



DEPARTMENT OF DEFENSE
ARMED FORCES EPIDEMIOLOGICAL BOARD
5109 LEESBURG PIKE
FALLS CHURCH, VA 22041-3258



REPLY TO
ATTENTION OF

AFEB (15-1a) 00-2

29 March 2000

MEMORANDUM FOR THE ASSISTANT SECRETARY OF DEFENSE (HEALTH AFFAIRS)
THE SURGEON GENERAL, DEPARTMENT OF THE ARMY
THE SURGEON GENERAL, DEPARTMENT OF THE NAVY
THE SURGEON GENERAL, DEPARTMENT OF THE AIR FORCE

SUBJECT: Armed Forces Epidemiological Board Final Report:
An Evaluation of the Scientific Literature as it Pertains to
Gulf War Illnesses, Volume II: "Pyridostigmine Bromide" and
an Assessment of Current DOD Supported Research Regarding
pyridostigmine bromide

1. The Board strongly endorses the report of its Special Committee transmitted herewith. Within the range of what we have come to expect over the years from the many RAND reports and reviews, the second volume in the Gulf War Series, dedicated to Pyridostigmine Bromide, is clearly an outlier:

- a. THIS IS NOT A CRITICAL REVIEW OF THE SCIENTIFIC LITERATURE. THERE IS NO EVIDENCE THAT THE AUTHORS APPLIED COMMON PROCEDURES FOR OBJECTIVELY ASSESSING THE SCIENTIFIC STRENGTH OF THE INDIVIDUAL STUDIES CITED, WHICH VARIED WIDELY IN DESIGN AND OTHER CRITICAL ASPECTS AFFECTING SCIENTIFIC VALIDITY. THE NARRATIVE PROCEEDS WITHOUT TRYING TO MAKE MUCH-NEEDED JUDGMENTS OR GIVING WEIGHT TO A REASONABLE PATH IN THE FACE OF CONFLICTING ALBEIT INCONCLUSIVE DATA. THE REPORT IS ESSENTIALLY AN INDISCRIMINATE CATALOG OF WHAT HAS BEEN PUBLISHED.
- b. THE REPORT INCLUDES SOME NEWS MEDIA AND INTERNET SOURCES, AS WELL AS TESTIMONY AT CONGRESSIONAL HEARINGS FROM ADVOCATES WHO HAVE SPECIFIC AGENDAS TO ADVANCE, INSTEAD OF BEING RESTRICTED TO STANDARD PEER-REVIEWED SCIENTIFIC PAPERS. DESPITE THE DISCLAIMER THAT UNPUBLISHED INFORMATION WAS OCCASIONALLY USED, BUT ONLY TO DEVELOP HYPOTHESES, INCLUSION OF THESE SOURCES AND THEIR PERSONAL TESTIMONY IN A REPORT ENTITLED, "A REVIEW OF THE SCIENTIFIC LITERATURE..."

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- b. (CONTINUED)
IMPLIES A DEGREE OF VALIDITY THAT IS ESPECIALLY
PROBLEMATIC IN LIGHT OF THE EMOTION AROUND GULF
WAR ILLNESS.
- c. THE REPORT INCLUDES RECOMMENDATIONS FOR MORE
EPIDEMIOLOGICAL STUDIES BASED ON CLEARLY
INADEQUATE RECALL MORE THAN NINE YEARS AFTER
THE FACT. SUCH STUDIES CAN RESOLVE NOTHING.
- d. THERE IS NO "FLAG" OR ANY EXPLICIT RECOGNITION
OF WHAT WE REFER TO AS AN "OVERARCHING ISSUE"
IN OUR REPORT. SINCE THIS ISSUE IS CRITICAL,
SOME ACKNOWLEDGMENT OF THE TOTAL LACK OF
ASSURED HUMAN EFFICACY IS SURPRISING AND HAS
NEGATIVE CONSEQUENCES FOR THE VALUE OF THE
RAND REPORT.
- e. THE QUALITY AND LEVEL OF INTERNAL AND EXTERNAL
REVIEW THAT RAND MAY HAVE PERFORMED IS NOT
APPARENT; THIS MAY BE A FACTOR IN THE WEAK,
UNEVALUATED INFORMATION AND RECOMMENDATIONS.

2. These shortcomings are so profound as to render the Document scientifically too weak for use in policy development. Furthermore, the document itself appears to have added to the burden of military decision-making through many person-hours already spent in efforts to clarify the quality of its information.

3. All of this is quite unfortunate, but is nonetheless an honest assessment of a report that is far less helpful than it could be. We cannot accept it and do not endorse its recommendations.

AFEB (15-1a) 00-2

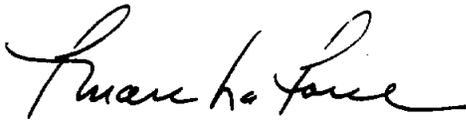
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4. The enclosed Special Subcommittee report details the AFEB's concerns with the RAND document. It also raises the overarching concern of assessing efficacy. The AFEB would like to point out that efficacy considerations and the side effect profile should inform future decision-making on pyridostigmine bromide.

5. The final report and its findings and recommendations were unanimously approved by the Board.

FOR THE ARMED FORCES EPIDEMIOLOGICAL BOARD:



F. MARC LaFORCE, M.D.
AFEB President



BENEDICT M. DINIEGA
Colonel, USA, MC
AFEB Executive Secretary

Copies Furnished:

Board Members

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HQ, USMC, PMO, CAPT Kenneth W. Schor

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CDR, WRAIR

CDR, USACHPPM, ATTN: MCHB-DC-C

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An Evaluation of
"A Review of the Scientific Literature as it Pertains to Gulf War Illnesses
Volume 2, Pyridostigmine Bromide"
And
An Assessment of Current DOD Supported Research
Regarding Pyridostigmine Bromide

FINAL REPORT

By

Special Subcommittee
of the
Armed Forces Epidemiological Board

28 January 2000

Approved by the Armed Forces Epidemiological Board at the February
2000 meeting.

FOR THE SPECIAL SUBCOMMITTEE:

A handwritten signature in cursive script, reading "Dennis M. Perrotta".

DENNIS M. PERROTTA
Chairman, Special Subcommittee

Background

The Environmental and Occupational Health Committee of the Armed Forces Epidemiological Board (AFEB) was requested to review a soon-to-be-released RAND document, "A Review of the Scientific Literature as it Pertains to Gulf War Illnesses, Volume III: "Pyridostigmine Bromide" (PB) (Appendix 1). Three members of the committee were selected to form a special subcommittee. The subcommittee met with basic and clinical scientists from the Uniformed Services University of the Health Sciences to answer and address the following tasks:

Evaluate the research and suggestions outlined in the report,
evaluate the research undertaken by the Department of Defense (DOD)
on this topic, and
identify the gaps in knowledge that should be addressed by further
research within DOD.

The subcommittee reviewed and discussed the report. Also, a synopsis of DOD supported research regarding PB currently underway or recently finished was reviewed. The subcommittee consisted of the following AFEB members:

Dennis M. Perrotta, PhD, CIC, President AFEB, Subcommittee Chair
Henry A. Anderson, MD, Chair, AFEB Environment Committee
Stanley I. Music, MD, DTPH (Lond.), AFEB Environment Committee.

Uniformed Services University of the Health Sciences staff that consulted with the subcommittee included:

LTC John Fontana, Anesthesiology
Professor John Sarvey, Pharmacology
COL David S. Dougherty, Neurology.

Over-arching Issue

A great deal of discussion occurred regarding the ability of a civilized society to test, on humans, the effectiveness of a pre-treatment to a chemical that reportedly is lethal and impervious to antidote within minutes of exposure. Since PB is considered an investigational drug for this use, what additional information would be required to approve it for use as a pre-treatment for exposure to soman? If human data are required, can we ethically recommend such tests that might require human exposure to a deadly substance? Is it reasonable, given the immense importance of valid safety and effectiveness information, to rely on surrogate measures in humans or animal experiments? Do issues of national security overcome ethical issues of human exposure?

The point of this discussion relates to our charge to assess the status of our knowledge regarding pyridostigmine bromide (PB) and Soman:

- a weapon of almost unimaginable horror exists – soman
- while it surely kills animals, there are little or no human exposure data to determine if it kills humans, at what dose it may kill, or even its

precise mechanism of action. Nonetheless, the available evidence compels us to view soman as a real threat, real enough that several hundred thousand American troops used PB as pretreatment during combat against Iraq in the Persian Gulf;

- the use of this pretreatment PB is complicated further by a set of facts:
 - there are no known human efficacy data for PB against soman,
 - the pretreatment use of PB (based upon animal experiments) indicates that effective postexposure therapy requires the maintenance of thrice-daily pretreatment dosing for the duration of the exposure-threat period (i.e., some PB blood level must be attained and maintained in advance of soman exposure for protection from soman),
 - the large scale use of PB during the Gulf War has itself been associated with reported adverse events whose prevalence is high, whose seriousness is not trivial and which may also be a cause of illness in Persian Gulf War veterans, and
 - while some humans have been given PB for relatively long periods of time, they are either people with myasthenia gravis (and an already abnormal neuro-muscular junction) or they were part of the previously referenced military operation wherein use was ordered but was not usefully documented. Compliance was unreliable with testimonials ranging from not taking PB, taking it sporadically, taking it as directed, and overdosing on PB.

The US military surgeons-general have formally requested guidance from the AFEB. The expected and customary response of the AFEB is to assemble selected persons with relevant experience and ask them to review the existing data. They are further requested to make the usual kind of recommendations, such as "do we know enough or are there some significant gaps in the research already completed or currently underway, etc." However, this is not the usual kind of situation as there are overarching ethical and moral issues that add discomforting dimensions and must be made explicit.

We must be able to defend our society against all possible threats, including aggressive warfare and terrorism. In considering the weapons that might be used against us, unlike explosive ordinance, such as bullets and bombs, whose killing potential can be reliably extrapolated from chickens and rats to humans, chemical agents like soman add an element of possibly unprecedented uncertainty. There is much we don't reliably know. And when we include defensive agents in our thinking, we escalate that uncertainty *precisely because there are no data upon which we can rely to be actually sure that a given therapy will actually protect humans.*

The subcommittee did not seek to answer the ethical conundrum, but recommends that this profoundly difficult issue be carefully considered at the appropriate leadership levels. We turned to the review of the document.

General Comments:

The characteristics of "aging" of the nerve agent soman, where it appears to rapidly and irreversibly bind to nerve cells, makes the use of some pretreatment a high priority for the safety of deployed military troops and the security of our country.

While the report represents an extensive, detailed review of the literature, in general there was little or no critical analysis of the reference material presented. No sense of critical judgement of the evidence was found and little weighting of the evidence was noted. The reader is left without a sense of which studies were well designed and carried out, and which were not and, hence, which might merit further study and which may not. The report states that it "cannot rule out PB as a contributor to illness in Persian Gulf War veterans" but it provides no critically evaluated information that leads the reader to conclude that it should be "ruled in" either. The same could be said for other purported causes of illnesses in PGW veterans (e.g. infectious diseases).

The report correctly points out that most, if not all, studies suffer from a lack of a consistent case definition for illness and no verifiable information as to who experienced any exposure to PB or other environmental exposures. From an epidemiological perspective, this lack of exposure and outcome data makes any study difficult to conduct and nearly impossible to interpret. Surrogate measures are often used in these situations, but no judgement as to which were validated was provided. In spite of this methodological minefield, a series of potential theories of causation were forwarded for consideration. Each section of the report that outlined a theory included "scientific recommendations". The subcommittee reviewed these recommendations.

Specific Research Questions

The committee strongly recommends that no additional epidemiologic studies of Persian Gulf War Veterans based solely on recall be supported or performed. Without verifiable exposure history and/or a measurable clinical case definition, no useful conclusions can be drawn. Self-reports of exposure provided 8 years after deployment are exquisitely susceptible to a variety of influences and biases that are difficult to measure in degree and direction. Likewise, self-reports of health conditions that have occurred since deployment to the Persian Gulf have been mostly categorized as "ill-defined signs and symptoms" and lend poorly to useful analysis and interpretation. No sense of exposures in the intervening years was provided. Only with the development of a standardized and verified biomarker would further epidemiological studies be warranted.

PB Use in the Persian Gulf War, page 55:

The recommendations address policy issues and the subcommittee supports all recommendations regarding the education of soldiers who may receive PB in future deployments. Further, the subcommittee recommends that effective health education approaches, appropriately targeting personnel that will receive PB, must be designed, field tested, and implemented at all levels. A thorough and complete tracking of who received instructions and PB should be of high priority.

While the use of animal models is problematic, the human subject issues described above suggest that testing of PB pretreatment in non-human primates (as described, page 56) is an important research tool that deserves further attention.

The subcommittee, restating what the full Board has recommended in the past, recommends that the accurate tracking of all medical treatments and troop movements during deployments, be among the highest priorities for DOD leadership. Environmental and disease/injury surveillance must be integral parts of every deployment strategy.

Blood-Brain Barrier Passage, page 80:

The question as to whether PB crosses the blood-brain barrier and if it does, whether it induces measurable untoward effects, is central to a number of other hypotheses proposed in this report. The subcommittee recommends that research be carried out to determine if PB exerts any detectable untoward effect in the brain and, if so, how that may relate to mission performance and risk for delayed or chronic adverse health conditions. The use of physostigmine (which does cross into the brain) as a model for what might occur if PB did reach the brain should be considered. Documentation of the effects (or lack thereof) of stress (more closely modeled to what a soldier might actually experience) on the permeability of the blood-brain barrier to PB will contribute important information. Radiolabeled bromine-containing PB in primates or volunteers could provide insight regarding the pharmacokinetics of PB under various environmental circumstances.

The question of passage of PB through the human blood-brain barrier is central to better understanding reported untoward effects by service members that consumed PB during the Persian Gulf War. To assist in formulating useful research suggestions, subcommittee members evaluated additional, recent literature related to stress, PB, and the integrity of the blood-brain barrier (See Appendix 3). These works demonstrated variability in the impact that stress has on the permeability of the blood-brain barrier depending on the animal and methodology used. Extrapolation from non-human mammalian experiments to the human experience remains problematic. Some human studies have failed to

include appropriate controls. With this extra information available, the Subcommittee believes that additional research is warranted as described above.

Individual Differences, page 104:

Because of the methodological issues described above, the subcommittee does not support further studies (as outlined in #1, page 104) that rely on self-reported illness and undocumented exposure history from 8 years past.

Recommendations #2 and 3 are of low priority since they should focus more on nerve agents and less on phenotypic issues. Until pre-deployment "profiling" is validated as a useful and accurate adjunct, recommendations #4 and 5 are premature.

Interactions, page 129:

Because of the methodological issues described above, the subcommittee does not support further studies that rely on self-reported illness and undocumented exposure history from 8 years past. This includes long-term follow-up of PGW veterans for signs of chronic illness when PB exposure has not been documented. Well-designed and implemented research which exposes animals to various compounds, alone, and in combination with PB may be of some utility. In general, there is little a priori reasoning suggesting that interactions between PB and other agents would occur.

Bromism, page 142:

The subcommittee agrees that there is insufficient evidence to warrant any research regarding bromism as a cause of illness in PGW veterans.

Multiple Chemical Sensitivities, page 161:

Without a useful, validated clinical case definition, and in the absence of documented PB exposure, studies of multiple chemical sensitivities in PGW veterans are not supportable. Interpretation of such studies would be impossible.

The use of SPECT scanning in individual diagnoses has not been validated and is not appropriate under these circumstances. There is a great deal of variation among tests and interpreters. Therefore, any study in an already potentially biased population without simultaneous normal controls, over time, would not be a powerful analytical piece of information.

The use of unpublished, non-peer reviewed research reports does not provide any useful additional information to an area of study fraught with subjectivity.

Neuromuscular Junction Effects, page 180:

Recommendations #1-6 are likely found in the neurosciences literature.

The subcommittee supports carefully designed and implemented studies of the effect of PB on muscle function in healthy volunteers as described in

recommendation #9. An example of a proposed protocol for this study is found in Appendix 2. Other recommendations are not supported because #9 provides more direct information.

Ethical considerations of volunteer studies must be addressed.

Neurotransmitter Dysregulation, page 207:

The issue of peripheral signaling is addressed in the previous section on neuromuscular junction effects. The issue of central effects is important only if research described in the section entitled "Blood-brain Barrier Passage" indicates that PB does cross the barrier. If it does not, then research on central signaling is not needed. If research shows that PB does cross the barrier, then carefully designed, controlled, and implemented research would be needed. The use of SPECT analysis is not validated and therefore its use is not supportable at this time.

Chronic Effects, page 237:

Sensitive testing techniques are only useful when exposure status can be documented and validated. The subcommittee did not support additional research on chronic conditions or symptoms unless they can be related to PB.

Recommendation #3 regarding prospective, pre-deployment testing deserves careful thought since they are subject to many influences other than chemical exposures. For example, just the rumor of deployment can measurably change neuropsychological findings and psychosomatic status of military personnel. This puts into question the ability to interpret any findings.

Other Considerations, page 263:

The subcommittee supports continuing work, done in collaboration with the U. S. Centers for Disease Control and Prevention regarding case definitions. None of the other recommendations in this section directly pertain to the effects of PB; the subcommittee made no deliberations or decisions pertaining to them.

Review of DOD Research

The subcommittee reviewed the document, "1999 Report to Congress (Draft) Summaries of DOD-Sponsored Studies Involving Pyridostigmine Bromide" dated 5 March 1999. This document provided brief overviews of DOD-supported studies in basic, applied, and clinical research.

The subcommittee judged the research to vary greatly in quality and applicability to answering priority questions about the potential impact of PB on human subjects.

For the same reasons stated above, any study that utilizes self-reported exposure to PB without documentation must be considered with extreme caution. While some researchers indicate that self-reports are "the only source of information" regarding PB exposure, that fact does not necessarily justify their use with a blind eye to the severe inherent limitations of these data.

Likewise, self-reported signs, symptoms, ill-effects and illnesses are limited in utility and subject to severe biases since exposure occurred over 8 years in the past (from current time). Timing of illnesses in relation to exposure, and the role of intervening exposures and illnesses further complicate the interpretation of these studies, no matter the sophistication of statistical manipulation.

If biomarkers are found and validated against documented exposures or illnesses, then carefully crafted study protocols may be considered. Otherwise, support for new epidemiologic studies was considered low priority at best.

Much of the basic research will contribute in small ways to the proposed topics for further attention noted above. Some will fall short in terms of usefulness and applicability. Work on the blood-brain barrier utilizing animals and volunteers (as suggested above) will contribute significantly if properly planned and implemented.

Studies that are underway should be completed. Specific recommendations for research that does, and does not merit further support are found within the report topics above.

Summary

The Special Subcommittee of the AFEB reviewed the RAND document on PB as well as brief reports of DOD-supported research on PB. The report was found to lack critical evaluation of available data. Without professional judgement to prioritize current knowledge, the subcommittee found the report to be of limited utility. It failed, in our opinion, to justify ruling PB out as a cause of illness in Persian Gulf War Veterans, or to justify ruling PB in.

Further epidemiologic investigations of an association between PB and ill effects experienced by Persian Gulf War veterans (in the absence of newly identified and validated biomarkers) are not supportable. Clinical research evaluating the effects of PB on the neuromuscular junction must be very carefully designed and implemented to consider the significant influences of the psychological environment of test subjects.

Further research into the potential passage of PB through the blood-brain barrier (and whether untoward effects are found if it does pass through) are worthy of continued support. The impact of stress on the permeability of the blood-brain barrier to PB is worthy of further study, only if the stress surrogate very closely

resembles what a soldier, sailor, marine, or airman would have experienced upon deployment to the Persian Gulf.

As always, the Armed Forces Epidemiological Board was pleased to provide this review for use in support of force health protection.

Appendix 1

Tasking Letter from Department of Defense (Health Affairs)



HEALTH AFFAIRS

OFFICE OF THE ASSISTANT SECRETARY OF DEFENSE
1200 DEFENSE PENTAGON
WASHINGTON, DC 20301-1200

James A. Zimble, M.D.
President
Uniformed Services University of the Health Sciences
4301 Jones Bridge Road
Bethesda, Maryland 20814-4799

06 JUL 1999

Dennis Perrotta, Ph.D.
President, Armed Forces Epidemiology Board
Chief, Bureau of Epidemiology
Texas Department of Health
1100 West 49th Street
Austin, Texas 78756

Dear Dr. Zimble and Dr. Perrotta:

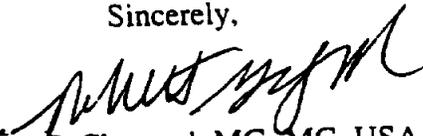
I would like to request that three members of the Armed Forces Epidemiology Board (AFEB) and a similar number of individuals from the Uniformed Services University of the Health Sciences (USUHS) faculty meet to review the Draft Rand Report entitled, **A Review of the Scientific Literature As It Pertains to Gulf War Illnesses, Volume III. Pyridostigmine Bromide**. Request that the AFEB chair the meeting and prepare the report.

This draft report, written by Beatrice Alexandra Golomb in September of 1998, makes a variety of suggestions about possible mechanisms of action of the pyridostigmine bromine, and whether or not it proved effective and safe as a product in the Gulf War.

The questions that I would like the group to address include an evaluation of: 1) the research and the suggestions made in the report; 2) the research that has been undertaken by the Department of Defense on this topic; and 3) gaps in the present knowledge that need to be addressed by further research within the Department of Defense. I am also asking that the group address, in light of this review of the report and the review of the DoD research program, whether alterations in the present position of the Department of Defense should be considered on this topic to address remaining unresolved issues.

I am hoping that such a meeting could be held within the next month or two with a report by September 1, 1999. If you can suggest one of more days that are convenient for such a meeting, Dr. Ruth Ellen Bulger, USUHS Vice President for Research, has volunteered to reserve a room at the USUHS for such a meeting. I am happy to provide a copy of the report and to answer any questions you may have on such a meeting. Please call me at (703) 681-1711. if I can provide additional information.

Sincerely,


Robert G. Claypool, MG, MC, USA
Deputy Assistant Secretary of Defense
(Health Operations Policy)

cc:

COL Benedict Diniega, Executive Secretary, AFEB
Ruth Ellen Bulger, Ph.D., USUHS Vice President for Research



HEALTH AFFAIRS

OFFICE OF THE ASSISTANT SECRETARY OF DEFENSE
1200 DEFENSE PENTAGON
WASHINGTON, DC 20301-1200

OCT 27 1999

Dennis Perrotta, Ph.D.
President, Armed Forces Epidemiology Board
Chief, Bureau of Epidemiology
Texas Department of Health
1100 West 49th Street
Austin, Texas 78756

Dear Dr. Perrotta:

I would like to thank the three members of the Armed Forces Epidemiology Board (AFEB) and the Uniformed Services University of the Health Sciences (USUHS) faculty who met to review the Draft Rand Report entitled, **A Review of the Scientific Literature As It Pertains to Gulf War Illnesses, Volume III. Pyridostigmine Bromide**. The AFEB chaired the meeting and prepared the Subcommittee report.

Thank you for providing us with a signed copy of the DRAFT Subcommittee report. As noted in the DRAFT report, this will be an agenda item for review and approval by the full AFEB at the February 2000 meeting.

If you provide us with the number of copies of the Rand study needed by Board members, I will ask the Office of the Special Assistant for Gulf War Illnesses to request sufficient copies from Rand. Rand has indicated that the author of the subject report, Dr. Beatrice Golomb, can be made available to respond to any questions or concerns raised by the Board. Dr. Bailey feels that the issues raised in this report should be addressed by outside experts and has specifically referenced the AFEB. Therefore, we anxiously await the results of your deliberations and your final report.

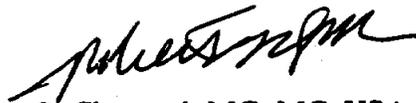
The questions that I asked the group to address included an evaluation of: 1) the research which the author reviewed and the suggestions made in the report; 2) the research that has been undertaken by the Department of Defense on this topic; and 3) gaps in the present knowledge that need to be addressed by further research within the Department of Defense. I also asked that the group address, in light of this review of the report and the review of the DoD research program, whether alterations in the present research course of the Department of Defense should be considered on this topic to address remaining unresolved issues.

The Subcommittee did not address the third question concerning current gaps in present knowledge that need to be addressed by further DoD research in this area. I will ask the US Army Medical Research and Materiel Command to provide that crosswalk. However, I would like to ask that you review new research, not considered by the Subcommittee, to be included in your final report. Specifically, I will attach two additional studies that I would like the Board to consider concerning PB crossing the Blood Brain Barrier. One is an article titled "Heat Stress, Even Extreme, Does Not Induce Penetration of Pyridostigmine into the Brain of Guinea Pigs" by Guy Lallement et al. And the other is a letter to the Editor of Nuclear Medicine & Biology, Vol. 26, pg. 249-250, by Frank W. Telang, et al. If additional articles are brought to our attention, I will forward them to your Executive Secretary.



Again please extend my sincere appreciation to the members of the Subcommittee who took time to review this in-depth Rand study. Please call me at (703) 681-1711, if I can provide additional information.

Sincerely,



Robert G. Claypool, MG, MC, USA
Deputy Assistant Secretary of Defense
(Health Operations Policy)

Attachments
As stated

cc:
COL Benedict Diniega, Executive Secretary, AFEB
Ruth Ellen Bulger, Ph.D., USUHS Vice President for Research

Appendix 2

Assessing the Impact of Pyridostigmine Bromide on the Neuromuscular Junction

Proposal for the Assessment of Pyridostigmine Bromide: Neuromuscular Effects

Healthy volunteers, ages 18-35 (typical active duty age), physiologically matched for pre-study physical activity. Both males and females. Enough statistical power to have an 80% chance of finding statistically significant indices of change in electrophysiologic measures. It should be double blinded with equal randomization to PB treatment and placebo treated groups.

BASELINE ASSESSMENT should include:

- History and physical with neurologic examination
- Baseline CBC, chemical profile, ANA, and saved sera for muscle antibody (to include anti-smooth, anti-striated, anti-acetylcholine receptor and others) serological comparisons.
- MMPI, also Beck's Depression or Hamilton Depression Scales
- Physical Fitness Scores, Semi-quantitative Strength Assessment
- Standard EMG/NCV
- Multipoint single motor unit stimulation (assesses single motor axons and attached muscle fiber pools)
- SFEMG (assesses neuromuscular junction integrity by measuring jitter)
- Intramuscular Electrical Stimulation (assesses muscle fibril conduction velocity).

Begin Blinded Treatment Phase: Standard per oral dosing of PB or placebo for 1, 2, and up to 4 weeks as determined by the need to carry out this far based on presumption of PB exposure.

- Monitor symptom profile continuously
- Continue Pre-Study Activity

POST STUDY ASSESSMENT

At completion:

- Repeat baseline assessment
- Muscle biopsy
- Caffeine stimulation for malignant hyperthermia

**Histopathological and electron microscopic analysis
Break the treatment code**

**At 6 months post completion:
Repeat baseline assessment
Reassess physical fitness scores.**

Ideally, this population could be found among the active duty population. A sufficiently non-threatening environment may be difficult to come by in the armed services. As a substitute, a college student population, appropriately compensated financially, may be the only other suitable population for study subjects.

Because the study questions of interest are clearly and largely impacted by subjective issues, the use of quantitative measures that do not rely on patient compliance will be an important part of this study protocol. Examples include the following.

Single Fiber Electromyography (SFEMG) is a non-compliance driven quantitative assessment of neuromuscular junction physiological effects used extensively in the diagnosis of abnormalities of the neuromuscular junction. There is a long track record of normative data from which to compare.

Intramuscular Electrical Stimulation is a similarly non-compliance driven quantitative assessment of muscle fibril electrical excitability and conduction velocity of muscle membrane activation.

Appendix 3

Additional Literature Reviewed Related to Blood Brain Barrier

1. **Lallemanet, G., Foquin, A., Baubicioni, D., Burckhart, M., Carpenter, P. & F. Canini. Heat Stress, Even Extreme, Does Not Induce Penetration of Pyridostigmine into the Brain of Guinea Pigs. Neurotox., 1998;(19)6:749-56**
2. **Telang, F.W., Ding, Y., Volkow, N., Molina, P. & S.Gatley. Letter to the Editor, Nuc Med Bio. 1999;26:249-250**
3. **Lundy, P. Stress and the Production of CNS Effects of Pyridostigmine: A Commentary. The ASA Newsletter. 1997;26:1,17-19.**
4. **Kaufer, D., Friedman, A. & H. Soreo. The vicious Circle of Stress and Anticholinesterase Responses. Neuroscientist. 1999;(5)3:173-183.**