macacas, recommends a 2 h orientation to the wheel. He began with 6 to 8 daily sessions (2 min exercise/5 min rest). Over a 5-week period, he gradually increased the duration of the work sessions to 10 min exercise/5 min rest, with 6 sessions per day and the minimal acceptable speed set to 3 mph (Curran et al., 1972). Avlonitou, working with patas monkeys, used a 2-h orientation to the wheel followed by this training schedule: 20 days--2 min exercise/4 min rest; 10 days--3 min/3 min; 10 days--4 min/2 min; 10 days--5 min/2 min, all for 6 sessions per day; 10 days--7 min/2 min, 5 sessions/day; 10 days--10 min/2 min, 4 sessions/day; 10 days--15 min/2 min, 3 sessions/day; and 20 days--20 min/2 min, 2 sessions/day. The minimum acceptable speed was 2 mph (Avlonitou, 1987; Elizondo, 1990).

With our Macacas, we used the following: 5 days--5 min exercise/3 min rest, 4 sessions/day, with the minimum acceptable speed initially set to 2.5 mph and increased to 2.7 mph; 4 days--5 min/3 min, 5 sessions/day; 4 days--5 mins/2 mins, 6 sessions/day, with the minimum acceptable speed increased to 2.8 mph and then to 3.0 mph; 1 day--6 mins/2 min, 6 sessions/day; 4 days--7 mins/2 mins, 6 sessions/day; 3 days--7 mins/1 min, 6 sessions/day; 1 day--8 mins/1 min, 6 sessions/day; 4 days--9 min/1 min, 6 sessions/day. The monkeys are currently running at 10 min/1 min, 6 sessions/day, with the minimal acceptable speed set to 3 mph. The monkeys run about 3.5 miles/day.

#### Metabolic and Physiological Data Collection

During most experiments, the Primate Exercise Wheel will be enclosed in a Plexiglas hood (volume = 1,600 liters), which will allow metabolic data to be collected. Room air will be drawn through the hood at a constant rate (>30 l/min) and  $\text{FEO}_2$  and  $\text{FECO}_2$  will be measured downstream by a Perkin-Elmer 11000 Medical Gas Analyzer. The total airflow will be measured by a Kurz 565-7A Mass Flowmeter that has been previously calibrated. The observed  $\text{FEO}_2$  ( $\text{FECO}_2$ ), flow, hood volume, and atmospheric pressure will be used to calculate  $\text{VO}_2$  ( $\text{VCO}_2$ ) following the methods and equations of Brown et al. (1984). Metabolic heat production will be calculated from  $\text{VO}_2$ ,  $\text{VCO}_2$ , and respiratory quotient values, with all volumes and flows corrected to STPD. Total evaporative heat loss will be determined by weighing the animal before and after the experiment (including any urine and feces produced during the experiment).

Core body temperature will be measured by a thermistor probe chronically inserted into the abdominal cavity and heart rate will be obtained from two electrocardiographic leads implanted subcutaneously over the thorax. Both of these parameters will be recorded via a Mini-Mitter Physiotel amplifier-transmitter implanted subcutaneously over the abdominal cavity and transmitted to a Mini-Mitter receiver located outside the Primate Exercise Wheel.

The outputs of the various devices described above will be connected to the Apple Macintosh II computer via National Instruments NB-MIO-16 boards and collected into a file under the control of a program written in the LabVIEW graphical programming language, Version 1.2. The file will have a header containing the title of the experiment, experimenter comments, the starting time of the experiment, and the date. The experimenter will enter a number of constants: barometric pressure, hood volume, and the animal's weight. The data will be displayed on the Macintosh monitor where the variables are updated at 30-s intervals and printed and saved on a disc at intervals set on the Macintosh control screen.

5. References

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## EXPERIMENTAL PROTOCOL

1. TITLE: Ergogenic Aids, Environmental And Physical Evaluation Model: Rest and Prior Exercise
2. PROJECT/TASK/WORK UNIT: 2729-04-30
3. PRINCIPAL CO-INVESTIGATORS/USERS:  
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5. USAF RELEVANCY AND INTERAGENCY DEPENDENCY: Military planners in all of the uniformed services require estimates of the potential effects of low-dose nerve agent exposure on the ability of personnel to carry out their missions. The effects on performance of proposed prophylactic and/or antidote drugs for nerve agent are also of great interest. USAFSAM/VNC is tasked to assess the interactions between such physiological burdens as thermal stress, physical work, and chronic, low dose exposure to nerve agents, prophylactics, and antidotes, as well as their combinations, in a primate model of human responses. This task includes specific protocols to evaluate the impact of proposed chemical defense drugs, prophylactic drugs, ergogenic aids, and physical stress on potential ground crew performance and airbase operability. A primate model that can be extrapolated to human responses will provide a means to investigate physiological functions under stress that is safe and ethical. The research will provide information to the Surgeon General and, ultimately, commanders that will help them make crucial decisions concerning the potential impact of chemical warfare on airbase operability.
6. SCIENTIFIC OBJECTIVES: The ultimate goal of these experiments will be to determine the biochemical and physiological effects of exposure to soman in an animal that is or is not pretreated with pyridostigmine, with or without postexposure treatment with atropine + 2-PAM in a moderate (25°C) or hot (35°C) environment. The primary focus of these experiments will be to describe the effects of these treatments on the thermal balance profile of chaired rhesus (Patas) monkeys.

Although the typical scenario calls for oral administration of a pretreatment drug, such as pyridostigmine, this is relatively difficult to accomplish in the monkey model because the monkey may reject the drug even when it is hidden in a favored food. Macacas, in particular, tend to rapidly place any food that is presented into their cheek pouch and then, sometimes hours later, remove it from the cheek pouch and chew and consume it or discard

it. Therefore, the monkeys will be implanted (subcutaneously) with an Alzet osmotic pump containing either vehicle or vehicle + physostigmine. This will allow a constant-rate infusion of physostigmine and this is a better model of what occurs in humans. Blood samples (about 2 ml) will be collected during any trial involving soman or pyridostigmine to determine cholinesterase activity. Blood samples will also be drawn at 15 min intervals and assayed for epinephrine and norepinephrine as a measure of adrenal and sympathetic activation, respectively.

7. TECHNICAL BACKGROUND: Soman, like other acetylcholinesterase inhibitors, produces a syndrome with a broad spectrum of individual symptoms that impact on the thermal balance and would tend to interfere with the ability to exercise or perform useful work. These include: 1) interference with normal control of muscle contractions, with muscle twitching that progresses to generalized muscle fasciculations which can develop into seizure-like activity and ultimately to paralysis of the skeletal muscles; 2) decrease in blood pressure and cardiac output, coupled with an increase in cardiac arrhythmias; 3) increase in respiratory rate (tachypnea), hyperexcitability, with increased amounts of secretions, followed by apnea (Anzueto et al., 1986); and 4) increase in rate of sweating and a decrease in skin and core temperature. The severity of these symptoms varies with dose, but the dose response curve is fairly steep. Chemical defense prophylactic and/or antidote drugs also have a significant impact on thermal balance.

Multiple perturbations, such as exercising in a hot environment ( $>30^{\circ}\text{C}$ ), even in the absence of these agents, places severe demands on the individual. Exercising in a hot ( $>30^{\circ}\text{C}$ ) environment places severe demands on the cardiovascular system -- demands that may be physiologically compromising. For example, the muscles demand increased blood flow to maintain an adequate supply of oxygen. If this demand is not met, work capacity will be attenuated.

Increased muscle activity causes heat production and this heat must be dissipated by increasing blood flow through the skin. If this demand is not met, hyperthermia (an abnormal rise in core temperature) will occur. Shunting blood to the cutaneous veins lowers cardiac filling pressure and stroke volume, so cardiac output is reduced just when demand is at a maximum. Heart rate rises sharply, up to 200 beats/min. Blood flow to the splanchnic and renal beds is reduced and the "second heart" (i.e., the action of contracting muscles) becomes a vital factor in maintaining ventricular filling pressure. An upright posture exacerbates these demands because 70% of the total blood volume is below the heart and 80% of this volume is in the veins (Rowell, 1986). Exhaustion is imminent at these high heart rates and core temperatures. But it is not clear to what extent exhaustion (and/or feelings of fatigue) occurs because of the massive amount of blood that is shunted to the cutaneous circulation and away from muscles, brain, etc., or because of the increase in core temperature.



If heat storage occurs, either as a result of being exposed to a hot ambient temperature, being enclosed in the chemical defense ensemble, or being exposed to a drug that modifies heat dissipation, it can lead to heat-related disorders. They can range from heat fatigue to heat exhaustion to heat stroke. Generally heat fatigue and heat exhaustion are self limiting if the individual can be allowed to rest in a cool environment and be provided with fluids.

If these conditions are untreated, however, they may lead to heat stroke. The prodromal signs and symptoms of heat stroke last from minutes to hours and include: dizziness, weakness, confusion and drowsiness, nausea and vomiting, anxiety and headache, disorientation and disassociation, tremors, twitches, convulsions, ataxia, and cerebellar dysfunction. Affect may range from apathy to irritability, with increased aggressiveness, irrationality, and mania or psychosis. The facial expression is typically apprehensive and "staring" (Bark, 1982). Sinus tachycardia, with peripheral circulatory failure (possibly caused by extensive cutaneous vasodilation), tachypnoea, proteinuria, and haemorrhagic diathesis are common (Kew et al., 1969; Shibolet et al., 1967). In exertion-induced heat stroke, the skin may be wet, but it is more commonly hot and dry (Knochel, 1974). These early symptoms rapidly progress to coma, where the person is unresponsive to painful stimuli, shock, and death.

Therefore, any treatment that alters sweating and/or blood flow through the skin or other sites will increase the risk of the development of heat-related disorders.

Atropine, which is generally accepted as an effective treatment for anticholinesterase (carbamate or organophosphate pesticides, chemical warfare nerve agents) exposure, has a significant impact on thermal balance. It competes with the neurotransmitter acetylcholine for binding sites at muscarinic receptors in smooth muscle, heart, and sweat glands, while having little, if any, effect on nicotinic receptors, such as those at the neuromuscular junction. One effect of this competition is to reduce sweating in humans and other primates by 40-60% (Avlonitou and Elizondo, 1988; Kolka et al., 1987; Sato and Sato, 1981; Craig, 1952). This suppression of thermoregulatory sweating and evaporative heat loss results in a net heat storage (Avlonitou and Elizondo, 1988) with a reduced heat tolerance and reduced normal exercise capacity (Craig, 1952; Cullumbine and Miles, 1956; and Davies et al., 1978). On the other hand, atropine also causes an "atropine flush" (i.e., an increase in cutaneous blood flow, Davies et al., 1978), but it is not known if this represents a mode of heat exchange. Atropine also causes an increase in heart rate, especially during exercise (Kolka et al., 1987; Avlonitou and Elizondo, 1988). It is not clear if this is an indirect result of a decrease in venous blood pressure due to blood being shunted to the skin (the "atropine flush"), to some direct effect on the heart (blocking parasympathetic inputs), or to another effect on the central nervous system.

When administering atropine, it is important to consider what other anticholinergics might be present in the system. These include a wide variety of over-the-counter and prescription drugs, such as antihistamines, cold medications, antidiarrheal medications, as well as minor (antidepressant) and major (anti-psychotic) tranquilizers, as these agents may act additively or synergistically with atropine (Matthew et al., 1986). The route of administration is also important; oral administration requires approximately twice the dose and twice the time to reach peak effectiveness (Mirakhor, 1978).

Pralidoxime chloride (2-PAM) is currently used as another antidote for organophosphate poisoning. If administered soon after organophosphate exposure, it reactivates bound peripheral acetylcholinesterase to allow hydrolysis of acetylcholine, which, in turn, allows synapses to regain normal function (Kolka et al., 1987). Therapeutic doses of 2-PAM do not cause any change in core temperature, skin temperature, heart rate, or whole body sweating in resting man (Robinson and McMichael, 1970), but do cause a small decrease in whole body sweating with moderate exercise in humans (Cummings et al., 1964) and monkeys (Kolka et al., 1987). Treatment with 2-PAM in the presence of higher sympathetic drive may cause sudden and dramatic increases in blood pressure as a result of increases in precapillary vascular resistance. Combining atropine and 2-PAM may augment the atropine-induced increase in body temperature (Kolka et al., 1987 and Cummings et al., 1964). Moreover, since 2-PAM does not cross the blood-brain barrier, it affords relatively little "reactivation" of central nervous system cholinesterase. Unfortunately, proPAM, which does cross the blood brain barrier, provides relatively little reactivation and causes some transient, but pronounced behavioral toxicity (Kenley et al., 1982).

Pyridostigmine is used as a prophylactic against organophosphate poisoning. Pyridostigmine "protects" cholinesterase by binding with it reversibly, preventing the irreversible binding and inhibition by nerve agents. Pyridostigmine is a quaternary amine and does not cross the blood brain barrier. It affects only peripheral sites (Matthew et al., 1988). Acutely, pyridostigmine causes trembling due to stimulation of nicotinic receptors in skeletal muscle. This can be controlled by administration of diazepam (Matthew et al., 1987), but diazepam does cross the blood brain barrier and does depress central cholinergic neurons by decreasing acetylcholine release. Pyridostigmine, when administered alone, causes an increase in sweating, with a decrease in skin and core temperatures (Avlonitou and Elizondo, 1988). The increase in sweating, with the concomitant water loss, may increase the cardiovascular stress. In Patas monkeys, this increased fluid loss does not seem to affect exercise tolerance (Elizondo, 1990), at least at modest (25-30%) cholinesterase inhibition. In rats, high levels (60%) of cholinesterase inhibition may compromise the ability to engage in moderate exercise, especially in a warm to hot environment (Francesconi et al., 1984). This may be due to the fact that the only route exercising rats have to dissipate heat is via their tail. But, even in

rats, modest (20-40%) cholinesterase inhibition does not appear to affect ability to exercise (Francesconi et al., 1986).

#### 8. EXPERIMENTAL METHOD/APPROACH:

##### a. Thermal Balance: Chaired Resting Monkey

Experimental Design: In a within-subject design, each of 6 adult female *Macaca mulatta* monkeys will be implanted with an Alzet pump containing vehicle or vehicle + pyridostigmine. The monkeys will then be allowed to equilibrate to either of two ambient temperatures (25 or 35°C) until changes in core temperature are less than 0.05°C over a 15-min period. This 15-min period will serve as the pretreatment baseline for subsequent manipulations. At the end of this 15-min baseline period, the monkey will receive an injection of vehicle or vehicle + soman and vehicle or vehicle + atropine + 2-PAM. The initial dose will be chosen based on consultation with SRL employees who are familiar with soman effects on behavior, but will be between .10 and .15 the LD<sub>50</sub>. If this dose causes a 25% decrease in heart rate, it will be used in the subsequent studies. Otherwise, the dose will be increased or decreased by 0.36 µg/kg and the experiment repeated until a 25% decrease is achieved. In addition to the data collected for the standard thermal balance profile, we will also note if and when any heart blocks occur and any noticeable changes in respiratory rate or depth. The dose of atropine will be 97 µg/kg and 2-PAM will be 17.1 mg/kg. These are the estimated rhesus monkey equivalent of the dose achieved by human injection of 2 combopens (Mattsson et al., 1981), while the dose level of pyridostigmine will be 150 µg/kg, which caused a 40% inhibition of cholinesterase in adult male monkeys (Blick et al., 1988).

The pyridostigmine will be administered via an Alzet osmotic pump (Alza Corp., Palo Alto, CA, Model 2ML1, 10 l/hr, 7 day) that will be implanted subcutaneously under sterile conditions in the operating rooms of the Research Support Section, 4th floor, Building 125, following the procedures of Blick et al. (RZB 88-01, "Interactions of Pyridostigmine and Soman During Chronic Exposure: Blood ChE and Performance Effects"). This will allow a constant-rate infusion of pyridostigmine. After a surgical level of anesthesia has been reached with ketamine (15 mg/kg. i.m.), a small (6-8 mm) skin incision will be made near the dorsal midline, between the scapulae. Blunt dissection will be used to open a subcutaneous pocket to accommodate the pump, which will be inserted, delivery orifice first, at body temperature. The incision will be closed with interrupted intradermal sutures and the monkey will be provided with postoperative analgesia (butorphenal tartrate, 0.25 mg i.m.). Lt Col John W. Fanton, DVM, USAFSAM/VSR, has agreed to serve as veterinary consultant for this routine implantation procedure. Cholinesterase activity will be monitored by drawing a baseline venous blood sample (2 ml) from a convenient leg vein before implantation and at 96 h after implantation and subjecting it to a standard assay. Each subject will be allowed a minimum of 1 week between subsequent treatments.

Statistics: The data will be analyzed by a 4-way ANOVA, where soman, temperature, pyridostigmine, and atropine + 2-PAM are the four factors. Separate analyses will be performed for each dependent variable. In the advent of equipment failure, loss of animals, etc., the data will be analyzed by individual 2 x 2 factorial within-subjects ANOVAs, where soman is one factor and temperature the other. In this case, separate analyses will be performed for each dependent variable for each drug (atropine and pyridostigmine).

Methods: A complete thermal profile will be conducted on 6 adult unanesthetized, nonheat-acclimated female Macaca mulatta monkeys. Each monkey will be housed individually in a standard stainless steel monkey cage. The room temperature will be maintained at  $24 \pm 2^\circ\text{C}$  with a 12:12 h light:dark cycle. The diet will consist of monkey chow supplemented with fresh fruit and water available ad libitum. All experiments will be carried out between 0800 and 1500.

The monkey will be trained to sit quietly in a Plexiglas primate restraining chair for the duration of the experimental session in a climatic chamber, isolated from external stimuli (VNC-89-07-C, "Training Program for Instrumentation, Telemetry, and Exercise Ergometry"). The dry bulb temperature of the chamber will be set to 25 or 35°C, with a relative humidity of 60%. Each animal will be allowed to equilibrate for approximately 2 h or until changes in core temperature are less than 0.05°C over a 15-min period. The core temperature will be measured by a thermal probe chronically implanted into the abdominal cavity (VNC-89-07-C, "Training Program for Instrumentation, Telemetry and Exercise Ergometry") and/or a probe inserted into the rectum approximately 10 cm from the sphincter. The rectal probe will be smooth and 5/32 in. in diameter. Lubricant (K-Y jelly) will be used to minimize discomfort. The data reported will be the individual steady-state values for each of the 6 animals averaged over a 60 to 120 min observation period, with each variable sampled at 30 s intervals, displayed on the MacIntosh II computer screen, printed, and stored on magnetic disks for later analysis.

b. Metabolic Heat Production: Room air will be drawn at a constant rate (8.5 l/min) through a Plexiglas hood enclosing the animal's head (Fig. 1). The flow will be adequate to ensure that no expired air can be blown out around the neck seal.  $\text{FEO}_2$  and  $\text{FECO}_2$  will be measured downstream by a Beckman analyzer. The total airflow will be measured using rotameters that have been previously calibrated. Metabolic heat production will be calculated from  $\text{VO}_2$ ,  $\text{VCO}_2$ , and respiratory quotient values, with all volumes and flows corrected to STPD.

c. Temperature: Core body temperature may be additionally measured by a thermistor probe chronically inserted into the abdominal cavity and recorded via a Mini-Mitter Physiotel amplifier-transmitter implanted subcutaneously over the abdominal cavity (VNC-89-07-C, "Training Program for Instrumentation, Telemetry,

and Exercise Ergometry") and a rectal probe as described earlier. Skin temperature will be measured by thermistors attached to the inner thigh, chest, back, calf, and tail.

d. Heat Loss: Total evaporative heat loss will be determined by weighing the animal before and after the experiment (including any urine and feces produced during the experiment). Respiratory water loss will be determined by calculating the difference between the absolute humidities of the air entering and leaving the hood multiplied by the total airflow. Estimation of the humidity will be done by employing two pairs of wet and dry bulb thermistors. The first pair will monitor the absolute humidity of the air flowing into the hood, while the second pair, located downstream from the hood, will monitor the absolute humidity of the air flowing out of the hood. Eccrine sweat rate will be calculated as the difference between total evaporative heat loss and respiratory water loss.

e. Heart Rate: Heart rate will be obtained from two electrocardiographic leads implanted subcutaneously and recorded via a Mini-Mitter Physiotel amplifier-transmitter (VNC-89-07-C, "Training Program for Instrumentation, Telemetry, and Exercise Ergometry") or a commercial cardiometer with standard external ECG leads. In all experiments involving soman, the ECG will be monitored to note the development of heart blocks.

The caloric equivalent will be calculated from the empirically derived formula (Johnson and Elizondo, 1979; Kolka and Elizondo, 1983):

$$K_e = 3.83 \pm 1.21 (RQ) \text{ Kcal/VO}_2.$$

Heat balance will be calculated (Johnson and Elizondo, 1979; Kolka and Elizondo, 1983):

$$\text{Heat Balance} = H + E_{\text{resp}} + E_{\text{sw}}$$

where heat balance,  $E_{\text{resp}}$ , and  $E_{\text{sw}}$  are determined experimentally and H is calculated.

f. Sympathetic Activation: Venous blood (2 ml) will be drawn from a convenient leg vein via an in-dwelling catheter (protected so the monkey cannot reach it) just before the animal is placed in the chamber, at the end of the 15-min control period and at no more than 15-min intervals for 60 to 120 min. The plasma will be assayed for epinephrine and norepinephrine, as a measure of adrenal and sympathetic nervous system activation, respectively. Level of sympathetic activation is inversely proportional to blood flow to viscera.

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10. DTIC LITERATURE SEARCH: A DTIC search has been conducted. The experiments proposed here complement ongoing research.

11. RESOURCE REQUIREMENTS:

a. Facilities: Initial housing for 12 rhesus monkeys and laboratory space for training PEW system in the Animal Resources Branch (USAFSAM/VSR).

b. Equipment: Components for the 2 PEW systems, 2 primate chairs, and recording equipment required for this work are being ordered and assembled.

c. Animals:

(1) Rhesus: Six rhesus (2.5-4.5 kg) will be trained in the PEW. An additional 6 rhesus will be used in the resting studies, no additional rhesus are anticipated except as possible replacement animals due to health or difficulty of training.

(2) Post-Experimental Disposition: The animals used in this study will be returned to the Veterinary Sciences Division (VS) for reassignment.

12. HAZARDS:

The hazards associated with handling animals are minimized by adherence to standard laboratory procedures, with which all applicable personnel have been familiarized.

All personnel will be briefed on the hazards of direct contact with the animals. Standard protective clothing, gloves (rubber and/or leather), and face masks will be worn during the handling of any animal. Personnel will have read USAFSAM Regulation 161-1 covering the control management for herpes virus simiae (B-Virus). Every possible attempt will be made to prevent scratches and bites.

13. ANIMAL USE:

a. Alternative Species: The rhesus monkey is the immediately available species of choice because of the large data base



on the species, including the area of exercise physiology, and the comparability to man.

b. Relief of Pain, Discomfort and Distress. The animals will be subjected to minimal discomfort from periodic blood sampling and brief electric shocks to motivate performance in the PEW.

c. Statement: The care and use of animals in these experiments will be in accordance with USAFSAM Regulation 169-2.

d. Veterinary Consultants:

Dr. Roger C. Harvey, USAFSAM/VSR, (512) 536-3477  
John W. Fanton, Maj, USAFSAM/VS, (512) 536-2078

e. Special Considerations: The monkey will be placed in the primate restraining chair by two experimenters and trained to sit quietly following the procedures outlined in VNC-89-07-C ("Training Program for Instrumentation, Telemetry, and Exercise Ergometry"). The animals will be in the chair no more than 6 h in any one session (most sessions will be 4 h or less) and there will be only 1 session in any given 5-day time period. The animals will be examined each time they are removed from the chair to determine if the chairing procedures caused decubital ulcers, contusions, or dependent edema. It is unlikely that these pathological processes will occur, but if they do, the chairing procedures will be temporarily suspended until the monkey is examined by the veterinary consultant.

14. SIGNATURE OF PRINCIPAL INVESTIGATORS AND DATE:

#### PROTOCOL ADDENDUM

1. TITLE: Ergogenic Aids, Environmental And Physical Evaluation Model: Rest and Prior Exercise

2. PROJECT/TASK/WORK UNIT: 2729-04-30

3. PRINCIPAL CO-INVESTIGATORS/USERS:

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Clifford J. Sherry, Ph.D., USAFSAM/VNC (SRL), (512) 536-3814

4. ASSOCIATE INVESTIGATORS:

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Susan H. Bomalaski, Maj, USAFSAM/VNC, (512) 536-3814

5. SCIENTIFIC OBJECTIVES: The ultimate goal of these experiments is to describe the thermal balance profile of chaired rhesus monkeys, during exposure to ambient temperatures of 25°C and 35°C at two absolute humidities (approximately 6 and 24 torr).

Dose response studies were to follow the current protocol in place; however, recent developments strongly suggest that it would be advantageous to do dose response studies prior to other procedures outlined in the original protocol. We therefore propose to conduct a dose response study for pyridostigmine to determine the effect of various pyridostigmine dosages on the thermal balance profile, as well as the possible effect on insulin-glucagon secretion and blood glucose levels.

6. TECHNICAL BACKGROUND: Pyridostigmine may tend to cause a decrease in blood glucose levels via vagal stimulation of the beta cells of the Islets of Langerhans of the pancreas, causing these cells to produce excess insulin. When blood glucose levels approach 50 mg%, there is a likelihood that a seizure will develop and if blood glucose levels remain low for a prolonged time period, it will likely cause permanent brain damage, especially in the cerebral cortex, diencephalon, and medulla. Death can occur as a result of respiratory failure (Guyton, 1976).

7. EXPERIMENTAL METHOD/APPROACH:

A. Thermal Balance: Chaired Resting Monkey

Experimental Design: From a pool of 12 rhesus or 10 patas monkeys, 6 rhesus or 6 patas monkeys will be randomly chosen and assigned to the experimental group. From this group, two rhesus or patas monkeys will be randomly assigned to each of three pyridostigmine dose levels: 1) 0.34 mg/kg/day (which causes approximately 25% inhibition of acetylcholinesterase); 2) 1.05 mg/kg/day (50% inhibition); and 3) 3.20 mg/kg/day (75% inhibition) (Kerenyi, 1989). Separate dose response curves will be collected for each set of environmental conditions. The order in which the dose response curves are conducted and the assignment of subjects to each dose level within each environmental condition are randomized and shown in Table 1. The order in which data will be collected on each subject for each dose response curve will also be randomized to minimize the effects of ordering and extraneous variables.

TABLE 1. SUBJECT ASSIGNMENTS

	<u>Pyrid-1</u>	<u>Pyrid-2</u>	<u>Pyrid-3</u>
25°C-24 torr	5,4	2,6	1,3
25°C-6 torr	1,5	3,2	4,6
35°C-6 torr	1,4	5,2	3,6
35°C-24 torr	3,2	4,1	6,5

The pyridostigmine will be administered via an Alzet osmotic pump (Alza Corp., Palo Alto, CA, Model 2ML1, 10 l/hr, 7 day) that will be implanted subcutaneously under sterile conditions in the operating rooms of the Research Support Section, following

the procedures of Blick et al. (RZB 88-01, "Interactions of Pyridostigmine and Soman During Chronic Exposure: Blood ChE and Performance Effects") under the supervision of Lt Col Fanton, Maj Harvey, or Maj Sauber. This will allow a constant-rate infusion of pyridostigmine. After a surgical level of anesthesia has been reached with ketamine (15 mg/kg. i.m.), a small (6-8 mm) skin incision will be made near the dorsal midline, between the scapulae. Blunt dissection will be used to open a subcutaneous pocket to accommodate the pump, inserted delivery orifice first, at body temperature. The incision will be closed with interrupted intradermal sutures and the monkey will be provided with postoperative analgesia (butorphenal tartrate, 0.25 mg i.m.). Lt Col John W. Fanton, DVM, USAFSAM/VSR, has agreed to serve as veterinary consultant for this routine implantation procedure. Cholinesterase activity will be monitored by drawing a baseline venous blood sample (2 ml) from a convenient leg vein before implantation, at 96 h after implantation, and subjecting it to a standard assay. Each subject will be allowed a minimum of 1 week between subsequent treatments.

On Day 5 after the Alzet pump has been implanted, the monkeys will be instrumented (as described in original protocol) and allowed to equilibrate to the assigned environmental condition until changes in core temperature are less than 0.05°C over a 15-min period. During this 15-min period and for three additional consecutive 15-min periods, steady-state observations will be collected for each selected dependent variable: core temperature, mean skin temperature, heart rate,  $VO_2$ ,  $VCO_2$ , respiratory quotient, MET, conductance, and respiratory evaporative heat loss. Dose response curves will be evaluated for each dependent variable for each environmental condition.

Insulin-glucagon-blood glucose: Venous blood (2 ml samples) will be drawn from a convenient leg vein. The appropriate blood fractions will be assayed for insulin, glucagon, and glucose using standard assay procedures.

b. Thermal Balance - Prior Exercise:

Experimental Design: From a pool of 12 rhesus or 10 patas monkeys, 6 rhesus or 6 patas monkeys will be randomly chosen and assigned to the experimental group. From this group, 2 rhesus or patas monkeys will be randomly assigned to each of 3 pyridostigmine dose levels: 1) 0.34 mg/kg/day (which causes approximately 25% inhibition of acetylcholinesterase); 2) 1.05 mg/kg/day (50% inhibition); and 3) 3.20 mg/kg/day (75% inhibition). Separate dose response curves will be collected for each set of environmental conditions. The order in which the dose response curves are conducted and the assignment of subjects to each dose level within each environmental condition are randomized and shown in Table 1. The order in which data will be collected on each subject for each dose response curve will also be randomized to minimize the effects of ordering and extraneous variables.

These monkeys will have been trained in the Primate Exercise

Wheel (PEW) to walk at a rate of 2-3 mph for 1-2 h, before the beginning of this experiment. On Day 5 after the Alzet pump has been implanted, the monkeys will be placed in the PEW and allowed to exercise for 1 h or until reaching criterion physiological measures (as described in original protocol). The monkey will then be removed from the PEW and allowed to equilibrate to the assigned environmental condition until changes in core temperature are less than 0.05°C over a 15-min period. During this 15-min period, and for three additional consecutive 15-min periods, steady-state observations will be collected for each selected dependent variable: core temperature, mean skin temperature, heart rate,  $VO_2$ ,  $VCO_2$ , respiratory quotient, MET, conductance, and respiratory evaporative heat loss. Dose response curves will be evaluated for each selected dependent variable for each environmental condition.

#### 8. REFERENCES:

1. Guyton, A. C. Textbook of Medical Physiology. Philadelphia: Saunders, 1978.
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#### 9. RESOURCE REQUIREMENTS:

a. Facilities: Initial housing for 12 rhesus monkeys and laboratory space for training PEW system in Building 185. Laboratory space and access to environmental chamber #5 in Building 160. Initial housing for 16 patas monkeys and laboratory space for training PEW system in Building 1001. The patas monkeys will be screened for simian hemorrhagic fever, which is extremely virulent and pathogenic in rhesus monkeys. In any experiments where rhesus monkeys will use equipment that has been used by patas monkeys, the equipment will be carefully cleaned with an appropriate disinfectant (as directed by USAFSAM/VS guidelines) and allowed to air-dry for at least 24 h (or longer, depending on guidelines generated by USAFSAM/VS personnel). Every effort will be made to minimize transitions from patas to rhesus monkeys.

#### b. Animals:

(1) From a pool of 12 trained rhesus monkeys (2.5-4.5 kg) or 10 trained patas monkeys (Protocol VNC-89-07-C), 6 rhesus and 6 patas monkeys will be transferred to this protocol. No additional monkeys are anticipated except as possible replacement animals due to health or difficulty of training.

(2) Post-Experimental Disposition: The animals used in this study will be returned to the Veterinary Sciences Division (VS) for reassignment.

## 10. ANIMAL USE:

a. **Alternative Species:** The rhesus monkey is the immediately available species of choice because of the large data base on the species, including the area of exercise physiology, and the comparability to man. The rhesus and patas monkeys are qualitatively similar and, like humans, sweat over the entire body surface. However, the evaporative heat loss that is due to sweating is 40% higher in patas. Because of the higher sweating capacity and other similarities with the human eccrine system, the patas is the most appropriate animal model for thermoregulatory studies. Alternatives in the form of lower species do not exist and the protocol does not unnecessarily duplicate previous experiments (Agricola/Animal Welfare Information Center, Biosis, Excerpta Medica, NTIS, MEDLARS-Bioethics).

### RESULTS

a. SRL employees drew blood for serum cholinesterase assays performed by Rothe Development personnel.

#### b. Preliminary Experiments:

(1) **Resting Experiments.** Preliminary results from the resting experiment at 25°C and 35°C are shown in Table 2.

TABLE 2. RESULTS OF RESTING EXPERIMENTS

	25.1*	25.2**	35.1**	35.2**
CORE TEMPERATURE	38.39 0.16	38.31 0.34	38.39 0.29	38.30 0.15
MEAN SKIN TEMPERATURE	34.13 0.75	34.67 0.49	36.65 0.35	36.32 0.75
HEART RATE	165.33 26.03	153.17 36.74	152.33 24.88	143.50 28.56
VO <sub>2</sub>	0.048 0.0038	0.045 0.0086	0.035 0.0056	0.037 0.0061

\* The animal equilibrated at 25°C. When core temperature changed < 0.05°C in a 15 min period, data were collected during this and 3 additional 15 min periods. Then the animal equilibrated at 35°C and data were again collected as described above.

\*\* The animal was exposed to 35°C first and then 25°C.

(2) Pyridostigmine Dose Response Curves. The results for animals chronically infused with pyridostigmine are shown in Figures 5, 6 and 7, and in Table 3.

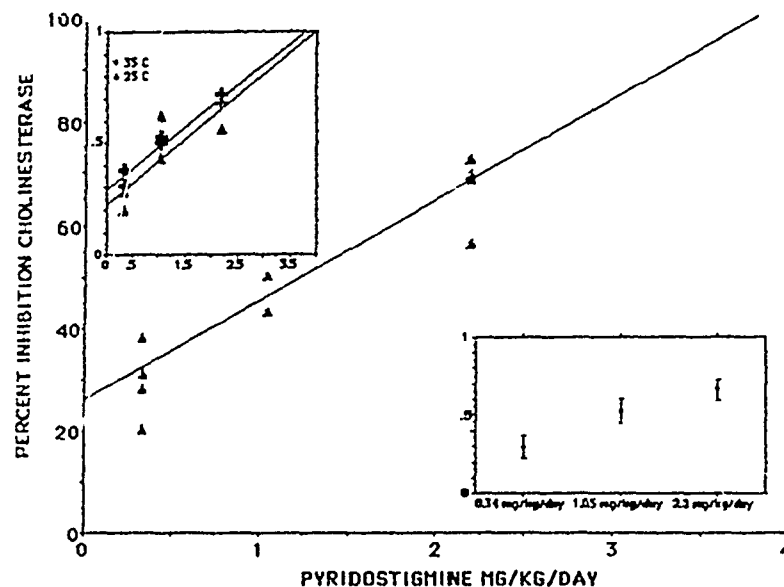


Figure 5. Dose-response curve for the effect of constant-rate infusion of pyridostigmine at three dose levels (0.34, 1.05, and 2.20 mg/kg/day) and at two ambient temperatures (25 or 35°C) on % cholinesterase inhibition. The lower insert shows that inhibition is dose dependent. The upper insert shows a (non-significant) shift in the dose-response curve (25°C  $\Delta$ , 35°C  $+$ ) associated with change in ambient temperature.

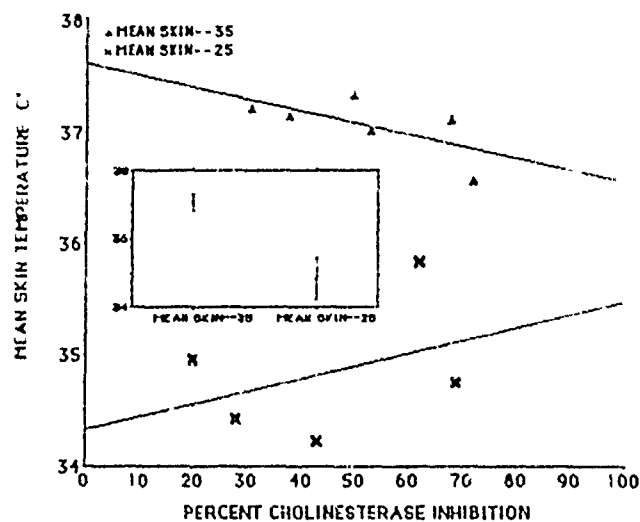


Figure 6. The dose response curve for the effect of cholinesterase inhibition at two ambient temperatures (25 or 35°C) on mean skin temperature. Insert shows that ambient temperature, but not cholinesterase inhibition, causes a significant change in mean skin temperature.

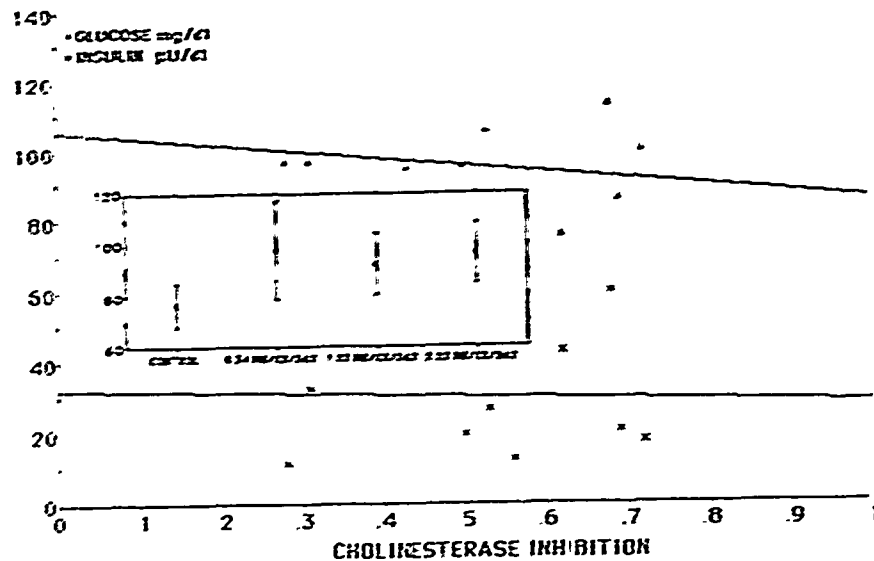


Figure 7. The dose-response curve for the effects of cholinesterase inhibition on blood glucose (▲) and insulin (X) levels. The insert shows that the higher two doses of pyridostigmine are associated with a tendency toward increasing glucose levels.

TABLE 3. RESULTS OF PYRIDOSTIGMINE EXPERIMENTS\*

	PYRIDOSTIGMINE DOSE (MG/KG/DAY)					
	0.34		1.05		2.2	
CORE TEMPERATURE	38.44	38.62	38.17	38.73	38.62	38.51
	0.04	0.097	0.038	0.094	0.06	0.048
MEAN SKIN TEMPERATURE	34.95	34.42	34.21	35.83	34.74	38.86
	0.129	0.246	0.27	0.09	0.581	0.122
HEART RATE	147	138	157	211	178	156
	14	28	12	21	17	17
VO <sub>2</sub>	0.053	0.048	0.051	0.054	0.073	0.045
	0.004	0.012	0.006	0.01	0.013	0.008
INHIBITION	20%	28%	43%	62%	69%	56%

\* Data from individual subjects, recorded as in Table 2, at 25°C.

## EXPERIMENTAL PROTOCOL

1. TITLE: Ergogenic Aids, Environmental and Physical Evaluation Model: Exercise Capacity
2. PROJECT/TASK/WORK UNIT: 2729-04-30
3. PRINCIPAL CO-INVESTIGATORS/USERS:  
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5. USAF RELEVANCY AND INTERAGENCY DEPENDENCY: Military planners in the uniformed services require estimates of the potential effects of low-dose nerve agent exposure of personnel on their ability to carry out their missions. The effects on performance of proposed prophylactic and/or antidote drugs for nerve agent are also of great interest. USAFSAM/VNC is tasked to assess the interactions between such physiological burdens as thermal stress and physical work and acute or chronic, low-dose exposure to nerve agents, prophylactics and antidotes, and their combinations, in a primate model of human physiological responses. This task includes specific protocols to evaluate the impact of proposed chemical defense drugs, prophylactic drugs, ergogenic aids, and physical stress on potential ground crew performance and airbase operability. A primate model that can be extrapolated to human responses will provide a means to investigate physiological functions under stress that is safe and ethical. The research will provide information to the Surgeon General, and ultimately, commanders that will help them make crucial decisions concerning the potential impact of chemical warfare on airbase operability.
6. SCIENTIFIC OBJECTIVES: The ultimate goal of these experiments is to determine the effects of pyridostigmine treatments with or without follow-up treatments with atropine and atropine + 2-PAM on the exercise tolerance of rhesus monkeys during exposure to ambient temperatures of 25°C and 35°C at two absolute humidities approximately 6 and 24 torr. Blood samples will be drawn to determine cholinesterase activity, to monitor epinephrine and norepinephrine levels (as a measure of adrenal and sympathetic activation, respectively), lactate, and to determine the possible effect on insulin-glucagon secretion and blood glucose levels.
7. TECHNICAL BACKGROUND: Low doses of nerve agents, as well as prophylactic and/or antidote drugs, can have an impact on the thermal balance of an exposed individual and ultimately on the ability to exercise or do useful work. Environmental variables, such as high ambient temperatures, also have a significant impact on the individual's thermal balance and ability to work.



Perturbations, such as exercising in a hot environment (>25°C), even in the absence of these agents, places adaptive demands on the individual. On one hand, exercising in a hot environment places severe demands on the cardiovascular system -- requirements that may be physiologically compromising. For example, the muscles demand increased blood flow to maintain an adequate supply of oxygen. If this demand is not met, work capacity will be attenuated. On the other hand, increased muscle activity causes heat production and this heat must be dissipated by increasing blood flow through the skin. If this demand is not met, hyperthermia (an abnormal rise in core temperature) will occur. If heat storage is excessive and/or prolonged, it can lead to general heat stress which covers a continuum of disorders from heat fatigue to heat exhaustion or heat stroke. Even in its milder forms, work is more fatiguing, decreased coordination reduces efficiency, judgment is impaired, and morale declines. Untreated, it can lead to heat stroke, where a life-threatening emergency can occur within minutes to hours (Augerson et al., 1986).

Shunting blood to the cutaneous veins lowers cardiac filling pressure and stroke volume, so cardiac pumping capacity is reduced just when demand is at a maximum. Heart rate goes up sharply, possibly to 200 beats/min. Blood flow to the splanchnic and renal beds is reduced and the "second heart" (i.e., the action of contracting muscles) becomes a vital factor in maintaining ventricular filling pressure. An upright posture exacerbates these demands because 70% of the total blood volume is below the heart and 80% of this volume is in the veins (Rowell, 1986). Exhaustion is imminent at these high heart rates and core temperatures. But it is not clear what causes the exhaustion (and/or feelings of fatigue); the massive amount of blood that is shunted to the cutaneous circulation and away from muscles, brain, etc., or because of the increase in core temperature alone. Any treatment (drugs, environmental conditions, clothing) that alters sweating and/or blood flow through the skin or other sites will act at least in an additive manner with these processes and ultimately decrease the time to work stoppage.

Atropine, the generally accepted treatment for anticholinesterase (carbamate or organophosphate pesticides, chemical warfare nerve agents) exposure, has a significant impact on thermal balance. It competes with the neurotransmitter acetylcholine for binding sites at muscarinic receptors in smooth muscle, heart, and sweat glands, while having little, if any, effect on nicotinic receptors, such as those at the neuromuscular junction. One effect of this competition is to reduce sweating in humans and other primates by 40-60% (Avlonitou and Elizondo, 1988; Kolka et al., 1987; Sato and Sato, 1981; Craig, 1952). This suppression of thermoregulatory sweating and evaporative heat loss results in a net heat storage (Avlonitou and Elizondo, 1988) with a reduced heat tolerance and reduced normal exercise capacity (Craig, 1952; Cullumbine and Miles, 1956; and Davies et al., 1978).

If heat storage is excessive and/or prolonged, as might be expected by being exposed to a "hot" environment, wearing personal protective equipment or the combination of exercise in a "hot" environment, it can lead to heat stroke (Leithead and Lind, 1964). In this situation, the liver may stop extracting lactate and may actually release lactate (Rowell et al., 1968). Ultimately, the brain itself may be damaged. On the other hand, atropine also causes an "atropine flush" (i.e., an increase in cutaneous blood flow, Davies et al., 1978), but it is not known if this represents a significant mode of heat exchange. Atropine also causes an increase in heart rate, especially during exercise (Kolka et al., 1987; Avlonitou and Elizondo, 1988). It is not clear if this is an indirect result of a decrease in venous blood pressure due to blood being shunted to the skin (the "atropine flush"), to some direct effect on the heart (blocking parasympathetic inputs), or to another effect on the central nervous system.

When administering atropine, it is important to consider what other anticholinergics might be present in the system. These include a wide variety of over-the-counter and prescription drugs, such as antihistamines, cold medications, antidiarrheal medications, as well as minor (antidepressive) and major (anti-psychotic) tranquilizers, as these agents may act additively or synergistically with atropine (Matthew et al., 1986). The route of administration is also important; oral administration requires approximately twice the dose and twice the time to reach peak effectiveness (Mirakhur, 1978)

Pralidoxime chloride (2-PAM) is currently used as another antidote for organophosphate poisoning. If administered soon after organophosphate exposure, it reactivates bound peripheral acetylcholinesterase to allow hydrolysis of acetylcholine, which, in turn, facilitates synapse return to normal function (Kolka et al., 1987). Therapeutic doses of 2-PAM do not cause any change in core temperature, skin temperature, heart rate, or whole body sweating in resting man (Robinson and McMichael, 1970), but do cause a small decrease in whole body sweating with moderate exercise in humans (Cummings et al., 1964; Kolka et al., 1987). Treatment with 2-PAM in the presence of higher sympathetic drive may cause sudden and dramatic increases in blood pressure as a result of increases in precapillary vascular resistance. Combining atropine and 2-PAM may augment the atropine-induced increase in body temperature (Kolka et al., 1987 and Cummings et al., 1964). Moreover, since 2-PAM does not cross the blood-brain barrier, it affords relatively little "reactivation" of central nervous system cholinesterase. Unfortunately, proPAM, which does cross the blood brain barrier, provides relatively little reactivation and causes some transient, but pronounced behavioral toxicity (Kenley et al., 1982).

Pyridostigmine is used as a prophylactic against organophosphate poisoning. Pyridostigmine "protects" cholinesterase by binding with it reversibly, preventing the irreversible binding and inhibition by nerve agents. Pyridostigmine is a quaternary

amine and does not cross the blood brain barrier. It affects only peripheral sites (Matthew et al., 1988). Acutely, pyridostigmine causes trembling due to stimulation of nicotinic receptors in skeletal muscle. This can be controlled by administration of diazepam (Matthew et al., 1987), but diazepam does cross the blood brain barrier and does depress central cholinergic neurons by decreasing acetylcholine release. Pyridostigmine, when administered alone, causes an increase in sweating, with a decrease in skin and core temperatures (Avlonitou and Elizondo, 1988). The increase in sweating, with the concomitant water loss, may increase the cardiovascular stress. In Patas monkeys, this increased fluid loss does not seem to affect exercise tolerance (Elizondo, 1990), at least at modest (25-30%) cholinesterase inhibition. In rats, high levels (60%) of cholinesterase inhibition may compromise the ability to engage in moderate exercise, especially in a warm to hot environment (Francesconi et al., 1984). This may be due to the fact that the only route exercising rats have to dissipate heat is via their tail. But, in rats, more modest (20-40%) cholinesterase inhibition does not appear to affect ability to exercise (Francesconi et al., 1986).

However, pyridostigmine may have an indirect effect on exercise tolerance in that it tends to cause a decrease in blood glucose levels via vagal stimulation of the beta cells of the Islets of Langerhans of the pancreas, which causes these cells to produce excess insulin. Low blood glucose levels have been associated with decreased work tolerance. When blood glucose levels approach 50 mg%, there is a likelihood that a seizure will develop and if blood glucose levels remain low for a prolonged time period, it will likely cause permanent brain damage, especially in the cerebral cortex, diencephalon, and medulla. Death can occur as a result of respiratory failure (Holt, 1968).

## 8. EXPERIMENTAL METHOD/APPROACH:

### A Exercise Tolerance:

a. Dose Response Curve: From a pool of 12 rhesus or 10 patas monkeys, 6 rhesus or 6 patas monkeys will be randomly chosen and assigned to the experimental group. From this group, 2 rhesus or patas monkeys will be randomly assigned to each of 3 pyridostigmine dose levels: 1) 0.34 mg/kg/day (which causes approximately 25% inhibition of acetylcholinesterase); 2) 1.05 mg/kg/day (50% inhibition); and 3) 3.20 mg/kg/day (75% inhibition), (Kerenyi, 1989). Separate dose response curves will be collected for each set of environmental conditions. The order in which the dose response curves are conducted and the assignment of subjects to each dose level within each environmental condition are randomized as shown in Table 1. The order in which data will be collected on each subject for each dose response curve will also be randomized to minimize the effects of ordering and extraneous variables.

TABLE 1. SUBJECT ASSIGNMENTS

	<u>Pyrid-1</u>	<u>Pyrid-2</u>	<u>Pyrid-3</u>
25°C-24 torr	5,4	2,6	1,3
25°C-6 torr	1,5	3,2	4,6
35°C-6 torr	1,4	5,2	3,6
35°C-24 torr	3,2	4,1	6,5

Although the typical scenario calls for oral administration of a pretreatment drug, such as pyridostigmine, this is relatively difficult to accomplish in some monkey models because the monkey may reject the drug even when it is hidden in a favored food. Macaques, in particular, tend to rapidly place any food that is presented into their cheek pouch and then, sometimes hours later, remove it from the cheek pouch and chew and consume it or discard it. Therefore, the monkeys will be implanted with an Alzet osmotic pump containing either vehicle (optional) or vehicle + pyridostigmine. This will allow a constant-rate infusion of pyridostigmine.

The Alzet osmotic pumps (Alza Corp., Palo Alto, CA, Model 2ML1, 10 l/hr, 7 day) will be implanted subcutaneously under sterile conditions in the operating rooms of the Research Support Section, following the procedures of Blick et al. (RZB 88-01 "Interactions of Pyridostigmine and Soman During Chronic Exposure: Blood ChE and Performance Effects") under the supervision of Lieutenant Colonel Fanton, Major Harvey, or Major Sauber. After a surgical level of anesthesia has been reached with ketamine (15 mg/kg, i.m.), a small (6-8 mm) skin incision will be made near the dorsal midline, between the scapulae. Blunt dissection will be used to open a subcutaneous pocket to accommodate the pump, which will be implanted, delivery orifice first, at body temperature. The incision will be closed with interrupted intradermal sutures and the monkey will be provided with postoperative analgesia (buprenorphine HCl, 0.01 mg/kg, i.m.). Cholinesterase activity will be monitored by drawing a baseline venous blood sample (2 ml) from a convenient leg vein before implantation and 96 h after implantation, subjecting it to a standard assay.

These subjects will have been trained in the Primate Exercise Wheel (PEW) to walk at a rate of 3 mph for 1-2 h before the beginning of this experiment (VNC-89-07-C, "Training Program for Instrumentation, Telemetry, and Exercise Ergometry").

Each trained monkey will exercise in a PEW which is enclosed by a Plexiglas hood. The PEW and metabolic hood will be placed in a climatic chamber, with the appropriate environmental conditions (temperature and humidity as determined above) and the minimum acceptable speed will be set to 3 mph. Following the methods of Elizondo (1989), the monkey will perform intermittent

exercise until it satisfies any one of the following criteria, at which point the experiment will terminate. The criteria are: 1) heart rates that approach the maximum for this species, threshold to be set at 300 beats/min; 2) core temperatures higher than 40°C; or 3) going underspeed (for more than 5 sequential sec and receiving mild shock) three times in two consecutive 15-min periods. (NOTE: If the speed of the PEW is less than the present minimal acceptable speed (3.0 mph), a yellow light turns on and if the monkey increases the speed of the PEW within 5 sec (grace period), the yellow light goes off. But, if it fails to increase the speed of the PEW within 5 sec, it will receive a mild shock (0.1 milliamp, 100 millisecond duration), at 1-sec intervals until it increases the speed of the PEW to the minimal preset speed. The PEW controller allows the experimenter to preset the maximum number of sequential shocks that the monkey can receive (not to exceed 10 [ten]). If the monkey receives this number of sequential shocks, the controller automatically turns the program off and the monkey cannot receive any additional shocks unless the experimenter manually restarts the program (VNC-89-07-C, "Training Program for Instrumentation, Telemetry, and Exercise Ergometry"). The total distance covered in miles and fractions of a mile, the average speed in miles/hour, the total exercise time, the number of grace period initiations, the cumulative grace period duration, and the total number of shocks will be recorded.

Thermal balance will be monitored while the monkey is exercising in the PEW during the environmental exposures in Building 160.

a. Metabolic Heat Production: Room air will be drawn at a constant rate (>30 L/min) through a Plexiglas hood enclosing the PEW.  $FEO_2$  (fraction expired oxygen) and  $FECO_2$  (fraction expired carbon dioxide) will be measured downstream by a Perkin-Elmer Model 1100 medical gas analyzer. The total airflow will be measured using Kurz Model 565-7A mass flowmeter that has been previously calibrated. Caloric equivalent heat production will be calculated from  $VO_2$  (oxygen uptake),  $VCO_2$  (carbon dioxide production), and respiratory quotient values, with all volumes and flows corrected to STPD (standard temperature pressure dry).

b. Temperature: Core body temperature will be measured by a thermistor probe chronically inserted into the abdominal cavity and recorded via a Mini-Mitter Physiotel amplifier-transmitter implanted subcutaneously over the abdominal cavity (VNC-89-07-C, "Training Program for Instrumentation, Telemetry, and Exercise Ergometry") in the operating rooms of the Research Support Section. After a surgical level of anesthesia is achieved (induction ketamine 10 mg/kg, i.m.; maintenance isoflurane) a small midline incision will be made under aseptic conditions near the ventral midline, over the abdominal cavity. Blunt dissection will be used to open a subcutaneous pocket to accommodate the amplifier-transmitter package. The two electrocardiographic leads will be tunneled under the skin, from the implantation site to sites on either side of the chest, which provide the best electrocardiographic signal (monitored during implantation). The temperature probe (13 cm long, 3 mm in diameter) will be

inserted into the abdominal cavity, via a small incision in the linea alba. Post operative analgesia will be by buprenorphine 0.025 mg/kg. All surgical procedures will be performed in the VS operating room under the supervision of the attending veterinarian.

The implanted amplifier-transmitter package is battery operated. The battery has a rated life of approximately 6 months (constant on). But this can be extended to 20+ months, by turning the amplifier-transmitter off, utilizing a built-in, three-position, magnetically activated switch (on, off, test), when the animal is not to be involved in an experiment for an extended time.

Subcutaneous implantation of the amplifier-transmitter and all leads will minimize the amount of human contact required -- limited to chairing the monkey to turn the amplifier-transmitter package on or off with a magnet.

c. Heat Loss: Total sweat loss will be determined by weighing the animal before and after the experiment (including any urine and feces produced during the experiment).

d. Heart Rate: Heart rate will be obtained from 2 electrocardiographic leads implanted subcutaneously and recorded via a Mini-Mitter Physiotel amplifier-transmitter (VNC-89-07-C, "Training Program for Instrumentation, Telemetry, and Exercise Ergometry").

The caloric equivalent will be calculated from the empirically derived formula (Johnson and Elizondo, 1979; Kolka and Elizondo, 1983):

$$K_e = 3.83 \pm 1.21 (RQ) \text{ Kcal/VO}_2$$

e. Sympathetic Activation: Venous blood will be drawn from a convenient leg vein just before the animal is placed in the chamber and at the end of the experiment. The plasma will be assayed for epinephrine and norepinephrine, as a measure of adrenal and sympathetic nervous system activation, respectively. Level of sympathetic activation is inversely proportional to blood flow to viscera.

f. Insulin-glucagon-blood glucose-lactate: Venous blood (2 ml samples) will be drawn from a convenient vein and the appropriate blood fractions will be assayed for insulin, glucagon, glucose, and/or lactate using standard assay procedures.

Dose response curves will be evaluated for each environmental condition for selected dependent variables: core temperature, heart rate,  $\text{VO}_2$ ,  $\text{VCO}_2$ , respiratory quotient, the total distance covered in miles and fractions of a mile, the average speed in miles/hour, the total exercise time, the number of grace period initiations, the cumulative grace period duration, and the total number of shocks.

b. Drug Interactions: Each of 6 rhesus or 6 patas monkeys will be implanted with an Alzet osmotic pump containing either vehicle (optional) or vehicle + pyridostigmine. The dose level of pyridostigmine will be 150  $\mu\text{g}/\text{kg}$ , which causes a 40% inhibition of cholinesterase in adult male monkeys (Blick et al., 1988).

Each trained monkey will exercise in a PEW which is enclosed in a Plexiglas hood. The PEW and metabolic hood will be placed in a climatic chamber, with the appropriate environmental conditions (temperature and humidity as determined previously) and the minimum acceptable speed will be set to 3 mph. Following the methods of Elizondo (1990), the monkey will perform intermittent exercise until it satisfies any one of the following criteria, at which point the experiment will terminate. The criteria are: 1) heart rates that approach the maximum for this species, threshold to be set at 300 beats/min; 2) core temperatures higher than 40°C; or 3) going underspeed (for more than 5 sequential sec and receiving mild shock) three times in two consecutive 15-min periods. While these monkeys are exercising, a partial thermal profile will be collected.

Just prior to being introduced into the PEW, each monkey will be injected with vehicle or atropine + 2-PAM. The dose of atropine will be 97  $\mu\text{g}/\text{kg}$  and 2-PAM will be 17.1 mg/kg. These are the estimated monkey equivalents of the dose that would be achieved by human self-injection of 2 combopens.

Statistics. The data will be analyzed by a 4-way ANOVA, where subjects, temperature, pyridostigmine, and atropine + 2-PAM are the factors, respectively. Separate analyses will be conducted for each dependent variable.

## B EXERCISE TOLERANCE - PRIOR HEAT STRESS

a. Dose Response Curve: From a pool of 12 rhesus or 10 patas monkeys, 6 rhesus or 6 patas monkeys will be randomly chosen and assigned to the experimental group. From this group, two rhesus or patas monkeys will be randomly assigned to each of three pyridostigmine dose levels: 1) 0.34 mg/kg/day (which causes approximately 25% inhibition of acetylcholinesterase); 2) 1.05 mg/kg/day (50% inhibition); and 3) 3.20 mg/kg/day (75% inhibition), (Kerenyi, 1989). The monkeys will have been trained to sit quietly in a Plexiglas primate restraining chair prior to the beginning of this experiment (VNC-89-07-C, "Training Program for Instrumentation, Telemetry, and Exercise Ergometry"). The chaired monkey will be placed in a climatic chamber, isolated from external stimuli for 1 h. The dry bulb temperature will be set to 35°C, humidity of 6 torr. The core temperature will be measured by a thermal probe chronically implanted into the abdominal cavity (VNC-89-07-C, "Training Program for Instrumentation, Telemetry, and Exercise Ergometry"). The monkey will then be removed from the chair and placed in the PEW and allowed to exercise under the same environmental conditions. Dose response curves will be evaluated for selected dependent variables: core

temperature, heart rate,  $VO_2$ ,  $VCO_2$ , respiratory quotient, the total distance covered in miles and fractions of a mile, the average speed in miles/hour, the total exercise time, the number of grace period initiations, the cumulative grace period duration, and the total number of shocks.

b. Drug Interactions: Each of 6 rhesus or 6 patas monkeys will be implanted with an Alzet pump containing vehicle or vehicle + pyridostigmine. The dose level of pyridostigmine will be 150  $\mu\text{g}/\text{kg}$ , which caused a 40% inhibition of cholinesterase in adult male monkeys (Blick et al., 1988). The monkeys will have been trained to sit quietly in a Plexiglas primate restraining chair prior to the beginning of this experiment (VNC-89-07-C, "Training Program for Instrumentation, Telemetry and Exercise Ergometry"). The chaired monkey will be placed in a climatic chamber, isolated from external stimuli for 1 h. The dry bulb temperature will be set to 35°C, relative humidity 6 torr. The core temperature will be measured by a thermal probe chronically implanted into the abdominal cavity (VNC-89-07-C, "Training Program for Instrumentation, Telemetry and Exercise Ergometry"). This 15-min period will serve as the pretreatment baseline for subsequent manipulations. At the end of this 15-min baseline period, the monkey will receive an injection of vehicle or atropine + 2-PAM. The dose of atropine will be 97  $\mu\text{g}/\text{kg}$  and 2-PAM will be 17.1 mg/kg. These are the estimated rhesus monkey equivalents of the dose achieved by human injection of 2 combopens (Mattsson et al., 1981). The dose level of pyridostigmine will be 150  $\mu\text{g}/\text{kg}$ , which caused a 40% inhibition of cholinesterase in adult male monkeys (Blick et al., 1988). The monkey will then be removed from the chair and placed in the PEW and allowed to exercise under the conditions previously described.

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10. DTIC LITERATURE SEARCH: A DTIC search (DTICT 43345, IR1027) has been conducted. The experiments proposed here complement ongoing research.
11. RESOURCE REQUIREMENTS:

a. Facilities: Initial housing for 12 rhesus monkeys and laboratory space for training PEW system in Building 185. Laboratory space and access to environmental chamber #5 in Building 160. Initial housing for 16 patas monkeys and laboratory space for training PEW system in Building 1001. The patas monkeys will be screened for simian hemorrhagic fever, which is extremely virulent and pathogenic in rhesus monkeys. In any experiments where rhesus monkeys will use equipment that has been used by patas monkeys, the equipment will be carefully cleaned with an appropriate disinfectant (as directed by USAFSAM/VS guidelines) and allowed to air-dry for at least 24 h (or longer, depending on guidelines generated by USAFSAM/VS personnel). Every effort will be made to minimize transitions from patas to rhesus monkeys.

b. Equipment: Components for the 2 PEW systems, including 1 PEW hood and recording equipment required for this work are being ordered and assembled.

c. Animals:

(1) From a pool of 12 trained rhesus monkeys (2.5-4.5 kg) or 10 trained patas monkeys (Protocol VNC-89-07-C), 6 rhesus and 6 patas monkeys will be transferred to this protocol. No additional monkeys are anticipated except as possible replacement animals due to health or difficulty of training.

(2) Post-Experimental Disposition: The animals used in this study will be returned to the Veterinary Sciences Division (VS) for reassignment.

12. HAZARDS:

The hazards associated with handling animals are minimized by adherence to standard laboratory procedures, with which all applicable personnel have been familiarized.

All personnel will be briefed on the hazards of direct contact with the animals. Standard protective clothing, gloves (rubber and/or leather), and face masks will be worn during the handling of any animal. Personnel will have read USAFSAM Regulation 161-1 covering the control management for herpes virus simiae (B-Virus). Every possible attempt will be made to prevent scratches and bites.

13. ANIMAL USE:

a. Alternative Species: The rhesus monkey is the immediately available species of choice because of the large data base on the species, including the area of exercise physiology, and the comparability to man. The rhesus and patas monkeys are qualitatively similar and, like humans, sweat over the entire body surface. However, the evaporative heat loss due to sweating is 40% higher in patas. Because of the higher sweating capacity and other similarities with the human eccrine system, the patas is

the most appropriate animal model for thermoregulatory studies. Alternatives in the form of lower species do not exist and the protocol does not unnecessarily duplicate previous experiments (Agricola/Animal Welfare Information Center, Biosis, Excerpta Medica, NTIS, MEDLARS-Bioethics).

b. Relief of Pain, Discomfort and Distress: The animals will be subjected to minimal discomfort from periodic blood sampling and brief electric shocks to motivate performance in the PEW.

c. Statement: The care and use of animals in these experiments will be in accordance with USAFSAM Regulation 169-2.

d. Veterinary Consultants: Dr. Roger C. Harvey/VSR/43477  
Dr. John W. Fanton/VSR/42078

e. Special Considerations: The monkey will be placed in the primate restraining chair by two experimenters and trained to sit quietly following the procedures outlined in VNC-89-07-C ("Training Program for Instrumentation, Telemetry, and Exercise Ergometry"). The animals will be in the chair no more than 4 h in any one session (most sessions will be 2-3 h or less) and there will be only 1 experimental session in any given 5-day time period. The animals will be examined each time they are removed from the chair to determine if the chairing procedures caused decubital ulcers, contusions, or dependent edema. It is unlikely that these pathological processes will occur, but if they do, the chairing procedures will be temporarily suspended until the monkey has been examined by the veterinary consultant.

14. SIGNATURE OF PRINCIPAL INVESTIGATORS AND DATE: