

Appendix A-1
Project Abstracts

INDEX - List of Persian Gulf Veterans' Illnesses Research Projects by Agency

Department of Defense (DoD)

DoD-1	Naval Health Study Program
DoD-1A	Epidemiologic Studies of Morbidity Among Gulf War Veterans: A Search for Etiologic Agents and Risk Factors; Study 1: A Study of Symptoms Among 1500 Seabees.
DoD-1B	Epidemiologic Studies of Morbidity Among Gulf War Veterans: A Search for Etiologic Agents and Risk Factors; Study 2: A comparative study of hospitalizations among Active-duty personnel who participated in the Gulf War and similar personnel who did not.
DoD-1C	Epidemiologic Studies of Morbidity Among Gulf War Veterans: A Search for Etiologic Agents and Risk Factors; Study 3: A comparative study of pregnancy outcomes among gulf war veterans and other active-duty personnel.
DoD-1D	Epidemiologic Studies of Morbidity Among Gulf War Veterans: A Search for Etiologic Agents and Risk Factors; Study 4: Infertility and Miscarriage in Gulf War Veterans.
DoD-1E	Epidemiologic Studies of Morbidity Among Gulf War Veterans: A Search for Etiologic Agents and Risk Factors; Study 5: Seabee Health Study.
DoD-1F	Epidemiologic Studies of Morbidity Among Gulf War Veterans: A Search for Etiologic Agents and Risk Factors; Study 6: A Comparison of Nonfederal Hospitalization Experience Among Veterans in California who have separated from active service: PGW vs. EV.
DoD-1G	Epidemiologic Studies of Morbidity Among Gulf War Veterans: A Search for Etiologic Agents and Risk Factors; Study 7: Prevalence of Congenital Anomalies Among Children of Persian Gulf War Veterans.
DoD-2	Physiological and Neurobehavioral Effects in Rodents from Exposure to Pyridostigmine, Fuels, and DEET
DoD-4	The General Well-Being of Gulf War Era Service Personnel from the States of Pennsylvania and Hawaii: A Survey
DoD-6	Program DoD-6
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DoD-6B	Combat Stress Diagnosis, PTSD Prevention
DoD-7A	Health Risk Assessment of Embedded Depleted Uranium: Behavior, Physiology, Histology, and Biokinetic Modeling
DoD-7B	Carcinogenicity of Depleted Uranium Fragments
DoD-8	Program DoD-8
DoD-8A	Serologic Diagnosis of Viscerotropic Leishmaniasis (VTL)
DoD-8B	Development of a Leishmania Skin Test Antigen (LSTA)
DoD-9	Identification of the Genetic Factors Which Control Tropism in Leishmania
DoD-10	Pyridostigmine Synergistic Toxicity Study
DoD-11	Male/Female Differential Tolerances to Pyridostigmine Bromide
DoD-12	Forward Deployable Diagnostics for Infectious Diseases
DoD-13	Effects of Persian Gulf War Service on Military Working Dogs
DoD-14	Risk Factors Among US Army Soldiers for Enrolling on the Department of Veterans Affairs Gulf War Registry
DoD-15	Comparative Mortality Among US Military Personnel Worldwide During Operations Desert Shield and Desert Storm
DoD-16	Kuwait Oil Fire Health Risk Assessment
DoD-17	Retrospective Studies Involving Military Use of Pyridostigmine as a Pretreatment for Nerve Agent Poisoning

DoD-18 Kuwait Oil Fires Troop Exposure Assessment Model (TEAM)

DoD-19 Persian Gulf Veterans Health Tracking System

DoD-20 A Statistical Study Correlating the Reported Cases of Gulf War Syndrome to Battlefield Locations of Afflicted US Army Personnel During the Iraq-Kuwait War; Part 1: Method to Related Troop Deployment and the Reported Cases of Gulf War Syndrome and Probable Incidence of Maladies Defined by ICD-9-CM

DoD-21 Study of Variability In Pyridostigmine Inhibition of Blood Cholinesterases in Healthy Adults and Individuals With Symptoms Following Participation in Operation Desert Storm

DoD-22 Chronic Organophosphorus Exposure and Cognition

DoD-23 Acute and Long-Term Impact of Deployment to Southwest Asia on the Physical and Mental Health of Soldiers and their Families

DoD-30 Epidemiological Studies Persian Gulf War Illnesses, PG Women's Health Linkage Study

DoD-31 Dysregulation of the Stress Response in the Persian Gulf Syndrome

DoD-32 Neuropsychological Functioning in Persian Gulf Era Veterans

DoD-33 Effects of Pyridostigmine in Flinders Line Rats Differing in Cholinergic Sensitivity

DoD-34 Characterization of Emissions from Heaters Burning Leaded Diesel Fuel in Unvented Tents

DoD-35 Feasibility of Investigating Whether There is a Relationship Between Birth Defects and Service in the Gulf War.

DoD-36 Fatigue in Persian Gulf Syndrome-Physiologic Mechanisms

DoD-37 Neurobehavioral and Immunological Toxicity of Pyridostigmine, Permethrin, and DEET in Male and Female Rats

DoD-38 Diagnostic Antigens of Leishmania tropica

DoD-39 A Controlled Epidemiological and Clinical Study into the Effect of Gulf War Service on Servicemen and Women of the United Kingdom Armed Forces

DoD-40 Psychological and Neurobiological Consequences of the Gulf War Experience

DoD-41 Evaluation of Muscle Function in Persian Gulf Veterans

DoD-42 The Symptomatic Persian Gulf Veterans Protocol: An Analysis of Risk Factors with an Immunologic and Neuropsychiatric Assessment

DoD-44 Investigation of Seminal Plasma Hypersensitivity Reactions

DoD-45 Physical and Emotional Health of Gulf War Veterans Women

DoD-46 Exploratory Data Analysis with the CCEP Database

DoD-47 Study of Mycoplasmal Infections in Gulf War Veterans

DoD-48 Assessment of Genomic Instability via Chromosome 7 Inversion Frequency in a Gulf-War Syndrome Cohort vs Selected Control Groups

DoD-49 Diagnosis and Dosemetry of Exposure to Sulfur Mustard: Development of Standard Operating Procedures and Exploratory Research on Protein Adducts

DoD-50 Toxicokinetics of O-Ethyl S-(2-Diisopropylaminoethyl) Methylphosphonothioate [(+)-VX] in Rats, Hairless Guinea Pigs and Marmosets - Identification of Metabolic Pathways

DoD-51 Transgenic Engineering of Cholinesterases: Tools for Exploring Cholinergic Responses

DoD-52 Female Gender and Other Potential Predictors of Functional Health Status Among Persian Gulf War Veterans

DoD-53 Long-Term Effects of Subclinical Exposures to Sarin

DoD-54 Assessment of Subchronic Neurobehavioral and Neuropathologic Effects in Rats

DoD-55 Low-Level Exposure to GB Vapor in Air: Diagnosis/Dosimetry, Lowest Observable Effect Levels, Performance-Incapacitation, and Possible Delayed Effects

DoD-56 Low-Level Sarin Neurotoxicity and Its Modulation by Pyridostigmine

DoD-57	Physiologic Effects of Stress in Gulf War Veterans
DoD-58	Illness Among Persian Gulf War Veterans: Case Validation Studies
DoD-59	Pyridostigmine-induced Neurodegeneration: Role of neuronal Apoptosis
DoD-60	Butyrylcholinesterase Genetic Variants in Persons with Gulf War Illness
DoD-61	Neurophysiologic and Neuropathologic Effects in Monkeys of Low Level Exposures to Sarin, Pyridostigmine, Pesticides, and Botulinum Toxoid
DoD-62	Sarin and Pyridostigmine Interaction under Physical Stress: Neurotoxic Effects in Mice
DoD-63	PGW Veterans: Epidemiological and Clinical Evidence for Residual Organophosphate Neurotoxicity
DoD-64	Individual Differences in Neurobehavioral Effects of Pyridostigmine
DoD-65	Multi-disciplinary pathophysiologic studies of neurotoxic Gulf War related syndromes leading to diagnosis and treatment
DoD-66	Testing for mycoplasmal infection replicability of nucleoprotein gene tracking and forensic polymerase chain reaction
DoD-67	Antibacterial Treatment method based upon the excretion of dead and decaying spherical bacteria
DoD-69	Five Year Follow-Up of arm Personnel Exposed to Chemical Warfare Agents

Department of Health and Human Services (HHS)

HHS-1	Health Assessment of Persian Gulf War Veterans from Iowa
HHS-2	Disease Cluster in a Pennsylvania Air National Guard Unit, EPI-AID 95-18
HHS-3	Biomarkers of Susceptibility and Polycyclic Aromatic Hydrocarbon (PAH) Exposure in Urine and blood Cell DNA from U.S. Army Soldiers Exposed to Kuwaiti Oil Well Fires
HHS-4	Suspected Increase of Birth Defects and health Problems Among Children Born to Persian Gulf War Veterans In Mississippi
HHS-5	Cognitive Function and Symptom Patterns in Persian Gulf Veterans
HHS-6	Defining Gulf War Illness

Department of Veterans Affairs (VA)

VA-1	Mortality Follow-up Study of Persian Gulf Veterans, First Update
VA-2	National Health Survey of Persian Gulf Veterans
VA-2A	VA National Survey of Persian Gulf Veterans - Phase I
VA-2B	VA National Survey of Persian Gulf Veterans - Phase II
VA-2C	VA National Survey of Persian Gulf Veterans - Phase III
VA-3	Use of Roster of Veterans Who Served in Persian Gulf Area
VA-4	Boston Environmental Hazards Research Center Program
VA-4Core	Boston Environmental Hazards Research Center Program
VA-4A	Evaluation of Cognitive Functioning of Persian Gulf Veterans
VA-4B	Evaluation of Neurological Functioning in Persian Gulf Veterans
VA-4C	Gulf War And Vietnam Veterans Cancer Incidence Surveillance
VA-4D	Evaluation of Respiratory Dysfunction Among Gulf War Veterans
VA-4E	The Aromatic Hydrocarbon Receptor (AhR) as a Biomarker of Susceptibility
VA-4F	Validity of Computerized Tests
VA-5	East Orange Environmental Hazards Research Center Program
VA-5Core	East Orange Environmental Hazards Research Center Program

VA-5A	Health and Exposure Survey of Persian Gulf Veterans
VA-5B	Physiological and Psychological Assessments of Persian Gulf Veterans
VA-5C	Effects of Exertion and Chemical Stress on Persian Gulf Veterans
VA-5D	Effects of Genetics and Stress on Responses to Environmental Toxins
VA-6	Portland Environmental Hazards Research Center Program
VA-6Core	Core Program: Portland Environmental Hazards Research Center: Environment, Veterans Health and the Gulf War Syndrome. Core Project for Clinical and Epidemiology Research
VA-6A	Psychosocial, Neuropsychological and Neurobehavioral Assessment (Project I)
VA-6B	Clinical and Neuroendocrine Aspects of Fibromyalgia (Project II)
VA-6C	Neurotoxicity of Environmental Pollutants and Warfare Agents (Project III)
VA-6D	DNA Damage from Chemical Agents and Its Repair (Project IV)
VA-6E	Clinical and Epidemiology Leishmania Research
VA-7	Desert Storm Reunion Survey
VA-8	Psychological Test Data of Gulf War Veterans Over Time
VA-9	Evaluation of Cognitive Functioning in Persian Gulf War Veterans Reporting War-related Health Problems
VA-10	Memory and Attention in PTSD
VA-11	Neuropsychological Functioning in Veterans
VA-12	Psychological Assessment of Operation Desert Storm Returnees
VA-13	Neurobehavioral Aspects of Persian Gulf Experiences: A Pilot Study
VA-15	Vaccine-Mediated Immunity Against Leishmaniasis
VA-16	Protective Immunity in Experimental Visceral Leishmaniasis
VA-17	Immunological Evaluation of Persian Gulf Veterans
VA-18	Chronic Gastrointestinal Illness in Persian Gulf Veterans
VA-20	Psychological Adjustment in Operation Desert Shield/Storm Veterans
VA-21	A Comparison of PTSD Symptomatology among Three Army Medical Units Involved in ODS
VA-36	Stress Symptoms and Their Causal Attribution in Desert Storm Veterans
VA-40	Musculoskeletal Symptoms in Gulf War Syndrome
VA-46	Diarrhea in Persian Gulf Veterans: An Irritable Bowel-Like Disorder
VA-47	Retrospective Verification of Mustard Gas Exposure
VA-48	Cross-Sensitization as a CNS Model for Gulf War Chemical Intolerance
VA-49	Sensitivity to Pyridostigmine Bromide: Persistent Neural Dysfunction
VA-50	Neuropsychological findings in a sample of Operation Desert Storm veterans
VA-51	Psychobiological Assessment of Desert Storm Veterans
VA-53	Spouses and Children Program

Department of Defense (DoD) and Department of Veterans Affairs (VA) Shared Funded Projects

VA/DoD-1D&1V	VA/DoD Multi-site treatment trial for Chronic Fatigue Syndrome and Fibromyalgia in Gulf War Veterans
VA/DoD-2D&2V	VA/DoD Core funding of the Medical Follow-up Agency
VA/DoD-2DA&2VA	Follow-Up investigation of troops exposed to nerve agents at Aberdeen Proving Ground
VA/DoD-2DB&2VB	Patterns of Pre-Persian Gulf War Illness and Health Care Seeking

List of Persian Gulf Veterans' Illnesses Research Projects by Research Areas

<i>Research type</i>	<i>Research Focus Area</i>		<i>Project No.</i>	<i>Project Title</i>
	<i>Secondary</i>	<i>Tertiary</i>		

Brain & Nervous System

Basic Research	<i>Treatment</i>		DoD-6A	Combat Stress Pharmacotherapy
Clinical Research			VA-12	Psychological Assessment of Operation Desert Storm Returnees
Clinical Research			VA-13	Neurobehavioral Aspects of Persian Gulf Experiences: A Pilot Study
Clinical Research			VA-20	Psychological Adjustment in Operation Desert Shield/Storm Veterans
Clinical Research			VA-21	A Comparison of PTSD Symptomatology among Three Army Medical Units Involved in ODS
Clinical Research			VA-50	Neuropsychological findings in a sample of Operation Desert Storm veterans
Clinical Research	<i>Chemical Weapons</i>		DoD-63	PGW Veterans: Epidemiological and Clinical Evidence for Residual Organophosphate Neurotoxicity
Clinical Research	<i>Symptoms/ General Health</i>	<i>Diagnosis</i>	DoD-67	Antibacterial Treatment method based upon the excretion of dead and decaying spherical bacteria
Clinical Research		<i>Diagnosis</i>	DoD-32	Neuropsychological Functioning in Persian Gulf Era Veterans
Clinical Research		<i>Diagnosis</i>	VA-4F	Validity of Computerized Tests
Clinical Research		<i>Diagnosis Prevention</i>	DoD-6B	Combat Stress Diagnosis, PTSD Prevention
Clinical Research	<i>Symptoms/ General Health</i>	<i>Diagnosis</i>	DoD-31	Dysregulation of the Stress Response in the Persian Gulf Syndrome
Clinical Research	<i>Symptoms/ General Health</i>		DoD-36	Fatigue in Persian Gulf Syndrome-Physiologic Mechanisms
Clinical Research	<i>Symptoms/ General Health</i>		DoD-40	Psychological and Neurobiological Consequences of the Gulf War Experience
Clinical Research	<i>Symptoms/ General Health</i>		DoD-41	Evaluation of Muscle Function in Persian Gulf Veterans
Clinical Research	<i>Symptoms/ General Health</i>		HHS-5	Cognitive Function and Symptom Patterns in Persian Gulf Veterans
Clinical Research	<i>Symptoms/ General Health</i>		VA-4A	Evaluation of Cognitive Functioning of Persian Gulf Veterans
Clinical Research	<i>Symptoms/ General Health</i>		VA-4B	Evaluation of Neurological Functioning in Persian Gulf Veterans
Clinical Research	<i>Symptoms/ General Health</i>		VA-6A	Psychosocial, Neuropsychological and Neurobehavioral Assessment (Project I)
Clinical Research	<i>Symptoms/ General Health</i>		VA-7	Desert Storm Reunion Survey

<i>Research type</i>	<i>Research Focus Area</i>		<i>Project No.</i>	<i>Project Title</i>
	<i>Secondary</i>	<i>Tertiary</i>		

Brain & Nervous System (cont.)

Clinical Research	<i>Symptoms/ General Health</i>		VA-8	Psychological Test Data of Gulf War Veterans Over Time
Clinical Research	<i>Symptoms/ General Health</i>		VA-9	Evaluation of Cognitive Functioning in Persian Gulf War Veterans Reporting War-related Health Problems
Clinical Research	<i>Symptoms/ General Health</i>		VA-10	Memory and Attention in PTSD
Clinical Research	<i>Symptoms/ General Health</i>		VA-11	Neuropsychological Functioning in Veterans
Clinical Research	<i>Symptoms/ General Health</i>		VA-51	Psychobiological Assessment of Desert Storm Veterans
Epidemiology Research			VA-36	Stress Symptoms and Their Causal Attribution in Desert Storm Veterans on the Physical and Mental Health of Soldiers and their Families
Epidemiology Research	<i>Symptoms/ General Health</i>		DoD-23	Acute and Long-Term Impact of Deployment to Southwest Asia
Epidemiology Research	<i>Symptoms/ General Health</i>		DoD-52	Female Gender and Other Potential Predictors of Functional Health Status Among Persian Gulf War Veterans

Chemical Weapons

Applied Research	<i>Diagnosis</i>		DoD-49	Diagnosis and Dosemetry of Exposure to Sulfur Mustard: Development of Standard Operating Procedures and Exploratory Research on Protein Adducts
Basic Research			DoD-50	Toxicokinetics of 0-Ethyl S-(2-Diisopropylaminoethyl) Methylphosphonothioate [(+)-VX] in Rats, Hairless Guinea Pigs and Marmosets - Identification of Metabolic Pathways
Basic Research	<i>Brain & Nervous System</i>		DoD-53	Long-Term Effects of Subclinical Exposures to Sarin
Basic Research	<i>Diagnosis</i>		VA-6D	DNA Damage from Chemical Agents and Its Repair (Project IV)
Basic Research	<i>Prevention</i>		DoD-51	Transgenic Engineering of Cholinesterases: Tools for Exploring Cholinergic Responses
Clinical Research	<i>Diagnosis</i>		VA-47	Retrospective Verification of Mustard Gas Exposure
Clinical Research	<i>Pyridostigmine Bromide</i>	<i>Prevention</i>	DoD-60	Butyrylcholinesterase Genetic Variants in Persons with Gulf War Illness
Epidemiology Research	<i>Symptoms/ General Health</i>		DoD-69	Five Year Follow-Up of arm Personnel Exposed to Chemical Warfare Agents
Epidemiology Research			VA/DoD- 2VA/DA	Follow-Up investigation of troops exposed to nerve agents at Aberdeen Proving Ground

Research type	Research Focus Area		Project No.	Project Title
	Secondary	Tertiary		

Depleted Uranium

Basic Research	<i>Environmental Toxicology</i>	DoD-7A	Health Risk Assessment of Embedded Depleted Uranium: Behavior, Physiology, Histology, and Biokinetic Modeling
Basic Research	<i>Environmental Toxicology</i>	DoD-7B	Carcinogenicity of Depleted Uranium Fragments

Diagnosis

Applied Research		DoD-12	Forward Deployable Diagnostics for Infectious Diseases
Applied Research		DoD-66	Testing for mycoplasmal infection replicability of nucleoprotein gene tracking and forensic polymerase chain reaction
Clinical Research		DoD-47	Study of Mycoplasmal Infections in Gulf War Veterans
Clinical Research	<i>Symptoms/ General Health</i>	HHS-6	Defining Gulf War Illness

Environmental Toxicology

Applied Research		DoD-16	Kuwait Oil Fire Health Risk Assessment	
Applied Research		DoD-18	Kuwait Oil Fires Troop Exposure Assessment Model (TEAM)	
Applied Research		DoD-19	Persian Gulf Veterans Health Tracking System	
Applied Research	<i>Interactions</i>	DoD-34	Characterization of Emissions from Heaters Burning Leaded Diesel Fuel in Unvented Tents	
Basic Research	<i>Brain & Nervous System</i>	<i>Chemical Weapons</i>	DoD-22	Chronic Organophosphorus Exposure and Cognition
Basic Research	<i>Prevention</i>	HHS-3	Biomarkers of Susceptibility and Polycyclic Aromatic Hydrocarbon (PAH) Exposure in Urine and blood Cell DNA from U.S. Army Soldiers Exposed to Kuwaiti Oil Well Fires	
Basic Research	<i>Prevention</i>	VA-4E	The Aromatic Hydrocarbon Receptor (AhR) as a Biomarker of Susceptibility	
Clinical Research	<i>Brain & Nervous System</i>	<i>Interactions</i>	VA-48	Cross-Sensitization as a CNS Model for Gulf War Chemical Intolerance
Clinical Research	<i>Symptoms/ General Health</i>	VA-4D	Evaluation of Respiratory Dysfunction Among Gulf War Veterans	
Epidemiology Research		DoD-13	Effects of Persian Gulf War Service on Military Working Dogs	
Epidemiology Research		VA-4C	Gulf War And Vietnam Veterans Cancer Incidence Surveillance	

<i>Research type</i>	<i>Research Focus Area</i>		<i>Project No.</i>	<i>Project Title</i>
	<i>Secondary</i>	<i>Tertiary</i>		

Immunology

Clinical Research			DoD-48	Assessment of Genomic Instability via Chromosome 7 Inversion Frequency in a Gulf-War Syndrome Cohort vs Selected Control Groups
Clinical Research	<i>Symptoms/ General Health</i>		VA-17	Immunological Evaluation of Persian Gulf Veterans
Clinical Research	<i>Brain & Nervous System</i>		VA-5B	Physiological and Psychological Assessments of Persian Gulf Veterans
Clinical Research	<i>Symptoms/ General Health</i>		VA-6B	Clinical and Neuroendocrine Aspects of Fibromyalgia (Project II)
Clinical Research	<i>Symptoms/ General Health</i>	<i>Environmental Toxicology</i>	VA-5C	Effects of Exertion and Chemical Stress on Persian Gulf Veterans

Interactions

Basic Research	<i>Brain & Nervous System</i>	<i>Chemical Weapons</i>	DoD-54	Assessment of Subchronic Neurobehavioral and Neuropathologic Effects in Rats
Basic Research	<i>Chemical Weapons</i>	<i>Pyridostigmine Bromide</i>	DoD-55	Low-Level Exposure to GB Vapor in Air: Diagnosis/Dosimetry, Lowest Observable Effect Levels, Performance-Incapacitation, and Possible Delayed Effects
Basic Research	<i>Chemical Weapons</i>	<i>Pyridostigmine Bromide</i>	DoD-56	Low-Level Sarin Neurotoxicity and Its Modulation by Pyridostigmine
Basic Research	<i>Chemical Weapons</i>	<i>Pyridostigmine Bromide</i>	DoD-61	Neurophysiologic and Neuropathologic Effects in Monkeys of Low Level Exposures to Sarin, Pyridostigmine, Pesticides, and Botulinum Toxoid
Basic Research	<i>Chemical Weapons</i>	<i>Pyridostigmine Bromide</i>	DoD-62	Sarin and Pyridostigmine Interaction under Physical Stress: Neurotoxic Effects in Mice
Basic Research	<i>Pyridostigmine Bromide</i>	<i>Brain & Nervous System</i>	DoD-2	Physiological and Neurobehavioral Effects in Rodents from Exposure to Pyridostigmine, Fuels, and DEET
Basic Research	<i>Pyridostigmine Bromide</i>	<i>Brain & Nervous System</i>	DoD-37	Neurobehavioral and Immunological Toxicity of Pyridostigmine, Permethrin, and DEET in Male and Female Rats

Leishmaniasis

Applied Research	<i>Diagnosis</i>		DoD-8A	Serologic Diagnosis of Viscerotropic Leishmaniasis (VTL)
Applied Research	<i>Diagnosis</i>		DoD-8B	Development of a Leishmania Skin Test Antigen (LSTA)
Applied Research	<i>Diagnosis</i>		DoD-38	Diagnostic Antigens of Leishmania tropica
Basic Research	<i>Prevention</i>		VA-15	Vaccine-Mediated Immunity Against Leishmaniasis
Basic Research	<i>Prevention</i>		VA-16	Protective Immunity in Experimental Visceral Leishmaniasis

<i>Research type</i>	<i>Research Focus Area</i>		<i>Project No.</i>	<i>Project Title</i>
	<i>Secondary</i>	<i>Tertiary</i>		

Leishmaniasis (cont.)

Basic Research	<i>Treatment</i>		DoD-9	Identification of the Genetic Factors Which Control Tropism in Leishmania
Clinical Research	<i>Diagnosis</i>	<i>Treatment</i>	VA-6E	Clinical and Epidemiology Leishmania Research

Mortality

Epidemiology Research			DoD-15	Comparative Mortality Among US Military Personnel Worldwide During Operations Desert Shield and Desert Storm
Epidemiology Research			VA-1	Mortality Follow-up Study of Persian Gulf Veterans, First Update

Pyridostigmine Bromide

Basic Research	<i>Brain & Nervous System</i>		DoD-59	Pyridostigmine-induced Neurodegeneration: Role of neuronal Apoptosis
Basic Research			VA-49	Sensitivity to Pyridostigmine Bromide: Persistent Neural Dysfunction
Basic Research	<i>Environmental Toxicology</i>		VA-6C	Neurotoxicity of Environmental Pollutants and Warfare Agents (Project III)
Basic Research	<i>Interactions</i>		DoD-10	Pyridostigmine Synergistic Toxicity Study
Basic Research	<i>Interactions</i>		VA-5D	Effects of Genetics and Stress on Responses to Environmental Toxins
Basic Research	<i>Prevention</i>		DoD-33	Effects of Pyridostigmine in Flinders Line Rats Differing in Cholinergic Sensitivity
Clinical Research	<i>Brain & Nervous System</i>	<i>Prevention</i>	DoD-64	Individual Differences in Neurobehavioral Effects of Pyridostigmine
Clinical Research			DoD-11	Male/Female Differential Tolerances to Pyridostigmine Bromide
Epidemiology Research			DoD-17	Retrospective Studies Involving Military Use of Pyridostigmine as a Pretreatment for Nerve Agent Poisoning
Epidemiology Research	<i>Prevention</i>		DoD-21	Study of Variability In Pyridostigmine Inhibition of Blood Cholinesterases in Healthy Adults and Individuals With Symptoms Following Participation in Operation Desert Storm

Reproductive Health

Clinical Research	<i>Symptoms/ General Health</i>	<i>Immunology</i>	DoD-44	Investigation of Seminal Plasma Hypersensitivity Reactions
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<i>Research Focus Area</i>	<i>Secondary</i>	<i>Tertiary</i>	<i>Project No.</i>	<i>Project Title</i>
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Reproductive Health (cont.)

Epidemiology Research			DoD-1C	Epidemiologic Studies of Morbidity Among Gulf War Veterans: A Search for Etiologic Agents and Risk Factors; Study 3: A comparative study of pregnancy outcomes among Gulf War veterans and other active-duty personnel.
Epidemiology Research			DoD-1D	Epidemiologic Studies of Morbidity Among Gulf War Veterans: A Search for Etiologic Agents and Risk Factors; Study 4: Infertility and Miscarriage in Gulf War Veterans
Epidemiology Research			DoD-1G	Epidemiologic Studies of Morbidity Among Gulf War Veterans: A Search for Etiologic Agents and Risk Factors; Study 7: Prevalence of Congenital Anomalies Among Children of Persian Gulf War Veterans.
Epidemiology Research			DoD-35	Feasibility of Investigating Whether There is a Relationship Between Birth Defects and Service in the Gulf War.
Epidemiology Research			HHS-4	Suspected Increase of Birth Defects and health Problems Among Children Born to Persian Gulf War Veterans In Mississippi

Symptoms/General Health

Clinical Research	<i>Brain & Nervous System</i>		DoD-57	Physiologic Effects of Stress in Gulf War Veterans
Clinical Research	<i>Brain & Nervous System</i>		DoD-58	Illness Among Persian Gulf War Veterans: Case Validation Studies
Clinical Research			DoD-1A	Epidemiologic Studies of Morbidity Among Gulf War Veterans: A Search for Etiologic Agents and Risk Factors; Study 1: A Study of Symptoms Among 1500 Seabees
Clinical Research			VA-4	Boston Environmental Hazards Research Center Program
Clinical Research	<i>Brain & Nervous System</i>		VA-4Core	Boston Environmental Hazards Research Center Core Program
Clinical Research			VA-5	East Orange Environmental Hazards Research Center Program
Clinical Research	<i>Brain & Nervous System</i>		VA-5Core	East Orange Environmental Hazards Research Center Core Program
Clinical Research			VA-18	Chronic Gastrointestinal Illness in Persian Gulf Veterans
Clinical Research			VA-40	Musculoskeletal Symptoms in Gulf War Syndrome
Clinical Research	<i>Brain & Nervous System</i>	<i>Reproductive Health</i>	DoD-45	Physical and Emotional Health of Gulf War Veterans Women
Clinical Research	<i>Immunology</i>	<i>Brain & Nervous System</i>	DoD-42	The Symptomatic Persian Gulf Veterans Protocol: An Analysis of Risk Factors with an Immunologic and Neuropsychiatric Assessment

Research type	Research Focus Area		Project No.	Project Title
	Secondary	Tertiary		

Symptoms/General Health (cont.)

Epidemiology Research			DoD-1B	Epidemiologic Studies of Morbidity Among Gulf War Veterans: A Search for Etiologic Agents and Risk Factors; Study 2: A comparative study of hospitalizations among Active-duty personnel who participated in the Gulf War and similar personnel who did not.
Epidemiology Research	<i>Reproductive Health</i>		DoD-30	Epidemiological Studies Persian Gulf War Illnesses, PG Women's Health Linkage Study
Epidemiology Research			DoD-1E	Epidemiologic Studies of Morbidity Among Gulf War Veterans: A Search for Etiologic Agents and Risk Factors; Study 5: Seabee Health Study.
Epidemiology Research			DoD-1F	Epidemiologic Studies of Morbidity Among Gulf War Veterans: A Search for Etiologic Agents and Risk Factors; Study 6: A Comparison of Nonfederal Hospitalization Experience Among Veterans in California who have separated from active service: PGW vs. EV.
Epidemiology Research			DoD-4	The General Well-Being of Gulf War Era Service Personnel from the States of Pennsylvania and Hawaii: A Survey
Epidemiology Research			DoD-14	Risk Factors Among US Army Soldiers for Enrolling on the Department of Veterans Affairs Gulf War Registry
Epidemiology Research			DoD-20	A Statistical Study Correlating the Reported Cases of Gulf War Syndrome to Battlefield Locations of Afflicted US Army Personnel During the Iraq-Kuwait War; Part 1: Method to Related Troop Deployment and the Reported Cases of Gulf War Syndrome and Probable Incidence of Maladies Defined by ICD-9-CM
Epidemiology Research			DoD-39	A Controlled Epidemiological and Clinical Study into the Effect of Gulf War Service on Servicemen and Women of the United Kingdom Armed Forces
Epidemiology Research			DoD-46	Exploratory Data Analysis with the CCEP Database
Epidemiology Research			HHS-1	Health Assessment of Persian Gulf War Veterans from Iowa
Epidemiology Research			VA-2A	VA National Survey of Persian Gulf Veterans - Phase I
Epidemiology Research			VA-2B	VA National Survey of Persian Gulf Veterans -Phase II
Epidemiology Research			VA-3	Use of Roster of Veterans Who Served in Persian Gulf Area
Epidemiology Research			VA-5A	Health and Exposure Survey of Persian Gulf Veterans
Epidemiology Research			VA-6Core	Core Program: Portland Environmental Hazards Research Center: Environment, Veterans Health and the Gulf War Syndrome. Core Project for Clinical and Epidemiology Research

<i>Research Focus Area</i>				
<i>Research type</i>	<i>Secondary</i>	<i>Tertiary</i>	<i>Project No.</i>	<i>Project Title</i>

Symptoms/General Health (cont.)

Epidemiology Research			VA-46	Diarrhea in Persian Gulf Veterans: An Irritable Bowel-Like Disorder
Epidemiology Research			VA/DoD-2DB/VB	Patterns of Pre-Persian Gulf War Illness and Health Care Seeking
Epidemiology Research		<i>Diagnosis</i>	VA-2C	VA National Survey of Persian Gulf Veterans-Phase III
Epidemiology Research	<i>Diagnosis</i>		HHS-2	Disease Cluster in a Pennsylvania Air National Guard Unit, EPI-AID 95-18

Treatment

Clinical Research			DoD-65	Multi-disciplinary pathophysiologic studies of neurotoxic Gulf War related syndromes leading to diagnosis and treatment
Clinical Research	<i>Symptoms/General Health</i>		VA/DoD-1D/V	VA/DoD Multi-site treatment trial for Chronic Fatigue Syndrome and Fibromyalgia in Gulf War Veterans

Persian Gulf Veterans' Illnesses Research Project Abstracts

Persian Gulf Veterans' Illnesses Research Database - Project No

DoD-1

Title: Naval Health Study Program

Project #: DoD-1 Agency: DoD

Study Location: Naval Health Research Center

P.I.: CDR Greg Gray, MC.

This is the parent Program for DoD projects 1A through 1G.

Title: Epidemiologic Studies of Morbidity Among Gulf War Veterans: A Search for Etiologic Agents and Risk Factors; Study 1: A Study of Symptoms Among 1500 Seabees
Project #: DoD-1A Agency: DoD Study Location: Naval Health Research Center
Project Status: Ongoing Research Type: Clinical Research
P.I.: CDR Greg Gray, MC, Research Focus: Symptoms/General Health
Start Date (CY):1994 Est. Completion (CY):1997

OVERALL PROJECT OBJECTIVE: To identify risk factors associated with reported symptoms in an effort to determine causes for possible Gulf War-related morbidity.

SPECIFIC AIMS: Are there differences in postwar morbidity between Gulf War veterans (GWV) and nondeployed veterans (NDV)?

METHODOLOGY: Seabees who were on Active-duty and had been so since September 1990 were eligible to participate. Seabees (Navy construction workers) were interviewed at two major Seabee bases, in Port Hueneme, CA, and Gulfport, MS. After signing a consent form, volunteers completed a questionnaire, provided blood and urine specimens, and had their height, weight, and hand-grip strength measured. A systematically selected subsample also performed a spirometry test. Whole blood, sera, and urine specimens were stored at -70°C. Sera were studied for evidence of infection while whole blood specimens may be characterized for genetic markers that may explain symptoms. Also, sera collected during this study will be compared to prewar sera, which are known to be available for a majority of the study population. Urine specimens may be used to rule out chronic diseases, such as adrenal insufficiency. Hand grip strength and spirometry results were compared among symptomatic and nonsymptomatic Seabees. The questionnaire responses will be used to compare the morbidity of GWV and NDV. Internal comparisons will be made among GWV using logistic regression modeling to determine if any specific exposures are associated with any symptoms or symptom complexes.

EXPECTED PRODUCTS (MILESTONES): A number of comparisons of morbidity between GWV and NDV Seabees have been performed. A summary manuscript is under journal review. Three supplemental manuscripts are in internal review or nearly ready for internal review: the role of pyridostigmine bromide on handgrip strength, the role of Mycoplasma fermentans in causing morbidity, and a factor analysis of symptom data.

STATUS/RESULTS TO DATE: Seabees (n=1498) were surveyed and studied. GWV Seabees self-reported a higher prevalence of exposures and symptoms, as well as higher scores for abnormal psychological variables. Gulf War Veterans were more likely to screen for post-traumatic stress disorder, had lower handgrip strengths, and higher serum ferritin assays, however, after numerous comparisons of these outcomes with various exposures, no exposure or group of exposures stood out as likely etiologic.

PUBLICATIONS: Kaiser KS, Hawskworth AW, Gray GC, et al. A comparison of self-reported symptoms among active duty seabees: Gulf war veterans versus era controls. 123rd Annual Meeting and Exhibition of the American Public Health Association, Session 2198, 1995, San Diego.

Gray GC, Kang HK, Barrett-Connor EL, Hyams KC, DeFraités RF, Cowan DN, et al. and Persian Gulf War Veteran Research Team. Methodological issues in epidemiologic studies of morbidity among Gulf War veterans. 123rd Annual Meeting and Exhibition of the American Public Health Association, Session 2198, 1995, San Diego.

Hawskworth AW, Knoke JD, Kaiser KS, Dlugosz LD, Gray GC. Effect of service in Operations Desert Shield/Storm on pulmonary function among US Navy Seabees. 1996 San Diego Biostatistics and Epidemiology Research Exchange, La Jolla, CA.

Title: Epidemiologic Studies of Morbidity Among Gulf War Veterans: A Search for Etiologic Agents and Risk Factors; Study 2: A comparative study of hospitalizations among Active-duty personnel who participated in the Gulf War and similiar personnel who did not.
Project #: DoD-1B Agency: DoD Study Location: Naval Health Research Center
Project Status: Ongoing Research Type: Epidemiology Research
P.I.: CDR Greg Gray, MC, Research Focus: Symptoms/General Health
Start Date (CY):1994 Est. Completion (CY):1998

OVERALL PROJECT OBJECTIVE: Screen Department of Defense (DoD) hospitalizations for association with Gulf War deployment; identify specific diseases or disease groups that merit further study.

SPECIFIC AIMS: Are hospitalization rates the same for Gulf War veterans (GWV) and nondeployed veterans (NDV)?

EXPECTED PRODUCTS (MILESTONES): These data have been proven very useful beyond their original intent in examining new hypothesis regarding specific Gulf War exposure and specific hospitalization outcomes. One study has been published in a leading journal, two more are in press and numerous abstracts have been presented (see below). Several more studies are under external review for publication:

STATUS/RESULTS TO DATE: In general, Gulf War veterans postwar hospitalization data are very similar to that of their nondeployed peers. Thus far, there has been little evidence on unexplained increased postwar hospitalization morbidity associated with Gulf War service.

PUBLICATIONS: Gray GC, Knoke JD Berg SW, Wignall FS, Barrett-Connor E. Response to suppositions and misunderstandings. *Amer J Epidemiol*, 1998;278 (in press).

Knoke JD, Gray GC. Hospitalizations for unexplained illnesses among US Persian Gulf War veterans. *Emerging Infectious Diseases*, 1998;4 (in press).

Smith TC, Hawksworth AW, Knoke JD, Gray GC. Does possible exposure to the destruction of Iraqi chemical munitions increase the likelihood of post war hospitalizations? 1997 Thirty-Eighth Navy Occupational Health and Preventive Medicine Workshop, Virginia Beach, Virginia.

Gray GC, Coate BD, Anderson CM, Kang HK, Berg SW, Wignall FS, Knoke JD, Barrett-Connor E. The postwar hospitalization experience of US Persian Gulf war veterans. *N Engl J Med* 1996; 335:1505-13.

Title: Epidemiologic Studies of Morbidity Among Gulf War Veterans: A Search for Etiologic Agents and Risk Factors; Study 3: A comparative study of pregnancy outcomes among Gulf war veterans and other active-duty personnel.

Project #: DoD-1C Agency: DoD Study Location: Naval Health Research Center

Project Status: Completed Research Type: Epidemiology Research

P.I.: CDR Greg Gray, MC, Research Focus: Reproductive Health

Start Date (CY):1994 Est. Completion (CY):1997

OVERALL PROJECT OBJECTIVE: To evaluate the risk of birth defects and other adverse pregnancy outcomes diagnosed in military medical treatment facilities (MTFs). Risk and relative risk of inpatient events will be assessed based on existing data.

SPECIFIC AIMS: Within the design parameters, are Persian Gulf War veterans (GWV) at higher risk of adverse pregnancy outcomes than nondeployed veterans (NDV)?

METHODOLOGY: This was an historical cohort study of reproductive outcomes among military personnel deployed to the Gulf War compared to military personnel who were not deployed.

Demographic and hospitalization data were available for approximately 500,000 GWV and 700,000 NDV from the Department of Defense Manpower Data Center. Substantial demographic information was available on all subjects, including race/ethnicity, proxy measures of socio-economic status (rank and education), and military occupation. Data on inpatient services received from MTFs were also available and diagnoses were recorded using ICD-9-CM codes. Risk and relative risks of major birth defects were evaluated. Potentially confounding factors such as maternal age were evaluated. Multivariate analysis was conducted to estimate relative risks, controlling for identified cofounders. Events among female service members were considered separately from those among spouses of male service members. In another study we measured the prevalence of Goldenhar syndrome among infants born in military hospitals to GWV and NDV. This project is now completed.

EXPECTED PRODUCTS (MILESTONES): Several manuscripts have been published, one is in press. No new studies are in progress. However, these data may have future use in testing additional hypotheses regarding specific subgroups of Gulf War Veterans and specific birth defect diagnoses.

STATUS/RESULTS TO DATE: Publications.

PUBLICATIONS: Cowan DN, Gray GC, DeFraités RF. Commentary: Responding to Dr. Haley's comments regarding birth defects among children of Gulf War veterans. *Amer J Epidemiol*, 1998;278 (in press).

Araneta MRG, Moore CA, Olney RS, Edmonds LD, Karcher JA, McDonough C, Hiliopoulos KM, Schlangen KM, Gray GC. Goldenhar Syndrome among infants born in military hospitals to Gulf War veterans. *Teratology*, 1997, 56:244-251.

Cowan DN, DeFraités RF, Gray GC, Goldenbaum M, Wishik S. The risk of birth defects among children of Persian Gulf War Veterans. *N Engl J Med*, 1997, 336:1650-56.

Title: Epidemiologic Studies of Morbidity Among Gulf War Veterans: A Search for Etiologic Agents and Risk Factors; Study 4: Infertility and Miscarriage in Gulf War Veterans
Project #: DoD-1D Agency: DoD Study Location: Naval Health Research Center
Project Status: Ongoing Research Type: Epidemiology Research
P.I.: CDR Greg Gray, MC, Research Focus: Reproductive Health
Start Date (CY):1994 Est. Completion (CY):1998

OVERALL PROJECT OBJECTIVE: To examine if there are differences between Gulf War veterans (GWVs) and nondeployed veterans (NDVs) in rates of adverse reproductive outcomes.

SPECIFIC AIMS: Do GWVs have higher rates of adverse reproductive outcomes such as miscarriages, early pregnancy loss, prematurity and low birth weight when compared to NDVs?

METHODOLOGY: A structured, self-completed, questionnaire was mailed to 17,166 service members (active duty, reserves, or National Guard) who were, on February 1, 1991, aged between 18-33 and either: married at that time; or currently married. Detailed demographic, military and deployment status, and reproductive outcome data through the end of 1995 were collected for the subject and their marital partner.

EXPECTED PRODUCTS (MILESTONES): Mailing of questionnaires completed (Fall, 1997); data cleanup and telephone callback interviews are in process (target completion: Spring, 1998). In another study, we are examining the characterizations of adverse reproductive and perinatal effects among conceptions, which occurred just prior to or during deployment to the Persian Gulf War theater.

STATUS/RESULTS TO DATE: Initial mailing to the randomly selected GWVs and NDVs was completed the summer of 1996; two follow-up mailings to increase the participation rate were completed in winter 1996 and summer 1997, respectively. Despite 3 attempts, use of locator services, and certified mail or military channels for non-active duty and active duty subjects respectively, questionnaires were undeliverable to 2,503 of the initial 17,166 mailed questionnaires. At the completion of the mailing phase, a total of 9,691 questionnaires had been returned, giving an overall participation rate of 66.1%. With respect to the telephone callback interviews to date, 750 (42.8%) are in process, 456 (26.1%) have been completed or were noncontactable/did not require contact, and 545 (31.1%) are pending/with locator services.

PUBLICATIONS: Poblete P, Morn CB, Araneta MR, Zau AC, Hiliopoulos KM, Kamens DR, Sato PA, Gray GC. Self-Administered Survey vs. Telephone Interview: A Reliability Study of Reproductive Outcomes. 1997 San Diego Biostatistics and Epidemiology Research Exchange, La Jolla, California.

Hiliopoulos KM, Calderon RL, Gray GC. The Persian Gulf war - reproductive outcomes study. 1996 San Diego Biostatistics and Epidemiology Research Exchange La Jolla, California.

Major JM, Anderson CM, Hiliopoulos KM, Gray GC. Finding people who have left the military. 1997 Thirty-Eighth Navy Occupational Health and Preventive Medicine Workshop, Virginia Beach, Virginia.

Title: Epidemiologic Studies of Morbidity Among Gulf War Veterans: A Search for Etiologic Agents and Risk Factors; Study 5: Seabee Health Study.

Project #: DoD-1E Agency: DoD Study Location: Naval Health Research Center

Project Status: Ongoing Research Type: Epidemiology Research

P.I.: CDR Greg Gray, MC, Research Focus: Symptoms/General Health

Start Date (CY):1996 Est. Completion (CY): 2013

OVERALL PROJECT OBJECTIVE: To study the health of Navy Seabees in relation to their deployments and the possible latent effects of Gulf War service.

SPECIFIC AIMS: 1. To determine if Gulf War Veterans have greater frequency of symptoms, illness, and propensity for making cognitive errors than other veterans; 2. to assemble a cohort for research on long-term effects of the Gulf War on morbidity.

METHODOLOGY: The proposed study is a nonconcurrent historical prospective study of all Seabees who were active duty for at least one month between August 2, 1990 and June 30, 1991. This group of approximately 17,500 current active duty, reservists, and former military personnel has been identified from records of the Defense Manpower Data Center. The study subjects will be mailed a survey to assess their current health status and their role (if any) in the Persian Gulf War. It is proposed that participants in the survey will be followed prospectively for 15 years with a follow-up survey every 5 years (in 2002, 2007, and 2012) to detect possible latent effects of the Gulf War on health. Potential sequelae include heart disease, diabetes, hypertension, & arthritis. To evaluate reliability of information, 400 of the survey participants will be interviewed by telephone and information on health and symptomatology from routine physicals will be collected from military medical records. Bivariate and multivariate analyses will be used to identify risk factors associated with the outcomes of interest and multivariate logistic regression modeling will be applied to evaluate relationships between risk factors and the health outcomes. Development of latent chronic disease will be monitored by calculating the cumulative prevalence rates after each 5-year survey follow-up and comparing the baseline prevalence.

EXPECTED PRODUCTS (MILESTONES): Expect to complete data collection by July 1998.

Baseline survey data showing initial symptom prevalences should be available by August 1998.

Subsequent analyses of symptom and morbidity data from written and telephone surveys are planned by March 2003, March 2008, and March 2013. A comprehensive report showing comparisons between Gulf War veterans and other veterans including a summary of risk factor associations with morbidity is projected for December 2013.

STATUS/RESULTS TO DATE: Pilot test of the survey instrument has been completed. The first mailing resulted in approximately 33 percent participation among those who received the survey. Presently, we are in the second mailing of the questionnaire.

PUBLICATIONS: Dlugosz LJ, Gray GC, Knoke J, Kang HK, Larson G. A prospective assessment of symptoms and illness in Gulf War veterans: the Seabee Health Study. 1996 San Diego Statistics and Epidemiology Research Exchange, La Jolle, California.

Title: Epidemiologic Studies of Morbidity Among Gulf War Veterans: A Search for Etiologic Agents and Risk Factors; Study 6: A Comparison of Nonfederal Hospitalization Experience Among Veterans in California who have separated from active service: PGW vs. EV.

Project #: DoD-1F Agency: DoD Study Location: Naval Health Research Center
Project Status: Ongoing Research Type: Epidemiology Research
P.I.: CDR Gregory Gray, MC, Research Focus: Symptoms/General Health
Start Date (CY):1995 Est. Completion (CY):1998

OVERALL PROJECT OBJECTIVE: To determine if the federal and non-federal hospitalizations rates differed among Gulf War Veterans (GWV) and nondeployed veterans (NDV) during the study period of 1 August 1991 to 31 December 1994.

SPECIFIC AIMS: The specific research questions this project will address include: Did the causes for hospitalization among GWV and NDV differ in (1) military and (2) nonfederal facilities? Did the proportional distribution of causes for hospitalization differ during the postwar follow-up period?

METHODOLOGY: The study population will consist of GWV and NDV who resided in California after the Gulf War. Military hospitalization and demographic data have been obtained from the Department of Defense Manpower Data Center (DMDC). Each hospitalization contains a principal diagnosis that is classified into 1 of the 14 major classifications of disease and injuries as defined by the ICD-9-CM. Information on nonfederal hospitalization has been obtained for California residents from two sources: (1) The Office of Statewide Planning and Development (OSHPD), which maintains the Patient Discharge Data Program, a database of hospital discharge information from all non-federally licensed hospitals in California and (2) the Department of Veterans Affairs Patient Treatment File (VAPTF) of all VA hospitalizations. Two hospitalization data links have been performed. The DMDC demographic and identification data will be linked with OSHPD data and the VAPTF to obtain nonfederal hospitalization data for GWV and NDV. The proportional distribution of causes for hospitalization for GWV and NDV during the postwar follow-up period will be compared.

EXPECTED PRODUCTS (MILESTONES): Expect study completion by September 1998.

STATUS/RESULTS TO DATE: A computerized linking program was designed by the California OSHPD to enable linkage of their database of nonfederal hospital discharge information to a NHRC database consisting of identifying demographic data for GWV and NDV. Negotiations for the terms of these collaboration were completed and data provided to OSHPD in Spring 1997. Data were linked and returned to NHRC in late November 1997. The data provided to the VA in Spring 1997 was linked and returned to NHRC in July 1997. The analysis of the data is in progress. A manuscript is under development.

PUBLICATIONS: none to date

Title: Epidemiologic Studies of Morbidity Among Gulf War Veterans: A Search for Etiologic Agents and Risk Factors; Study 7: Prevalence of Congenital Anomalies Among Children of Persian Gulf War Veterans.

Project #: DoD-1G Agency: DoD Study Location: Naval Health Research Center

Project Status: Ongoing Research Type: Epidemiology Research

P.I.: CDR Gregory Gray, MC, Research Focus: Reproductive Health

Start Date (CY):1995 Est. Completion (CY):1998

OVERALL PROJECT OBJECTIVE: To determine if the prevalence of congenital anomalies in the infants born to Gulf War Veterans (GWV) exceed those of infants of (1) nondeployed veterans (NDV) and (2) the nonmilitary population.

SPECIFIC AIMS: The specific research questions this project will address include: (1) Did the prevalence of congenital anomalies differ among infants of GWV, NDV, and the nonmilitary population prior to and after the Persian Gulf War? (2) Did the types of congenital anomalies differ among infants of GWV, NDV, and the nonmilitary population prior to and following the Persian Gulf War? (3) What were the pathologic patterns of congenital anomalies among infants born to GWV? How did these characteristics differ by military subpopulations (e.g., service, occupation, geographic location)?

METHODOLOGY: The Department of Defense Manpower Data Center (DMDC) maintains a database of demographic, military and hospitalization data among military personnel. The state health departments of Arizona, Arkansas, California, Georgia, Hawaii, Iowa, and Oklahoma conduct active surveillance of congenital anomalies through the initial 12 months of life. Live births and fetal deaths occurring after 20 weeks of gestation are recorded in Birth and Death Certificate Records of the Vital Statistics Registries of these states.

Two registry matches will be performed. The DMDC database will be matched with Vital Statistics Registry of each of these seven states to identify live births and fetal deaths occurring among conceptions of GWV and NDV between 1989 and 1993. Names and other personal identifiers common to the DMDC and Vital Statistics Registries will be matched with the Birth Defects Registry of each state. Overall and diagnostic-specific rates of congenital anomalies will be calculated and compared among infants of GWV and NDV and the nonmilitary population of each state. Univariate analysis will be applied to compare rates between subpopulations. Multivariate techniques will be applied to identify characteristics associated with congenital malformations. Cluster analysis will be performed to detect interrelations among selected anomalies and subpopulations.

EXPECTED PRODUCTS (MILESTONES): The overall as well as diagnostic-specific prevalence rates of congenital anomalies among infants of GWV and NDV will be determined. Pathologic patterns of congenital malformations among infants of GWV will be described, and may serve useful in developing hypotheses about genetically or environmentally-induced birth defects for future investigations.

STATUS/RESULTS TO DATE: Data provided by the Hawaii Birth Defects Monitoring Program is being analyzed to compare overall and diagnostic-specific rates of birth defects. Birth defect data among infants of GWV and NDV in Arizona are complete for 1989-1991; the Arizona Birth Defect Monitoring Program is still collecting data for 1992-1993 births. Identification of births to military personnel in Metropolitan Atlanta is complete; linkage to the Metropolitan Atlanta Congenital Defects Program is in progress to identify military infants with birth defects. Identification of births to military personnel in California and Arkansas are in progress. Collaborative agreements are being negotiated with Iowa. Seeking participation from Oklahoma.

PUBLICATIONS: Araneta H, Anderson C, Karcher JA, Schlangen KM, Gray GC. The use of deterministic matching to identify births to military personnel using Department of Defense data sets and the Hawaii Birth Certificate Registry. 1996 San Diego Biostatistics and Epidemiology Research Exchange, La Jolle, California.

Title: Physiological and Neurobehavioral Effects in Rodents from Exposure to Pyridostigmine, Fuels, and DEET

Project #: DoD-2

Agency: DoD

Study Location: Tri-Svc Tox Progr, NMRI-TD

Project Status: Completed

Research Type: Basic Research

P.I.: CDR J Rossi, Ph.D.

Research Focus: Interactions

Start Date (CY):1994 Est. Completion (CY):1997

**Pyridostigmine Bromide
Brain & Nervous System**

OVERALL PROJECT OBJECTIVE: This project has evaluated the potential of a simulated Gulf War (GW) exposure consisting of multiple chemicals, alone and in conjunction with an imposed psychological stressor, to induce biological effects in Sprague-Dawley rats. Effects were for similarity to symptoms and effects reported by exposed Gulf War veterans (e.g., "Gulf War Veterans' Illnesses" (GWVI)). No animal model for GWI currently exists; therefore, the project investigated if the rodent model can reproduce the symptoms reported in GWI.

SPECIFIC AIMS: This study tested the hypothesis that simultaneous exposure to a combination of chemical stressors, each well tolerated individually, causes subtle physiological changes when coupled with psychological stress. The study used Sprague-Dawley rats as an animal model to identify effects reported by humans similarly exposed during the GW. The study also attempted to identify biomarkers for use in evaluating the extent of exposure and of effect in humans.

METHODOLOGY: The chemical stressors and routes of exposure evaluated were: inhalation of fuel vapor (diesel, jet fuel) and their combustion products, dermal absorption of the insect repellent, N, N-diethyl-m-toluamide (DEET), and oral (or i.p.) administration of pyridostigmine bromide (PB).

Psychological stress was simulated by intermittent administration of a mild electrical shock. The shock was administered randomly by a computer controlled apparatus, so that it would be uncontrollable and unpredictable, functioning as a traumatic stressor. Male Sprague-Dawley rats were exposed by inhalation to diesel and/or jet fuel vapor, orally to PB, and dermally to DEET, alone, and in various combinations. Half of the animals received a periodic, random, mild electrical shock as psychological and neurobehavioral changes by standard tests, immediately after exposure and after a 60-day latent period. The study investigated physiological and neurobehavioral effects, neurotransmitter levels, and changes in protein composition in serum/plasma, liver, kidney, testes, and brain from exposed rats.

EXPECTED PRODUCTS (MILESTONES): This study evaluated the potential of a simulated Gulf War (GW) exposure consisting of multiple chemical exposures, alone and exacerbated by an imposed psychological stressor, to induce toxic effects in Sprague-Dawley rats which may have some similarities to the symptoms reported by Gulf War veterans. This study investigated, in a rodent model, factors that may have contributed to the development of symptoms reported in GWI.

Representative chemical exposures from the GW theater, with and without an imposed psychological stressor, were investigated individually and in combinations to determine possible interactive effects.

STATUS/RESULTS TO DATE: Almost all of the significant differences in this study involved animals exposed to JP4 fuel vapor only, or in combinations of JP4 or footshock and pyridostigmine bromide and DEET. Differences between groups in the 14 day neurobehavioral study were found only for startle (JP4, stress, stress & JP4) and for appetite reinforcer approach (JP4). In the 60 day study, differences were observed in a variety of measures on the Navy Neurobehavioral Toxicity Assessment Battery (NTAB) for combinations of stressors which included JP4 or footshock. The results suggest the JP4 fuel vapor and/or JP4 in combination with footshock stress affect a variety of neurobehavioral tests. A manuscript is being prepared for peer-reviewed publication of these results.

PUBLICATIONS: Nelson RA. Determining Types of Health Effects to Persian Gulf Veterans Due to Exposure to Occupational Hazards, Thesis No. AFIT/GEE/ENV/95D-13, Air University, USAF Institute of Technology, Wright-Patterson Air Force Base, OH. 1995.

Rossi J, MacMahon K, Ritchie G, Wolfe R, Leahy H, Nordholm A, Caldwell D. Neurobehavioral changes in rats exposed to a simulated Persian Gulf War environment. Society of Toxicology meeting, Cincinnati, Ohio, March 1997 (abstract).

MacMahon K, Rossi J, Wolfe R, Leahy H, Narayanan L, Witzmann F, Eggers J, Ritchie G, Nordholm A. Physiological responses of Sprague-Dawley rats exposed to low doses of pyridostigmine bromide, N,N-diethyl-m-toluamide (DEET), JP-4 jet fuel, and stress. Society of Toxicology meeting, Cincinnati, Ohio, March 1997 (abstract).

Title: The General Well-Being of Gulf War Era Service Personnel from the States of Pennsylvania and Hawaii: A Survey
Project #: DoD-4 Agency: DoD Study Location: WRAIR, Wash. DC
Project Status: Completed Research Type: Epidemiology Research
P.I.: Marlowe Research Focus: Symptoms/General Health
Start Date (CY):1992 Est. Completion (CY):1994

OVERALL PROJECT OBJECTIVE: Assess the general sense of well-being of Gulf War era veterans in the States of Hawaii and Pennsylvania.

SPECIFIC AIMS: Identify groups within the population reporting physical or mental distress and highlight probable causes or contributing factors. Ascertain whether any group within this population was at risk for future development of PTSD related to their Gulf War era experiences. Evaluate the status of the 14th Quartermaster Detachment. Assess the impact of the casualties of the 14th Quartermaster Detachment on the community of Greensburg, Pennsylvania.

METHODOLOGY: Citizens of Pennsylvania and Hawaii who served during the period of Operation Desert Shield/Storm were identified and asked to participate in a survey involving questionnaires and interviews (approx. 14,100). Study materials were designed and administered; 4,334 responses were obtained. Data was integrated with responses from an additional 25,000 individuals being studied as part of a larger assessment. Survey instruments and methods included a questionnaire, Impact of Events Scale, Brief Symptom Inventory, PTSD Risk Algorithm, and records/reports from Pacific Center for PTSD and from PTSD Clinical Team, VA Medical Center, Pittsburgh, PA. The distribution of psychological and illness symptoms in the samples were analyzed, and differences in psychological and physical symptoms between deployers and nondeployers were examined.

EXPECTED PRODUCTS (MILESTONES): An assessment of the general physical and mental well-being of the subject population. Information to assist Congressional determination of the need for appropriated funds for specialized counseling and support services to counter any adverse effects of service during Operation Desert Shield/Storm. Information addressing potential links between service in the Gulf and subsequent health and adjustment difficulties upon return.

STATUS/RESULTS TO DATE: The studied population was generally well-adjusted, with subgroups experiencing either physical symptoms, high levels of stress, or both (about 15% of the population). Among those deployed and who reported physical symptoms, neither exposure to combat nor its aftermath bear much relationship to their distress. Only the fact of deployment explains the higher rate of physical symptoms compared those who were not deployed. About 15% of those deployed were experiencing both stress and physical symptoms at the time of the study. Many of these seem at risk for development of further difficulty, particularly PTSD.

PUBLICATIONS: Stretch RH, Bliese PD, Marlowe DH, et al. Physical health symptomatology of Gulf War-era service personnel from the states of Pennsylvania and Hawaii. *Military Medicine*, 1995, 160:131-136.

Stretch RH, Bliese PD, Marlowe DH, et al. Psychological health of Gulf War-era military personnel. *Military Medicine*, 1996, 161:257-261.

Stretch RH, Marlowe DH, Wright KM, et al. Post-traumatic stress disorder symptoms among Gulf War veterans. *Military Medicine*, 1996, 161:407-410.

Title: Program DoD-6

Project #: DoD-6

Agency: DoD

Study Location: WRAIR, Wash. DC

P.I.: Meyerhoff

This is the parent Program for DoD projects DoD-6A through DoD-6B.

Title: Combat Stress Pharmacotherapy

Project #: DoD-6A

Agency: DoD

Study Location: WRAIR, Wash. DC

Project Status: Ongoing

Research Type: Basic Research

P.I.: Meyerhoff

Research Focus: Brain & Nervous System

Start Date (CY): 1988 Est. Completion (CY): 1999

Treatment

OVERALL PROJECT OBJECTIVE: Combat stress reaction (CSR) and posttraumatic stress disorder (PTSD) are thought to be part of a common spectrum of disorders. It is essential to develop animal models for combat stress reaction, to identify neurochemical mechanisms of, and pharmaceutical countermeasures for combat stress effects. Emphasis is on rapidly acting countermeasures which could be administered far forward, and which would allow the stress casualty to remain on duty and prevent the development of PTSD. Pharmaceutical countermeasures must be free of unwanted side effects, such as sedation or impairment of memory or performance.

SPECIFIC AIMS: Evaluate clinical and pre-clinical pharmaceuticals to prevent/reverse conditioned defeat (CD). Use acoustic startle, the swim test, neuroendocrine markers and telemetric monitoring of heart rate in behaving, unrestrained animals exposed to threat, develop technology for predicting vulnerability to conditioned defeat; thus enhancing evaluation of possible preventive measures.

EXPECTED PRODUCTS (MILESTONES): FY 97: Examine the effect of valium on CD in another species (mice) to test the generality of the finding that valium may exacerbate CD (and might be contraindicate in combat stress reaction). Extend testing to include benzodiazepine antagonists and inverse agonists in mice. In addition, begin testing Buspirone, a clinically available, non-sedating, non-benzodiazepine anxiolytic drug in mouse CD model. Will also test its more potent pre-clinical analogs - Gepirone, ipsapirone, and tiaspirone. The startle response has been reported to be exaggerated in both CSR and PTSD. Accordingly, we will test the effect of CD on acoustic startle in the mouse. To identify potential aids to diagnosis of risk for CSR, we will further characterize hormonal responses to CD. FY 98: Begin testing other behaviorally active clinically-available drugs in mouse CD model: (a) beta blockers (atenolol); (b) antidepressants used in panic disorders (Prozac, tricyclics); (c) stimulants such as caffeine and amphetamine. Evaluate all listed compounds for capability to reverse CD-induced deficits in the swim test. FY 99: Begin testing novel pre-clinical, non-benzodiazepine anxiolytics in the mouse CD model, including cholecystokinin antagonists; corticotrophin releasing hormone antagonists; and Neuropeptide Y (NPY) agonists. Evaluate potential therapeutic effects of thyrotropin releasing hormone (TRH) - an endogenous peptide stimulant successfully employed to reverse learned helplessness. Target drug intervention at specific brain sites, study viable brain slices from defeated mice, including amygdala and hippocampus, to evaluate agents for selective effects on specific circuits involved in CD. Study the effects of CD on cognitive function and neurodegeneration.

STATUS/RESULTS TO DATE: By establishing a streamline model of conditioned defeat (CD), we have met the requirement of developing an animal model for testing diagnostics for component processes of combat stress reaction (CSR) and screening drugs for safe, rapid treatment. We have shown that CD markedly decreases activity in the swim test - a measure used in the pharmaceutical industry to screen antidepressants. In an effort to develop reliable predictors and clinical indicators for CSR, we have characterized the dramatic responses to CD of hormones stimulated via the anxiogenic peptide, corticotrophin releasing hormone (CRH). We have shown that acoustic startle response (ASR) in hamsters was exacerbated by administration of CRH. This is significant because exaggerated acoustic startle response has been validated as a biological marker for PTSD and reported as a symptom of CSR as well. We found that valium is clearly counterproductive as a treatment for hamsters, as it markedly exacerbated CD. Thus, the CD model affords us the capability of identifying drugs which might be contraindicated, as well as those which might be beneficial. By way of confirmation, an Israeli colleague is reporting that benzodiazepines fail to block the development of PTSD following trauma. We have optimized the CD model as a test bed for screening drugs by modifying it to deliver rapid, high-volume, low-cost throughout.

PUBLICATIONS: Hebert M, Potegal M, Moore T, Evenson AR, Meyerhoff J. Diazepam exacerbates conditioned defeat in hamsters (*Mesocricetus auratus*). *Pharmacology, Biochemistry and Behavior*, 1996 (in press).

Potegal M, Ferris C, Hebert M, Skaredoff L, Meyerhoff J. Attack priming in female Syrian golden hamsters is mediated by a c-fos coupled process within the corticomedial amygdala. *Neuroscience*, 1996 (in press).

Potegal M, Hebert M, deCoster M, Meyerhoff J. Brief, high frequency stimulation of the corticomedial amygdala induces a delayed and prolonged increase of aggressiveness in male Syrian golden hamsters. *Behavioral Neuroscience*, 1996, 110(2):1-12.

Title: Combat Stress Diagnosis, PTSD Prevention

Project #: DoD-6B Agency: DoD Study Location: WRAIR, Wash. DC

Project Status: Ongoing Research Type: Clinical Research

P.I.: Meyerhoff Research Focus: Brain & Nervous System

Start Date (CY): 1987 Est. Completion (CY): 1998
**Diagnosis
Prevention**

OVERALL PROJECT OBJECTIVE: Develop diagnostic instruments for far-forward, early diagnosis of soldiers at risk for combat stress reaction (CSR) and posttraumatic stress disorder (PTSD) to allow early intervention to prevent chronic PTSD.

SPECIFIC AIMS: Use combined neuroendocrine and psychophysiologic techniques to develop rapid, reliable and inexpensive means of determining soldiers' level of stress, fitness for duty and risk of CSR/PTSD.

METHODOLOGY: We will measure salivary levels of cortisol, testosterone, and dehydroxyepiandrosterone sulfate (DHEAS) as stress indices. We will critically evaluate the reliability of voice frequency modulation as a stress index. In collaboration with the New Haven VA Medical Center we will conduct a prospective study on risk of stress reactions/PTSD in Special Operations Forces.

EXPECTED PRODUCTS (MILESTONES): FY 96: Correlate salivary cortisol and Soldier of the Month Board performance; measure effect of stress on salivary testosterone and DHEAS. FY 97: Determine effect of psychological stress on voice frequency modulation spectrum and correlate with performance as well as physiological and hormonal changes. FY 98: Complete prospective study of changes in acoustic startle and dexamethasone suppression tests (using salivary cortisol measurements) in soldiers subjected to stressful deployments.

STATUS/RESULTS TO DATE: Ongoing. Because the "Soldier of the Month (SOM) Board" is a uniquely robust physiological stressor, we have been using it as a test bed to develop and validate field-ready stress measures, such as a battery of non-invasive (salivary) hormonal measures. We will use these measures in stress studies in soldiers at risk for combat stress reaction and/or PTSD during military operations in the field. We have characterized the psychoendocrine and autonomic profile of operational stress, using the SOM Board as a model. Elevated plasma and salivary cortisol levels were measured in soldiers after an SOM Board experience. We have identified several plasma hormones which correlate positively with anxiety, and have demonstrated that anticipatory anxiety predicts poor performance.

PUBLICATIONS: Pitman RK, Orr SP, van der Kolk BA, et al. Analgesia: a new dependent variable for the biological study of PTSD, In Post Traumatic Stress Disorder: Etiology Phenomenology and Treatment (ed by Wolf ME). American Psychiatric Press, 1990:141-147.

Title: Health Risk Assessment of Embedded Depleted Uranium: Behavior, Physiology, Histology, and Biokinetic Modeling

Project #: DoD-7A

Agency: DoD

Study Location: AFRRRI, Bethesda, MD

Project Status: Ongoing

Research Type: Basic Research

P.I.: Terry Pellmar, Ph.D.

Research Focus: Depleted Uranium

Start Date (CY):1994 Est. Completion (CY):1998

Environmental Toxicology

OVERALL PROJECT OBJECTIVE: Evaluate health risks associated with tissue-embedded depleted uranium (DU) fragments by studying the behavioral, physiological, and histological consequences of implanted DU in a rodent model.

SPECIFIC AIMS: Evaluations in the DU-implanted rodent model included: behavioral tests of motor activity and memory, conduction studies of peripheral nerve function, measurement of central nervous system excitability in brain slice preparations, biochemical assessments of renal function, histological assessment of local tissue damage and capsule formation, measurement of tissue concentrations of uranium, and development of a biokinetic model describing the distribution of uranium over time.

METHODOLOGY: Appropriate doses to be used for analysis of DU effects were defined and then a study of 325 rats was conducted to obtain toxicity data. Rats were randomly assigned to 5 treatment groups: 1) low-dose DU, 2) medium-dose DU, 3) high-dose DU, 4) tantalum (Ta) controls, and 5) non-implanted sham-surgical controls. In the low-dose and medium-dose groups, Ta was substituted for a fraction of the DU pellets to keep the total number of implanted fragments constant. Rats were evaluated, euthanized and tissue samples taken at 1, 6, 12, or 18-months following fragment implantation. The investigators determined that sample sizes of 15 rats per group were necessary to achieve statistical significance, and additional animals (20 rather than 15) were implanted for the 18-month time point with the expectation of a 20-25% natural mortality. Each rat was thoroughly evaluated for changes in behavior (functional battery, motor activity, passive avoidance test), peripheral nerve function (conduction velocity measurements), CNS excitability (hippocampal brain slice electrophysiology), renal function (plasma and urine biochemistries) and tissue histology including capsule formation. In addition, data on tissue uranium levels (measured by kinetic phosphorescence analysis) from a subgroup of rats was used to develop a biokinetic model to predict uranium distribution. All measured parameters were compared among groups through analysis of variance and significance accepted at the $P < 0.05$ level.

EXPECTED PRODUCTS (MILESTONES): Determine appropriate doses for a DU toxicity study in the rat: July 1995. Complete toxicity assessment at the one-month point: Dec 1995. Complete toxicity assessment at the six-month point: Dec 1996. Complete toxicity assessment at the 12-month point: June 1997. Complete toxicity assessment at the 18-month point: Dec 1997. Complete biokinetic modeling for distribution of DU fragments: Jan 1998.

STATUS/RESULTS TO DATE: As of the 12-month time point, examination of the pellets in situ revealed fibrous tissue adhering to the DU but not the Ta pellets. Uranium levels were high and dose-dependent in kidney, urine, and bone. Despite high uranium levels in kidney, no renal toxicity was evident. After 23 weeks, body weight in high-DU dose animals was significantly lower than controls. Unexpectedly, uranium was found in the brain of DU-implanted animals. No behavioral neurotoxicity was evident. However, excitability of hippocampal neurons was altered in the high-DU dose animals at 6 months and in all of the DU groups at 12 months. These data suggest that kidney toxicity may be less of a hazard than was anticipated but that cognitive deficits need to be further considered. Data for the 18-month time point are currently being collected.

PUBLICATIONS: Hogan J, Benson KA, Landauer M, Pellmar TC. Toxicity of embedded Depleted Uranium (DU) in the rat. *The Toxicologist*, 1997, 36:17.

Pellmar TC, Hogan J, Benson KA, Landauer M. Health Risk Assessment of Embedded Depleted Uranium (DU) 6-Month Evaluation Point, AFRRRI Special Publication Report, 1997, 97-4.

Title: Carcinogenicity of Depleted Uranium Fragments

Project #: DoD-7B Agency: DoD Study Location: Inhal Tox Lab, Albuquerque NM

Project Status: Ongoing Research Type: Basic Research

P.I.: Fletcher Hahn, Ph.D. Research Focus: Depleted Uranium

Start Date (CY): 1995 Est. Completion (CY): 1998 Environmental Toxicology

OVERALL PROJECT OBJECTIVE: Assess the carcinogenic risks associated with long-term exposure to DU-containing shrapnel in wounds using a rodent model.

SPECIFIC AIMS: Determine the carcinogenicity of radioactive DU fragments in tissues relative to nonradioactive (tantalum) foreign-body fragments, and correlate urine and kidney concentrations of uranium with time after implantation of DU fragments.

METHODOLOGY: Relative carcinogenicity of embedded DU fragments was determined using test metals, in the form of flat squares, surgically implanted into the muscles of rats. Thorotrast was used as a positive control radioactive material. These animals will be observed for two years for the onset, incidence, and biological characteristics of tumors developing at the site of implantation. The incidence of subcutaneous tumors will be compared among dose groups by using a Cox proportional hazards model.

Renal toxicity of chronic uranium exposure will be studied after determination of the time course to achieve a steady-state renal DU concentration from an implanted source. Urinalysis will include U concentration and biochemical indicators of kidney function. Rats will be examined for lesions, with particular attention to the urinary system. Kidneys will be examined histologically for lesions related to U toxicity. U content in the kidney and skeleton and remainder of the carcass will be determined. Laser phosphorimetry is being used to analyze tissue and urine samples for U.

EXPECTED PRODUCTS (MILESTONES): The carcinogenic hazard of radioactive DU fragments relative to nonradioactive fragments is being determined so that informed judgements can be made about the clinical management of veterans with DU fragments embedded in their soft tissues.

Milestones of this study are: May 1997: Initiation of the long-term study of potential carcinogenic effects of implanted DU fragments. May 1999: Completion of the long-term carcinogenesis of DU fragments.

STATUS/RESULTS TO DATE: A preliminary 60-day study determined that the solubility of implanted DU was less than DU alloyed with Titanium (DUTi) in both rats and mice, and early histological responses to implanted DU were less than DUTi. The surface of both metals was visibly roughened after 30 days. These features indicated that the subcutaneous implantation model of foreign body carcinogenesis, as described by Brand et al (1975) is not appropriate to study the carcinogenesis of implanted DUTi. Accordingly, a revised protocol was used for the long-term study: Du (0.75% Ti) fragments in 3 sizes were implanted into the leg muscles of rats. Using 3 sizes resulted in rats receiving a mass of metal that ranged across a factor of 20 and a surface radioactivity that ranged a factor of 10. In addition, a group of rats received tantalum fragments of similar sizes to serve as negative control animals, and a group of rats received Thorotrast injections to serve as positive control animals for radioactive materials.

PUBLICATIONS: Hahn FF, Lundgren DL, Hoover MD, Guilmette RA. DU Distribution and Carcinogenesis Studies. In Proceedings of Depleted Uranium Workshop, held at Armed Forces Radiobiology Research Institute, November 15, 1996 (submitted for publication in Military Medicine).

Title: Program DoD-8

Project #: DoD-8

Agency: DoD

Study Location: WRAIR, Wash. DC

P.I.: COL D Gordon

This is the parent Program for DoD projects DoD-8A through DoD-8B.

Title: Serologic Diagnosis of Viscerotropic Leishmaniasis (VTL)

Project #: DoD-8A Agency: DoD Study Location: WRAIR, Wash. DC

Project Status: Completed Research Type: Applied Research

P.I.: COL D Gordon Research Focus: Leishmaniasis

Start Date (CY):1993 Est. Completion (CY):1996 Diagnosis

OVERALL PROJECT OBJECTIVE: Develop a reliable serologic test for viscerotropic leishmaniasis. **SPECIFIC AIMS:** The goal was to identify an antibody (Ab)-based serologic assay to detect active infection with *Leishmania* parasites causing the clinical syndrome of VTL using a standard format such as enzyme-linked immunosorbent assay (ELISA).

METHODOLOGY: Use of ELISA-based assays to diagnose active infection with an infectious agent is a widely accepted and useful diagnostic intervention. Currently, there are no commercially available serologic assays to detect *Leishmania* infection. Some specialty labs use the indirect immunofluorescence technique (IFAT) format to detect antibodies to surface proteins of the promastigote (insect vector form of the parasite); however, the IFAT is not useful in VTL. Once the parasite is injected into humans by the sand-fly vector, it transforms into an amastigote (the form found in mammals). The amastigote is a genetically different morphologic form which expresses a unique repertoire of antigens. This effort is based on the hypothesis that these amastigote dominant antigens hold the key to serologic diagnosis of VTL. Using recombinant molecular biology techniques, proteins which are recognized by VTL sera can be made in large quantities and used as capture antigens in ELISA tests.

EXPECTED PRODUCTS (MILESTONES): Identification of reactive clones made from screening a genomic library of *Leishmania* DNA, cloning and sequencing the recombinant proteins and using the recombinant proteins to screen sera from patients with viscerotropic leishmaniasis (VTL).

STATUS/RESULTS TO DATE: A genomic library was made from a Desert Storm isolate of *Leishmania*. Parasite DNA was sheared and introduced into *E. coli* via phage virus. Bacterial clones with parasite DNA were transferred to nitrocellulose and then incubated with radioiodinated protein labeled VTL sera. Reactive clones were then isolated, induced, and protein was sequenced. Patients with VTL appear to recognize a 210 kd recombinant protein, called Lt-1, the carboxy third of the protein is made of repeats. Smaller recombinant proteins and synthetic peptides based on the repeats have been made. To date, none of these peptides reliably discriminates infected from noninfected samples.

PUBLICATIONS: Dillon DC, Day CH, Whittle JA, Magill AJ, Reed SG. The molecular and immunological characterization of *Leishmania tropica* antigens: serological and cellular responses by viscerotropic Leishmaniasis Patients. VIII Intern Congress of Parasitology, 1994.

Dillon DC, Day CH, Whittle JA, Magill AJ, Reed. The Molecular and immunological characterization of *Leishmania tropica* antigens: serological and cellular responses by Viscerotropic Leishmaniasis Patients. 43rd Annual Mtg AM SocTrop Med & Hygiene, 1994.

Dillon DC, Day CH, Whittle JA, Magill AJ, Reed. Characterization of a *Leishmania tropica* antigen that detects immune responses in Desert Storm Viscerotropic Leishmaniasis patients. Proc. Natl Acad Sci, 1995, (92):7981-82.

Title: Development of a Leishmania Skin Test Antigen (LSTA)
Project #: DoD-8B Agency: DoD Study Location: WRAIR, Wash. DC
Project Status: Ongoing Research Type: Applied Research
P.I.: COL D Gordon Research Focus: Leishmaniasis
Start Date (CY):1993 Est. Completion (CY):2000 Diagnosis

OVERALL PROJECT OBJECTIVE: Develop a reliable skin test for Leishmania infection.

SPECIFIC AIMS: The goal is to identify a safe, potent, and non-sensitizing LSTA; manufacture it under cGMP; and obtain an IND for its use in phase 1 and phase 2 clinical trials, and ultimately, a commercially available, FDA-licensed product.

METHODOLOGY: Skin tests are widely accepted diagnostic interventions for diagnosis of prior infection with an infectious agent (e.g., tuberculosis). Currently there is no Leishmania skin test licensed for use in the USA or available under an IND. Once required phase 1 and phase 2 studies are completed in humans, studies could be performed in Gulf War veterans with confirmed and suspected leishmaniasis.

EXPECTED PRODUCTS (MILESTONES): Completion of the IND application by 4th Quarter FY96 and completion of phase 1 study by 2nd Quarter FY97. Phase 2 trials in FY97-98. Phase 3 trials in 98. Licensure by 2000.

STATUS/RESULTS TO DATE: Microfluidized-lysate (MFL)-LSTA, Lot 0172, was manufactured under cGMP in May 1995. Pre-clinical testing in guinea pigs and non-human primates has proved it to be safe and potent. A similar product has sensitized naive guinea pigs on repetitive testing but whether this will occur in humans is not known. SDS-PAGE analysis of product substance, final container product and following heat treatment has shown the MFL-LSTA to be stable. IND submitted to FDA on 6/2/96 and approved on 16 Nov 96. Recruitment for phase 1 clinical trial began in December 96 and the phase 1 clinical trial was performed between 18 Feb 97 and 20 May 97. Sixty-six doses of MFL-LSTA, lot 0172, were given to 15 volunteers. The product proved to be safe and well tolerated in the volunteers; however, 2 volunteers exhibited a type 1 allergic cutaneous response to the antigen and placebo formulation. The most likely cause is pre-existing hypersensitivity to dextran, a component of the lyophilization buffer. A reformulation LSTA is currently being made to eliminate the need for lyophilized product with dextran. The reformulated placebo was manufactured under cGMP in June 97 and the antigen was made in Aug 97. Final container testing is ongoing and revised IND information was submitted to the FDA in Nov 97. An abbreviated (one dose) phase 1 clinical trial is being performed at WRAIR in Jan 98. Phase IIA dose ranging and potency trials are planned for Peru, Brazil, and Kenya in calendar year 98. Formal sensitization clinical trials must await completion of phase IIA trials.

PUBLICATIONS: Stitler JM, Ballou WR, Eckels KH, Magill AJ. Good Manufacturing Practices Production of Leishmania Skin Test Antigen: 1. Protocol Requirements for Investigational New Drug (IND) Application. 43rd Annual Mtg of AM Soc Tropical Med & Hygiene, 1994.

Stitler JM, Ballou WR, Eckels KH, Wellde BT, Topper MJ, Rowton ED, Magill AJ. Good Manufacturing Practices Production of Leishmania Skin test antigen: 2. Production of a Microfluidized Lysate. 44th Annual Mtg of AM Soc Tropical Med & Hygiene, 1995.

Stitler JM, Ballou WR, Eckels KH, Wellde BT, Topper MJ, Rowton ED, Magill AJ. Current good manufacturing practices (cGMP) : production of a microfluidized lysate Leishmania skin test antigen (MFL-LSTA). 45th Annual Meeting of the American Society of Tropical Medicine and Hygiene, Baltimore, Maryland, 1996.

Title: Identification of the Genetic Factors Which Control Tropism in Leishmania

Project #: DoD-9

Agency: DoD

Study Location: WRAIR, Brazilia

Project Status: Ongoing

Research Type: Basic Research

P.I.: Dr. Grogl/Callahan

Research Focus: Leishmaniasis

Start Date (CY):1994 Est. Completion (CY):1998

Treatment

OVERALL PROJECT OBJECTIVE: Identify the gene(s) that control tropism in Leishmania and determine its (their) sequence and function.

SPECIFIC AIMS: The goal of this research is to identify the gene(s) that control tropism in Leishmania. The identification of a "tropism" gene will enable the development of specific gene probes (primers) to be used in a patient screening program to identify those at risk of reactivation of latent infections; address the fundamental question of infectious disease pathophysiology, namely why an organism infects a particular cell; optimize treatment regimens according to Leishmania species and the immune status of the host. The identification of the genetic factor(s) involved in the visceralization of Leishmania will require development in vitro and in vivo models of tropism to facilitate the study of viscerotropic leishmaniasis (diagnosis, therapy, prevention) and Leishmania tropism.

METHODOLOGY: Develop in vitro and in vivo models of Leishmania tropism to use for the determination of the genetic factors controlling tropism: a promastigote temperature sensitivity model; and a cutaneous and a visceral animal model of Leishmania tropism. Create Leishmania mutants with altered tropism in the in vitro and in vivo models of tropism: temperature sensitive parasites changed to resistant and visa versa; and cutaneous parasites (in the animal model) changed to visceral and visa versa. Use recently developed molecular genetic techniques to restore the original (wild-type) phenotype to the Leishmania temperature and tropism mutants by transfection with the appropriate Leishmania cosmid DNA libraries. Identify the gene(s) controlling temperature sensitivity and tropism present in the cosmid(s) which restore(s) a wild-type phenotype to the Leishmania cells. Retest the function of these genes in both models using transfection methodology.

EXPECTED PRODUCTS (MILESTONES): Molecular characterization includes Identification of the genes present in the cosmids which restore a wild-type phenotype to the Leishmania mutants, sequencing the genes by 1998, and determining the homologies with genes in the database by 1998.

STATUS/RESULTS TO DATE: An in vitro promastigote temperature model was developed and the investigators determined that temperature sensitivity in vitro correlates with Leishmania tropism in vivo. There is no absolute temperature requirement for dermatotropism, but there is a minimum temperature resistance requirement for visceralization. The parasite strains that have an unusual tropism in the human host (to include the viscerotropic L. tropica from Desert Storm) also show an unexpected temperature sensitivity in the model. Two animal models were developed where the tropism of the Leishmania strain is known, uniform, and reproducible (all organisms are found in one location, either visceral or cutaneous) when inoculated in the skin (sc) to mimic a sand fly bite, including a cutaneous model with no visceralization, and a visceral model with no skin lesions. Leishmania temperature sensitive and resistant mutants have been obtained. Laboratory techniques were developed and standardized for transfection and for the Leishmania cosmid DNA library. An L. donovani and two L. tropica temperature-resistant strains were selected in vitro. An L. mexicana strain was chemically mutagenized and a tropism mutant was selected in our hamster model. Wild-type DNA cosmid libraries were made of all the Leishmania strains (4) that will be mutagenized and selected for altered tropism in the hamster model.

PUBLICATIONS: Callahan HL, Portal IF, Grogl M. Development of an in vivo Leishmania temperature sensitivity model: preliminary studies with strains with aberrant tropism in vivo. 44th Annual Meeting of Am Soc Tropical Med & Hygiene, 1995, 53(2):215.

Callahan HL, Portal IF, Bensinger SJ, Grogl M. The temperature sensitivity of Leishmania promastigotes in vitro: a model for tropism in vivo. Exp Parasit, 1996, 84:400-9.

Callahan HL, Grogl M. Development of animal models to study Leishmania tropism at the molecular level. Am J Tropical Med. (in press).

Title: Pyridostigmine Synergistic Toxicity Study

Project #: DoD-10 Agency: DoD Study Location: CHPPM, Aberdeen MD

Project Status: Completed Research Type: Basic Research

P.I.: Dr. McCain Research Focus: Pyridostigmine Bromide

Start Date (CY):1994 Est. Completion (CY):1994 Interactions

OVERALL PROJECT OBJECTIVE: Determine potentially toxic interactions when pyridostigmine bromide, permethrin, and DEET are given concurrently to male rats by gavage.

SPECIFIC AIMS: Phase I determined the acute oral lethal dose response relationship of each compound with the propylene glycol solution in which it was delivered. Phase II was a dose response study using results from Phase I as well as calculated dosage solutions of the three materials in combinations. This study was motivated by concerns about the possible synergism of pyridostigmine taken by the servicemembers in ODS to protect them against potential nerve agent exposure and permethrin and DEET, insecticides which were used at the same time.

METHODOLOGY: The first phase was the development of acute oral lethal dose-response relationship of each compound with the vehicle, determine dosage levels for the second phase. Part 1 of Phase II served as a positive control for the interaction portion of the study and verified data obtained from Phase I. The Part 1 dose response study used dosing developed from Phase I. Part 2 of Phase II was similar to the control portion except that the vehicle contained the calculated LD16 of the other two compounds. The Phase I study protocol used 16 groups of 10 animals each, with one group of 10 receiving only the propylene glycol vehicle. Gross necropsy was performed on all animals as soon as possible after death. Animals surviving the 14 day study period were sacrificed and examined for gross pathological lesions. The Phase II study used 15 groups of six animals each for the positive control portion and 18 groups of 10 animals each for the interaction portion. Animals were necropsied and examined for gross pathological lesions.

EXPECTED PRODUCTS (MILESTONES): Report on toxicity produced by high dose interactions of military materiel and medical pretreatments.

STATUS/RESULTS TO DATE: The principal finding was that at extraordinarily high doses, there is an increased mortality in rats given pyridostigmine bromide, permethrin, and DEET simultaneously by gavage when compared to expected additive lethal effect of the individual compounds. The direct significance of these findings to ground forces who served in the Gulf War is uncertain because these doses and route of exposure do not correspond to any suspected human exposures.

PUBLICATIONS: US Army Center for Health Promotion and Preventive Medicine Report. Acute Oral Toxicity Study of Pyridostigmine Bromide, Permethrin, and DEET in the Laboratory Rat. Toxicological Study, 1995, 75-48-2665.

Title: Male/Female Differential Tolerances to Pyridostigmine Bromide

Project #: DoD-11 Agency: DoD Study Location: S.FL Drug Res Miami FL

Project Status: Ongoing Research Type: Clinical Research

P.I.: Dr. K Lasseter Research Focus: Pyridostigmine Bromide

Start Date (CY):1994 Est. Completion (CY):1997

OVERALL PROJECT OBJECTIVE: Determine if males and females have different tolerances to doctrinal dose (30 mg every 8 hours) of pyridostigmine bromide.

SPECIFIC AIMS: To evaluate the tolerance of pyridostigmine bromide (30 mg every 8 hours for 21 days); to evaluate multiple dose kinetics; and to evaluate the effect of weight in males and females upon drug tolerance.

METHODOLOGY: Double-blind study.

EXPECTED PRODUCTS (MILESTONES): Final Report.

STATUS/RESULTS TO DATE: In-life portion of the study and the one year follow-up have been completed. Results from this study indicate that pyridostigmine is safe. Expected side effects, primarily gastrointestinal, were observed. Other events did not appear to be related to the drug since the reporting incidence was equal in active drug and placebo groups. No difference in side effects were found related to gender or weight.

PUBLICATIONS: none to date

Title: Forward Deployable Diagnostics for Infectious Diseases

Project #: DoD-12 Agency: DoD Study Location: WRAIR, Wash. DC

Project Status: Ongoing Research Type: Applied Research

P.I.: COL D Jarboe Research Focus: Diagnosis

Start Date (CY):1993 Est. Completion (CY):2001

OVERALL PROJECT OBJECTIVE: Develop a series of simple diagnostic assays suitable for forward deployed preventive medicine teams, Area Medical, and Forward Laboratories.

SPECIFIC AIMS: Develop rapid and simple diagnostic tests to permit the identification of the causes of infection outbreaks before they reach the epidemic state. Serological assays are being developed for typhus fever, dysenteries caused by shigella and other bacteria, and leptospirosis. Tests utilizing polymerase chain reaction (PCR) technology are being explored for diagnosing dengue fever. Fluorescent assays for malaria and other parasitic diseases are under investigation. Hand-held chromatographic immunoassays are being developed for dengue fever. Future applications include campylobacter, malaria, and arboviral and rickettsial disease agents. Dipstick technology is being developed for dengue fever as well as rickettsial disease agents responsible for scrub and endemic typhus, and spotted fever group diseases.

METHODOLOGY: Technologies used in this research program center on hand-held chromatographic immunoassays, electrochemiluminescence (ECL) detection, polymerase chain reaction assays, and dip-stick assays.

EXPECTED PRODUCTS (MILESTONES): Forward deployable assays using immunochemical-based assays against dengue, hanta-, and selected hemorrhagic fever viruses are expected to reach Milestone I in 4QFY98. Confirmatory polymerase chain reaction assays with high specificity and sensitivity are expected to reach Milestone I in 4QFY98 were conducted.

STATUS/RESULTS TO DATE: Sensitive and specific enzyme-linked immunoassays are currently available for the detection of selected antigens, or anti-agent IgG/IgM antibodies for a select group of infectious diseases. Application of chromatographic assay technology occurred in FY96 with completed products available in FY98. Prototype polymerase chain reaction assays are currently available for dengue virus, malaria, shigella, ETEC, hemorrhagic fever viruses, and hantaviruses. New PCR technology that will allow hand held detection is currently being explored. Evaluation of fieldable reagents and validation of the assays will continue at least until FY98. Initial laboratory development is completed for dip-stick assays for scrub and endemic typhus; dip-sticks for rickettsial spotted fever group and dengue fever disease agents are in progress.

PUBLICATIONS: Kelly DJ, Chan CT, Paxton H, Thompson K, Howard R, Dasch GA. Comparative evaluation of a commercial enzyme immunoassay for the detection of human antibody to Rickettsia typhi. *Clinical & Diagnostic Laboratory Immunology*, 1995, 2(3):356-60.

Weddle JR, Chan TC, Thompson K, Paxton H, Kelly DJ, Dasch GA, Strickman D. Effectiveness of a dot-blot immunoassay of anti-Rickettsia tsutsugamushi antibodies for serologic analysis of scrub typhus. *Am J Tropical Med & Hygiene*, 1995, 53(1):43-6.

Title: Effects of Persian Gulf War Service on Military Working Dogs

Project #: DoD-13 Agency: DoD Study Location: AFIP, Wash. DC

Project Status: Ongoing Research Type: Epidemiology Research

P.I.: LTC R Moeller Research Focus: Environmental Toxicology

Start Date (CY):1994 Est. Completion (CY):2001

OVERALL PROJECT OBJECTIVE: The possibility of exposure to environmental factors and endemic diseases exist for the population of military working dogs (MWDs) that deployed to the Persian Gulf (PG) theater of war. The question to be answered is: In the final analysis, what are the differences in diagnoses between the PG MWD cohort and a matched (on the basis of age, sex and breed) comparison group which never deployed to Southwest Asia (SWA).

SPECIFIC AIMS: The (null) hypotheses to be tested is that in the final analysis there will be no differences in the diagnoses between the PG MWD cohort and the comparison group which never deployed to SWA. Should this hypothesis not be supported: 1) the possibility exists that differences in diagnoses between the two groups may be the result of deployment to SWA; and 2) dates of deployment and location in theater will be compared among the PG MWDs, and conceivably to those of PG veterans.

METHODOLOGY: The PG MWD cohort was identified after the cessation of hostilities and subsequent redeployment. The inclusive deployment dates for this population are 1 August 1990 to 31 December 1991. MWDs receive semi-annual physical examinations throughout their active duty lives, which include clinical evaluations and routine panels of hematologic, serologic, and blood chemical analyses. The results of these tests are recorded in the animal's medical record. Those MWDs that are euthanized will have peripheral blood samples collected prior to euthanasia for the above tests. When natural death occurs, the most recent blood test will be used. Test results are to be included in the dog's permanent medical record. Necropsies are performed in accordance with a standard protocol contained in TB Med 283. Medical records from all deceased MWDs in the Department of Defense are archived at the DODMWDVS, Lackland AFB, Texas.

Based on the assumptions of a condition with 10% prevalence in the population, looking for a relative risk of 2.5 in the exposed group, setting the alpha level at 0.05 and the beta level at 0.2, a minimum of 112 animals of each group must be included in the study. Therefore, the medical and training records of those 118 MWDs which deployed to the Persian Gulf, and 472 non-deployed MWDs matched four to one based on age, gender and breed, will be abstracted during the study period for the following variables: animal identification; age at death; date of death; breed; gender; location during the time frame 1 August 1990 to 31 December 1991; duration of deployment; neurologic illness; orthopedic illness; dermatological illness; gastrointestinal illness; infectious diseases; parasitism; neoplasms; behavioral changes after 1 August 1990; pathologic diagnoses of biopsy specimens and pathologic diagnoses of autopsy specimens. These data will be electronically stored in a database for statistical analysis using the SPSSR analysis program.

Those 30 Persian Gulf deployed MWDs still living, and 120 age, gender, breed matched non-deployed control MWDs will be transported to the DODMWDVS, Lackland AFB when the responsible Veterinary Corps officer has determined the animal is no longer physically fit for duty and in need of humane euthanasia. Upon arrival, the medical record will be screened to determine the cohort of assignment. The MWD will receive a complete physical exam, to include the following: CBC; serum chemistry panel; serum acetylcholinesterase activity levels; urinalysis; fecal exam for parasites; canine thyroid hormone measurements (T4, cTSH); electrocardiography; radiography; a neurologic examination and a behavioral assessment.

The MWD will be anesthetized according to a standard approved protocol. Radiographs of elbows, stifles, coxofemoral joints and spine will be obtained if not present in the record. Electromyograms and nerve conduction studies will then be conducted on the anesthetized dog, to determine neuromuscular function.

Euthanasia of the dog will be completed with a standard approved injectable euthanasia agent (Beuthanasia) and immediately necropsied in accordance with the TB Med 283. At necropsy, gross changes are described and an extensive set of tissues collected and forwarded to the Department of Veterinary Pathology, Armed Forces Institute of Pathology, Washington, D.C. Muscle biopsies of the biceps femoris and triceps brachii; and nerve biopsies of the tibial and radial nerves will be

collected for analysis at Auburn University. Additionally, 6 gram samples of liver, kidney, lung, brain and fat will be collected for ultra low temperature freezing and stored at the AFIP or DoD Veterinary Laboratory until toxicological procedures may be performed, if indicated. Formalin fixed tissues will be processed for histopathologic examination resulting in a detailed final pathology diagnostic case report consisting of a list of pathologic findings and an interpretation of these findings. Remaining wet tissues, paraffin blocks, microslides and case folder materials will be archived. The pathology report will be forwarded to the DODMWDVS, Lackland AFB, TX for inclusion in the MWD's medical record.

All clinical and pathological information collected during the final examination procedures will be electronically stored in a database for statistical analysis using the SPSSR statistical program. Upon completion of initial data and records collection data, a multivariate analysis of collected variables will be accomplished to determine the effects of age, gender and breed on those conditions commonly occurring in the entire population of MWDs. Odds ratios and ninety-five percent confidence intervals will be calculated on all conditions occurring more frequently in one cohort to determine the effects of the exposure status on those conditions. Fishers's exact p values will be calculated to determine statistical significance of any conditions occurring more frequently in one cohort.

EXPECTED PRODUCTS (MILESTONES): See Objectives.

STATUS/RESULTS TO DATE: Ongoing. We have identified one hundred eighteen MWDs that deployed to various locations in the PG Theater in support of operations within the inclusive dates listed above. The AFIP funded a pilot protocol enabling the early implementation of a data collection system that includes pathologic (including surgical and post-mortem morphologic changes), demographic, and clinical findings from initially identified PG MWD cohort and matched comparison MWDs. The pilot protocol funding expired in 1995.

The collection and analysis of epidemiological data is the primary responsibility of the US Army Veterinary Corps Officer currently stationed at the DODMWDVS, Lackland AFB, TX. Records have been collected and reviewed on 78 Persian Gulf cohort animals and approximately 400 additional dogs that will serve as a comparison group.

In January 1997, a panel of civilian veterinary medical experts from eight different academic and industrial institutes met, as an advisory body, with investigators from the DODMWDVS and AFIP to discuss the project.

The Department of Veterinary Pathology, Armed Forces Institute of Pathology, along with a database management consultant with expertise in SNOWMED International is near completion of the database system. This database system should be completed and ready for data entry in February 1998. Computer software and hardware have been obtained to support this process. The AFIP and the DOD Veterinary Laboratory at Fort Sam Houston, TX are storing MWD tissue specimens for toxicological analysis, if indicated. Electrophysiological myoneural diagnostic evaluation is ongoing in cohort animals already at DODMWDVS. Muscle biopsies of the biceps femoris and triceps brachii; and nerve biopsies of the tibial and radial nerves are currently being collected for electron microscopic analysis at Auburn University. Approximately 75 % of the PG MWD cohort are deceased; however, information and records must be collected, collated and abstracted. Clinical and pathological findings must be entered into the database management system and analyzed in order to draw conclusions and compare the two cohorts.

PUBLICATIONS: none to date

Title: Risk Factors Among US Army Soldiers for Enrolling on the Department of Veterans Affairs Gulf War Registry
Project #: DoD-14 Agency: DoD Study Location: WRAIR, Wash. DC
Project Status: Completed Research Type: Epidemiology Research
P.I.: Jim Writer Research Focus: Symptoms/General Health
Start Date (CY):1993 Est. Completion (CY):1997

OVERALL PROJECT OBJECTIVE: Determine the presence of unique characteristics (demographics, aptitude test scores, self-reported health behaviors, past hospitalizations) of Army personnel enrolled in the VA Registry of Persian Gulf veterans..

SPECIFIC AIMS: There were two specific aims of this hypothesis-generating study. The first was to investigate logistical, epidemiological, and statistical methodologies for obtaining, combining, and analyzing records. These techniques will be needed for future studies on reported post-deployment illnesses in soldiers who served in the Gulf War, and will also be applicable in evaluating the health effects of future deployments. The second aim was to determine which characteristics (to include demographics, past medical history, and health risk behaviors) of soldiers who have been enrolled in the VA Gulf War registry differ from those who have not enrolled. Ultimately, this information can be used to identify soldiers at a high-risk of reporting post deployment medical problems. These soldiers may be targeted for interventions before or after their deployment to reduce the risk adverse health effects attributable to service in a war zone.

METHODOLOGY: Using case-control methodology the investigators studied the association between Gulf War veterans' various demographic, medical, or health behavior characteristics and their probability of enrolling in the Gulf War registry. The study population consisted of individuals on the VA database and controls from the Defense Manpower Data Center's (DMDC) roster of Gulf War participants. Characteristics evaluated included demographic factors, recruit applicant aptitude test scores, pre-deployment self-reported health behaviors, and pre-deployment health care utilization as are represented by hospitalizations in military hospitals. These data are available in already existing Army or DoD databases, however, all data may not be available for all study subjects.

EXPECTED PRODUCTS (MILESTONES): A data base of US Army Gulf War veterans, which includes health indicators and demographic data from the pre, during, and post deployment phases of their military careers was generated. The study determined if existing data sources can be used to predict a soldier's likelihood of having significant post-deployment adverse health outcomes or a high level of concern about potential health effects of the deployment.

STATUS/RESULTS TO DATE: Preliminary data analysis presented at the Armed Forces Epidemiology Board's December 1996 meeting. Manuscript is being prepared.

PUBLICATIONS: none to date

Title: Comparative Mortality Among US Military Personnel Worldwide During Operations Desert Shield and Desert Storm

Project #: DoD-15

Agency: DoD

Study Location: WRAIR, Wash. DC

Project Status: Completed

Research Type: Epidemiology Research

P.I.: Jim Writer

Research Focus: Mortality

Start Date (CY):1994 Est. Completion (CY):1995

OVERALL PROJECT OBJECTIVE: Determine if military personnel deployed to Operations Desert Shield/Desert Storm have higher death rates than military personnel who did not deploy.

SPECIFIC AIMS: Characterize the disease and non-battle injury (DNBI) mortality experience of US military personnel during ODS/DS. Determine whether US military personnel deployed to Southwest Asia (SWA) had a higher rate of death than US military personnel who did not deploy.

METHODOLOGY: Casualty reports were used to calculate cause-specific mortality rates among all personnel on active duty in the US Armed Forces (including Reserve and National Guard) over a one-year interval that included the Persian Gulf War. Death rates among active duty personnel deployed to SWA at any time during this period were compared to rates for all military personnel on active duty but not deployed to SWA. Each death was characterized as to have been most likely caused by disease or trauma. Trauma deaths were additionally classified as due to unintentional (accidental) injury, battle injury, self-inflicted injury, or homicide.

EXPECTED PRODUCTS (MILESTONES): Determination of whether US military personnel deployed to SWA had a higher death rate than non-deployed military personnel.

STATUS/RESULTS TO DATE: Except for hostile action deaths, the overall mortality experience among US military personnel who deployed did not significantly differ from that observed among US military personnel who did not deploy to SWA. No evidence of clusters of unexpected deaths were found, except for two accidents. The number and circumstances of nonbattle deaths among Persian Gulf troops were typical for the U.S. military population.

PUBLICATIONS: Walter Reed Army Institute of Research Report. Comparative Mortality Among US Military Personnel Worldwide During Operations Desert Shield and Desert Storm. January 1995.

Writer JV, DeFraités RF, Brundage JF. Comparative mortality among US military personnel in the Persian Gulf region and worldwide during Operations Desert Shield and Desert Storm. *JAMA*, 1996, 275(2):118-121.

Title: Kuwait Oil Fire Health Risk Assessment

Project #: DoD-16 Agency: DoD Study Location: CHPPM, Aberdeen MD

Project Status: Completed Research Type: Applied Research

P.I.: Dr. J Heller Research Focus: Environmental Toxicology

Start Date (CY):1991 Est. Completion (CY):1994

OVERALL PROJECT OBJECTIVE: To characterize both the carcinogenic and noncarcinogenic health risks to DOD troops and civilian employees exposed to the environment affected by the oil fires during and after Operation Desert Storm.

SPECIFIC AIMS: 1. Environmental Monitoring - The environmental monitoring study attempted to characterize the concentration of pollutants that DOD personnel were exposed to during their deployment in the Gulf region. Air monitoring and soil sampling data were used to calculate exposure point concentrations for individual Health Risk Assessments in the final report. 2. Industrial Hygiene Sampling Study - The industrial hygiene air survey monitored and characterized occupational exposures of DOD personnel who had potential high risk exposure to oil fire emissions. The focus was on individuals working outdoors and on worst-case situations within the oil fields next to Kuwait City. 3. Biological Surveillance Initiative - The Biological Surveillance Initiative was conducted to refine and corroborate the results from the Health Risk Assessment. This was accomplished by a collection of objective biological measurements of exposure and effect in real-time, and by establishment of any observable biologic effect or marker of exposure to oil fire pollutants in a cohort of U.S. soldiers.

METHODOLOGY: 1. Sampling and Analysis - Sampling and analytical methodologies for ambient air, soil, industrial hygiene air, biologic samples, and quality assurance procedures are detailed in the final report. 2. Risk Assessment - The methodology used for this Health Risk Assessment was the EPA guidance for the Comprehensive Environmental Response, Compensation and Liability Act sites (CERCLA), also known as "Superfund" sites. The methodology did not determine an individual's health outcome or include the use of reported health effects data. The components of the risk assessment process included data collection and evaluation, exposure assessment, toxicity assessment, and risk characterization and uncertainty analysis.

EXPECTED PRODUCTS (MILESTONES): Reports

STATUS/RESULTS TO DATE: Completed. The potential for significant long-term adverse health effects for the exposed DOD troop or civilian employee populations is minimal. The total predicted excess carcinogenic risk both in Kuwait and in Saudi Arabia did not exceed 3 excess cancers per 1,000,000 population exposed. The predicted carcinogenic risk levels are well within the EPA range of acceptable excess carcinogenic risks. The majority (>99%) of noncarcinogenic risk at all monitoring sites is predicted to be from the inhalation of volatile organic compounds and is assessed as low. The results of the Biological Surveillance Initiative support the conclusion that noncarcinogenic risk levels indicate minimal potential adverse health effects.

As of January 1998, the DoD Persian Gulf registry unit movement database contains location data for 3,198 Army units, 248 Navy sea and ground units, and 1,021 Marine Corps units. A location code is annotated for Air Force personnel in the DoD Persian Gulf personnel database. The location code replaces the unit identification code (UIC) for tracking purposes. To date, 84,925 Air Force personnel contain location codes.

The U.S. Armed Services Center for Research of Unit Records (CRUR) reviewed approximately 6 million pages of documents and has conducted fourteen S-3/G-3 conferences. The S-3/G-3 conferences consist of former Operation Desert Shield / Desert Storm operations officers (at the brigade and division levels) meeting at the CRUR to review existing unit movement and location records. From the reviews, enhancements were made to the database to include data gap-fills, unit additions, and unit movement location data down to the company and detachment level. The database currently contains approximately 700,000 grid coordinates, latitudes/longitudes and names of base camp locations. It is important to note that the database will continue to increase in size when additional records are found. The resolution of the database will further increase because additional S-3/G-3 conferences are scheduled in 1998.

PUBLICATIONS: US Army Environmental Hygiene Agency, Final Report. Kuwait Oil Fire Health Risk Assessment. No. 39-26-L192-91, 18 February 1994.

Title: Retrospective Studies Involving Military Use of Pyridostigmine as a Pretreatment for Nerve Agent Poisoning

Project #: DoD-17

Agency: DoD

Study Location: WRAIR, Wash. DC

Project Status: Completed

Research Type: Epidemiology Research

P.I.: D. Clawson

Research Focus: Pyridostigmine Bromide

Start Date (CY):1991 Est. Completion (CY):1992

OVERALL PROJECT OBJECTIVE: To obtain safety data for New Drug Application with the Food and Drug Administration (FDA).

SPECIFIC AIMS: To perform retrospective evaluation of effects of pyridostigmine use in the Persian Gulf.

METHODOLOGY: Retrospective evaluation.

EXPECTED PRODUCTS (MILESTONES): Reports.

STATUS/RESULTS TO DATE: Complete. Three surveys were completed, reports prepared, and forwarded to the FDA. Results indicated that pyridostigmine continues to be safe. Side effects reported were primarily gastrointestinal in nature. Headaches were also reported by the respondents.

PUBLICATIONS: Three Survey Reports consolidated Into an Amendment to the FDA Investigational New Drug Application.

Title: Kuwait Oil Fires Troop Exposure Assessment Model (TEAM)

Project #: DoD-18 Agency: DoD Study Location: CHPPM, Aberdeen MD

Project Status: Completed Research Type: Applied Research

P.I.: Jeffrey Kirkpatrick Research Focus: Environmental Toxicology

Start Date (CY):1993 Est. Completion (CY):1996

OVERALL PROJECT OBJECTIVE: To respond to Public Law 102-190 (Section 734: Troop Registry from exposure to oil well fires in Operation Desert Storm) by characterizing the potential carcinogenic and non-carcinogenic health risks to U.S. military personnel exposed to the environment affected by the oil well fires during and after Operation Desert Storm.

SPECIFIC AIMS: The site-specific Kuwait Oil Fire Health Risk Assessment addressed the risks to DOD troops located at the eight fixed air/soil sampling sites where actual environmental data were collected and analyzed. This study will incorporate actual site data with the modeled air concentrations, exposure data, and the Troop Location Registry data, to determine the risk to U.S. military personnel that were not located at fixed sampling sites for their entry tour of duty and for periods of time when no sampling occurred.

METHODOLOGY: Using a geographic information system (GIS) that is capable of mapping troop location and movement over time, in conjunction with the troop movement database, the locations of all US military personnel are being mapped during Operation Desert Storm. Once troop locations have been determined and mapped, the GIS determines troop exposure to oil fire pollution using satellite images, modeled plume boundaries, oil field crude composition data, oil field emission rates, modeled pollutant concentrations, and actual field data. When troop location and exposure have been determined, the potential health risks resulting from the exposure will be determined.

EXPECTED PRODUCTS (MILESTONES): The outcomes expected from this study will be the determination of exposure and potential health risks associated with oil fire pollution for U.S. military personnel participating in Operation Desert Storm.

STATUS/RESULTS TO DATE: This project is ongoing. As of January 1998, the U.S. Army Center for Health Promotion and Preventive Medicine (CHPPM) is currently processing and analyzing daily oil fires exposures in support of both the oil fires Public Laws (102-190; 102-585) and the Office of the Special Assistant to the Deputy Secretary of Defense for Gulf War Illnesses (OSAGWI) oil fires case narrative. A brief summary of completed and current tasks follows.

Several data sets have been obtained, developed, and integrated to characterize the potential carcinogenic and non-carcinogenic health risks to U.S. military personnel exposed to the environment affected by the oil fires during and after Operation Desert Storm (ODS). The data sets include daily satellite images and modeled superplume boundaries (February - October 1991); toxicological factors; exposure factors; Kuwaiti crude oil compositions; pollutant specific emission factors; the Defense Manpower Data Center's ODS personnel roster; and the U.S. Armed Services Center for Research of Unit Records (CRUR) daily unit movements. A series of computer programs have processed the data sets into listings of ODS units exposed to the oil fires. The exposures are characterized by duration (i.e., number of days) and the predicted excess cancer and non-cancer values and associated ranges are provided. The ODS veterans' attached with the exposed units are currently being identified with applicable exposure data linked to the veterans' database record.

PUBLICATIONS: US Army Environmental Hygiene Agency, Final Report. Kuwait Oil Fire Health Risk Assessment. No. 39-26-L192-91, 18 Feb 1994. This report describes the eight fixed air/soil sampling sites study and presents the preliminary results of the KOFTEAM pilot project.

Title: Persian Gulf Veterans Health Tracking System

Project #: DoD-19 Agency: DoD Study Location: CHPPM, Aberdeen MD

Project Status: Ongoing Research Type: Applied Research

P.I.: Dr. Jack M. Heller Research Focus: Environmental Toxicology

Start Date (CY):1996 Est. Completion (CY):1997

OVERALL PROJECT OBJECTIVE: To respond to Public Law 102-585 (Section 702: Scientific research using Troop Health Registry) by characterizing all potential exposures (e.g., oil fires, vaccines, chemical/biological warfare agents, pesticides, pyridostigmine bromide, etc) to U.S. military personnel participating in Operation Desert Storm, and to assess the potential health consequences of those exposures.

SPECIFIC AIMS: The site-specific Kuwait Oil Fire Health Risk Assessment and the Troop Exposure Assessment Model (TEAM) address the potential risks to U.S. military personnel from potential exposure to airborne contaminants from oil well fires. This study attempts to characterize other potential exposures experienced by U.S. military personnel during Operation Desert Storm and to assess the potential health risks/consequences of those potential exposures.

METHODOLOGY: The Persian Gulf Veterans Health Tracking System uses a geographical information system (GIS) to assess potential exposures and medical outcomes for U.S. military personnel who participated in Operation Desert Storm. Once troop locations are determined and mapped (from the Troop Location Registry data), the GIS can help determine potential troop exposure to any number of different chemicals, environmental threats, or other materials. When troop locations and potential exposures have been determined, the potential health risks resulting from the potential exposures can be determined. In addition to examining potential exposure, databases on medical outcomes (e.g., Comprehensive Clinical Evaluation Program (CCEP), the Veterans Administration Mortality Study, etc.) can be evaluated by studying potential exposures, locations, movements, and relationships of the troops in these databases for epidemiological significance.

EXPECTED PRODUCTS (MILESTONES): Characterization of the potential exposures and health risks/outcomes associated with service by U.S. military personnel in Operation Desert Storm.

STATUS/RESULTS TO DATE: Ongoing. As of January 1998, several environmental, toxicological, epidemiological, and demographic / unit movement databases have been entered into the TEAM system. The environmental data sets include daily satellite images and modeled oil fires superplume boundaries; exposure factors; Kuwaiti crude oil compositions; pollutant specific emission factors; modeled plume boundaries from the Khamisiyah Pit Demolition; and locations of suspected Iraqi CW/BW sites. The toxicological data include the IRIS and HEAST databases produced by the EPA and pertinent exposure factors data published by the U.S. Army and the EPA. The DODs Comprehensive Clinical Evaluation Program (CCEP) database along with limited unit identification code data from the VA comprises the TEAM epidemiologic database. The epidemiologic database has been used to support medical outcome studies for the Khamisiyah Case Narrative published by the OSAGWI. The demographic / troop unit movement databases include the Defense Manpower Data Center's ODS personnel roster and the U.S. Armed Services Center for Research of Unit Records (CRUR) daily unit movements and locations.

The CHPPM is continuing to work with DoD agencies and research groups investigating Gulf War Illnesses.

PUBLICATIONS: none to date

Title: A Statistical Study Correlating the Reported Cases of Gulf War Syndrome to Battlefield Locations of Afflicted US Army Personnel During the Iraq-Kuwait War; Part 1: Method to Related Troop Deployment and the Reported Cases of Gulf War Syndrome and Probable Incidence of Maladies Defined by ICD-9-CM

Project #: DoD-20 Agency: DoD Study Location: USAR Lab, APG, MD
Project Status: Completed Research Type: Epidemiology Research
P.I.: Dr. Gluckman Research Focus: Symptoms/General Health
Start Date (CY):1994 Est. Completion (CY):1995

OVERALL PROJECT OBJECTIVE: To devise a procedure for counting the collections of symptoms and diagnoses of veterans' illnesses and relating them to the U.S. military grid system locations in the Kuwait-Iraq-Saudi Arabian theater of operations.

SPECIFIC AIMS: To identify the geographic locations by date of the Army personnel who served in the Gulf War and whose names are recorded in the VA register as experiencing severe health problems. The units to which these personnel were assigned are tracked across the Iraq-Kuwait region, in order to determine whether epidemiological commonalities can be detected based upon location at certain dates.

METHODOLOGY: VA malady codes of afflicted personnel related to unit identification codes were identified. Malady information was regrouped and coded into crisp and fuzzy numbers. For the Part 1, only crisp numbered malady information was used. The DOD Register was used to identify geographic coordinate locations of Army units. A mathematical algorithm was developed to compare geographic location to maladies and possible cause(s) of each of the maladies. The algorithm is based on the ratios of the number of combatants reporting illnesses (in any grid) to the total number of combatants (occupying the grid) as discrete probabilities. These are considered to be the probabilities of occurrence of a health condition for the total time period that a military unit occupies a grid. A production model needs to be completed to compute statistical measures that are concerned with Gulf War veterans' illnesses.

EXPECTED PRODUCTS (MILESTONES): A mathematical procedure to count the collections of symptoms and diagnoses over the various map grids of the region.

STATUS/RESULTS TO DATE: Completed.

PUBLICATIONS: Army Research Laboratory Technical Report ARL-TR-800, July 1995.

Title: Study of Variability In Pyridostigmine Inhibition of Blood Cholinesterases in Healthy Adults and Individuals With Symptoms Following Participation in Operation Desert Storm
Project #: DoD-21 Agency: DoD Study Location: WRAIR, Wash. DC
Project Status: Completed Research Type: Epidemiology Research
P.I.: Bhupendra Doctor, Ph.D. Research Focus: Pyridostigmine Bromide
Start Date (CY):1995 Est. Completion (CY):1997 Prevention

OVERALL PROJECT OBJECTIVE: Assess individual differences in blood cholinesterase regulation and response to pyridostigmine.

SPECIFIC AIMS: Determine if individuals have different sensitivities to inhibition of blood cholinesterases by pyridostigmine and/or differences in the rate of spontaneous reactivation of cholinesterases after pyridostigmine exposure. Determine if there is a difference in the cholinesterases of Gulf War veterans who took pyridostigmine and seen at the Gulf War Health Center compared to age and sex matched controls.

METHODOLOGY: Blood (in EDTA) is obtained from Gulf War veterans seen at the Gulf War Health Center at Walter Reed Army Medical Center and from age and sex matched controls. Blood samples were assigned code numbers when drawn in the Gulf War Health Center, and the code was broken for the first 40 samples only after results from the data analysis were complete. Estimated spontaneous reactivation times after in vitro pyridostigmine inhibition, red cell acetylcholinesterase activity, and plasma butyrylcholinesterase activity were compared in blood samples from 20 veterans of Operation Desert Storm and 20 control subjects, matched for sex and age. Determination of plasma butyrylcholinesterase phenotypes was done at the Eppley Institute, University of Nebraska, Omaha, NE.

EXPECTED PRODUCTS (MILESTONES): Complete initial studies of the first 40 volunteers by FY96; and from 100 volunteers by FY97.

STATUS/RESULTS TO DATE: In the initial study of 40 volunteers, 18 of the 20 veterans in the study (90%) presented to the WRAMC Comprehensive Clinical Evaluation Program with post-war symptoms. All Gulf War veterans indicated they had taken pyridostigmine as a pretreatment drug for nerve agent exposure; 15 of 20 recalled having side effects after taking pyridostigmine. Mean red cell lysate acetylcholinesterase for all subjects was 5.7 U/ml +/- 0.7; mean plasma butyrylcholinesterase was 4.9 U/ml +/- 1.1. Mean spontaneous reactivation time (t-1/2 at 2.5 microM pyridostigmine) for all subjects was 42.6 min +/-5.4; mean for veterans was 43.2 +/- 6.2; mean for controls was 42.1 min +/- 4.6. Statistical analysis of reactivation times (repeated measures ANOVA) revealed no statistically significant differences between controls and veterans. However, significant differences in reactivation times (p=0.01) were observed between males and females across both groups. Differences in cholinesterase activities between controls and veterans were not statistically significant, but a differences between sexes was again significantly different. The occurrence of the UU (homozygous, usual, wild type) and UA (heterozygous, usual, atypical) phenotypes of butylcholinesterase in this study population [95% (38 of 40) and 5% (2 of 40), respectively] is close to the expected frequency, 96% and 4%. All Gulf War veterans in the study had the homozygous, usual, wild-type (UU) allele. Symptoms exhibited by these individuals do not appear to be related to either altered ability of red cell acetylcholinesterase to be inhibited by pyridostigmine, to extended spontaneous reactivation time after pyridostigmine exposure, or to a genetic mutation in the butyrylcholinesterase phenotype. In the study of the remaining 60 volunteers, another 40 samples have been collected to date. The coding of these samples will not be examined until the remaining 20 blood samples have been analyzed.

PUBLICATIONS: In vitro pyridostigmine inhibition of red cell acetylcholinesterase: a comparison in Gulf war veterans and normal controls. 1996 Medical Defense Bioscience Review, May 1996, 1254-61. Baltimore, Maryland. Proceedings in press.

A comparison of blood cholinesterases, in vitro pyrdostigmine inhibition, and butyrylcholinesterase phenotypes in Gulf War veterans and controls. The Second Chemical and Biological Medical Treatment Symposium, July 1996, 359-64.

Title: Chronic Organophosphorus Exposure and Cognition

Project #: DoD-22 Agency: DoD Study Location: Med Coll of GA Res Inst., Augusta

Project Status: Ongoing Research Type: Basic Research

P.I.: Jerry J. Buccafusco, Research Focus: Environmental Toxicology

**Start Date (CY):1995 Est. Completion (CY):1998 Brain & Nervous System
Chemical Weapons**

OVERALL PROJECT OBJECTIVE: Evaluate the effects of low-level sub-chronic exposure to an organophosphorus cholinesterase inhibitor on normal cognitive function in animal models. The long term goals are to identify the underlying mechanisms of organic brain damage related to environmental toxins and to develop novel treatment strategies to improve memory/cognitive performance in affected patients.

SPECIFIC AIMS: This study will address four main questions: 1. Does low-level sub-chronic exposure to an organophosphorus (OP) cholinesterase inhibitor produce learning or memory deficits in rats and non-human primates trained to perform various memory tasks; and will prior exposure to atropine sensitize animals to OP agents? 2. Is memory impairment to an OP agent associated with alterations in the presynaptic regulation of cholinergic neurons in relevant brain regions in rats; and/or is there an alteration in the ability of the brain adrenergic system to regulate presynaptic cholinergic function? 3. Is memory impairment to an OP agent associated with alterations in the postsynaptic regulation of cholinergic or adrenergic receptors? Can these alterations be reflected in the genes encoding the various muscarinic or nicotinic receptor subtypes? 4. Can new therapies (reversible cholinesterase inhibitors, nicotinic agonists, adrenergic agonists) which improve memory deficits in Alzheimer's patients also improve cognitive function in rats or monkeys with OP-induced memory impairment?

METHODOLOGY: Protocol 1: Three groups of 12 rats each are administered 0.25, 0.5, or 1.0 mg/kg of DFP once daily over 2 weeks. The status of brain muscarinic receptors and blood cholinesterase is determined and 2 days after the last dose of DFP they will be examined for performance in the Morris water maze. Monkeys will be well-trained in the delayed matching-to-sample (DMTS) task. The highest dose of diisopropylfluorophosphate (DFP) which does not elicit overt signs of anticholinesterase toxicity or which will not decrease DMTS performance will be employed in the study. Protocol 2: Rats are administered a 14-day DFP regimen. Samples of regional brain tissue are taken on 4 occasions during treatment from randomly selected rats (6 per time point) to determine the status of choline acetyl transferase (ChAT) activity and for the estimation of ACh turnover rates. We examine the ability of clonidine to inhibit presynaptic cholinergic function using biochemical techniques. Finally, these experiments are repeated in rats subjected to atropine sensitization. Protocol 3: Rats are administered a 14-day DFP regimen. Samples of regional brain tissue are taken on four occasions during treatment to determine the status of muscarinic, nicotinic, and alpha-adrenergic receptor subtypes in several brain regions using quantitative receptor autoradiography. Small samples of tissue are removed for quantitative RT-PCR. Protocol 4: The monkeys which have been evaluated in Protocol 1 for production of long-term decrements in DMTS performance are employed. They receive increasing doses of drugs designed to enhance cognitive performance. The experiments occur concomitantly with a similar series performed in rats tested using the Morris water maze.

EXPECTED PRODUCTS (MILESTONES): This research will elucidate mechanisms of permanent memory dysfunction produced by exposure to a relevant organophosphorus agent DFP in non-human primate and rat models. Also, the effects of reversing this memory impairment using classical and novel agents developed for patients with Alzheimer's disease will be evaluated.

STATUS/RESULTS TO DATE: Chronic, low-level exposure to acetylcholinesterase (AChE) inhibitor organophosphate (OP) insecticides or chemical warfare agents produces abnormalities in CNS acetylcholine (ACh) function, and in humans, may be associated with impaired cognitive function well after withdrawal from such exposure. The purpose of the present study is to identify the severity of impairment in spatial learning of rats and monkeys following protracted withdrawal from chronic, low-level exposure to the OP agent DFP. Assessment of spatial learning began either 3 or 17 days after completion of a 14 day DFP treatment regimen (50, 250, or 500 mg/kg). During the 14 day treatment regimen, prior to withdrawal, spontaneous activity and olfactory behaviors were initially suppressed during DFP exposure, effects which became tolerant with repeated exposure to the 250

mg/kg dose regimen. Performance of a standard water maze task was impaired for up to 21 days after withdrawal from treatment with a 250 mg/kg dose of DFP. Latencies to find a hidden platform were increased by 30-40% above control levels throughout testing and no overt signs of DFP toxicity were evident. All animals did, however, demonstrate the ability to eventually learn the task. AChE activity in the frontal cortex and hippocampus was suppressed to 41% and 51% of control levels, respectively, after 14 days of DFP (250 mg/kg) treatment. By 7 days after withdrawal from treatment, frontal cortex AChE activity had recovered to 84% of control levels, whereas hippocampal AChE activity had recovered to only 64.40%. By 21 days after withdrawal from treatment, AChE in both brain regions had recovered to near-control levels. Performance of a previously well-learned delayed matching task by monkeys was not affected by DFP regimens after withdrawal of the drug. Chronic, low-level OP exposure, therefore, produces protracted impairment of working memory after drug withdrawal that is not associated with continued suppression of AChE activity. This impairment may be attributed to the decrease in recovery of AChE in the hippocampus, relative to the cortex. This decreased rate of enzyme recovery may contribute to hippocampal toxicity underlying protracted impairment of working memory. In 3 rhesus monkeys, well trained in the performance of a delayed response task, chronic daily exposure to 10-20 mg/kg of DFP produced severe toxicity and task impairment after 8 weeks of treatment. However, task performance was at near control levels by 6-10 days after DFP withdrawal. Withdrawal from chronic exposure to low levels of DFP may compromise working memory or learning of a new task, but fails to inhibit the performance of a well learned task if no toxicity is present.

PUBLICATIONS: Gattu M, Boss K, Terry A, Buccafusco J. Reversal of Scopolamine-Induced Deficits in Navigational Memory Performance by the Seed Oil of *Celastrus paniculatus*, 1997, (57):4:793-799.

Prendergast M, Terry A, Buccafusco J. Chronic, low-level exposure to diisopropylfluorophosphate causes protracted impairment of spatial navigation learning. *Psychopharmacology*, 1997, 129:183-191.

Title: Acute and Long-Term Impact of Deployment to Southwest Asia on the Physical and Mental Health of Soldiers and their Families

Project #: DoD-23

Agency: DoD

Study Location: WRAIR, Wash. DC

Project Status: Ongoing

Research Type: Epidemiology Research

P.I.: MAJ John Stuart

Research Focus: Brain & Nervous System

Start Date (CY):1993 Est. Completion (CY):1998

Symptoms/General Health

OVERALL PROJECT OBJECTIVE: Determine the acute and long-term impact of deployment to SWA on the physical and mental health of soldiers and their families.

SPECIFIC AIMS: Determine the acute and long-term impact of deployment to SWA on the physical and mental health of soldiers organizational, and environmental factors within military units that produce psychiatric casualties. Identify the critical indicators resulting in increased risk and assess the long-term consequences of exposure to trauma. Develop recommendations on post-deployment interventions. Determine impacts of Army family support policies and programs on active duty and reserve component soldiers and their families.

METHODOLOGY: Interviews and surveys of soldiers and leaders participating in ODS were conducted several times prior to the ground war, immediately after the ground war, and at intervals following return home. Soldiers and their spouses were surveyed and interviewed in order to evaluate the social, psychological, organizational, and environmental factors within military units that produce psychiatric casualties, lead to dysfunctional behavior and decrements in military performance, generate psychomatic illness and increased susceptibility to physical illness. The family-community research team performed field surveys of spouses of active duty and reserve component (USAR) and (ARNG) soldiers from units deployed to the Persian Gulf for ODS on deployment-related stressors and psychological symptoms, spouse support-seeking behavior, family perceptions of Army support and assistance, stress-coping responses, and family well-being during reunion with returning soldiers and in the aftermath of ODS. Surveys and interviews of selected Reserve and National Guard units and their families have been conducted during the period following ODS, while additional interviews and surveys were conducted with soldiers and families participating in Operation Just Cause and Sinai peacekeeping deployments.

EXPECTED PRODUCTS (MILESTONES): Develop recommendations affecting post-combat interventions. Determine the impact of Army family support policies and programs on both active duty and research component soldiers and families.

STATUS/RESULTS TO DATE: An assessment of psychological factors related to deployment stress and family separation during Operation Desert Shield/Storm was conducted with a stratified sample of married and single U.S. Army Individual Ready Reserve soldiers two years post ODS. Analyses were performed in 1994 with final report completed in spring of 1995 and submitted to Army Deputy Chief of Staff for Personnel. Deployment stress and adaptation was studied on a large sample of U.S. Army National Guard and U.S. Army Reserve Unit soldiers who activated and deployed for Operation Desert Shield/Storm. Physiological stress, distress, coping and factors related to current deployed effects of family separation were assessed. Physiological well-being was studied with respect to deployment location factors. Persian Gulf Region analyses were performed in summer of 1995 and resulted in a manuscript on the long term effects of Desert Storm on the Army Reserve and National Guard soldiers and their families.

PUBLICATIONS: Epidemiological considerations regarding the health and effectiveness of women in the armed forces. Technical Report, AD#ADA278202.

Combat service support survey results: A light infantry division and a mechanized infantry division. Technical Report AD#ADA278203.

The Long Term Effects of Operation Desert Storm on the Psychological Well-Being of US Army Reserve and National Guard Veterans. Manuscript under review by editors of J Applied Social Psychology.

Title: Epidemiological Studies Persian Gulf War Illnesses, PG Women's Health Linkage Study
Project #: DoD-30 Agency: DoD Study Location: Klemm Analyses Group, Wash DC.
Project Status: Ongoing Research Type: Epidemiology Research
P.I.: Rebecca J. Klemm, Research Focus: Symptoms/General Health
Start Date (CY):1996 Est. Completion (CY):1999 Reproductive Health

OVERALL PROJECT OBJECTIVE: Compare the incidence, prevalence, general health outcomes, and risks of women deployed in the Gulf War theater of operations with Gulf War era women who were not deployed to the Gulf.

SPECIFIC AIMS: The primary focus of this project is to link Persian Gulf exposures to subsequent physical and mental health outcomes.

METHODOLOGY: Self-reported survey information from a sample of 20,000 veterans will be linked to DoD tracking files and other mapping files and medical records.

EXPECTED PRODUCTS (MILESTONES): The survey will be fielded in early 1998. Medical record followup will begin during spring 1998. Data analysis will occur in the summer of 1998.

STATUS/RESULTS TO DATE: Data which is necessary to start the pilot study is being obtained from the Defense Manpower Data Center.

PUBLICATIONS: Klemm RJ. The Persian Gulf Health Linkage Study: a longitudinal study designed to assess short-term and long-term effects of Gulf War Service. American Public Health Association (APHA) annual meeting, November 1997.

Title: Dysregulation of the Stress Response in the Persian Gulf Syndrome

Project #: DoD-31 Agency: DoD Study Location: Georgetown Univ & VAMC Wash DC
Project Status: Ongoing Research Type: Clinical Research
P.I.: Daniel Clauw, M.D. Research Focus: Brain & Nervous System
Start Date (CY):1996 Est. Completion (CY):1999 Symptoms/General Health

OVERALL PROJECT OBJECTIVE: Test the hypothesis that Gulf War veterans who report persistent unexplained symptoms do not represent a discrete illness but instead fall within a continuum of stress-related syndromes commonly seen in the general U.S. population, including fibromyalgia (FM), chronic fatigue syndrome (CFS), multiple chemical sensitivity (MCS), and somatoform disorder (SD).

SPECIFIC AIMS: Specific hypotheses to be tested include: (1) Gulf War veterans with unexplained symptoms display centrally mediated disturbances in autonomic tone, and this leads to vasomotor instability and smooth muscle dysmotility, and symptoms such as irritable bowel syndromes and migraine headaches, (2) these same individuals display diffuse disturbances in nociception (pain threshold) that are partly responsible for many of the pain-related symptoms seen in this condition such as muscle and joint pain and persistent sore throat,(3) the same neuroendocrine changes seen in FM, CFS, and post-traumatic stress disorder (PTSD) which are characterized by blunted responses in the hypothalamic-pituitary axis, are seen in these Gulf War veterans and may also be associated with fatigue symptoms.

METHODS: This study is a collaborative effort between Georgetown University Medical Center and the Washington D.C. Veterans Administration Medical Center (VAMC). The results of neurophysiological and neuropsychological testing will be compared between a group of Gulf War veterans and a group of individuals with FM/CFS. Participants are admitted to the Clinical Research Center at Georgetown for two days following their routine admission to the VAMC, and undergo a series of studies to evaluate physiologic and biochemical parameters. The physiologic studies measure qualitative and quantitative aspects of symptoms, including specialized testing of peripheral and visceral nociception, and smooth muscle motility. Biochemical analyses include evaluation of multiple indices of autonomic function, including neurohormone levels at baseline and after standardized stressors, and cerebral spinal fluid levels of neurotransmitters.

EXPECTED PRODUCTS (MILESTONES): A better understanding of common underlying pathophysiologic mechanisms in fibromyalgia, chronic fatigue syndrome, multiple chemical sensitivity and the undiagnosed illnesses of Gulf War veterans should lead to both more effective treatment of Gulf War veterans and produce effective strategies to avoid these problems in future deployments.

STATUS/RESULTS TO DATE: Six Gulf War veterans have been studied; nearly all of the comparison group patients with FM/CFS and healthy normal controls have been recruited and the data analyzed. These data reveal differences between patients and controls, which indicates that the hypotheses about Gulf War veterans will be testable. Recent efforts have been undertaken to enhance subject recruitment, such as more frequent communication with the VAMC and attempts to accommodate the Gulf War veteran patients during their off-days from the VAMC. Subject recruitment has fallen short of the original plan but the investigators are hopeful that their intensified recruitment efforts will increase subject recruitment in the second year of the study. Sample size is still too small to report any preliminary results.

PUBLICATIONS: none to date

Title: Neuropsychological Functioning in Persian Gulf Era Veterans

Project #: DoD-32 Agency: DoD Study Location: VAMC Boston

Project Status: Ongoing Research Type: Clinical Research

P.I.: Roberta White, Ph.D. Research Focus: Brain & Nervous System

Start Date (CY): 1996 Est. Completion (CY): 1999 Diagnosis

OVERALL PROJECT OBJECTIVE: To evaluate the neuropsychological functioning of Persian Gulf War (PGW)-era veterans who are seeking treatment or diagnostic evaluation for any type of health or adjustment complaint. The group of patients who were deployed to the Gulf are being compared to a treatment seeking patient group who were not deployed to the Gulf. Data from these groups are also being compared with those from a (non-treatment seeking) research sample of PGW veterans who are being studied using the same neuropsychological instruments. In addition, all of these patients and research subjects are being administered a standardized set of questionnaires and semi-structured interviews that will identify their current health symptoms, pre-existing physical and mental health condition, sociodemographic variables, Gulf experiences, and Post PGW-era stressors. These instruments also permit post-traumatic stress disorder (PTSD), multiple chemical sensitivity (MCS), chronic fatigue syndrome (CFS), and other psychiatric disorders to be diagnosed. **SPECIFIC AIMS:** The specific aim of this study is to determine whether veterans of the Persian Gulf War (PGW) show cognitive impairments suggestive of central nervous system damage at a greater rate than PGW-era veterans who were not deployed to the Gulf.

METHODOLOGY: Two hundred subjects will be examined in three years; 100 veterans deployed to the Gulf during the PGW and 100 serving in the military during that era who were not deployed to the Gulf. These subjects will be recruited from among those seeking treatment or diagnostic evaluation at the Boston VAMC through all outpatient treatment services. It is anticipated that approximately 40 subjects in each group will be recruited in each of years 1 and 2, and 20 in each group in year 3 of the proposed period of support. The recruitment procedure has been fully outlined. It has been devised to reduce selection bias that would reduce the comparability of Gulf-deployed and non-deployed veterans.

a. **Subjects.** All treatment seeking veterans discharged from the military since the beginning of the PGW will be considered potential subjects. A list of veterans who have been admitted to any outpatient clinical service (with the exclusion of the substance abuse clinic) at the Boston VAMC in Jamaica Plain, the outpatient clinic at Causeway Street, and the Lowell VAMC will be generated by the supervisor of outpatient clinics and will be updated periodically. This list will include names, social security numbers, and telephone numbers (where available) or addresses (if telephone numbers are not available). As a preliminary step, the lists will be cross-checked with the Persian Gulf War Registry to determine the approximate proportions of the PGW veterans and non-deployed PGW-era veterans available. However, the personnel involved in the recruitment of subjects will not be aware of the status of potential subjects with regard to deployment to the Gulf and steps will be taken to reduce the likelihood that this information will become known to project staff during the recruitment process.

b. **Initial Contact.** The first contact with potential subjects will be by telephone (or by mail, if there is no telephone number available). At the beginning of this contact, subjects will be requested to not indicate whether or not they served in the Gulf. They will be told that, if they should decide to participate in the research project, information concerning deployment will be obtained at a later time. The initial contact will consist of a description of the project, including types of assessment, time required, and financial compensation. Subjects will have an opportunity to ask questions about the procedure. They will be informed that whether or not they participate will have no bearing on their medical care and that, if they choose to participate, that they may withdraw at any time without prejudice. They will be asked to indicate whether they wish to participate, wish not to participate, or wish to defer this decision. An appointment at the Boston VAMC will be scheduled for patients agreeing to participate.

c. **Methods.** Patients will first be seen for a brief screening interview and patients retained in the study sample will be presented the study consent form for signature. Subjects will complete a battery of neuropsychological tests, a set of questionnaires, and 2 semi-structured interviews (one will encompass their current health and past environmental exposure history; the other will be done to

determine psychiatric and/or PTSD diagnoses).

EXPECTED PRODUCTS (MILESTONES): To isolate factors that may differentiate those who served in the Gulf who are seeking treatment for health complaints from their treatment-seeking counterparts who were not deployed to the Gulf. This is a step toward understanding the Gulf War Syndrome.

STATUS/RESULTS TO DATE: Recruitment for this study is fully underway. To date, over 100 subjects have participated in the study. We expect to complete data collection in the Summer of 1998. Data entry and management are on-going.

PUBLICATIONS: none to date

Title: Effects of Pyridostigmine in Flinders Line Rats Differing in Cholinergic Sensitivity
Project #: DoD-33 Agency: DoD Study Location: Univ. NC, Chapel Hill
Project Status: Ongoing Research Type: Basic Research
P.I.: David H. Overstreet, Research Focus: Pyridostigmine Bromide
Start Date (CY): 1996 Est. Completion (CY): 1998 Prevention

OVERALL PROJECT OBJECTIVE: Test the hypothesis that a genetically based cholinergic supersensitivity might underlie the increased sensitivity of vulnerable human populations, similar to the condition labeled Multiple Chemical Sensitivity (MCS).

SPECIFIC AIMS: Determine strain-dependent effects of acute and chronically administered pyridostigmine. Determine whether chronic pyridostigmine will protect agonists effects of other anticholinesterase agents. This will be accomplished by developing an animal model with cholinergic supersensitivity which is also more sensitive to a variety of drugs and other chemical agents.

METHODOLOGY: Rats will be treated with pyridostigmine acutely or chronically and growth hormone, body temperature and locomotor activity will be assessed. The Flinders Line rats developed at Flinders University in Australia by selective breeding for differential responses to the anticholinesterase, diisopropyl fluorophosphate (DFP). These rats have been shown to be more sensitive to directly acting muscarinic agonists, as well as a variety of other drugs including alcohol, diazepam, and nicotine. The heightened sensitivity of this special line of rats to a variety of drugs suggests that they will also be more sensitive to the effects of pyridostigmine, an anticholinesterase drug which was given to soldiers during ODS.

EXPECTED PRODUCTS/MILESTONES: Ultimately, this may lead to the development of challenge test to predict cholinergic sensitivity in soldiers.

STATUS/RESULTS TO DATE: The results of initial experiments indicate that there are no line differences in telemetrically monitored hypothermia or general activity after pyridostigmine (4, 12, or 36 mg/kg, orally administered), even though there were substantial differences in hypothermia induced by the muscarinic agonist, oxotremorine. These negative findings were expected because pyridostigmine does not exert central cholinergic effects necessary to induce these changes.

Growth hormone concentrations, the parameter most likely to change after pyridostigmine treatment, is still being analyzed.

PUBLICATIONS: Overstreet DH, Rezvani AH, Yang Y, Hamed H, Janowsky DS. Animal model of chemical sensitivity involving cholinergic agents. Presented at Toxicology in Risk Assessment Symposium held in Bethesda, MD, 1997.

Overstreet DH, Yang Y, Hamed M, Janowsky DS, Rezvani AH. Strain- and Gender-dependent effects of oxotremorine and pyridostigmine. Presented at Society for Neuroscience, New Orleans, 1997.

Title: Characterization of Emissions from Heaters Burning Leaded Diesel Fuel in Unvented Tents

Project #: DoD-34 Agency: DoD Study Location: USDept. Energy, Albuquerque, NM
Project Status: Ongoing Research Type: Applied Research
P.I.: Hsu-Chi Yeh, Ph.D. Research Focus: Environmental Toxicology
Start Date (CY):1996 Est. Completion (CY):1998 Interactions

OVERALL PROJECT OBJECTIVE: Estimate the exposure to in-tent pollutants during the Gulf War deployment by simulation of human exposure to aerosols in unvented tents heated by leaded diesel fuel.

SPECIFIC AIMS: Perform physical and chemical characterization of aerosols produced by heaters that burned leaded diesel fuel in an unvented tent, and estimate exposure to lead, combustion gases (such as CO₂, CO, NO_x, and SO₂), and organic compounds (such as PAHs).

METHODOLOGY: Using Army tents and heaters, four heaters will be evaluated for variability. Aerosols and vapors will be characterized physically and chemically using a Tenax sampler (vapors), the Lovelace Multi-jet Cascade Impactor/Parallel Flow Diffusion Battery (aerosol size distribution, concentration, and chemical composition vs. particle sizes), an electrostatic precipitator sampler (particle morphology), and filter samples (aerosol concentration). These samples will provide chemical compositions, lead content, time-averaged aerosol size distribution, and concentration. Real-time aerosol size distribution and concentration as a function of time will be obtained using a Quartz Crystal Microbalance or a Differential Mobility Particle Sizer.

EXPECTED PRODUCTS/MILESTONES: This research will provide detailed information on pollutants produced in unvented tents from heaters that burn leaded diesel fuels. This information is needed to assess potential exposure of service personnel who served in the Persian Gulf War.

STATUS/RESULTS TO DATE: The investigators performed an extensive review of the appropriate materiel (tents, heaters, and fuels) in order to provide the most accurate simulation of conditions present during the Gulf War, including extensive communications with materiel developers at Natick and Fort Belvoir. An Army GP-medium tent and several tent heaters and fuels have been obtained. Several studies have been conducted using SF-6 trace gas methods to determine critical study parameters including the air exchange rate of the tent and the tent volume. The experimental protocol has been approved and preparations are underway for gas analyses.

PUBLICATIONS: none to date

Title: Feasibility of Investigating Whether There is a Relationship Between Birth Defects and Service in the Gulf War.

Project #: DoD-35 Agency: DoD Study Location: March of Dimes, Sacramento, CA

Project Status: Ongoing Research Type: Epidemiology Research

P.I.: John A. Harris, M.D. Research Focus: Reproductive Health

Start Date (CY):1996 Est. Completion (CY):1998

OVERALL PROJECT OBJECTIVE: Conduct a feasibility study to determine if Reserve participants in the Persian Gulf War can be linked to vital statistics and Birth Defects Registry data.

SPECIFIC AIMS: Determine if DoD computerized information is accurate compared to the hospital medical records and determine the feasibility of investigating birth defects in the children of Gulf War veterans.

METHODOLOGY: Information will be obtained from DOD on all children born to active duty Gulf War veterans posted to or stationed in California between May 1991 and May 1994. From this, a random sample of 500 births per year between 1991 and 1994 (approximately 20% of births to active duty military personnel) will be drawn and an attempt will be made to locate their hospital medical records. All records will be reviewed and abstracted for data on congenital abnormalities using criteria established by the California Birth Defects Monitoring Program (CBDMP). This information will be compared to current DOD computerized information about offspring of active Gulf War veterans. An additional objective will be to link California birth certificates and CBDMP files.

EXPECTED PRODUCTS (MILESTONES): The review of an estimated total 1500 birth records is expected to yield 2.8% or 42 cases with structural congenital abnormalities. This number will be adequate to quantitatively estimate the concurrence with DOD computerized information on congenital abnormalities.

STATUS/RESULTS TO DATE: The investigators have just received approval from the Army Human Subjects Research Review Board, permitting them to begin the study after a protracted negotiation concerning patient confidentiality concerns.

PUBLICATIONS: none to date

Title: Fatigue in Persian Gulf Syndrome-Physiologic Mechanisms

Project #: DoD-36 Agency: DoD Study Location: Univ. TX, SW

Project Status: Ongoing Research Type: Clinical Research

P.I.: Ronald G. Haller, M.D. Research Focus: Brain & Nervous System

Start Date (CY): 1996 Est. Completion (CY): 1998 Symptoms/General Health

OVERALL PROJECT OBJECTIVE: Test the hypothesis that mechanisms used by muscle cells to use oxygen and produce energy for work may be impaired in patients suffering abnormal muscle fatigue and the inability to exercise.

SPECIFIC AIMS: On the basis of preliminary results of evaluation of symptoms of abnormal fatigability in Gulf War veterans, the researchers hypothesize that the common complaint of abnormal fatigue and exercise intolerance in these patients is attributable to impaired energy production via oxidative phosphorylation. Under this general hypothesis, the researcher will address four specific questions: (1) Is there an abnormality in muscle oxygen utilization or oxygen transport to muscle during exercise in affected individuals? (2) Is there exaggerated metabolic muscle fatigue in exercise consistent with impaired energy production? (3) Is the metabolic and physiologic response to aerobic physical conditioning impaired in these patients? (4) Is there a specific pattern of impaired activities of mitochondrial oxidative enzymes or respiratory chain complexes to account for impaired oxidative metabolism on attenuated increases in oxidative capacity in response to physical training.

METHODOLOGY: Exercise protocols and non-invasive monitors of oxygen transport and utilization as well as detailed muscle biochemistry will be used to identify specific causes of exercise intolerance in patients. The investigators will employ forearm and cycle exercise to determine maximal work and oxidative capacity and to compare fatigue and metabolic responses to similar workloads among patients and age- and weight-matched sedentary control subjects. The study will compare muscle metabolic and physiologic responses to aerobic training in patients and matched control subjects. The oxidative mechanism in blood will be monitored by measurements of blood levels of diffusible metabolites that reflect oxidative capacity; measurement of intramuscular metabolites using ³¹-phosphorus magnetic resonance spectroscopy; and by using infrared spectroscopy.

EXPECTED PRODUCTS (MILESTONES): This study will evaluate the hypothesis that oxidative limitations detected with non-invasive testing are attributable to impaired function of the mitochondrial respiratory chain as assessed biochemically in biopsied muscle.

STATUS/RESULTS TO DATE: none reported.

PUBLICATIONS: none to date

Title: Neurobehavioral and Immunological Toxicity of Pyridostigmine, Permethrin, and DEET in Male and Female Rats

Project #: DoD-37

Agency: DoD

Study Location: Univ. FL, Gainesville

Project Status: Ongoing

Research Type: Basic Research

P.I.: Frans van Haaren, Ph.D.

Research Focus: Interactions

Start Date (CY):1996 Est. Completion (CY):1999

**Pyridostigmine Bromide
Brain & Nervous System**

OVERALL PROJECT OBJECTIVE: Determine if neurobehavioral toxicity and immune response alterations are produced by small doses of medical pretreatments and insecticides, alone or in combination, in male and female rats.

SPECIFIC AIMS: This study will test the hypothesis that administration of pyridostigmine bromide (PB), permethrin (PERM) and DEET as single agents, or in combination, results in neurobehavioral toxicity and an altered immune response. It will also test the hypothesis that females are significantly more sensitive to the effects of these agents than are males.

METHODOLOGY: Chemical analyses will determine (1) the metabolism and elimination of PB, permethrin and DEET, alone and in combination, for male and female subjects who participated in the neurobehavioral experiments, and (2) how PB, permethrin and DEET, alone and in combination, affect cholinesterase activity in these same subjects. This information will allow the correlation of plasma concentrations of PB, permethrin and DEET with the behavioral observations. Experiments have been designed to measure various aspects of CNS functioning in the presence of sub-toxic doses of PB, PERM, and DEET in adult male and female rats. Doses for PB will be 3/10/30 mg/kg, for PERM will be 15/30/60 mg/kg and for DEET will be 50/200/500 mg/kg. In addition, plasma concentrations of PB, PERM, and DEET will be determined and their effects on cholinesterase activity will be correlated with CNS functioning. The neurobehavioral analyses will be complemented by an assessment of the immune response in the same subjects, as well as in lymphocytes from health human volunteers. The immunological experiments will be conducted to determine the role of PB, permethrin and DEET and (1) Suppression of the first and second signal transduction pathways in T-lymphocytes, (2) Cytokine expression, and (3) B-lymphocyte function. The neurobehavioral test battery is designed to measure critical aspects of CNS functioning related to symptoms of generalized fatigue, gastro-intestinal disturbance, muscle and joint pain, headaches, and memory loss. The neurobehavioral test battery includes an assessment of spontaneous locomotor behavior and an analysis of overall CNS functioning, motivation and memory performance.

EXPECTED PRODUCTS (MILESTONES): This study extends observations from previous experiments in male rats exposed to toxic doses of pyridostigmine or permethrin to female rats and to the effects of PB, permethrin and/or DEET in combination. This study will also provide additional information on the motivational and memory aspects of CNS functioning.

STATUS/RESULTS TO DATE: none reported.

PUBLICATIONS: none to date

Title: Diagnostic Antigens of Leishmania tropica

Project #: DoD-38 Agency: DoD Study Location: Inf. Dis. Res. Instit., Seattle, WA

Project Status: Ongoing Research Type: Applied Research

P.I.: Steven G. Reed, Ph.D. Research Focus: Leishmaniasis

Start Date (CY):1996 Est. Completion (CY):1998 Diagnosis

OVERALL PROJECT OBJECTIVE: Develop a sensitive method to detect infection with *Leishmania tropica*, or related species, in military personnel.

SPECIFIC AIMS: There are currently no defined antigens for use in the diagnosis of leishmaniasis. There is an urgent need for sensitive methods to detect infection with *Leishmania tropica*, or related species, in military personnel. The researchers have developed such tests for other *Leishmania* species, and have isolated and characterized *L. tropica* antigens reactive with sera from *L. tropica* infected Gulf War veterans. This study will attempt to develop similar tests for *L. tropica* using these recombinant *L. tropica* antigens. Effort will also be directed at cloning additional *L. tropica* antigens reactive with *L. tropica* infection sera and antigens that stimulate T-cells from individuals with *L. tropica* infection.

METHODOLOGY: Emphasis will be given to expressed genes which are highly sensitive. Inserts will be sequenced for determination of epitopes which may be produced synthetically. Recombinant or synthetic peptides will be evaluated by ELISA for their ability to detect antibody in patient sera. In related studies, cloned gene products will be evaluated for their ability to elicit patient T-cell response in vitro. These evaluations will include proliferation and cytokine production. Emphasis will be placed on finding antigens which are shared between *Leishmania* species having the ability to elicit strong T-cell responses. Such antigens will be candidates for skin test antigens and vaccine production.

EXPECTED PRODUCTS (MILESTONES):

STATUS/RESULTS TO DATE: Several new peptides of Lt-1 antigen have been made and evaluated them as serological reagents. Also, screening of *L. tropica* genomic libraries for new antigens have begun.

PUBLICATIONS: none to date

Title: A Controlled Epidemiological and Clinical Study into the Effect of Gulf War Service on Servicemen and Women of the United Kingdom Armed Forces

Project #: DoD-39 Agency: DoD Study Location: King's Coll., UK

Project Status: Ongoing Research Type: Epidemiology Research

P.I.: Simon Wessely, M.D. Research Focus: Symptoms/General Health

Start Date (CY):1996 Est. Completion (CY):1999

OVERALL PROJECT OBJECTIVE: Assess the prevalence of unexplained illnesses, including chronic fatigue-like symptoms, in members of the United Kingdom Armed Forces who were deployed to the Persian Gulf during the Gulf War and who have served, and are serving in Bosnia.

SPECIFIC AIMS: This study is a two stage cohort study. Stage 1 consists of a postal health screening questionnaire to be sent to three groups who were/are in the Armed Forces; those who served in the Persian Gulf, those who served in Bosnia, and those who served in neither the Persian Gulf or Bosnia. The second stage involves performing neuropsychological tests on cases identified by stage 1 and a control group.

METHODOLOGY: This epidemiological study of the prevalence of unexplained illnesses in the populations at risk will use a two-stage design. Stage 1 will be a questionnaire survey of 3000 Gulf War veterans selected at random, an equivalent sample of Bosnia veterans, and appropriate control groups for each. Stage II will involve interview, examination, and testing of all those (approximately 10%) in Stage I who fall above a cutoff defining subjective health. Information gathered at Stage II will be used to estimate the prevalence of diagnosed and unexplained morbidity, including chronic fatigue symptoms, in UK service personnel, and to calculate whether there is an excess associated with Gulf War and/or Bosnia service. If there is, then the researchers will be able to examine pre-morbid and psychosocial factors which may be implicated in such an increase, as well as identify avenues for further biological and psychosocial research.

EXPECTED PRODUCTS (MILESTONES): Reports and publications. This epidemiological study should ascertain if service in the Gulf War by UK armed forces personnel was associated with an increase in physical and/or psychological morbidity, and if so, if there is evidence of an increase in either new or ill-defined conditions such as chronic fatigue syndrome, or an illness peculiar to Gulf War service.

STATUS/RESULTS TO DATE: The questionnaire has been devised and a detailed pilot study has been undertaken, with resulting revisions to the questionnaire. The main body of the study is currently in progress with 12,750 questionnaires being sent to currently serving and ex military personnel. Tracing procedures have been extensively investigated and algorithms devised. This research effort is currently being coordinated with a new initiative by the British government (since this grant was awarded) to ensure that all UK research into Gulf War Illnesses proceeds in an integrated fashion.

PUBLICATIONS: none to date

Title: Psychological and Neurobiological Consequences of the Gulf War Experience
Project #: DoD-40 Agency: DoD Study Location: VAMC West Haven, CT
Project Status: Ongoing Research Type: Clinical Research
P.I.: Steven Southwick, M.D. Research Focus: Brain & Nervous System
Start Date (CY):1998 Est. Completion (CY):2001 Symptoms/General Health

OVERALL PROJECT OBJECTIVE: Determine the nature of memory for traumatic events as they relate to PTSD symptoms.

SPECIFIC AIMS: To examine the course of memory for traumatic events over time and the brain areas believed to be altered due to trauma.

METHODOLOGY: Longitudinal follow-up of a cohort of veterans of Operation Desert Storm (e.g. questionnaires, ratings scales). MRI study of 30 Desert Storm veterans with PTSD. This research will continue to follow the course of symptoms of Post-Traumatic Stress Disorder in a population of Gulf War veterans. This study of how such symptoms, as well as memory function, change over time in the veterans will lead to a better understanding of PTSD and the elements of risk that would cause and continue PTSD symptoms over time. The study is in two parts. The first part is a longitudinal descriptive study of trauma-related symptomatology in Gulf War veterans. The second part is an investigation of memory function and hippocampal volume in Gulf War veterans who meet criteria for posttraumatic stress disorder (PTSD). Results of the researchers' previous work with a cohort of Gulf War veterans indicated that there was an overall increase in PTSD symptomatology in the veterans over the first two years following the Gulf War. With regard to memory testing, there were many instances of inconsistent recall for events that were objective and highly traumatic in nature. The data do not support the position that traumatic memories are fixed or indelible and suggest that as PTSD symptomatology increases, so does amplification of memory for traumatic events. This study continues the original methodology for the fifth, sixth and seventh post-war years. It is anticipated that a high percentage of subjects who have already participated in this research can be recruited to continue in this project and that at least 100 new subjects can also be recruited. It is anticipated that a better understanding of the longitudinal course of trauma-related symptomatology and risk factors for the development and maintenance of these symptoms would have implications for treatment. The second part of the study will examine the possibility that memory deficits in the subject population are relatively broad-based and reflect problems at several levels of information processing, including acquisition, retention and/or retrieval and that these memory deficits may be related to decreased hippocampal volume. Results of this study would make it possible to determine whether these abnormalities are present in a variety of combat populations and whether such abnormalities can be detected at earlier stages than in the previously-studied Vietnam veteran population. Potential relationship to clinical symptomatology and psychosocial functioning will also be investigated.

EXPECTED PRODUCTS/MILESTONES: The investigators expect to show that memory for traumatic events is not consistent over time, and that there may be associated decrease in hippocampal volume in PTSD.

STATUS/RESULTS TO DATE: none reported.

PUBLICATIONS: none to date

Title: Evaluation of Muscle Function in Persian Gulf Veterans

Project #: DoD-41 Agency: DoD Study Location: Univ. PA

Project Status: Ongoing Research Type: Clinical Research

P.I.: Krista Vandenberg, Ph.D. Research Focus: Brain & Nervous System

Start Date (CY): 1996 Est. Completion (CY): 1999 Symptoms/General Health

OVERALL PROJECT OBJECTIVE: Investigate the etiology of ongoing chronic fatigue and muscle weakness in Gulf War veterans with unexplained illnesses by evaluating abnormalities in skeletal muscle function.

SPECIFIC AIMS: Evaluate skeletal muscle in Persian Gulf veterans with severe chronic fatigue and Persian Gulf veterans with no medical complaints (healthy controls); determine the relationship between the severity of chronic fatigue in Persian Gulf veterans and the degree of muscle dysfunction; and compare muscle function in Persian Gulf veterans with severe chronic fatigue with muscle function in healthy sedentary civilians and civilians with chronic fatigue.

METHODOLOGY: The goal of this proposal is to evaluate skeletal muscle in Persian Gulf veterans. Our major hypothesis is that Persian Gulf veterans who present a symptom profile consistent with chronic fatigue suffer from muscle dysfunctioning. In addition, we hypothesize that the severity of chronic fatigue in this population is related to the degree of muscle dysfunctioning. To test these hypotheses, a battery of tests will be performed on Persian Gulf veterans with severe chronic fatigue (n=50) and on Persian Gulf veterans who were deployed but who have no medical problems (n=50). The evaluation will consist of the following measurements: 1. Functional assessment as reflected in isometric and isokinetic measures; 2. In vivo metabolic measures as determined by ³¹P-Magnetic Resonance spectroscopy (MRS); 3. In vivo morphological measures as determined via Magnetic Resonance Imaging (MRI); 4. Histological analyses of muscle biopsies; 5. Electromyographic evaluation of motor unit recruitment; 6. Muscle enzyme levels; 7. Genetic screening.

EXPECTED PROJECTS (MILESTONES): This project runs over three years. The first 6 months serve to recruit the subjects and to train the necessary personnel. The following two years will be used to collect all the data at a rate of approximately 1 subject/week (at the end of the first year 13 Persian Gulf veterans with chronic fatigue and 13 healthy veterans will have been tested). At the end of the second year, 24 more subjects with chronic fatigue and 24 healthy controls will have been tested. For the first 6 months of the third year the final 13 veterans with chronic fatigue and 13 controls will be tested. Data collection will be completed at the end of 2.5 years. The final 6 months of this 3 year period will be used to analyze and interpret the data.

STATUS/RESULTS TO DATE: In the process of acquiring data.

PUBLICATIONS: Vandenberg K, Walter G, Lenrow D, Leigh JS, Fishman AP. Skeletal muscle in Persian Gulf veterans. J Chronic Fatigue Syndrome, 1996, 2(2/3):137-138.

Title: The Symptomatic Persian Gulf Veterans Protocol: An Analysis of Risk Factors with an Immunologic and Neuropsychiatric Assessment

Project #: DoD-42 Agency: DoD Study Location: VAMC Birmingham

Project Status: Ongoing Research Type: Clinical Research

P.I.: Warren Blackburn, Jr. M.D. Research Focus: Symptoms/General Health

**Start Date (CY):1996 Est. Completion (CY):2000 Immunology
Brain & Nervous System**

OVERALL PROJECT OBJECTIVE: Determine if there are fundamental neuropsychiatric or immunologic abnormalities in Gulf War veterans which might be associated in a case control study with their service in the Gulf.

SPECIFIC AIMS: The specific goals of this proposal are to: 1) evaluate self-reported conditions in Persian Gulf veterans to determine if they represent described and accepted medical conditions and/or are associated with objective findings which would validate these symptoms; 2) evaluate symptomatic Gulf war veterans to determine risk factors which may identify veterans who have reported symptoms; and, 3) evaluate in a controlled fashion symptomatic, but undiagnosed, Persian Gulf war veterans to determine if there is a definable immunologic or neuro-psychiatric abnormality.

METHODOLOGY: This study will determine whether specific immunological or neuropsychiatric abnormalities can be detected in GW participants who are symptomatic, but in whom no specific diagnosis has as yet been made. These studies may uncover an immunological basis for some of the broad types of symptoms reported by GW veterans. This proposal attempts to get at the heart of the problem cited in the Institute of Medicine (IOM) report, that GW veterans' mystery illnesses may not be a single illness and that it is important to study various possible causes and the interactions between causes. The Birmingham VAMC has been very active in evaluating patients returning from the Persian Gulf war. Approximately 1400 individuals have been seen and have had evaluations. Of these, about 10-15% are asymptomatic. Of the remaining 85%, approximately 90% have a diagnosable illness. A number of Persian Gulf veterans, which are conservatively estimated as 100 of those seen at the BVAMC, are symptomatic and do not have defined illnesses. The use of advanced methodology in the imaging, immune, mycoplasma and oxidant injury study is important. Sample sizes are adequate and support for statistical analysis is an integral part of the proposal.

EXPECTED PRODUCTS (MILESTONES): Reports and publications.

STATUS/RESULTS TO DATE: None reported.

PUBLICATIONS: none to date

Title: Investigation of Seminal Plasma Hypersensitivity Reactions

Project #: DoD-44 Agency: DoD Study Location: Univ. Cincinnati MSC

Project Status: Ongoing Research Type: Clinical Research

P.I.: Jonathan Bernstein, M.D. Research Focus: Reproductive Health

Start Date (CY): 1996 Est. Completion (CY): 1999 Symptoms/General Health Immunology

OVERALL PROJECT OBJECTIVE: The purpose of this project is to investigate Persian Gulf War (PGW) veterans and their sexual partner(s) who are experiencing a burning sensation after contact with semen since returning from the Persian Gulf.

SPECIFIC AIMS: Military and veteran sources will be surveyed to obtain a population of women who developed vaginal burning syndrome after their sexual partners returned from the Persian Gulf.

METHODOLOGY: The initial phase of this project has concentrated on identifying the population and characterizing the nature of their symptoms using screening questionnaires. Questionnaires assessing for post-traumatic stress disorder were also included. Subjects were identified through local and regional veterans hospital Gulf War screening physicians, in response to media press releases pertaining to this investigation and through a "Burning Semen Syndrome" (BSS) web page on the internet. A pilot project was conducted to more thoroughly investigate a small group of PGW veterans in local proximity to the Cincinnati Veterans Administration Hospital (VAH). This evaluation included more detailed questionnaires related to their medical history before and after their tour of duty in the Persian Gulf, potential exposures while there and the nature of their burning semen symptoms. Screening blood tests were performed on both the PGW veteran and their sexual partner to exclude underlying etiologies such as sexually transmitted diseases. Semen was obtained from each male participant and cultured to exclude viral, bacterial and fungal infections. Each female underwent a pap smear with cervical and vaginal cultures to exclude viral, bacterial and fungal infections. Both subjects were skin tested to perennial and seasonal allergens to assess their atopic state. Each subject was also skin tested to the male's whole semen to diagnose or exclude an immediate type allergic response. Serum from each subject was also used to determine if IgG, IgE and IgA specific antibodies were being produced in response to seminal plasma proteins using an ELISA method.

EXPECTED PRODUCTS (MILESTONES): If the hypotheses are correct, 1) women with seminal plasma hypersensitivity and vaginal burning syndrome will demonstrate immediate cutaneous reactivity to seminal plasma protein fractions; 2) one or more seminal plasma proteins that cause localized vaginal symptoms in our civilian group of women will also cause similar symptoms in women with Gulf War vaginal burning syndrome; and 3) the ability of seminal plasma proteins to regulate (either stimulate or inhibit) the humoral, cellular or secretory immune responses after exposure to physical and chemical factors encountered in the Gulf War.

STATUS: Preliminary findings: A total of 46 individuals responded to the screening questionnaires. All of the respondents were male; 41 of 46 of their female sexual partners experienced a burning sensation after contact with semen while only 15 of the males experienced burning after contact with their own semen. The completion rate of the questionnaires proportionally decreased as they became lengthier and more complex. There did not appear to be any correlation between BSS and post-traumatic stress disorder among those that completed all of the questionnaires. Six male PGW veterans and five of their respective sexual partners participated in the pilot study (one of the PGW veteran's female sexual partner declined participation as she was asymptomatic). Four of the six male PGW veterans and two of the five female sexual partners exhibited positive skin tests consistent with atopy. None of the participants or their sexual partners elicited positive skin test reactions to their whole semen nor were specific serum IgG, IgA or IgE antibodies to seminal plasma proteins detected using an ELISA method. Three women had *Ureaplasma Urealyticum* isolated from their cervical cultures, one grew streptococcus Group B from their cervical culture and one had an active yeast infection. Two of the women had significant ANA titers and one had an increased sedimentation rate. Work is in progress utilizing polymerase chain reaction methodology to identify *ureaplasma urealyticum* in the semen of PGW veterans which could not be isolated by culture. Investigation is also centered on identifying whether bacteriostatic proteins and other bacteriostatic compounds (i.e. Zinc sulfate) are deficient in their semen making their sexual partner more susceptible to infection.

Active recruitment will continue during the next two years to identify a larger number of PGW couples with BSS who will undergo extensive medical evaluation similar to that conducted on first year pilot study participants. Cohort control groups consisting of asymptomatic PGW veterans deployed and non-deployed to the Persian Gulf are also being recruited to participate in this project. These initial findings suggest that infection may be playing a significant role in causing burning semen symptoms. All subjects with evidence of infection will be offered treatment with appropriate antibiotics and/or antifungals to determine if the burning symptoms are attenuated. More detailed evaluation of a larger population of PGW veterans with BSS and cohort control groups is necessary before linkage of BSS to service in the Persian Gulf can be established.

PUBLICATIONS: Bernstein JA, Martin RLM, Lummus ZL. Localized Human Seminal Plasma Hypersensitivity: A Potential Model for Gulf War "Burning Semen Syndrome." *Fundamental and Applied Toxicology*, 1997, 36:201.

Title: Physical and Emotional Health of Gulf War Veterans Women

Project #: DoD-45 Agency: DoD Study Location: Univ. MI, Ann Arbor

Project Status: Ongoing Research Type: Clinical Research

P.I.: Penny F. Pierce, Ph.D., Research Focus: Symptoms/General Health

**Start Date (CY):1996 Est. Completion (CY):1999 Brain & Nervous System
Reproductive Health**

OVERALL PROJECT OBJECTIVE: This study will compare the prevalence of general and gender specific health problems in 900 Air force women deployed to the Gulf and 900 Air Force women deployed elsewhere.

SPECIFIC AIMS: To assess whether the prevalence of health problems remain elevated in women deployed to the Gulf at six years post deployment, after controlling for potentially confounding factors such as age, reproductive history, sexual history, and lifestyle factors.

METHODOLOGY: This research is to describe the incidence, prevalence, and nature of symptoms associated with Persian Gulf War service within a randomized sample of Air Force women. Our design is developed on previous experience with two studies of Gulf War veteran women as well as the recommendations of numerous military and civilian scientific groups established to address this body of research. Specifically, in previous studies we have used a comparison group of women deployed to sites other than the theater of the war under the assumption they were physically fit for duty and would provide a better comparison than those who did not deploy perhaps for physical health reasons. Further sampling strategies will follow the recommendations for both the National Institutes of Health (NIH, 1994) and the Institute of Medicine (IOM, 1995; Roberts, 1995), who both call for well-designed epidemiologic studies. In both our previous studies we used a randomized stratified factorial design (theater vs. non-theater; parent vs. non-parent; and component (active, guard, and reserve). Similar sampling procedures will be used in this study in order to control for potentially confounding effects of component and paternal status. This study will also include detailed information on sexual, reproductive, and menstrual history to allow for more complete analyses of findings.

Sample size and power will detect differences observed in previous studies. Data collection involves recruiting the study participants and maximizing the response rate while minimizing attrition. A single comprehensive questionnaire will provide basic demographic, socioeconomic, and background information and include measures of general physical health and measures of gender-specific health. Mental health measures include assessment of depression, somatization, and 8 PTSD specific to Gulf War service and will include a stressful life event measure. Data processing includes all appropriate range and logic checks. Statistical analyses include descriptive statistics, cross tabulations with chi-square and t-tests, distributions, and multiple regression analyses for health outcomes and deployment status.

EXPECTED PRODUCTS (MILESTONES): Three project years: 1) all planning and pre-testing during year 1; one-half of data collection (mail administration) in year 1 and the second half in year 2; all coding and data entry in year 2; and the final report and electronic data file as deliverables.

STATUS: Data collection is in progress.

PUBLICATIONS: none to date

Title: Exploratory Data Analysis with the CCEP Database

Project #: DoD-46

Agency: DoD

**Study Location: Naval Post-Graduate School,
Monterey, CA**

P.I.: Dr. Hemant Bhargava

Research Focus: Symptoms/General Health

Start Date (CY):1995 Est. Completion (CY):1997

OVERALL PROJECT OBJECTIVE: To systematically examine the database for interesting relationships, and to find, with a high level of confidence, the most interesting ones without having to do an exhaustive search.

SPECIFIC AIMS: 1) to develop a generic computer package to do exploratory data analysis using genetic algorithms to decide which attributes to consider in the analysis; 2) to apply this package on a number of specific application runs relevant to the Gulf War Syndrome problem; 3) to produce, analyze, and comprehensively document the results of these runs, and produce a set of findings and conclusions.

METHODOLOGY: For the search problem over the space of attributes found in the CCEP database, the main search methodology is based on genetic algorithms. The overall solution methodology consists of exploratory data analysis and knowledge discovery in databases.

EXPECTED PRODUCTS (MILESTONES): 1. The computer software: a) a hypothesis generator module; b) a filtering module; and c) a results presentation and interpretation module.

STATIS/RESULTS TO DATE: Version 1.0 of the Hypothesis Generator Module is complete, tested, and applied in a series of production runs.

PUBLICATIONS: none to date

Title: Study of Mycoplasmal Infections in Gulf War Veterans
Project #: DoD-47 Agency: DoD Study Location: WRAIR, Wash. DC
Project Status: Ongoing Research Type: Clinical Research
P.I.: Raymond Chung, MD, Research Focus: Diagnosis
Start Date (CY):1995 Est. Completion (CY):1998

OVERALL PROJECT OBJECTIVE: This study is designed to find evidence for mycoplasmal infections in symptomatic Operation Desert Storm veterans, by comparing the rate of seroconversion in symptomatic veterans as compared to healthy Gulf veterans and by culturing for Mycoplasma organisms in symptomatic and healthy Gulf veterans.

SPECIFIC AIMS: See objectives.

METHODOLOGY: This study has two parts: (1) Sera from 200 symptomatic Gulf veterans and 200 healthy Gulf veterans, stored in the U.S. Armed Forces HIV Repository are used. Sera prior to and after deployment to South West Asia are tested for antibody to *M. fermentans*, *M. genitalium*, and *M. penetrans*. (2) Blood, urine, oropharyngeal, and rectal samples in 100 consecutive veterans undergoing evaluation at WRAMC and 100 asymptomatic veterans are tested for evidence of mycoplasmal infection by culture, serology and PCR.

EXPECTED PRODUCTS (MILESTONES): August 1996 - Initial Part One results; March 1997 - Addendum Part One results; August 1997 - Part Two complete.

STATUS/RESULTS TO DATE: Part one of the study was done using 151 cases and 151 age and gender matched controls. There was no statistically significant difference in the seroconversion rate to any of the aforementioned Mycoplasma. However the seroconversion rate was unexpectedly low: 1.5% for controls and 3.0% in cases for *M. fermentans*. The odds ratio (OR) for cases compared to controls was 2.0 with a confidence interval of 0.4 - 10.9. In order to be able to detect an OR of 2 with a background seroconversion rate of 1 - 2% with 80% power, a larger study needs to be done. An addendum to the protocol has been submitted. Part two of the study is still ongoing, and as of 9 Sep 1996, 41 symptomatic veterans and 18 healthy controls have entered the study. No significant difference in the seroconversion rate to Mycoplasma has been found when comparing symptomatic to healthy Gulf veterans. Because of the low seroconversion rate, the power of part one of the study is low and a larger sample size will allow a better estimation of seroconversion to Mycoplasma in this population.

PUBLICATIONS: none to date

Title: Assessment of Genomic Instability via Chromosome 7 Inversion Frequency in a Gulf-War Syndrome Cohort vs Selected Control Groups

Project #: DoD-48 Agency: DoD Study Location: WRAIR, Wash. DC

Project Status: Completed Research Type: Clinical Research

P.I.: William N. Fishbein, Research Focus: Immunology

Start Date (CY):1995 Est. Completion (CY):1997

OVERALL PROJECT OBJECTIVE: This study is designed to determine the frequency of chromosome 7 inversions in blood lymphocytes of four groups of volunteers: 1) symptomatic Gulf War veterans with findings consistent with a diagnosis of autoimmune or collagen vascular disease, 2) Gulf War veterans without abnormalities, 3) symptomatic volunteers with autoimmune or collagen vascular disease, and 4) healthy volunteers with no Gulf War exposure.

SPECIFIC AIMS: See objectives.

METHODOLOGY: Blood will be obtained from twenty five consecutive cases of each of the four groups. The samples are examined for the chromosome 7 inversion blindly in the laboratory of Dr. William Fishbein, an investigator from the AFIP. After the 100 samples the investigators will be unblinded and a statistical analysis will be done.

EXPECTED PRODUCTS (MILESTONES): March 1997 - collection complete; April 1997 - Analysis complete.

STATUS/RESULTS TO DATE: This study of 102 cases has now been completed, after data distribution, breaking of the code, and statistical analysis. There was adequate matching of age, sex, and race, and no evidence was found for genetic instability among symptomatic Gulf War veterans, or any of the other groups tested; a preliminary report will be presented at the Experimental Biology meeting in San Francisco in April, 1998.

PUBLICATIONS: none to date

Title: Diagnosis and Dosemetry of Exposure to Sulfur Mustard: Development of Standard Operating Procedures and Exploratory Research on Protein Adducts
Project #: DoD-49 Agency: DoD Study Location: TNO Prins Maruits Lab, Rijswijk, Netherlands
Project Status: Ongoing Research Type: Applied Research
P.I.: Hendrick P. Benschop, Research Focus: Chemical Weapons
Start Date (CY):1996 Est. Completion (CY):2000 Diagnosis

OVERALL PROJECT OBJECTIVE: Develop procedures to assess mustard vesicant agent levels (which are highly reactive and show little persistence in vivo) in patients, based on estimation of exposure levels from levels of sulfur mustard adducts to DNA and proteins in tissue samples. The principal objective of this research is to develop methodologies that can be readily adapted for use in the field.

SPECIFIC AIMS: Three quantitative assessment procedures will be developed and tested in one set of animal experiments for mutual assay validation. There are: 1) immunochemical assays of sulfur mustard adducts to DNA in human blood and skin; 2) GC/MS determination of the N7-adduct of sulfur mustard with guanine (N7-HETE-Gua) in human blood, skin, and urine; and, 3) GC/MS determination of the sulfur mustard adduct to the N-terminal valine in blood hemoglobin. The practical applicability of the assays will be confirmed by demonstrating the assays at an independent institute.

METHODOLOGY: The feasibility of this project was proved in the previous research where it was proposed to use ³⁵S-sulfur mustard to isolate the adducts. This technique was fruitful with protein adducts in research with hemoglobin. It has been effective in labeling loss of heterozygosity. There is no reason to believe it would not work with albumin and keratin.

EXPECTED PRODUCTS (MILESTONES): Laboratory research to be performed 10/1/96-9/30/99; 10/1/99-2/28/00 will be report preparation and review.

STATUS/RESULTS TO DATE: See expected products.

PUBLICATIONS: none to date

Title: Toxicokinetics of 0-Ethyl S-(2-Diisopropylaminoethyl) Methylphosphonothioate [(+)-VX] in Rats, Hairless Guinea Pigs and Marmosets - Identification of Metabolic Pathways
Project #: DoD-50 Agency: DoD Study Location: TNO Prins Maruits Lab, Rijswijk, Netherlands
Project Status: Ongoing Research Type: Basic Research
P.I.: Hendrick P. Benschop, Research Focus: Chemical Weapons
Start Date (CY):1996 Est. Completion (CY):1999

OVERALL PROJECT OBJECTIVE: Develop methodology for and clarify the toxicokinetics of VX stereoisomers in laboratory animals.

SPECIFIC AIMS: Develop methodology for and clarify the toxicokinetics of VX stereoisomers in laboratory animals.

METHODOLOGY: VX enantiomers will be resolved using a detection apparatus to be developed early in the study. The sensitivities of gas chromatographic analysis in combination with mass spectrometry will be compared with that for micro-liquid chromatography employed with a chiral stationary phase. Thermionic and/or flame photometric detection limit of 10 pg per enantiomer. Once selected, the system will be used to identify and quantify +/- VX metabolites generated in vitro in blood, and from liver and skin homogenates from animals, and, if possible, human tissue. In vivo toxicokinetics and metabolism will be determined in the blood of anesthetized, atropinized, and artificially ventilated hairless guinea pigs. The initial time frame for blood sampling will be up to 60 minutes after intoxication and will be modified as appropriate.

EXPECTED PRODUCTS (MILESTONES): Successful completion of this research would enhance the understanding of VX toxicokinetics and metabolic elimination. This information is important because of uncertainties in current pretreatment strategies stemming from differences between VX and its G agent counterparts. Among military chemical agents, VX is the most specific for acetylcholinesterase and is the most toxic. It is also persistent in the field and offers the greatest hazard to military personnel following direct exposure or indirect exposure from the handling of contaminated individuals or materials. It is anticipated that the toxicokinetics of VX will differ from its G agent counterparts.

STATUS/RESULTS TO DATE: Laboratory research from 1/1/95 - 11/30/97; report preparation and review 12/1/97 - 4/30/98.

PUBLICATIONS: none to date

Title: Transgenic Engineering of Cholinesterases: Tools for Exploring Cholinergic Responses
Project #: DoD-51 Agency: DoD Study Location: Hebrew Univ of Jerusalem, Israel
Project Status: Ongoing Research Type: Basic Research
P.I.: Hermona Soreq, Ph.D. Research Focus: Chemical Weapons
Start Date (CY): 1996 Est. Completion (CY): 2000 Prevention

OVERALL PROJECT OBJECTIVE: To assess the potential of transgenic cholinesterases to protect organisms against anticholinesterase neurotoxicity.

SPECIFIC AIMS: Our previous work has demonstrated that overexpression of a specific alternative variant of the acetylcholine-hydrolyzing enzyme acetylcholinesterase (AChE) in brain protects transgenic mice against the acute toxicity of organophosphate (OP) poisons. However, we also observed that chronic overexpression of AChE in the central nervous system (CNS) may promote delayed impairments in cognitive and neuromotor function. Our objectives, therefore, are to determine the specific cholinesterase isoforms and mode(s) of expression conferring the greatest level of protection against OP poisoning with the least side effects. To this end, we aim to develop animal models for testing the protective value and potential side-effects of transgenic overexpression of various human cholinesterases. In addition, we shall search for genetic factors conferring variability in the susceptibility of individuals to OP intoxication.

METHODOLOGY: To study the protective effects of overexpressed AChE on the nervous system, we established transgenic mice carrying two copies of the human gene encoding the synaptic form of AChE. These mice display elevated levels of AChE in central cholinergic neurons normally expressing this enzyme and reduced sensitivity to cholinergic intoxication. However, despite apparently normal embryonic and early post-natal development, AChE-transgenic mice exhibit delayed-onset, progressive impairments in central cognitive functions such as learning and memory, and in neuromotor function. To determine the protective value of alternative AChE isoforms with different subcellular and tissue-specific patterns of expression, and to dissect the mechanism(s) through which excess AChE mediates late onset neuropathologies, we are establishing additional lines of transgenic mice. Using these mice, we wish to determine whether soluble or partially amphiphilic forms of the protein confer greater protection, in which tissues these enzymes should be expressed, whether the long-term effects of overexpression are dependent on the catalytic activity of the enzyme, and whether specific alternative C-terminal peptide domains may exert more active roles in mediating neurodegenerative processes. To investigate possible genetic polymorphisms affecting sensitivity to anticholinesterases we are studying upstream regulatory sequences in the human AChE gene promoter.

EXPECTED PRODUCTS (MILESTONES): This project is designed to select the cholinesterase variant(s) with the greatest potential to protect an organism from the acute toxicity of OP poisons, while elucidating the mechanisms of delayed neuropathologies induced by chronic overexpression of AChE in the CNS. These studies will therefore allow us to develop strategies for the safe use of transgenic cholinesterases as part of our chemical defense arsenal.

STATUS/RESULTS TO DATE: The investigators have constructed and expressed a variety of catalytically active and inactive variants of in *Xenopus* oocytes and embryos and characterized their biochemical properties in the amphibian. They have identified neurite growth-promoting activities of some variants, and shown this activity to be independent of acetylcholine hydrolysis. These constructs were then used to establish new lines of transgenic mice which are currently being characterized for expression of the transgenes. An additional line of transgenic mice carrying DNA encoding an antisense RNA targeted against rodent AChE is being established as a first step in the long-term project of establishing mice expressing exclusively or predominantly the human enzyme. Once the spatiotemporal expression pattern(s) of the various transgenes are characterized, the sensitivity of the different lines of mice to OP poisons will be tested. At the same time, long-term performance of these mice in tests of cognition and neuromotor function will be examined. DNA samples are also being collected from human subjects presenting hypersensitivity to anticholinesterases used in medical or agricultural settings and searching for possible allelic variations in either the AChE or BCHE gene which might be correlated with adverse reactions to these compounds. To date, the investigators have identified a single point mutation in the human BCHE gene coding sequence and a rare polymorphism in the human AChE gene upstream

promoter region that may confer hypersensitivity to a variety of cholinesterase inhibitors.

PUBLICATIONS: Beerl R, Andres C, Lev-Lehman LE, Timberg R, Huberman T, Shani M, Soreq H. Transgenic expression of human acetylcholinesterase induces progressive cognitive deterioration mice. *Curr Biol*, 1995, 5:1063-71.

Friedman A, Kaufer D, Shemer J, Hendler I, Soreq H, Tur-Kaspa I. Pyridostigmine brain penetration under stress enhances neuronal excitability and induces early immediate transcriptional response. *Nature Med*, 1996, 2:1382-1385.

Andres C, Beerl B, Friedman A, Lev-Lehman E, Henis S, Timberg R, Shani M, Soreq H. Acetylcholinesterase-transgenic mice display embryonic modulations in spinal cord choline acetyltransferase and neurexin 1B gene expression followed by late onset neuromotor deterioration. *Proc Natl Acad Sci*, 1997, 94:8173-8178.

Title: Female Gender and Other Potential Predictors of Functional Health Status Among Persian Gulf War Veterans

Project #: DoD-52

Agency: VA

Study Location: VAMC Boston

Project Status: Ongoing

Research Type: Epidemiology Research

P.I.: Jessica Wolfe

Research Focus: Brain & Nervous System

Start Date (CY):1995 Est. Completion (CY):1998

Symptoms/General Health

OVERALL PROJECT OBJECTIVE: The primary objective of this study is to identify and describe the effects of potential predictors on the functional health status and health perceptions of male and female veterans approximately four years after their deployment to the Persian Gulf; and to examine if and how identified risk factors differ between female and male veterans. A secondary objective of the proposed project is to ascertain the prevalence of multiple chemical sensitivity (MCS)-like symptoms reported among this population, and to explore risk factors for the development of this syndrome.

SPECIFIC AIMS: The more specific goal of this study is to use the obtained data to significantly advance DoD screening and intervention efforts aimed at enhancing positive military and post-deployment adaptation among soldiers, especially women. With women's rapidly increasing representation in the U.S. Armed Forces, there is a pressing need to investigate these relationships, and to understand factors that might be distinctively associated with women's well-being, both during and following war-time deployment.

METHODOLOGY: By using cross-sectional and longitudinal data from an existing, carefully followed military cohort and by expanding predefined measures of health symptoms, and health perceptions, this investigation is conducting analyses that focus directly on: (a) defining the set of reported environmental and psychosocial combat-theater exposures and physical health variables associated with female and male soldiers recent deployment; and, (b) describing the relationship of these variables to functional status and self-reported physical health. A primary emphasis is on investigating the role or impact of gender, specifically, whether female gender is a significant factor in predicting either functional health status or health perceptions. Data analyses will proceed through a series of three major stages. The initial stage involves generation of descriptive analyses for all variables of interest and will permit comparison of responses between women and men. The second stage involves the determination of bivariate odds ratios of gender, PTSD diagnostic status, and environmental exposure on functional health status. The third stage involves multivariate regression procedures to examine the effects of gender, PTSD and reported environmental exposure on functional status and on self-reported health.

In addition, an exploratory analysis will be conducted of the comprehensive symptom review results with respect to the diagnosis of Multiple Chemical Sensitivity. One of the first steps in ascertaining the prevalence of MCS-like symptoms among our study population is developing a survey instrument that can be used to identify subjects with MCS-like symptoms. To date, there is only one survey instrument that has been validated to assess MCS symptoms. However, due to its length, we are currently testing the validity of a shortened version of this existing questionnaire on patients referred to the Massachusetts Respiratory Hospital, and controls recruited by participating patients. Depending on the prevalence of individuals who meet criteria for MCS-like symptoms, multivariate models similar to those for functional status with MCS-like symptoms as the dependent variable will be conducted.

EXPECTED PRODUCTS (MILESTONES): To develop a shorter questionnaire than the one currently used to identify MCS, scientific publications on Persian Gulf War illnesses (including the impact of stress and trauma on outcome, annual reports, gender models of health outcomes.

STATUS/RESULTS TO DATE: A manuscript reporting the development of a short MCS questionnaire is currently under review (Hu, Stern, Rotnitzky et al.). After 3 mailings of the Time 4 questionnaire, approximately 43% of the original Devens cohort of 2949 PGW veterans have returned completed surveys. Additional phone surveys are planned to sample non-responders and assess the extent to which they may differ from responders. Appropriate weighting procedures can then be applied to statistically minimize the effects of any response bias.

PUBLICATIONS: Proctor SP, Wolfe J, Friedman M. Evaluation of health symptoms reported by Gulf War veterans. Paper presented at the Annual Meeting of the International Society

for Environmental Epidemiology, Research Triangle Park, NC. September 1994.

Wolfe J, Brown PJ, Bucsela ML. Symptom responses of female Vietnam veterans to Operation Desert Storm. *American Journal of Psychiatry*, 1992, 149, 676-679.

Wolfe J, Brown P, Kelley J. Reassessing war stress: exposure and the Gulf War. *Journal of Social Issues*, 1993, 1993.

Title: Long-Term Effects of Subclinical Exposures to Sarin

Project #: DoD-53 Agency: DoD Study Location: Albuquerque, NM

Project Status: Ongoing Research Type: Basic Research

P.I.: Rogene Henderson, Research Focus: Chemical Weapons

Start Date (CY): 1997 Est. Completion (CY): 2000 Brain & Nervous System

OVERALL PROJECT OBJECTIVE: The overall project objective is to determine the toxicokinetics of inhaled sarin on F344 rats and to determine if single or repeated (5X and 10X) exposures to low levels of sarin results in changes in the quantity of cholinergic synaptic markers.

SPECIFIC AIMS: 1. Determine the dosimetry of inhaled tritiated-sarin. 2. Determine the effect of subclinical exposures to sarin on cholinergic synaptic markers.

METHODOLOGY: Specific Aim I will be accomplished in 3 steps: Step 1: Expose rats by inhalation to two levels of tritiated sarin; Step 2. Analyze brain slices and critical areas of the brain for tritium at three time points after exposure; Step 3. Determine clearance time for sarin and its metabolites. Specific Aim II will be accomplished in 2 steps: Step 1. Expose rats to a high and a low dose of sarin; Step 2. Determine muscarinic and nicotinic receptor densities as well as acetylcholine esterase and choline acetyl transferase activities and choline uptake mechanisms in the brains of exposed rats at one (1) day and one (1) month after exposure. Results will illustrate the degree of persistence of the effects.

EXPECTED PRODUCTS (MILESTONES): In this study we expect to determine the dosimetry to the brain of different inhalation exposure concentrations of sarin and how long the agent remains in critical areas of the brain. We expect to complete these studies during the first 18 months of the study. We expect to complete the studies on the effect of single and repeated exposures to sarin on the quantity of cholinergic synaptic markers during the last two years of the study.

STATUS/RESULTS TO DATE: The lab has been set up to meet safety and security requirements. The synthesis of tritiated sarin has begun.

PUBLICATIONS: none to date

Title: Assessment of Subchronic Neurobehavioral and Neuropathologic Effects in Rats

Project #: DoD-54 Agency: DoD Study Location: Battelle, Columbus, OH

Project Status: Ongoing Research Type: Basic Research

P.I.: Carl Olson, Ph.D. Research Focus: Interactions

**Start Date (CY): 1997 Est. Completion (CY): 1999 Brain & Nervous System
Chemical Weapons**

OVERALL PROJECT OBJECTIVE: The primary objective is to determine if exposure to low levels of sarin (GB) create neurobehavioral or neuropathologic effects. Another objective is to assess the effect of low-level GB exposure on possible toxic effects produced by other chemicals, especially chemicals that have a mechanism of action similar to that of GB.

SPECIFIC AIMS: The specific aim is to determine if subtle abnormal neurobehavioral or neuropathologic effects are detected as a result of short term exposure to low levels of chemicals, or combinations of chemicals, to which U.S. servicemen were exposed in the Persian Gulf area. A total of 9 groups of rats, vehicle control; GB; PB/DEET; CPF; Positive control; GB+PB/DEET; GB+CPF; CPF+PB/DEET, and GB+CPF+PB/DEET, with 24 rats per group will be evaluated in stages. Results will be analyzed to determine if any effects are statistically significant.

METHODOLOGY: The study is designed to evaluate neurobehavioral and neuropathologic alterations that may result from exposure to GB alone and from exposure to GB and a combination of chemicals, PB, DEET, and CPF, at dose levels which, when given alone, may produce a measurable effect (e.g., blood acetylcholinesterase [AChE] inhibition) but do not produce observable signs of exposure. The test system is a mature, outbred rat strain which is capable of exhibiting neuropathologic alteration following exposure to organophosphorus compounds known to cause delayed neuropathy. Pilot Studies are conducted to establish a dose of each chemical, given over a 4 day period, that may produce a measurable effect but does not produce clinical signs of intoxication. These doses will be used in the Subchronic Study in which animals are exposed to chemicals, as well as chemical combinations, over a 4 day period and monitored for up to 30 days. Neurobehavioral assessments (Functional Observational Battery [FOB] and evaluation of motor activity) are performed prior to and three times following exposures. At the time of sacrifice (30 days post-exposure), specimens from the central and peripheral nervous system are taken for neuropathologic evaluation. During both phases, blood AChE activities are assessed. A control compound, such as carbaryl, is used to produce neurobehavioral abnormalities.

EXPECTED PRODUCTS (MILESTONES): The milestones will be completion of the Pilot Studies, completion of the Subchronic Study neurobehavioral evaluations, completion of the neuropathology evaluations, statistical analyses of the data, and report preparation.

STATUS/RESULTS TO DATE: The contract for this proposal was signed September 29, 1997.

MREF Protocol 134 was prepared and reviewed and approved by both Battelle's Institutional Animal Care and Use Committee (IACUC) and the U.S. Army MPMC Animal Use Review Officer, MAJ David L. Ruble. The use of GB already at the MREF was approved by USAMPMC. DEET and PB have been ordered and received, and 5 g of CPF were requested and received from Dow Elanco.

A shipment of 146 Sprague-Dawley rats approximately 3 months of age was received in December 1997. This is the oldest age at which rats are available, although a 6 month old rat is required for testing in subchronic studies. A technique for periodic blood sampling of these animals without the use of anesthesia is being developed. Rat blood is being analyzed for cholinesterase activity using both dithiobis(2-nitrobenzoic acid)(DTNB) with erythrocytes and dithionitric acid (DTNA) with whole blood in order to determine relationships and to attain better accuracy in measuring low levels of cholinesterase inhibition.

PUBLICATIONS: none to date

Title: Low-Level Exposure to GB Vapor in Air: Diagnosis/Dosimetry, Lowest Observable Effect Levels, Performance-Incapacitation, and Possible Delayed Effects

Project #: DoD-55 Agency: DoD Study Location: Prinz Maurits Lab, Netherlands

Project Status: Ongoing Research Type: Basic Research

P.I.: Herman vanHelden, Ph.D. Research Focus: Interactions

**Start Date (CY):1997 Est. Completion (CY):2000 Chemical Weapons
Pyridostigmine Bromide**

OVERALL PROJECT OBJECTIVE: To investigate the relationship between C.t-values, internal dose and adverse effects on performance in guinea pigs and marmoset monkeys which will be exposed to low levels of sarin (GB).

SPECIFIC AIMS: (1) At which C.t-value (t £ 5 h) of GB exposure does an internal dose of GB become measurable, i.e., what is the lowest observable effect level of exposure (LOEL)?; (2) At which C.t-value (t £ 5 h) of GB exposure and internal dose do these (systemic) effects of exposure start to have adverse effects on the performance of military personnel (LOAEL)?; (3) What are the consequences of continuous carbamate pretreatment during low level exposure to GB, i.e., will unexpected adverse effects on performance emerge through this combination of two cholinesterase inhibitors?; (4) What is the time course of adverse effects on performance following GB exposure and are there delayed effects?

METHODOLOGY: A number of central nervous system effects and miosis will be measured as sensitive and relevant parameters of incapacitation.

EXPECTED PRODUCTS: In this study we expect to get answers to the above-mentioned practically relevant questions.

STATUS/RESULTS TO DATE: A number of Technical Objectives are being conducted which are necessary to be able to execute the proposed experiments: (1) A glass exposure chamber for whole-body exposure of unanaesthetized guinea pigs and marmosets (one animal per chamber) to low levels of GB vapor in air, has been designed and constructed, which is at the implementation stage. This equipment should meet a number of requirements in order to measure a number of parameters on-line during exposure in a safe way: respiration, EEG and miosis. Moreover, blood samples for toxicokinetic analysis and measuring ChE-activities should be taken during exposure; (2) The implementation of the telemetric method for measuring EEG-signals and the VER (visual evoked response) signal is making progress; (3) Different types of cameras have been tested for real-time measurement of miosis on both eyes in guinea pigs from outside the exposure chamber. The most suitable digital camera has been chosen; (4) The dosimetry method to determine the internal dose of GB bound to BuChE in guinea pigs is operational by now.

PUBLICATIONS: none to date

Title: Low-Level Sarin Neurotoxicity and Its Modulation by Pyridostigmine

Project #: DoD-56 Agency: DoD Study Location: U of CA

Project Status: Ongoing Research Type: Basic Research

P.I.: Barry Wilson, Ph.D. Research Focus: Interactions

Start Date (CY): 1997 Est. Completion (CY): 2001 Chemical Weapons

Pyridostigmine Bromide

OVERALL PROJECT OBJECTIVE: To "Conduct animal studies designed to assess the possible long-term or delayed clinical effects of low-level or subclinical exposures to chemical warfare agents." The nerve agent sarin (GB) will be assessed for its ability to induce peripheral neuropathy and myopathy in the presence and absence of co-exposure to pyridostigmine bromide (PB). Sensitive morphological methods will be used to examine the delayed effects of low-level and sub-clinical exposures to sarin on the structure of the hen and mouse brain, spinal cord, peripheral neuromuscular and peripheral sensory system.

SPECIFIC AIMS: TASK ONE: Baseline DATA 1) Determine baseline levels (in the hen and mouse) of: acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and Neuropathy Target Esterase (NTE) in brain, RBC and plasma (the avian erythrocyte lacks AChE activity): AChE and BChE in the pectoral muscle; creatine kinase (CK) in blood. Establish the kinetics of inhibition of blood AChE/BChE to the test agents sarin, DFP, paraoxon and PB in vitro. Determine a high, non-lethal dosage and dosages that correspond to selected levels (i.e. 10%, 25%, %50%) of blood and brain AChE inhibition. Determine the differential agent sensitivity of hens and mice, and the induction of axonal degeneration in the spinal cord and peripheral nerves of DFP-treated mice. 2) Determine whether a single large dose of sarin (versus DFP and parathion) induces neurobehavioral signs of AChE-induced myopathy (e.g. proximal limb weakness and histopathology) and subsequent signs of OPIDN (distal weakness), and to correlate these findings with biochemical and morphological indices of nerve-fiber damage. TASK TWO: Multiple Exposures - Assess the effects of multiple sarin exposures using a strategy of stepping down the dosages of sarin progressively until no effects are detected upon morphological assay of spinal cord, peripheral nerve and muscle of hens, and these tissues plus brain regions and sensory and motor terminals in mice. TASK THREE: Pyridostigmine Bromide (PB) - Establish the highest no-effect dosage (HNED) of multiple doses of PB for use in Task Four. Experiments outlined in Task One and Task Two will be repeated, except that PB will be substituted for sarin. A single large dose of PB, and multiple smaller doses of PB, are expected to induce sub-junctional myopathy in vulnerable muscles. Absence of myopathic changes in animals treated with multiple doses of PB will provide the end-point for determination of the highest no-effect dosage of PB. TASK FOUR: Combined Effects of Sarin and PB - The final task is to determine whether the HNED of PB administered some days before, or shortly after sarin administration, causes the HNED of sarin to induce changes in biochemical or morphological parameters not present or greater than those found in animals treated with the same dose of sarin alone.

METHODOLOGY: The delayed effects of exposure to sub-lethal doses of sarin may include changes in: (1) brain; (2) muscle and spontaneous activity; (3) peripheral nerves/spinal cord and spontaneous activity. Compounds known to induce specific toxicities will be used as positive controls for #2 (parathion) and #1 and #3 (DFP). Substances will be dissolved in saline, which will serve as a negative control agent. A stepwise series of experiments will be performed with avian and mammalian species. White Leghorn laying hens and male Swiss albino mice will be used: both species are reported to be sensitive to the acute and delayed neurotoxic effects of DFP and sarin. Biochemistry - ChE Determinations: Red blood cell acetylcholinesterase (AChE) and plasma non-specific cholinesterase (BChE) in mouse blood are differentiated by separating RBCs and plasma or by using an inhibitor of non-specific cholinesterase. Most samples are measured using the colorimetric method of Ellman, et al. (1961) modified for use with an automatic microplate reader. Samples with very low activity or samples from PB treated animals may be measured using the radiometric method of Johnson and Russell (1975).

Neuropathy Target Esterase Determinations: Brain neuropathy target esterase (NTE) activity is determined by the colorimetric method of Johnson (1977) as modified by Wilson's laboratory (Mackay, et al., 1996) from that of Correll and Ehrich (1991).

Creatine Kinase Determinations: CK in plasma or muscle will be measured using the method of Hess, et al. (1968).

Protein Determinations - Protein is measured by the method of Lowry (1951).

Cytochemistry - Examination of muscle fibers will be carried out on frozen sections for morphology (hematoxylin and eosin) and cholinesterases with routine histological techniques modified from Karnovsky and Roots (1964) using acetylthiocholine as substrate (Wilson et al., 1990).

Morphological and Morphometric Analyses - At designated time-points, animals will be anesthetized, heparinized, and perfused. Tissue samples will be obtained from the following areas: frontal cortex, hippocampus, basal ganglia, cerebellum, medulla oblongata, mid-lumbar spinal cord, lumbosacral roots and dorsal root ganglia, proximal, mid-level and distal regions of nerves, and terminal-rich regions of sampled muscles. Tissues will be fixed and embedded in epoxy resin.

One-micrometer-thick sections will be stained and examined by bright-field microscopy. Thin sections will be prepared as needed from sensory and motor nerve terminals, treated and examined with a transmission electron microscope. Morphometric assessment will be carried out on perfect cross-sections of identical regions of distal nerves that show the earliest changes of DFP neuropathy. Focus will be placed on myelinated fibers which are most vulnerable in OP and other toxic neuropathies. Numbers of myelinated fibers will be counted on light photomicrographs (final magnification X600). Sizes of myelinated axons will be estimated by tracing the perimeter of the inner aspect of the myelin sheath. Axonal diameters and shape factor will be calculated from these measurements. The outer perimeter of the myelin sheath will be traced and used to determine nerve fiber perimeter, area and diameter. Myelin sheath thickness (MST) will be calculated.

EXPECTED PRODUCTS (MILESTONES): Task one (year 1) will consist of "scoping" trials to establish appropriate dose/response ranges for sarin, DFP and paraoxon. Task Two (years 1, 2) will determine thresholds and relative dose-effect levels for biochemical and morphological end-points. Tasks Three and Four (year 3) will examine whether pyridostigmine bromide (PB) induces responses to sub-threshold doses of sarin or DFP when the carbamate is given days before administration of the organophosphate (OP). The results will indicate how much and how often exposure to sarin is needed to produce sub-clinical (i.e. structural and functional) neurotoxic effects, which regions of the nervous system are most sensitive to sarin, and whether PB is able to modulate (increase/decrease) the neurotoxic potency of sarin.

STATUS/RESULTS TO DATE: Facilities are being readied for nerve agent work. Monitoring procedures and US Army approvals are being finalized.

Animals: 60 male mice (strain Cd1) were received from Charles River Co. They have been acclimated to our animal facility. 60 female chicks (white leghorn) were received from Hyline. They are being raised to the appropriate age for our studies.

The assay conditions for measuring cholinesterase in mouse are being standardized. The existing assay condition (1 mM acetylthiocholine substrate and pH 8 assay buffer) seem satisfactory.

Cholinesterase activity levels in mouse RBC and plasma were 1.37 ± 0.14 and 0.55 ± 0.013 nmol/min/ml respectively. In comparison acetylcholinesterase (AChE) and non-specific cholinesterase (BChE) levels in chicken plasma were 61.8 ± 11.2 and 551 ± 62.7 in a past study conducted for the USAMRDC (B.W. Wilson et al., 1987, The actions of repeated exposures of VX on chickens). Birds do not have RBC cholinesterase; proportions of AChE and BChE vary by species.

PUBLICATIONS: none to date

Title: Physiologic Effects of Stress in Gulf War Veterans

Project #: DoD-57 Agency: DoD Study Location: Georgetown Univ., Wash DC

Project Status: Ongoing Research Type: Clinical Research

P.I.: Daniel Clauw, M.D. Research Focus: Symptoms/General Health

Start Date (CY):1997 Est. Completion (CY):2000 Brain & Nervous System

OVERALL PROJECT OBJECTIVE: To address the association between Persian Gulf War Illness (PGWI) and biological stress responses among women who served in the Persian Gulf War (PGW), to determine if individuals with PGWI have responses similar to that of individuals with fibromyalgia and Chronic Fatigue Syndrome, specifically targeting the causal relationship between PGWI and stress.

SPECIFIC AIMS: 1. To perform clinical and psychological evaluation on a sampling of symptomatic and asymptomatic individuals deployed to the PGW; 2. To determine if PGWI patients show evidence of a low central corticotropin releasing hormone (CRH) state by administering IL-6 and evaluating ACTH response; 3. To demonstrate that PGWI patients show evidence of impaired activation of the adrenomedullary and sympathoneural components of the sympathetic nervous system evidenced by biological responses to dolorimeter testing, tilt table testing and computer testing; 4. To demonstrate that PGWI patients have evidence of a decreased peripheral responsiveness to catecholamines as seen in biological responses to tilt table testing; 5. To show that significant abnormalities to these components of the stress response can be noted in most PGWI patients and that the nature of the abnormality can predict the predominant clinical symptoms.

METHODOLOGY: The first year of research will involve finalizing project plans, developing a sampling frame, and recruiting and evaluating 35 participants, including 35 patients and 25 controls; and generating mid-term and quarterly reports of progress. Patient recruitment will continue into the third year to include 20 patients and 5 controls and data analysis will begin. Correlation will be made between self-reported medical information and clinical data, and data from this study will be linked to the Persian Gulf Women's Health Linkage Study for further analysis.

EXPECTED PRODUCTS (MILESTONES): To gain a better understanding of the pathophysiology of PGWI in order to improve recommendations for treatment of patients.

STATUS/RESULTS TO DATE: The final stages of project planning are currently underway and the early stages of patient recruitment have begun.

PUBLICATIONS: none to date

Title: Illness Among Persian Gulf War Veterans: Case Validation Studies
Project #: DoD-58 Agency: DoD Study Location: Univ of Iowa
Project Status: Ongoing Research Type: Clinical Research
P.I.: Bradley Doebbeling, Research Focus: Symptoms/General Health
Start Date (CY):1997 Est. Completion (CY):2001 Brain & Nervous System

OVERALL PROJECT OBJECTIVE: To compare the true rate of confirmed disease among samples of veterans deployed to the Gulf with and without these predefined conditions, versus true rate of confirmed disease among samples of veterans not deployed, with and without these self-reported conditions.

SPECIFIC AIMS: The purpose of the current project is to compare the rates of false positive reports of illness among both deployed and non-deployed veterans. Because of the magnitude of the difference in prevalence between these groups, we feel it is important to explore and characterize their cognitive deficits, depression, and multisystemic conditions. These studies will be performed by comparing the true rate of confirmed disease among samples of veterans deployed to the Gulf with and without these self-reported conditions versus the true rate of confirmed disease among samples of veterans not deployed with and without these self-reported conditions. Furthermore, we plan to also compare risk factors including medical and family history, psychological factors (such as major lifetime events or stress, personality traits, and social support), and occupational and environmental exposures for validated illness in a series of nested case-control studies for each illness outcome.

METHODOLOGY: A sample of veterans deployed to the Gulf and of non-deployed veterans who met pre-defined criteria for one of the four conditions of interest will be invited to participate in the follow-up studies. Additionally, a sample of subjects who did not meet criteria for any of the telephone survey conditions selected from among those deployed and not deployed to the Gulf will serve as controls. Selected subjects will be invited to participate in detailed personal interviews, physical examinations, structured neuropsychological, neurophysiological, psychiatric, and other laboratory testing. STUDY 1 is comprised of various tests for cognitive dysfunction. STUDY 2 is comprised of specific psychiatric testing for depression. STUDY 3 is comprised of testing batteries examining for Multisystemic Conditions.

EXPECTED PRODUCTS (MILESTONES): In this study, we plan to determine if the rate of false positives of the deployed Iowa Persian Gulf Veterans' self-reports of cognitive dysfunction (CD) are the same as the rate of false positives of the nondeployed PGW veterans self-reports of CD. We will also determine if the rate of false positives of the deployed Iowa Persian Gulf Veterans' (PGW veterans) self-reports of depression are the same as the rate of false positives of the nondeployed PGW veterans self-reports of depression. Finally, we will determine if the rate of false positives of the deployed Iowa Persian Gulf Veterans' (PGW veterans) self-reports of multisystemic conditions (fibromyalgia, chronic fatigue syndrome, multiple chemical sensitivity) are the same as the rate of false positives of the nondeployed PGW veterans self-reports of multisystemic conditions.

STATUS/RESULTS TO DATE: The grant funding just began September 25, 1997. Thus far, we have begun the process of recruiting the Study Coordinator and Programmer/Analyst. We have also begun developing and revising data collection forms, and obtaining final human subjects approval from the Department of the Army and the University of Iowa.

PUBLICATIONS: Schwartz DA, Doebbeling BN, Merchant JA, et al. Self-reported illness and health status among Gulf War veterans: A population-based study. JAMA 1997, 277:238-45.

Crispino MR, Goerdt C, Burmeister LF, Doebbeling BN. Evaluation of health-related quality of life in Persian Gulf War Veterans. J Gen Intern Med, 10(4) (Supplement):41, 1995.

Doebbeling BN, Rohrer JE, Woolson RF, Torner JC, Saag KG, Merchant JA, Bentler S, Schwartz DA. Health services utilization among Persian Gulf War and activated non-deployed veterans. Annual Meeting of the American Public Health Association, Indianapolis, IN, (poster) November 11, 1997.

Title: Pyridostigmine-induced Neurodegeneration: Role of neuronal Apoptosis
Project #: DoD-59 Agency: DoD Study Location: Purdue Res Foundation
Project Status: Ongoing Research Type: Basic Research
P.I.: Gary E. Isom, Ph.D. Research Focus Pyridostigmine Bromide
Start Date (CY): Est. Completion (CY):2000 Brain & Nervous System

OVERALL PROJECT OBJECTIVE: Characterize the neurotoxic response to pyridostigmine by determining the exposure conditions required to induce neurodegeneration in the rat brain and examine the underlying mechanism in a neuronal cell model. This will be accomplished by administering varying doses of pyridostigmine to rats and at specific times, the brains will be examined histologically and by molecular biology procedures for apoptotic cell death. Cultured cells from the brain area(s) that undergo degeneration will be used to determine the molecular processes that pyridostigmine activates to initiate the brain cell death. Another objective is to determine the involvement of neuronal oxidative stress in the response and to determine if antioxidants will attenuate the degeneration in animals and cultured cell models.

SPECIFIC AIMS: 1. Characterize the dose-response of pyridostigmine-induced neuronal apoptosis in the rat brain and the ability of antioxidants to alter the neurotoxicity; 2. Detailed biochemical and molecular analysis of pyridostigmine-induced apoptosis in cultured cerebellar granule cells; 3. Determine the mechanism by which pyridostigmine stimulates the generation of intracellular reactive oxygen species and the linkage to apoptosis.

METHODOLOGY: Apoptosis will be documented in brain areas by the Apotage (TUNEL) method, electron microscopy morphometric analysis and DNA (fragmentation) laddering on gel electrophoresis. Mechanistic studies will include measurement of redox transcription activation by EMSA, reactive oxygen species generation by microfluorescence procedures, and mitochondrial dysfunction by use of fluorescence dyes.

EXPECTED PRODUCTS (MILESTONES): In this study, we expect to show that pyridostigmine induces neurodegeneration by initiating apoptosis cell death in the brain. The mechanism of this brain injury will determine by use of neuronal cell models.

STATUS/RESULTS TO DATE: We are completing the dose-response study to pyridostigmine in rats and determining the duration of treatment required to produce neurodegeneration. We have documented by use of multiple analyses that 4 days of pyridostigmine treatment (0.5 to 1.85 mg/kg) induces apoptotic cell death in the cortex, hippocampus and striatum.

PUBLICATIONS: none to date

Title: Butyrylcholinesterase Genetic Variants in Persons with Gulf War Illness
Project #: DoD-60 Agency: DoD Study Location: Univ of Nebraska Med Center
Project Status: Ongoing Research Type: Clinical Research
P.I.: Oksana Lockridge, Ph.D. Research Focus: Chemical Weapons
Start Date (CY):1997 Est. Completion (CY):1998 Pyridostigmine Bromide Prevention

OVERALL PROJECT OBJECTIVE: If this hypothesis is correct we expect to find a correlation between low levels of butyrylcholinesterase or between genetically abnormal butyrylcholinesterase and Gulf War Illness. Butyrylcholinesterase is a scavenger of nerve agents. A person with low levels of this enzyme or with a genetically defective enzyme would be less protected from nerve agents with the result that more of the agent would reach the nerves.

SPECIFIC AIMS: 1. To obtain blood samples from healthy and sick Gulf War veterans; 2. To obtain histories from the subjects detailing their exposure and symptom; 3. To measure the activity levels and phenotype of butyrylcholinesterase; 4. To determine the genotype of the butyrylcholinesterase; 5. To see if Gulf War Illness correlates with butyrylcholinesterase activity level or genotype.

METHODOLOGY: Gulf War veterans are recruited from the Omaha area. In addition, blood samples have been obtained from a collaborator at Walter Reed Army Research Institute. Histories are obtained by interviewing the subjects. Blood samples are tested for butyrylcholinesterase activity and phenotype with a spectrophotometric assay. The genotype is determined by amplifying the butyrylcholinesterase gene and sequencing the DNA.

EXPECTED PRODUCTS (MILESTONES): We expect to have collected 100 samples from the Omaha area and to have completed 100 interviews by spring 1998. Analysis of blood samples is ongoing.

STATUS/RESULTS TO DATE: All veterans interviewed to date believe there is a Gulf War Illness, regardless of their own health status. In answer to the question "do you think you have Gulf War Illness?" most veterans reply "I don't know." Even those who consider themselves healthy wonder whether a symptom they have is a consequence of their service in the Gulf. The veterans attribute their illness to oil fire smoke, pyridostigmine pills, non-FDA approved shots, or possible exposure to nerve agents. Of the blood samples we have tested to date, the frequency of genetic variants was the same in veterans who claimed to have Gulf War Illness and in controls.

PUBLICATIONS: none to date

Title: Neurophysiologic and Neuropathologic Effects in Monkeys of Low Level Exposures to Sarin, Pyridostigmine, Pesticides, and Botulinum Toxoid

Project #: DoD-61

Agency: DoD

Study Location: Battelle

Project Status: Ongoing

Research Type: Basic Research

P.I.: Carl T. Olson, Ph.D.

Research Focus: Interactions

Start Date (CY): 1997 Est. Completion (CY): 2000

Chemical Weapons

Pyridostigmine Bromide

OVERALL PROJECT OBJECTIVE: The primary objective is to determine if exposure to low levels of GB creates subtle enzymatic, neurophysiologic or neuromuscular pathologic effects. Another objective is to assess the effect of low-level GB exposure on possible toxic effects produced by other chemicals, especially chemicals that have a mechanism of action similar to that of GB.

SPECIFIC AIMS: To determine if subtle abnormal enzymatic, neurophysiologic or neuromuscular effects or neuropathology are caused as a result of short term exposure to low levels of chemicals, or combinations of chemicals, to which U.S. servicemen were exposed in the Persian Gulf area. A total of 5 groups of rhesus monkeys, a saline control; a positive control, GB/BotTox, PB/DEET/CPF; GB/BotTox+PB/Deet/CPF, with 8 monkeys per group are evaluated in two stages. Results are analyzed to determine if any effects are statistically significant.

METHODOLOGY: The study is designed to evaluate enzymatic, neurophysiologic and neuropathologic alterations that may result from exposure to GB/BotTox alone and from exposures to GB/BotTox and a combination of PB, DEET, and CPF, at dose levels which, when given alone, may produce a measurable effect (e.g., blood acetylcholinesterase [AChE] inhibition) but do not produce observable signs of intoxication.

Pilot Studies are conducted to establish a dose of each chemical, given over a 14 day period, that may produce a measurable effect but not produce clinical signs. These doses are used in the Subchronic Study in which animals are exposed over a 14 day period and evaluated for up to 170 days. Periodic neurophysiologic and electromyographic examinations of monkeys are performed. In addition, tissue specimens are examined for pathology at study termination. During the study, blood AChE, neurotoxic esterase (NTE), creatine kinase (CK) and alanine aminotransferase (ALT) activities are assessed. A control compound, diisopropyl fluorophosphate (DF), is used to produce neurophysiologic and neuropathologic abnormalities.

EXPECTED PRODUCTS (MILESTONES): The completion of the Pilot Studies, completion of the Subchronic Study neurophysiologic evaluations at 24 hr, at 60 days, at 90 days, at 180 days, and at 270 days, completion of the neuropathology evaluations, completion of the statistical analyses of the data, and completion of a final report.

STATUS/RESULTS TO DATE: The contract for this proposal was signed September 29, 1997.

MREF Protocol 135 was prepared, and reviewed and approved by both Battelle's Institutional Animal Care and Use Committee (IACUC) and the U.S. Army MPMC Animal Use Review Officer, MAJ David L. Ruble. Botulinum toxoid was requested from USAMPMC and received. The use of GB already at the MREF was approved by USAMPMC. DFP, DEET, and PB have been ordered and received, and 5 g of chlorpyrifos was requested and has been received from Dow Elanco.

A source of 60 male rhesus monkeys weighing approximately 3.5 to 6 kg was located, and a Purchase Order signed. These monkeys are to be shipped to the MREF in early 1998. Monkey blood is being analyzed for cholinesterase activity using both dithiobis(2-nitrobenzoic acid) (DTNB) with erythrocytes and dithionitric acid (DTNA) with whole blood in order to determine relationships and to attain better accuracy in measuring low levels of cholinesterase inhibition.

PUBLICATIONS: none to date

Title: Sarin and Pyridostigmine Interaction under Physical Stress: Neurotoxic Effects in Mice

Project #: DoD-62 Agency: DoD Study Location: S. Ill Univer

Project Status: Ongoing Research Type: Basic Research

P.I.: Satu M. Somani, Ph.D. Research Focus: Interactions

**Start Date (CY):1997 Est. Completion (CY):1999 Chemical Weapons
Pyridostigmine Bromide**

OVERALL PROJECT OBJECTIVE: To elucidate whether physical stress amplifies the neurotoxic effects and neuromuscular abnormalities after administration of pyridostigmine, sarin and the combination of these two agents.

SPECIFIC AIMS: (i) To evaluate the neurotoxic effects of low dose sarin in mice using behavioral (muscular weakness, ataxia, and motor dysfunction), electrophysiological (neuromuscular function), biochemical (inhibition of cholinesterase, neurotoxic esterase (NTE), and enhanced lipid peroxidation) in brain, spinal cord, platelets, and sciatic nerve and histopathological (light and electron microscopy) (axonal degeneration and demyelination in spinal cord and sciatic nerve and neuromuscular damage) analyses. (ii) To compare the above neurotoxic effects induced by low dose sarin exposure in pyridostigmine treated mice with untreated mice so as to determine the possible interactive effects of pyridostigmine with sarin-induced neurotoxicity. (iii) To evaluate the effects of physical stress on low dose sarin, pyridostigmine, and the combination of two to determine the above parameters and correlate with morbidity associated with Gulf War veterans.

METHODOLOGY: First year, we are doing the experiments to investigate the interactive effects of exercise and pyridostigmine on neuro-behavioral (muscular weakness, motor dysfunction), electrophysiological (neuromuscular dysfunction), biochemical (cholinesterase, neurotoxic esterase, creatine phosphokinase) and histopathological (axonal degeneration) changes in NIH Swiss mice.

EXPECTED PRODUCTS (MILESTONES): In this study we expect to determine that physical exercise amplifies the neuromuscular abnormalities in pyridostigmine treated mice.

STATUS/RESULTS TO DATE: Sixty NIH Swiss mice were ordered and they have been divided into four groups of 15 animals. Animals in group III and VI are being exercised on treadmill. The experiments are in progress. After 10 weeks of various treatments, the animals will be observed for behavioral changes and neuromuscular dysfunction. They will be sacrificed after 10 weeks and biochemical and histopathological changes in blood, brain, spinal cord and sciatic nerve will be recorded. The data will be correlated with the morbidity associated with Gulf War Veterans.

PUBLICATIONS: none to date

Title: PGW Veterans: Epidemiological and Clinical Evidence for Residual Organophosphate Neurotoxicity

Project #: DoD-63

Agency: DoD

Study Location: Oregon Health Sciences Univ

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Peter Spencer, Ph.D.

Research Focus: Brain & Nervous System

Start Date (CY):1997 Est. Completion (CY):2000

Chemical Weapons

OVERALL PROJECT OBJECTIVE: We seek definitive evidence to support or refute the proposal that Gulf War veterans have nervous-system deficits consistent with prior exposure to organophosphorus (OP) chemicals. Focus will be placed on Desert Storm soldiers within a 50 km radius of Khamisiyah, Coalition-Occupied Iraq, who may have been exposed to sarin/cyclosarin following detonation of Bunker 73 in March 1991.

SPECIFIC AIMS: 1. Compare the neurobehavioral and neurophysiological status of PGW veterans who served exclusively in Desert Shield versus those who served in Desert Storm and who were present in the Khamisiyah area during the first two weeks of March 1991. 2. Search for evidence of neurological damage consistent with the long-term sequelae of symptomatic exposure to OP compounds. 3. Determine the potential of developing an inexpensive neurobehavioral screening test to predict neurological damage associated with OP poisoning.

METHODOLOGY: Desert Storm soldiers who were proximate to Khamisiyah during and following detonation of shells containing sarin/cyclosarin will be given neuropsychological and neurophysiological examinations tailored for the detection of OP-induced deficits. Comparison groups will include other Desert Storm veterans, and veterans who only served in Desert Shield. Baseline data will be obtained from PGW-era non-deployed veterans. Civilians with a documented history of non-convulsive symptomatic exposure to OP insecticides will serve as positive controls. Symptoms, functional neurobehavioral performance, and neurophysiological measures will be assessed in study groups. The fit between the spatial-temporal location of veterans, known releases of OP nerve agents, and evidence of neurological deficits will be determined.

EXPECTED PRODUCTS (MILESTONES): 1. Characterize OP-related nervous system changes in civilian positive controls; 2. Obtain database from the Department of Defense (DoD) of the DoD-estimated 20,000 individuals in the Khamisiyah cohort (50 km radius only) with possible sarin/cyclosarin exposure. Complete sampling plan; 3. Locate deployed and nondeployed subjects and carry out exposure/health interviews; 4. Carry out and analyze results of neurobehavioral testing (Phase 1); 5. Carry out and analyze results of neurophysiological examination (Phase 2).

STATUS/RESULTS TO DATE: 1) Items that have been developed include: the study protocol; introductory letter; the script introducing the computer-assisted telephone interview (CATI), including the consent statement that will be read; a draft of the study CATI questionnaire; and details of the neurophysiological test battery. These are under mandatory review by the Human Subjects Review Board of the Office of the Surgeon General. 2) Human studies will commence upon approval from the Human Subjects Review Board of the Office of the Surgeon General. 3) Bids have been solicited for the CATI.

Note: Activation of human subject research has been delayed by several months because of an unadvertised requirement for review of this IRB-approved proposed human research by the Office of the Surgeon General.

PUBLICATIONS: Spencer PS, Schaumburg, HH, Ludolph AC. Experimental and Clinical Neurotoxicology, 2nd Edition. Oxford Univ Press, New York (in press).

Spencer and the Portland Environmental Hazards Research Center Team. Health effects of exposures in the Gulf War. VIIIth International Congress on Toxicology, Paris, France, July 1998, in press.

Title: Individual Differences in Neurobehavioral Effects of Pyridostigmine
Project #: DoD-64 Agency: DoD Study Location: Midwest Res Institute
Project Status: Ongoing Research Type: Clinical Research
P.I.: Mary R. Cook, Ph.D. Research Focus: Pyridostigmine Bromide
Start Date (CY):1997 Est. Completion (CY):2001 Brain & Nervous System Prevention

OVERALL PROJECT OBJECTIVE: To provide the U.S. Army with a more complete body of knowledge for optimal use of pyridostigmine as a prophylactic organophosphate-defense agent if a future large-scale deployment is needed.

SPECIFIC AIMS: 1) Conduct a study to quantify any physiological or performance consequences of the use of pyridostigmine; 2) determine whether exposure to heat exacerbates any effects found; and, 3) evaluate individual differences in cholinesterase inhibition that might predict deleterious response to pyridostigmine.

METHODOLOGY: In the first study, two groups of subjects (at least 18 men and 18 women per group) will be randomly assigned to take 30 or 60 mg PYR for 13 doses at 8-hour intervals. Each subject will also take 13 doses of placebo, and order of PYR and placebo will be counter-balanced. Testing will occur on days 4 and 5 of each drug regimen. The test battery to be administered includes physiological, sensorimotor, and cognitive measures. In the second study, men and women will follow the same pyridostigmine regimen, but will be tested in the heat on those physiological and performance tasks that showed effects in the first study.

EXPECTED PRODUCTS (MILESTONES): Study 1 should be completed by September 1999.

STATUS/RESULTS TO DATE: Data collection for Study 1 should begin in March or April 1998.

PUBLICATIONS: none to date

Title: Multi-disciplinary pathophysiologic studies of neurotoxic Gulf War related syndromes leading to diagnosis and treatment

Project #: DoD-65 Agency: DoD Study Location: Univ of TX SW Med Cntr at Dallas

Project Status: Ongoing Research Type: Clinical Research

P.I.: Robert Haley, M.D. Research Focus: Treatment

Start Date (CY):1994 Est. Completion (CY):2000

OVERALL PROJECT OBJECTIVE: 1) Demonstrate the specific sites and nature of neurologic damage associated with the symptoms comprising the case definition; 2) Develop a two stage system of testing and confirming the diagnosis of neurological impairment in Gulf War veterans for use in cost-effective screening of large populations of Gulf War veterans; 3) Provide insights into the pathophysiology of the neurologic impairment of Gulf War veterans upon which to base approaches to treatment.

SPECIFIC AIMS: 1) Extend the battery of tests for detecting neurotoxic brain and nerve damage; 2) Enlarge the sample of cases and controls from the prior sample of 46 to 100; 3) Apply the new battery to all 100 cases and controls; 4) Plan and conduct a population survey of Gulf War veterans to estimate the prevalence of the case definition of Gulf War-related neurologic syndrome; 5) Corroborate the pathophysiology of the neurologic dysfunction found in the population survey.

METHODOLOGY: In the first six months, we will perform the extended battery of tests on the original 46 cases and controls, and plan the population replication survey. In the second six months, we will perform the extended battery of tests on the 54 cases and controls added to the sample, and conduct the population replication survey and analyze the results. In the second year, we will perform the most predictive subset of the extended battery of tests on cases and controls selected from the population survey.

EXPECTED PRODUCTS (MILESTONES): By the end of the first year, we expect to have demonstrated the extent and nature of neurotoxic damage responsible for symptoms satisfying the case definition of the Gulf War-related neurologic syndrome, have demonstrated the pathophysiologic basis for many of the symptoms, have identified a subset of tests most useful for identifying it, and have estimated the prevalence of the syndrome in the deployed and nondeployed populations of Gulf War-era veterans. By the end of the second year, we expect to have demonstrated whether the veterans satisfying the case definition in the population sample have the same neurologic basis as those in the initial sample.

STATUS/RESULTS TO DATE: 1. Conducted an epidemiologic survey of a reserve Seabees battalion, developed a case definition of Gulf War-related neurologic dysfunction (6 syndrome variants), demonstrated that cases satisfying the case definition had organic neurologic dysfunction compared with matched controls, conducted a further case-control analysis showing that the cases were more likely (RR 4-8) than controls to report wartime exposure to being present during a chemical weapons alarm, being present near Khafji on January 19 or 20, 1991, having advanced adverse reactions to pyridostigmine bromide, using large amounts of government-issue insect repellent, wearing pet tick and flea collars, and working as a security guard. These findings supported the theory that the Gulf War-related neurologic syndrome was caused by exposure to combinations of organophosphate-like chemicals in the war. 2. We designed experiments to test this theory in hens, carried them out in collaboration with Duke and Kansas State Universities and EPA, and demonstrated that combinations of DEET, pyridostigmine, permethrin, and chlorpyrifos act synergistically to cause delayed, chronic neurotoxicity. 3. Completed a study of the neuropsychologic evidence of organic brain dysfunction in ill Gulf War veterans. 4. Completed a study of the audiovestibular evidence of brainstem dysfunction in ill Gulf War veterans. 5. Conducted a literature review of 19 published reports of posttraumatic stress disorder in Gulf War veterans demonstrating that the apparent prevalence of PTSD was entirely due to errors in measurement and that there is no basis for the contention that Gulf War veterans are ill from PTSD and combat stress. 6. Reanalyzed the evidence in three epidemiologic studies comparing the prevalence of mortality, hospitalization and birth defects in deployed versus nondeployed Gulf War-era populations and demonstrated that the original conclusions of no war-attributable effects were due to bias from incorrect calculation of confidence intervals, selection bias from the "healthy-warrior effects," and selection bias from incomplete followup of veterans who separated

from the service.

PUBLICATIONS: Haley RW, Kurt TM, Hom J. Is there a Gulf War syndrome? Searching for syndromes by factor analysis of symptoms. JAMA 1997; 277:222.

Haley RW, Kurt TM, Roland PS, et al. Evaluation of neurologic function in Gulf War veterans: a blinded case-control study. JAMA 1997; 277:223-230.

Haley RW, Kurt TL. Self-reported exposure to neurotoxic chemical combinations in the Gulf War: a cross-sectional epidemiologic study. JAMA 1997; 277:231-237.

Title: Testing for mycoplasmal infection replicability of nucleoprotein gene tracking and forensic polymerase chain reaction

Project #: DoD-66 Agency: DoD Study Location: WRAIR Medical Center

Project Status: Ongoing Research Type: Applied Research

P.I.: Charles Engel, Jr., M.D. Research Focus: Diagnosis

Start Date (CY):1998 Est. Completion (CY):1998

OVERALL PROJECT OBJECTIVE: To determine the reliability of three tests for *Mycoplasma fermentans* (incognitus strain) in human pure blood mononuclear cells, "usual" polymerase chain reaction procedure (UPCR), an optimized chelex polymerase chain reaction (OCPCR) procedure, and a nucleoprotein gene tracking (NGT) procedure.

SPECIFIC AIMS: 1) determine and compare the test-retest reliability of the usual PCR, optimized PCR, and NGT procedures at a laboratory experienced in these testing procedures; and 2) determine and compare inter-lab reliability of the usual PCR, optimized PCR, and NGT procedures at four different laboratories.

METHODOLOGY: 60 symptomatic Gulf War veterans (30 previously untested, 10 previously tested positive on UPCR, 10 previously tested negative on OCPCR, and 10 previously tested negative on NGT) will have blood drawn, aliquoted, and shipped overnight to 4 different laboratories for mycoplasmal testing. One of the labs, experienced in the 3 testing procedures, will retest each subject. Each aliquot will receive a separate identification code so that all labs are completely blinded as to what subject a given aliquot came from and whether any two aliquots are from the same subject. Cohen's Kappa will be used to determine the rate of agreement for each test adjusting for chance agreement.

EXPECTED PRODUCTS (MILESTONES): Information pertaining to the relative utility of these three mycoplasmal testing procedures. A manuscript summarizing the research will be submitted for peer-reviewed publication.

STATUS/RESULTS TO DATE: Participating labs were trained in the testing procedures to be used in the research. The protocol has completed external peer-review and internal institutional review board and human use committee reviews. Contracting and subcontracting arrangements are being completed, and subjects are currently being identified.

PUBLICATIONS: none to date

Title: Antibacterial Treatment method based upon the excretion of dead and decaying spherical bacteria

Project #: DoD-67

Agency: DoD

Study Location: Louisiana Med Foundation

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Edward Hyman, M.D.

Research Focus: Brain & Nervous System

Start Date (CY):1997 Est. Completion (CY):1999

**Symptoms/General Health
Diagnosis**

OVERALL PROJECT OBJECTIVE: To establish the relation of Desert Storm Syndrome (DSS) to Systemic Coccoal Disease (SCD), to characterize the infectious agent(s) involved, and to evaluate the effectiveness of the diagnostic and treatment methods we have used in civilian SCD for the management of veterans with DSS, to train VA and/or DoD medical personnel in the use of the methods.

SPECIFIC AIMS: see objectives.

METHODOLOGY: The primary clinical study is a two arm, randomized, patient and evaluator blinded, placebo controlled, pilot study of an already-tested diagnostic/therapeutic regiment for the treatment of DSS.

The methods include: 1) demonstration of bacteremia by the urinalysis method of Dr. Hyman (ref. 1,2,3 in 13 vide infra); 2) randomization of patients to treatment or control group; 3) quantitative evaluation of the manifestations of DSS by a blinded evaluating team at S.U.N.Y.-Stony Brook; 4) suppression or elimination of bacteremia by antibiotic treatment, controlled and modified by continuing surveillance of cocciuria (ref 3); 5) re-evaluation of patients (treated or placebo treated) by the blinded team at S.U.N.Y.; 6) treatment of placebo group as in D; 7) re-reevaluation of the now treated, previously placebo, patients.

EXPECTED PRODUCTS (MILESTONES): We expect to establish whether the treatment is or is not effective in improving fatigue, cognitive dysfunction, joint and somatic pain, headache, dermatitis, GI dysfunction. We expect to characterize the infecting organisms.

STATUS/RESULTS TO DATE: The code has not yet been broken. No results are available.

PUBLICATIONS: Hyman ES. Improved microscopic detection of bacteriuria. Biotech Histochem 1992, (27)1:1-8.

Hyman ES. A computer algorithm offers a comprehensive view of quantitative bacteriuria. Nephron, 1993, 65:549-558.

Hyman ES. A Urinary Marker for Occult Systemic Coccoal Disease. Nephron, 1994; 68:314-326.

Title: Five Year Follow-Up of arm Personnel Exposed to Chemical Warfare Agents
**Project #: DoD-69 Agency: DoD Study Location: Institute of Medicine/Medical
Follow-up Agency**
Project Status: Ongoing Research Type: Epidemiology Research
P.I.: Dr. R Miller Research Focus: Chemical Weapons
Start Date (CY):1997 Est. Completion (CY):1999 Symptoms/General Health

OVERALL PROJECT OBJECTIVE: This epidemiology study of morbidity and mortality outcomes of potential low-level exposure to chemical warfare agents at Khamisiyah, Iraq, and changes in the patterns of seeking health care following notification of possible exposure will be done at the request of the Department of the Army. Health outcomes and health care use patterns in the personnel of the two engineer battalions (approximately 1,000 individuals) will be compared with those of personnel of control engineer battalions. The study results have implications for the health and health care of other veterans of the Persian Gulf War.

Specific objectives are to ascertain morbidity and mortality outcomes in active duty and former active duty soldiers using passive records-based methods and compare the outcome rates with controls with varying likelihood of having been exposed to chemical warfare agents. VA and DoD hospitalizations and outpatient data will be obtained. Patterns of health perception and health care use will be compared before and after notification of possible chemical warfare agent exposure.

SPECIFIC AIMS: see objectives.

METHODOLOGY: The study and control battalions will be selected in collaboration with DASG-HS-PM. The unit identifier codes (UIC) of these battalions will be used to identify individuals, using the rosters made available by the VA Environmental Epidemiology Service. Two battalions from the Khamisiyah site will form study group #1; two comparable battalions from within the 50 km circle surrounding Khamisiyah will constitute study group #2. Control group #1 will be selected from comparable battalions deployed to the PGW theater but were never within the 50 km circle; control group #2 will consist of comparable battalions not deployed to the PGW theater. The entire study and control populations are estimated to include approximately 5,000 to 7,000 individuals.

EXPECTED PRODUCTS (MILESTONES): Findings and conclusions will be published jointly with DoD and VA investigators in a peer-reviewed professional journal.

STATUS/RESULTS TO DATE: none reported.

PUBLICATIONS: none to date

Title: Health Assessment of Persian Gulf War Veterans from Iowa

Project #: HHS-1 Agency: HHS Study Location: CDC and state of IA

Project Status: Ongoing Research Type: Epidemiology Research

P.I.: Drue Barrett, Ph.D. Research Focus: Symptoms/General Health

Start Date (CY):1994 Est. Completion (CY):1998

OVERALL PROJECT OBJECTIVE: To assess the prevalence of self-reported symptoms and illnesses among military personnel deployed during the Persian Gulf War and to compare the prevalence of these conditions with the prevalence among military personnel on active duty at the same time, but not deployed to the Persian Gulf.

SPECIFIC AIMS: This study addressed the following specific hypotheses: (1) Persian Gulf War (PGW) veterans experienced more self-reported health problems in the post-war period than did Persian Gulf-era military personnel who did not serve in the Persian Gulf region; (2) active duty military personnel with service in the Persian Gulf region experienced more self-reported health problems in the post war period than did active duty military personnel who did not serve in the Persian Gulf region; (3) National Guard and reserve personnel with service in the Persian Gulf region experienced more self-reported health problems in the post-war period than did National Guard and reserve personnel who did not serve in the Persian Gulf region; and (4) National Guard and reserve personnel with service in the Persian Gulf region experienced more self-reported health problems than active duty military personnel with comparable service.

METHODOLOGY: Data on self-reported health outcomes was collected through a telephone survey with a random sample of Persian Gulf War veterans and Persian Gulf War-era controls who listed Iowa as their home of record. Interviews were completed with 3,695 eligible study subjects (76%).

EXPECTED PRODUCTS (MILESTONES): Initial report published in JAMA on January 15, 1997.

STATUS/RESULTS TO DATE: Initial results indicate that compared with non-PGW military personnel, PGW veterans report a significantly higher prevalence of symptoms of depression, PTSD, chronic fatigue, cognitive dysfunction, bronchitis, asthma, fibromyalgia, alcohol abuse, anxiety, sexual discomfort, and health related quality of life. Additional data analysis is ongoing. A protocol has been developed for collecting physical exam data on a subset of the telephone survey participants to validate self-report of asthma. The protocol received OMB approval on 1/2/98. It is anticipated that data collection will begin in April.

PUBLICATIONS: Iowa Persian Gulf Study Group. Self-reported illness and health status among Gulf War veterans: a population-based study. JAMA 1997; 277:238-245.

Title: Disease Cluster in a Pennsylvania Air National Guard Unit, EPI-AID 95-18
Project #: HHS-2 Agency: HHS Study Location: CDC and states of PA & FL
Project Status: Ongoing Research Type: Epidemiology Research
P.I.: William C. Reeves, MD Research Focus: Symptoms/General Health
Start Date (CY):1994 Est. Completion (CY):2000 Diagnosis

OVERALL PROJECT OBJECTIVE: At request of VA, DoD, Pennsylvania Health Department, CDC investigators conducted an EPI-AID to evaluate reported cluster of illness in the Pennsylvania 193rd Air National Guard.

SPECIFIC AIMS: Characterize patients, determine if there was a cluster of illness, determine if the illness was unique to the 193rd ANG, related to residence in Pennsylvania, or related to PGW service, characterize the illness and derive a working case definition, and identify risk factors for illness.

METHODOLOGY: Stage 1 case series; stage 2 cross sectional population-based interview; stage 3 physical exam, psychometric testing, clinical laboratory, research laboratory assessment and nested case control study.

STATUS/RESULTS TO DATE: Field studies are complete. Preliminary report is published. Manuscript describing case definition, analysis of epidemiologic and infectious disease risk factors has been tentatively accepted pending satisfactory revision. Manuscript describing associations between deployment stressors and chronic multisymptom illness is in final stages of preparation. Collaborations are in place to provide serum specimens to other Gulf War Illness investigators for testing and to participate in a prospective study of the cohort. Collaborations are also in place for multicenter factor analysis of symptom data. Finally, the illness we identified in this group of Gulf War (GW) veterans is similar to chronic fatigue syndrome (CFS) and specimens will be tested by techniques under development by the CFS program to identify unique infectious agents (not detected by usual protocols), evidence of immune stimulation, and neurotransmitters.

PUBLICATIONS: Morbidity and Mortality Weekly Report: Unexplained Illness Among Persian Gulf War Veterans in an Air National Guard Unit, Preliminary Report, August 1990-March 1995. MMWR, 44(23):443-447, 1995.

Title: Biomarkers of Susceptibility and Polycyclic Aromatic Hydrocarbon (PAH) Exposure in Urine and blood Cell DNA from U.S. Army Soldiers Exposed to Kuwaiti Oil Well Fires
Project #: HHS-3 Agency: HHS Study Location: NIH/NCI/DCE/LCTP/IVP
Project Status: Completed Research Type: Basic Research
P.I.: Miriam Poirier, Ph.D. Research Focus: Environmental Toxicology
Start Date (CY):1992 Est. Completion (CY):1998 Prevention

OVERALL PROJECT OBJECTIVE: To compare PAH level in soil and air with biomarkers of exposure (urinary metabolites), biologically-effective dose (DNA adducts) and individual susceptibility (genetic polymorphisms) for this group of 62 soldiers. This project is technically not a research project. It was an analysis requested by the U.S. Army Environmental Hygiene Agency, now known as the Center for Health Promotion and Preventive Medicine, as a part of their ongoing exposure assessments.

SPECIFIC AIMS: To determine the extent of exposure and the correlation between these various biomarkers.

METHODLOGY: Soldiers participating in a USAEHA surveillance project in which ambient PAH monitoring and biomonitoring were performed. Samples of blood and urine were taken in Germany in June (1991), in August, eight weeks after deployment to Kuwait, and in October after the return to Germany. PAH-DNA adducts were measured by benzo[a]pyrene-DNA dissociation-enhanced lanthanide fluoroimmunoassay (DELFLIA) and aromatic DNA adducts were measured by ³²P-postlabeling in blood cell DNA. Urinary benzo[a]pyrene metabolites have been determined by immunaffinity chromatography and fluorescence. Glutathione-s-transferase and cytochrome P450 1A1 polymorphisms have been determined by PCR amplification of the DNA.

EXPECTED PRODUCTS (MILESTONES): The anticipated findings were higher pollution in Kuwait accompanied by higher levels of DNA adducts and urinary metabolites.

STATUS/RESULTS TO DATE: By both methodologies, blood cell DNA adduct measurements were lower in Kuwait than in Germany in June and October. The increase in adduct levels in October (compared to August in Kuwait) was statistically significant. Levels of urinary 1-hydroxy-pyrene-glucuronide (-OH-PG) were also lower in Kuwait than in Germany but the differences were not statistically significant. No differences in biomarker levels were associated with any metabolic polymorphism. The data suggest that the location of the soldiers' duty stations in Kuwait represented a cleaner working environment than Germany. Actual PAH measurements of air and soil in Kuwait showed very low levels of contamination. Literature values for ambient PAH measurements for Germany at the same time are much higher.

PUBLICATIONS: Poirier MC, Weston A, Schoket B, et al. Biomonitoring of U.S. Army soldiers serving in Kuwait in 1991. Manuscript accepted by the journal "Cancer Epidemiology Biomarkers and Prevention." (in press).

Title: Suspected Increase of Birth Defects and health Problems Among Children Born to Persian Gulf War Veterans In Mississippi
Project #: HHS-4 Agency: HHS Study Location: CDC, Jackson, MS
Project Status: Completed Research Type: Epidemiology Research
P.I.: Alan Penman, MBChB, Research Focus: Reproductive Health
Start Date (CY):1994 Est. Completion (CY):1996

OVERALL PROJECT OBJECTIVE: To determine the nature and extent of a reported increase of birth defects and health problems among children born to Persian Gulf War veterans from two units of the Mississippi National Guard.

SPECIFIC AIMS: To calculate the number and rate of birth defects and health problems in children born to Persian Gulf War veterans from two units of the MS National Guard after their return from overseas service, and determine whether an excess of birth defects/health problems occurred.

METHODOLOGY: Descriptive survey by medical record review, supplemented by parent/physician interview as required. Comparison of observed numbers/rates with expected numbers/rates from birth defect surveillance systems, epidemiological studies, etc.

EXPECTED PRODUCTS (MILESTONES): Final report published by CDC on 12/19/94 (Field Epidemiology Report #95-01).

STATUS/RESULTS TO DATE: The total number of all types of birth defects was not greater than expected. The frequency of premature birth, low birth weight, and other health problems appeared similar to that in the general population.

PUBLICATIONS: Penman AD, Tarver RS. No evidence of increase in birth defects and health problems among children born to Persian Gulf War veterans in Mississippi. *Military Medicine*, 1996, 161(1):1-5.

Title: Cognitive Function and Symptom Patterns in Persian Gulf Veterans

Project #: HHS-5 Agency: HHS Study Location: Boston Univ School of Public Health

Project Status: Ongoing Research Type: Clinical Research

P.I.: Drue H. Barrett, Ph.D. Research Focus: Brain & Nervous System

Start Date (CY):1997 Est. Completion (CY):2000 Symptoms/General Health

OVERALL PROJECT OBJECTIVE: 1) Delineation of a neuroanatomical/neurophysiological basis, if any, for the reported cognitive dysfunction in some Gulf War veterans; 2) Delineation of symptom patterns shown by some individuals who served in the Gulf War; and 3) verification of research findings in an independent cohort of soldiers who served in the Gulf shortly after cessation of hostilities.

SPECIFIC AIMS: 1) Examine differences in degree of activation present on fMRI scans in specific neuroanatomical regions within the frontal lobe, particularly the cingulate gyrus area and the dorsolateral prefrontal cortex, in Gulf War veterans challenged with a test of working memory; 2) Use a mathematical technique of Logical Analysis of Data (LAD) to analyze the relationships of symptom responses in Gulf-deployed and non-Gulf-deployed subjects; and 3) Replicate symptom prevalence comparisons and neuropsychological tests in a Danish cohort of Gulf-deployed and non-Gulf-deployed soldiers.

METHODOLOGY: 1) Both conventional MRI and functional imaging will be used to assess changes in brain activation, to examine symptom profile, and to examine the relationship between functional activation and clinical state. Subjects will be selected from an ongoing Boston Environmental Hazards Center study of neuropsychological function and will include 40 high-symptom Gulf-deployed veterans, 40-low-symptom Gulf-deployed veterans, and 40 Germany-deployed veterans. 2) LAD will be used to examine previously collected symptom data from Gulf-deployed and Germany-deployed veterans (n=300) to see if there is a set of complaints characteristic of service in the Gulf region useful for determining etiology or for case definition. 3) Neuropsychological test results and symptom prevalence measures will be replicated and verified in a cohort of Danish armed forces. Neuropsychological function and symptom prevalence of Danish troops deployed to the Gulf (n=200) will be compared with non-deployed Danish troops (n=200).

EXPECTED PRODUCTS (MILESTONES):

STATUS/RESULTS TO DATE: Final proposal developed and submitted for IRB approval; OMB clearance package currently being developed by CDC.

PUBLICATIONS: none to date

Title: Defining Gulf War Illness

Project #: HHS-6

Agency: HHS

**Study Location: CDC, Univ of Med & Dent of NJ-
Robert Wood Johnson Med School**

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Drue H. Barrett, Ph.D.

Research Focus: Diagnosis

Start Date (CY):1997 Est. Completion (CY):2000

Symptoms/General Health

OVERALL PROJECT OBJECTIVE: To characterize and compare alternative classifications for symptoms and functional disability which remain medically unexplained in Gulf War veterans.

SPECIFIC AIMS: 1) Phase I - To assess persistence and stability of symptoms over time, as well as to compare the performance of data-driven case definitions derived from two samples: the New Jersey Center for Environmental Hazards Research (NJCEHR) sample of Department of Veterans Affairs Gulf War Veterans Registry participants and a CDC Air Force cohort. Standard definitions for medically unexplained symptoms will also be evaluated; 2) Phase 2 - To assess generalizability of derived and existing case definitions in a random sample of deployed and non-deployed Gulf War era veterans; 3) To assess psychiatric distress among Phase I and Phase II participants at risk for having a psychiatric diagnosis.

METHODOLOGY: All subjects from the previously studied NJCEHR cohort (N= 1,161) and a sample of the CDC Air Force cohort (N= 1,400) will be administered a symptom questionnaire. The stability of symptoms will be assessed by comparing the previous assessment of symptoms (Time 1) with the current assessment (Time 2). Case definitions derived using factor analytic procedures with Time 1 data will be compared to case definitions derived using Time 2 data. Generalizability of the case definitions will be determined by administering the symptom questionnaire on a new randomly selected national sample of Gulf War veterans and era controls (N=3,000). A standardized telephone interview will be used to assess psychiatric conditions on a sample of Phase I and Phase II participants who are identified by the symptom questionnaire as having high levels of psychologic distress (number of subjects not to exceed 600).

EXPECTED PRODUCTS (MILESTONES):

STATUS/RESULTS TO DATE: The protocol is currently being finalized.

PUBLICATIONS: none to date

Title: Mortality Follow-up Study of Persian Gulf Veterans, First Update
Project #: VA-1 Agency: VA Study Location: VAMC Washington DC
Project Status: Ongoing Research Type: Epidemiology Research
P.I.: Han Kang, Dr. P.H. Research Focus: Mortality
Start Date (CY):1996 Est. Completion (CY):1998

OVERALL PROJECT OBJECTIVE: VA's Environmental Epidemiology Service is undertaking a retrospective follow-up mortality study of Persian Gulf veterans that will compare the overall mortality rates and cause-specific mortality rate of all 697,000 servicemembers who served in the Persian Gulf theater of operations anytime between August 1990 and May 1991 with those of a comparison group of 803,526 service members randomly selected from various subsets of all servicemembers who were in service anytime between August 1990 and May 1991 but who were not deployed to the Persian Gulf theater.

SPECIFIC AIMS: Are there excess deaths among Persian Gulf veterans from various natural and external causes in comparison to their non-Persian Gulf veteran counterparts?

METHODOLOGY: A retrospective cohort mortality study; 697,000 study subjects, 800,000 controls; Cox regression model, SMR analysis. Vital status has been determined using a VA database known as the Beneficiary Identification and Record Locator Subsystem (BIRLS) and a Social Security Administration file for deaths reported through December 1995. Death certificates have been requested from VA regional offices, Federal Records Centers or from state vital statistics offices. Cause of death are coded by a qualified nosologist using the International Classification of Diseases, 9th revision, without the knowledge of whether the decedent served in the Persian Gulf area. As the first mortality follow-up of Persian Gulf veterans, this study represents an important first step in addressing the health concerns of Persian Gulf veterans. The study results as reflected in mortality should provide an early warning for any possible life threatening health conditions as a consequence of Persian Gulf service. The study will be periodically updated to ascertain any long-term consequences of Persian Gulf service on mortality outcomes.

EXPECTED PROEJCTS (MILESTONES): Data collection is complete. Preliminary results were presented at the 125th Annual APHA meeting in November 1997.

STATUS/RESULTS TO DATE: The original study indicates that the overall mortality rate is higher in Persian Gulf veterans compared to non-deployed era veterans. However, when causes due to accident, suicide and homicide are accounted for, there is no difference in mortality rate between the two groups. The updated mortality follow-up through December 1995 continues to show that excess mortality in Gulf veterans are due to external causes. This study will be periodically updated to ascertain any long-term consequence of Persian Gulf service on mortality outcomes.

PUBLICATIONS: Kang H, Bullman TA. Mortality among U.S. veterans of the Persian Gulf war. The New England Journal of Medicine, 1996, 335:1498-1504.

Title: National Health Survey of Persian Gulf Veterans

Project #: VA-2

Agency: VA

Study Location: VAMC Washington DC

Project Status: Ongoing

Research Type: Epidemiology Research

P.I.: Han Kang, Dr. P.H.

Research Focus: Symptoms/General Health

Start Date (CY):1994 Est. Completion (CY):2001

OVERALL PROJECT OBJECTIVE: To estimate prevalence of various symptoms and other health outcomes for Persian Gulf War veterans.

SPECIFIC AIMS: To estimate and compare prevalence of various symptoms, medical conditions, and unexplained illnesses in Persian Gulf war veterans and those of non-Persian Gulf veterans.

METHODOLOGY: A population-based health survey. Several studies are currently underway that investigate the incidence, prevalence, and nature of illnesses in Gulf War veterans. The National Health Survey of Persian Gulf Veterans and Their Family Members is conducted in three phases. VA-2A through VA-2C describe the different phases of this program. .

EXPECTED PROJECTS (MILESTONES): Publication in a peer-reviewed journal.

STATUS/RESULTS TO DATE: The proposed study protocol and questionnaire were peer-reviewed and approved by an external scientific oversight committee in April 1995. The proposed survey questionnaire was approved by the Office of Management and Budget (OMB) in September 1995.

The initial questionnaires were mailed in November 1995 and the first supplemental questionnaires were mailed in January 1996 to 20,426 non-respondents and individuals with new mailing addresses.

Phase I of the survey was completed in June 1996. Phases II and III are in process.

PUBLICATIONS: none to date

Title: VA National Survey of Persian Gulf Veterans - Phase I

Project #: VA-2A

Agency: VA

Study Location: VAMC Washington DC

Project Status: Completed

Research Type: Epidemiology Research

P.I.: Han K. Kang, Dr. P.H.

Research Focus: Symptoms/General Health

Start Date (CY):1995 Est. Completion (CY):1996

OVERALL PROJECT OBJECTIVE: To estimate prevalence of various symptoms and other health outcomes for Persian Gulf War veterans.

SPECIFIC AIMS: To estimate and compare prevalence of various symptoms, medical conditions, and unexplained illnesses in Persian Gulf war veterans and those of non-Persian Gulf veterans.

METHODOLOGY: A population-based health survey. Several studies are currently underway that investigate the incidence, prevalence, and nature of illnesses in Gulf War veterans. The National Health Survey of Persian Gulf Veterans and Their Family Members is conducted in three phases. In this Phase I, a questionnaire was mailed to each of 30,000 veterans (15,000 Persian Gulf Veterans; 15,000 non-Persian Gulf Veterans). Multiple follow-up mailings were made to increase the response rate.

EXPECTED PROJECTS (MILESTONES): Publication in a peer-reviewed journal.

STATUS/RESULTS TO DATE: Phase I of the survey was completed in June 1996.

PUBLICATIONS: none to date

Title: VA National Survey of Persian Gulf Veterans - Phase II

Project #: VA-2B Agency: VA Study Location: VAMC Washington DC

Project Status: Ongoing Research Type: Epidemiology Research

P.I.: Han K. Kang, Dr. P.H. Research Focus: Symptoms/General Health

Start Date (CY):1996 Est. Completion (CY):1998

OVERALL PROJECT OBJECTIVE: To estimate prevalence of various symptoms and other health outcomes for Persian Gulf War veterans.

SPECIFIC AIMS: To estimate and compare prevalence of various symptoms, medical conditions, and unexplained illnesses in Persian Gulf war veterans and those of non-Persian Gulf veterans.

METHODOLOGY: A population-based health survey. Several studies are currently underway that investigate the incidence, prevalence, and nature of illnesses in Gulf War veterans. The National Health Survey of Persian Gulf Veterans and Their Family Members is conducted in three phases. In this Phase II, a sample of 8,000 non-respondents will be randomly selected and a telephone interview will be attempted using a CATI questionnaire which includes a question on reasons for refusal.

Telephone interviews with the non-respondents will assist in assessing potential non-respondent bias and will supplement the postal survey data. In addition, during Phase II, selected self-reported data collected by the postal questionnaire will be validated through records review for 2,000 veterans from each group. Phase II will be completed by June 1998.

EXPECTED PROJECTS (MILESTONES): Publication in a peer-reviewed journal.

STATUS/RESULTS TO DATE: Phase II is in process.

PUBLICATIONS: none to date

Title: VA National Survey of Persian Gulf Veterans - Phase III

Project #: VA-2C

Agency: VA

Study Location: VAMC Washington DC

Project Status: Ongoing

Research Type: Epidemiology Research

P.I.: Han K. Kang, Dr. P.H.

Research Focus: Symptoms/General Health

Start Date (CY):

Est. Completion (CY):

**Brain & Nervous System
Diagnosis**

OVERALL PROJECT OBJECTIVE: To estimate prevalence of various symptoms and other health outcomes for Persian Gulf War veterans.

SPECIFIC AIMS: To estimate and compare prevalence of various symptoms, medical conditions, and unexplained illnesses in Persian Gulf war veterans and those of non-Persian Gulf veterans.

METHODOLOGY: A population-based health survey. Several studies are currently underway that investigate the incidence, prevalence, and nature of illnesses in Gulf War veterans. The National Health Survey of Persian Gulf Veterans and Their Family Members is conducted in three phases. In this Phase III, the same 2,000 veteran respondents and their family members from each group will be invited to participate in a comprehensive physical examination under a uniform comprehensive clinical examination protocol at a VA medical facility. Implementation of Phase III will begin in the near future.

EXPECTED PROJECTS (MILESTONES): Publication in a peer-reviewed journal.

STATUS/RESULTS TO DATE: Phase III will begin in the near future.

PUBLICATIONS: none to date

Title: Use of Roster of Veterans Who Served in Persian Gulf Area

Project #: VA-3 Agency: VA/DoD Study Location: VAMC Washington DC

Project Status: Ongoing Research Type: Epidemiology Research

P.I.: Han K. Kang, Dr. P.H. Research Focus: Symptoms/General Health

Start Date (CY):1994 Est. Completion (CY):1998

OVERALL PROJECT OBJECTIVE: To properly address the issue of possible exposure to environmental hazards, all individuals who served in the Persian Gulf region need to be identified.

SPECIFIC AIMS: To prepare a computer file of over 697,000 troops assigned to the Persian Gulf area during Operation Desert Shield/Desert Storm.

METHODOLOGY: The U.S. Army and Joint Services Environmental Support Group (ESG) is in the process of computerizing troop unit locations in the Persian Gulf Theater.

EXPECTED OUTCOMES (MILESTONES): Complete description of unit locations in the Persian Gulf Theater.

STATUS/RESULTS TO DATE: The personnel database is now operational (about 750,000 individuals). Troop movement software is now operational. Documenting troop movements from Combat Records and from individuals who served in the Gulf is continuing. This database has been made available to VA's Environmental Epidemiology Service (EES), giving VA access to demographic data on troops stationed in the Persian Gulf. The risk assessment data are obtained, and oil field emissions modeling data are synthesized.

RESULTS: The initial troop roster and troop unit locations were available July 1996.

PUBLICATIONS: none to date

Title: Boston Environmental Hazards Research Center Program

Project #: VA-4 Agency: VA Study Location: VAMC Boston

P.I.: D. Ozonoff, M.D./R. White, Ph.D.

This is the parent program for Projects VA-4A through VA-4F.

Title: Boston Environmental Hazards Research Center Program

Project #: VA-4Core Agency: VA Study Location: VAMC Boston

Project Status: Ongoing Research Type: Clinical Research

**P.I.: D. Ozonoff, M.D./R White, Ph.D. Research Focus: Symptoms/General Health
Brain & Nervous System**

The primary purpose of the BEHC Core is to support pilot projects concerned with PGW veterans' health issues, and thus, provides funding to initiate projects that then will be developed into externally funded projects. Additionally, there are various administrative and organizational tasks that are central to the Center's function.

Therefore, the Core budget reflects the above-described purposes. Specifically, it supports pilot or new start-up projects not currently part of one of the Center's six formal projects, administrative expenses, and partial salary support for personnel who work on specific tasks central to the Center functioning. These tasks include administration (i.e., budget work, coordinating reports and annual updates), computer set-up and data management, and consultation.

PUBLICATIONS: see VA-4 subprojects

Title: Evaluation of Cognitive Functioning of Persian Gulf Veterans

Project #: VA-4A

Agency: VA

Study Location: VAMC Boston

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Jessica Wolfe, Ph.D

Research Focus: Brain & Nervous System

Start Date (CY): 1994 Est. Completion (CY): 1999

Symptoms/General Health

OVERALL PROJECT OBJECTIVE: The primary focus of this research project is to determine whether specific cognitive deficits or deficit patterns are associated with the presence of self-reported health complaints and/or with hazardous environmental exposures in the Gulf. (Exposure information will be obtained either from self-report, exposure modeling, and DoD data on troop locations). In examining these associations, the influence of war-zone stress, PTSD symptomatology, psychological distress, and post-deployment life stressors is being evaluated. **SPECIFIC AIMS:** The specific objectives of this study are: (1) to observe and determine whether cognitive performance deficits are associated with self-reported health complaints stemming from Persian Gulf deployment; (2) to determine the relationship of environmental exposure (self-report and based on an independent exposure protocol) during deployment to cognitive performance; (3) to characterize subsets of cognitive patterns or deficits among exposure classes; (4) to delineate the association of cognitive alterations or deficits with levels of combat exposure, combat stress, and PTSD symptomatology; and (5) to conduct a preliminary examination of the relationship among health symptoms, hazardous environmental exposure, and other war-zone experiences, specifically traumatic war-time stressors and ensuing trauma symptomatology (Sutker, Uddo, Brailey, & Allain, 1993; Wolfe, Keane, & Young, in press). Identification of diagnostic predictors and their relationship to outcomes of interest will help establish preliminary models related to important components of the military experience. The development of these models will aid Department of Veterans Affairs (VA) and Department of Defense (DoD) activities related to veterans' health care by: (1) facilitating symptom recognition and diagnosis in areas of growing concern (environmental exposure and traumatic stress following war), (2) emphasizing interdisciplinary approaches, (3) contributing to treatment planning, and (4) providing a baseline against which cognitive and mental status change over time can be gauged. Observations and findings from this study can lead to more focused investigation of cognitive functions that may be directly associated with defined exposures and/or PTSD symptomatology.

METHODOLOGY: Subjects in this face-to-face study are contacted by phone and asked to participate in as many parts of the protocol as they would like. The protocol consists of an in-depth questionnaire which asks about background information relating to job, education, family history, medical and psychological problems, an interview with an Environmental Health specialist about experiences and exposures while in the Gulf, a psychological interview with a clinical psychologist asking about readjustment issues and psychiatric stress since the war, and neuropsychological tests examining primarily memory, attention, and learning abilities.

EXPECTED PRODUCTS (MILESTONES): Multiple scientific manuscripts, annual reports, annual reports of Boston Environmental Hazards Center, longitudinal data on stress and health.

STATUS/RESULTS TO DATE: We completed the data collection phase of Project #1 in the Fall of 1996. A total of 220 subjects from a stratified, random sampling from the larger Devens cohort participated. In addition, 73 PGW veterans from the New Orleans area (in collaboration with Drs. Pat Sutker and Jennifer Vasterling); and 50 controls (veterans deployed to Germany but not the Gulf) have completed the Project #1 protocol.

Cleaning and merging all the Project #1 data has been completed and data analyses and manuscript writing are in progress. Analyses of health symptom reporting by the Devens, New Orleans, and Germany-deployed subjects have been carried out specifically examining the effect of self-reported environmental exposure on health symptom reporting, controlling for war-zone exposures and traumatic stress. A manuscript has been written describing these results and it has been submitted for consideration for publication. In addition, a "Letter to the Editor" to the American Journal of Epidemiology (in response to a recent article by Dr. Haley about PTSD) has been accepted for publication and is in press. Several other manuscripts are in progress (topics include: neuropsychological test performance, PTSD and psychiatric status, and exposure assessment). (A paper describing health symptom reporting in the Devens cohort at Time 2 was recently published in

the American Journal of Industrial Medicine.)

In order to substantiate and corroborate information reported to us by veterans regarding potential hazardous exposures in the Gulf, we are working on incorporating all the collected epidemiological data into a Geographic Information System (GIS) format so that temporal and spatial characteristics can be modeled and integrated into data analytic procedures. We are also working with the US Army's Center for Health Promotion and Preventative Medicine (CHPPM) and the US Army's Center for Research of Unit Records (RUR) to incorporate some of their information on oil fire smoke exposure estimates and unit-level troop locations into our analyses. When we have worked out the technical issues regarding modeling location information, we will conduct analyses to compare individual troop location and exposure information from our study subjects to information contained in the RUR computer database for our particular units of study and their effect on health symptom patterns, neuropsychological test performance, and psychological stress.

PUBLICATIONS: Wolfe J, Proctor SP, Davis JD, Borgos MS, Friedman M. Health symptoms reported by Persian Gulf War veterans two years after return. American Journal of Industrial Medicine 1998, 33:104-113.

Proctor SP, White RF, Wolfe J, Heeren T, Borgos MS, Davis JD, Vasterling J, Sutker P. Health status of Persian Gulf War veterans: Self-reported health symptoms. Presented as part of a symposium "Persian Gulf War Veterans' Health Complaints- Psychological Studies" at the Annual Meeting of the American Psychological Association, Chicago, IL; Organizer: RF White, Ph.D., 1997.

Wolfe J, Brown P, Kelley J. Reassessing war stress: exposure and the Gulf War. J Soc Issues, 1993, 49, 15-31.

Title: Evaluation of Neurological Functioning in Persian Gulf Veterans
Project #: VA-4B Agency: VA Study Location: VAMC Boston
Project Status: Ongoing Research Type: Clinical Research
P.I.: Robert Feldman, M.D. Research Focus: Brain & Nervous System
Start Date (CY): 1994 Est. Completion (CY): 1999 Symptoms/General Health

OVERALL PROJECT OBJECTIVE: This study evaluates neurological functioning in returning Persian Gulf War (PGW) veterans using clinical examinations and neuroimaging techniques capable of detecting subtle changes in central nervous system functioning (functional magnetic resonance imaging [fMRI]). The following study description reflects a change from the original proposal.

SPECIFIC AIMS: The specific objectives of this study are: (1) to objectively describe Persian Gulf War veterans' neurological status using standard clinical methodology, (2) to observe and determine patterns of central nervous system dysfunction in PGW veterans and PGW-era veterans and (3) to explore whether similar patterns of particular exposure characteristics exist within the groups and whether distinct patterns exist between the groups.

METHODOLOGY: Subjects for clinical neurological exams are veterans from the subjects evaluated in Project 1. These subjects are also being examined as part of the laboratory and pulmonary function study in Project #4, VA-4D.

EXPECTED PRODUCTS (MILESTONES): In this study, we expect to determine if there are differences in subtle neurological functioning between PGW veterans and PGW-era veterans.

STATUS/RESULTS TO DATE: Fifty subjects have undergone neurological exams. Pilot functional magnetic resonance imaging (fMRI) studies are currently in progress. This past year has been devoted to establishing the paradigms and setting up the equipment necessary to perform and analyze the functional imaging studies, as well as to begin scanning normal controls. Preliminary neuropsychological testing (from Project #1) has revealed several areas of behavior where these subject groups differ from normal controls. One of those areas is working memory. We have therefore created a visual working memory task that can be used for fMRI studies. In addition, we will examine motor function (Finger Tapping) and attention (Continuous Performance Test) in fMRI studies. We have also completed the computer programs necessary to analyze the fMRI imaging studies.

In the summer of 1997, we submitted a grant application to establish a collaborative agreement project with CDC in which we used the preliminary information collected on fMRI (described above) and the work done on establishing the test paradigms to enlarge the scope of the above described fMRI project. This new study project was funded on November 1, 1997 (for 3 years). Thus, the fMRI work will now be incorporated into the new CDC grant's scope.

PUBLICATIONS: none to date

Title: Gulf War And Vietnam Veterans Cancer Incidence Surveillance

Project #: VA-4C

Agency: VA

Study Location: VAMC Boston

Project Status: Ongoing

Research Type: Epidemiology Research

P.I.: Richard Clapp, D.Sc.

Research Focus: Environmental Toxicology

Start Date (CY):1994 Est. Completion (CY):1999

OVERALL PROJECT OBJECTIVE: To examine the pattern of cancer diagnosed in Massachusetts Vietnam veterans as compared with Vietnam-era veterans for the time period 1988-1993. This linkage will be accomplished by linking a computer tape of Massachusetts veterans who received differential bonuses to the Massachusetts Cancer Registry master file and analyzing the resulting linked cases, as was done for the years 1982-1988 in prior study by the Principal Investigator. Another objective is to establish a database linkage for New England area Persian Gulf War veterans so as to be able to examine cancer incidence in this cohort at a later point in time.

SPECIFIC AIMS: 1. Monitor cancer incidence in Gulf War and Vietnam veterans of New England states, as part of the overall surveillance of disease in veterans exposed to environmental hazards; 2. Extend previous studies of cancer incidence and mortality by collecting and analyzing data on Massachusetts Vietnam veterans diagnosed in the years 1988-1993; 3. Analyze the results of the Massachusetts veterans cancer incidence linkage in a nested case-control design incorporating information on military and civilian occupations, branch of service and, where possible, location of service; 4. Establish a roster of Gulf War veterans in New England states, with identifying information such as names, dates of birth, and social security numbers, resulting in a computer file of Gulf War veterans that can be used in future linkage studies examining cancer incidence and mortality from various diseases; 5. Provide a database from which to plan additional clinical and preventative interventions to reduce morbidity and mortality from targeted diseases.

METHODOLOGY: In Year 1, we plan to initiate the linkage. In years 2-5, we plan to sort the cases into cancers of interest and other (auxiliary) cancers, analyze the data as a "nested" case-control study, and present odds ratios for specific cancers in Vietnam veterans compared with Vietnam-era veterans. Also in years 2-5, we plan to devise a system for linking the roster of Gulf War veterans in the New England states with cancer registries in all states which have population-based data.

EXPECTED PRODUCTS (MILESTONES): In this study we expect to determine cancer incidence rates in Massachusetts Vietnam veterans for the period 1988-1993. At completion of year 2 of the study, we expect to have preliminary results based on analysis of this database. Results pertaining to cancer incidence in Gulf War veterans is not expected for 10 years or so due to the latency period in cancer development.

STATUS/RESULTS TO DATE: Dr. Clapp has recently completed his update of cancer incidence in Massachusetts Vietnam veterans compared to Vietnam-era veterans. This was done using methods described in a previously published study by Dr. Clapp and colleagues (Clapp et al. Intl. J. Epidemiol., 1991; 20:7-12). Findings were that of the 245 cancers in Vietnam veterans diagnosed between the ages of 35 and 64 during the years 1988 to 1993, there were substantially increased numbers of nasal/nasopharyngeal cancer and oral cancer and smaller increases in other types of cancer. A Letter to the Editor to the International Journal of Epidemiology was published in June 1997.

The method of doing surveillance of cancer in Vietnam veterans is planned for Gulf War veterans. The technique of linking the roster of Gulf War veterans to State Cancer Registries were presented at a meeting of Central Cancer Registries in April 1997 and the plan to begin cancer surveillance will be developed and implemented in the coming years.

PUBLICATIONS: Clapp, R. Update of cancer surveillance of veterans in Massachusetts. Letter to the Editor, International Journal of Epidemiology; 26(3):679-681, 1997.

Clapp R. Cancer surveillance of veterans in Massachusetts 1988-1993. Abstract presented at the North American Association of Central Cancer Registries, 1997 Annual Conference held in Boston, MA, April 1-3, 1997.

Clapp R. Cancer surveillance of Vietnam veterans in Massachusetts, USA 1982-1993. Abstract submitted for presentation at the 12th Symposium of the

Scientific Committee on Epidemiology in Occupational Health of the International Commission on Occupational Health held in Zimbabwe, September 16-19, 1997.

Title: Evaluation of Respiratory Dysfunction Among Gulf War Veterans
Project #: VA-4D Agency: VA Study Location: VAMC Boston
Project Status: Ongoing Research Type: Clinical Research
P.I.: Lewis Pepper M.D. Research Focus: Environmental Toxicology
Start Date (CY): 1994 Est. Completion (CY): 1999 Symptoms/General Health

OVERALL PROJECT OBJECTIVE: To assess respiratory status in relation to exposure to oil fire pollution in Gulf War veterans and evaluate the relationship between measures of pulmonary dysfunction and respiratory symptoms and exposure in subjects recruited for Project #1.

SPECIFIC AIMS: 1. Create a cumulative personal exposure estimate for each subject utilizing the U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM) exposure assessment data for their unit combined with geographic specific information which maps each unit's location in the Persian Gulf War theater; 2. Assess respiratory status using standardized respiratory questionnaire; 3. Assess respiratory function with physical examination and pulmonary function testing; 4. Assess pre-existing asthma risk with total serum IgE levels; 5. Assess non-specific bronchial responsiveness (BHR) as a measure of early outcome and as a potential risk factor in the development of lung disease by using methacholine challenge testing (initiating in Year 2 of the study); 6. Examine possible predictors of respiratory function (pulmonary function tests (PFT) and methacholine challenge test) using cumulative environmental exposure assessment variable, respiratory medical history, occupational history, and smoking status variables; 7. Develop a database from which to plan preventative interventions to reduce morbidity and mortality from respiratory diseases.

METHODOLOGY: Subjects will be veterans from the subjects evaluated in Project 1. Each will complete a standardized respiratory questionnaire, systematic cardio-pulmonary physical exam, and pulmonary function testing. Spirometric measures will include forced expiratory volume in one second (FEV1), forced vital capacity (FVC) and FEV1/FVC in accordance with American Thoracic Society recommendations. Subject data will be adjusted for age, height and gender and transformed to percentage predicted values using Knudson predictive equations. Blood will be drawn by venapuncture at the time of the visit and total serum IgE, complete blood count and differential and quantitative immunoglobulin levels will be measured. Additional blood samples will be drawn and stored for future laboratory tests (e.g., DNA analyses, infectious diseases). Bronchial responsiveness will be determined via methacholine challenge testing starting in Year 2. Nonspecific bronchial hyperresponsiveness has been suggested as a risk factor for, or an early event in the development of both acute and chronic occupational lung diseases. Occupational exposures to irritant substances at concentrations similar to the Gulf War exposures have been shown to cause airway hyperresponsiveness and it is believed that these exposures might be associated with the development of asthma.

EXPECTED PRODUCTS (MILESTONES): In this study, we expect to determine if there are differences in respiratory function (PFTs) in Gulf War veterans associated with cumulative personal exposure estimates obtained from exposure modeling by the U.S. Army Hygiene Agency. At the completion of Year 3 of the study, we expect to have preliminary results based on analyses of the data collected at that time.

STATUS/RESULTS TO DATE: We have expanded the study protocol to include members of a unit in an air ambulance company who were activated and sent to Germany during the Gulf War during December 1990 through August 1991. This group was added as a comparison group as it was mobilized but not deployed to the Persian Gulf. It consists of medics, helicopter pilots, flight crews, mechanics, communications specialists, and administrative support personnel whose intended mission was handling and transport of wounded US soldiers evacuated from the Gulf. Due to low US military casualties, the unit assisted German civilian missions. Fifty subjects from this unit have participated in Project #1.

In order to conduct the planned methacholine challenge testing, we have relocated the study test site to the Pulmonary Lab at Boston Medical Center. The relocation has resulted in a delay in starting up this phase of the study. At this time a total of 88 subjects have completed the pulmonary questionnaire and 65 have come in for pulmonary function testing (these subjects have also participated in Project #1). We plan to call these people back in to complete the study with the

methacholine challenge testing. Recruitment of study subjects who will receive methacholine challenge as well as conventional pulmonary function testing, physical exam, and blood tests was initiated in January 1998.

PUBLICATIONS: none to date

Title: The Aromatic Hydrocarbon Receptor (AhR) as a Biomarker of Susceptibility
Project #: VA-4E Agency: VA Study Location: VAMC Boston
Project Status: Ongoing Research Type: Basic Research
P.I.: David Sherr, Ph.D. Research Focus: Environmental Toxicology
Start Date (CY): 1994 Est. Completion (CY): 1999 Prevention

OVERALL PROJECT OBJECTIVE: The objective of this project is to test the hypothesis that the aromatic hydrocarbon receptor (AhR) is a molecular biomarker for polycyclic aromatic hydrocarbon (PAH) susceptibility. Veterans of the Persian Gulf war were potentially exposed to relatively high doses of PAHs in smoke emanating from Kuwaiti oil well fires. The high incidence of physical complaints by Gulf War veterans may reflect previously documented PAH toxicities including suppression of the immune system and neurologic dysfunction.

SPECIFIC AIMS: 1. Confirm that AhR expression levels correlate with PAH bioactivity; 2. Optimize the use of single strand conformational polymorphism (SSCP) analysis to detect AhR gene polymorphism and mutations in mice; 3. Apply results to human studies. Results obtained in the fulfillment of aims #1 and 2 will define the conditions for employing AhR levels and gene polymorphism as molecular biomarkers of PAH susceptibility.

METHODOLOGY: The first aim of the project is to confirm that the level of AhR in mice correlates with PAH toxicity and carcinogenicity. To do this first we propose genetically engineering AhR transgenic mice in which elevated AhR expression is directed to lymphocytes and then assessing a) PAH-induced immunosuppression as a representative indicator of organ toxicity and b) lymphocyte and lung transformation as measures of cancer risk in AhR transgenic mice.

To prepare for human studies, we will evaluate AhR polymorphism in mice using single strand conformational polymorphism (SSCP) analysis. Double stranded DNA from samples of veterans' blood is separated by heating followed by rapid cooling on ice. The three-dimensional conformation reassumed by each strand is determined by its nucleic acid sequence. Single nucleotide changes can be detected when the reannealed DNA is run on 6% acrylamide gels.

To apply results to humans, polyclonal and monoclonal antibodies to human AhR (and/or ARNT) will be produced and employed in conventional or PCR-based enzyme-linked immunosorbent assays (ELISA) of AhR protein levels in peripheral blood lymphocytes from Persian Gulf War veterans who complete Projects #1 and 4.

EXPECTED PRODUCTS (MILESTONES): This project will result in the production of new strains of mice which differ in their responsiveness to environmental pollutants. These mice will represent invaluable tools in investigating the effects of these pollutants in humans. We will also develop the technology to screen veterans of the Persian Gulf war for genetic factors which could put them at risk for immune defects and cancer.

STATUS/RESULTS TO DATE: The primary goal of aim # 1 is to develop mutant mice that express various levels of AhR. We have now successfully established an AhR knock out mouse colony and are evaluating the effects of PAH on bonemarrow stromal cells, preB cells and the thymic epithelial cells isolated from these mice. The results obtained so far provide evidence that AhR expression and activation are essential for PAH-induced cell death by apoptosis in preB cells grown over the stromal cells. This mouse line will be further helpful in deciphering the role of other molecules/signals involved in the PAH-induced apoptosis of lymphocytes. These studies will involve the analysis of the expression and activation of the transcription factors and oncogenes known to be regulated by AhR activation.

We have also generated the AhR transgenic mouse line, in which full length AhR is expressed at higher levels in lymphoid tissues. This was done by subcloning the whole AhR reading frame into an expression vector containing IgH enhancer (Em) and promoter (Pm) elements. 2 founder lines are positive for AhR transgene as detected by Southern blotting and polymerase chain reaction (PCR) techniques. We have also performed the analysis for the AhR expression at the protein level in the lymphoid tissues (e.g., thymus and spleen) in the southern/PCR transgene-positive litters and compared that with wild type litters either from the same litter batch or from animals outside of this line. Immunoblotting results show Southern/PCR transgene-positive litters express high levels of AhR in their thymus and spleen as compared to wild type litters of the same age and background. These mouse lines will be used to confirm the role of AhR in PAH-induced immunotoxicity and the effect of

AhR hyper expression on the expression and activation of signal molecules involved in the PAH-induced immunotoxicity. The results obtained from the AhR transgenic mice will be compared with those of AhR knock out mice to give us a better understanding of AhR bioactivity. Specific aim #3 was to identify human AhR gene polymorphisms in the functional domains of the DNA binding (bHLH) and AhR-ligand binding (PAS) domain. Two polymorphic forms were detected in the bHLH domain by SSCP analysis. Sequencing revealed one of the polymorphic forms has several changes in this region, but interestingly, none of these changes resulted in any amino acid variation indicating the conservative nature of the DNA binding sequence of AhR. By contrast, multiple forms of the AhR PAS domain were observed and DNA sequencing revealed five nucleotide changes in the human AhR. Four of these five mutations resulted in changed codons giving rise to multiple alleles in this domain. Some of the alleles represent significant amino acid changes. Unlike significant polymorphisms in mouse AhR, we did not detect any length polymorphisms in the human AhR. As a next step, DNA fragments will be cloned into the human AhR expression vector obtained from Dr. C. Bradfield. The chimeric vectors will be transfected into AhRnull or AhR defective cell lines and tested for their responsiveness to AhR ligands (e.g., TCDD or PAH). For this purpose we have already standardized the AhR binding assays using the highly sensitive radioisotope analysis of the sucrose gradient fractions of AhR ligand treated cell cytosol.

PUBLICATIONS: Vaziri C, Shneider A, Sherr DH, Faller D. Expression of the aryl hydrocarbon receptor is regulated by serum and mitogenic growth factors in murine 3T3 fibroblasts. 1996. *J. Biol. Chem.* 271, 25921-25927.

Yamaguchi K, Near RI, Matulka RA, Shneider A, Toselli P, Trombino AF, Sherr DH. Activation of the aryl hydrocarbon receptor/transcription factor and bone marrow stromal cell-dependent preB cell apoptosis. *J Immunol.* 1997; 158:2165-2173.

Yamaguchi K, Near R, Shneider A, Cui H, Ju S-T, Sherr DH. 1996 Fluoranthene induced apoptosis in murine T cell hybridomas is independent of the aromatic hydrocarbon receptor. *Toxicol. Appl. Pharmacol.* 139:144.

Title: Validity of Computerized Tests

Project #: VA-4F

Agency: VA

Study Location: VAMC Boston

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Roberta White, Ph.D.

Research Focus: Brain & Nervous System

Start Date (CY):1994 Est. Completion (CY):1999

Diagnosis

OVERALL PROJECT OBJECTIVE: The objective of this project is to continue the validation process of the Neurobehavioral Evaluation System (NES), a battery of computer-assisted behavioral tests developed for the purpose of detecting brain dysfunction secondary to exposure to neurotoxicants, with an expanded battery of subtests. The NES tests are derived from standard neuropsychological tests that have been well characterized in terms of brain-behavior relationships, but differ in input and output modality and in other respects from traditional tests. Therefore, the validity of the NES subtests can only be verified by testing patients with known brain damage and showing that such damage affects performance on those tests. The sensitivity of the NES to neurotoxicant exposure cannot be fully interpreted, in terms of CNS mechanisms, without such validation. Because separate funding was received for a wide scale validity study of the NES3 (HSR&D Cooperative Study #29, PI: Roberta F. White, Ph.D.), this project has been curtailed to include one especially relevant clinical group for effects of toxicants, patients with Parkinson's Disease (PD). This reflects a change from the original proposal.

SPECIFIC AIMS: 1. Assess the validity of the expanded battery of NES tests within a patient group with Parkinson's Disease) studied in the previous phase of this study. 2. Correlate performance on the new NES subtests with performance on the standard paper-and-pencil tests on which they are based. 3. Determine the sensitivity of the NES subtests to the progression of neurologic disease by longitudinal testing of PD patients.

METHODOLOGY: Subjects are being recruited from both the Boston VAMC and Boston University Medical Center. Each subject will have an initial interview to provide demographic data followed by administration of the Mini-Mental Status Examination and WAIS-R Information and Picture Completion subtests (40 minutes). Subjects meeting inclusion criteria will then be administered the expanded NES battery (approximately 100 minutes).

EXPECTED PRODUCTS (MILESTONES): This study has important clinical and research implications: (1) to facilitate the assessment of patients with suspected subtle brain damage secondary to exposure from neurotoxicants by providing a sensitive, relatively brief battery of tests of CNS function that can be easily administered; (2) to provide a test battery for which brain-behavior relationships are known (providing measures which will be useful in hypothesis-driven research on the cerebral localization of neurotoxicant effects); (3) to provide tests with known diagnostic validity for application to clinical situations.

STATUS/RESULTS TO DATE: Data analysis and manuscript writing have proceeded describing the validity of NES2 in various patient groups. Two papers have been written and published. The NES3 battery fully has been developed. Subject recruitment is anticipated to start in Winter 1998.

PUBLICATIONS: Kregel M, White RF, Diamond R, Letz R, Cyrus P, Durso R. A comparison of NES2 and traditional neuropsychological tests in a neurologic patient sample. *Neurotoxicology and Teratology* 1996; 18:435-439.

White RF, Diamond R, Kregel M, Lindem K, Feldman RG: Validation of the NES2 in Patients with Neurologic Disorders. *Neurotoxicology and Teratology* 1996; 18: 441-448.

Title: East Orange Environmental Hazards Research Center Program
Project #: VA-5 Agency: VA Study Location: VAMC East Orange
P.I.: B. Natelson, M.D./J. Ottenweller, Ph.D.

This is the parent Program for VA projects VA-5A through VA-5D.

Title: East Orange Environmental Hazards Research Center Program

Project #: VA-5Core Agency: VA Study Location: VAMC East Orange, NJ

P.I.: B. Natelson, M.D./J. Ottenweller, Ph.D.

NJCEHR funds that support all center projects, rather than specific ones, are allocated under the "CORE". This includes salaries for the Administrator, Scientific Coordinator, Statistician, Statistical Aide, Biomedical Engineers, Systems Analyst, and Associate Research Director. The support of these staff members is required for all Center projects. In addition, core funds cover miscellaneous expenses such as investigator travel, subject reimbursement, subject meals and office supplies & equipment. The percentages of these costs that are allocated for each project varies according to which projects are active during each fiscal year. During this year, approximately 66% of these resources are devoted to Project 3. Thirty-four percent is devoted to a continuation of Project 2. The only major deviation from this allotment of resources is that the Statistician and Statistical Aide are currently devoting their time to analyses of data generated in project 2.

PUBLICATIONS: see VA-5 subprojects

Title: Health and Exposure Survey of Persian Gulf Veterans
Project #: VA-5A Agency: VA Study Location: VAMC East Orange
Project Status: Ongoing Research Type: Epidemiology Research
P.I.: Howard Kipen, M.D. Research Focus: Symptoms/General Health
Start Date (CY):1994 Est. Completion (CY):1998

OVERALL PROJECT OBJECTIVE: To survey 2800 Persian Gulf War registry veterans randomly sampled from the Northeast quadrant of the U.S. to determine risk factors and their relationships to symptom clusters that could represent significant health problems for Gulf veterans.

SPECIFIC AIMS: 1. To provide health and medical histories of registry veterans who served in the Persian Gulf; 2. To characterize the medical and combat experiences of these veterans during the Gulf war; 3. To perform case control comparisons for various categories and combinations of symptoms with respect to individual and environmental risk factors identified in Aims 1 and 2.

METHODOLOGY: The survey was mailed from Environmental Epidemal Service to veterans who served in the Gulf. This survey was mailed to 2800 veterans on the Persian Gulf Registry, including 1,935 selected at random from the registry. This survey includes a symptom checklist, an environmental risk factor survey, a family and social support survey, and anxiety and depression scales. Multivariate analysis of the resulting information will include cluster analysis, principal components factor analysis, and multidimensional scaling. From these analyses we expect to be able to develop one or more case definitions for the unexplained illnesses afflicting Persian Gulf veterans.

EXPECTED PRODUCTS (MILESTONES): We have found a high prevalence of Chronic Fatigue and Chemical Sensitivity among registry veterans. We also expect to delineate a series of risk factors for the development of these illnesses, and for Post-traumatic Stress Disorder which may cluster with one or both of these illnesses.

STATUS/RESULTS TO DATE: Data collection is complete. We have received 60% of our questionnaires back, including 1,161 (60%) from the random group. Symptom data has been preliminarily analyzed. The mean number of endorsed symptoms out of 48 total was 24, with headaches, difficulty remembering, and fatigue being the most bothersome. Factor analysis has identified five factors that characterized those in poor health and a preliminary definition has been presented. Confirmatory factor analyses are now being done, and case control analyses will follow.

PUBLICATIONS: Self-reported health status and exposure among Gulf war registry veterans. *Epidemiology* July 1997, Vol. 8, No. 4 Supplement.

Self-reported health status among Gulf War registry veterans, presented at APHA Annual Meeting 11/97.

Title: Physiological and Psychological Assessments of Persian Gulf Veterans
Project #: VA-5B Agency: VA Study Location: VAMC East Orange
Project Status: Ongoing Research Type: Clinical Research
P.I.: Benjamin Natelson, M.D. Research Focus: Immunology
Start Date (CY): 1994 Est. Completion (CY): 1998 Brain & Nervous System

OVERALL PROJECT OBJECTIVE: This project will determine if some of the unexplained medical illnesses in Persian Gulf veterans may be similar to Chronic Fatigue Syndrome (CFS) or Multiple Chemical Sensitivity (MCS) in civilian populations. It will include viral/immunological studies, neuropsychological studies, and studies of physiological responses to a number of physical and cognitive challenges. A series of psychosocial factors will be evaluated to determine whether they are risk factors for the illnesses suffered by these veterans.

SPECIFIC AIMS: 1. To determine whether viral or cytokine patterns may suggest an infectious agent as a cause of illness in Persian Gulf veterans; 2. To determine if these veterans suffer chronic fatigue or chemical sensitivity; 3. To determine psychosocial risk factors that might have predisposed some veterans to develop their medical problems; 4. To determine if there are neuropsychological deficits in Persian Gulf veterans; 5. To determine if these veterans have abnormal physiological responses to physiological and cognitive challenges.

METHODOLOGY: Persian Gulf veterans who have chronic fatigue, chemical sensitivity, or a combination of these and healthy control veterans are recruited for these studies. Blood samples are drawn, peripheral lymphocytes are isolated, and polymerase chain reactions are used to quantify herpes, epstein-barr and other viruses and to assess the level of interleukins and tumor necrosis factor-alpha. The subjects fill out a series of psychosocial instruments and environmental exposure surveys. A battery of neuropsychological tests are given in conjunction with magnetic resonance imaging of the brain. To evaluate the possibility of early peripheral neuropathy, neurological evaluation is supplemented with test of threshold for fine touch in the distribution of the radial nerve in the hand using Semmes-Weinstein monofilaments. Finally, we perform comprehensive testing of autonomic nervous system function and evaluate the veteran's ability to respond appropriately to behavioral challenges.

EXPECTED PRODUCTS (MILESTONES): We expect a number of the unexplained illnesses in Persian Gulf veterans will be similar to CFS and MCS. Data on civilians with CFS suggest that some cytokines and some of the herpes viruses might be elevated. Our own data on civilians with CFS reveal neuropsychological deficits in memory tasks and occasional non-specific white matter changes in the MRI's. Finally, we expect abnormal responsiveness to some of the physiological stimuli, but it remains unclear whether there will be hyper- or hyporesponsiveness.

STATUS/RESULTS TO DATE: Persian Gulf veterans seen here at the East Orange VA Medical Center were mailed a screening form to evaluate their suitability for entry into studies. Additional veterans from an epidemiological survey of 2,800 Persian Gulf veterans in the northeast United States have also been screened for entry into the studies in this project. As of 1/1/98, 166 subjects have been enrolled in our studies. After evaluation, a substantial number of veterans have fulfilled criteria for CFS, MCS, or CFS with MCS. At present, analyses have been completed for several studies and 15 manuscripts are currently in preparation. Results of our Psychological study suggest that the illness reported by all PG veterans cannot simply be attributed to an underlying psychiatric disorder. Symptomatic veterans examined often had either no Axis I diagnosis or had the same diagnoses found in our healthy veteran comparison group. Thus, it is possible that PG veterans' complaints of fatigue, pain and chemical sensitivity may either be due to exposure of environmental toxins during their service in the Gulf or may be a somatic response to the stresses of combat. Neuropsychological comparisons between healthy PG veterans and PG veterans with fatiguing illness point to at least two areas of subtle cognitive impairment in Persian Gulf War Veterans: 1) Complex attention/information processing, and 2) simple attention. Additionally, we completed an investigation examining disability and health related quality of life in Gulf War Veterans. Results from this study indicate that Gulf War veterans with fatiguing illness suffer from significant disability and functional decline. Those with a comorbid psychiatric illness suffer the most extensive disability. Tests of autonomic function indicated less responsive homeostatic reflexes, including reduced baroreceptor reflex sensitivity, in a pattern that indicated autonomic dysregulation involving sympathetic nervous

system. When challenged to perform stressful cognitive tasks (speech and arithmetic), veterans with CFS displayed significantly reduced blood pressure increases that were attributable to inadequate cardiac output. Interestingly, the most substantial hypotensive effect was in the subgroup of veterans with concurrent Axis 1 diagnosis. No significant differences were found during the cold pressor test -- an aversive sensory stressor. Hyporesponsiveness to cognitive but not to sensory stressors suggests a possible disease of brain areas interfacing high level cognitive processing and autonomic components of stress response.

PUBLICATIONS: Lange G, Tiersky L, Scharer J, Fiedler N, Pollet C, Morgan J, DeLuca J, Natelson BH, Gulf War Illness is a heterogenous disorder. Abstract for American Psychological Association, 1997 Symposium.

Fiedler N, Lange G, Tiersky L, DeLuca J, Policastro T, McNeil K, Natelson BH. Fatiguing Illness and the Gulf War veteran: The New Jersey Environmental Hazards Research Center, Abstract for American Psychological Association, 1997 Symposium.

Title: Effects of Exertion and Chemical Stress on Persian Gulf Veterans

Project #: VA-5C Agency: VA Study Location: VAMC East Orange

Project Status: Ongoing Research Type: Clinical Research

P.I.: Nancy Fiedler, Ph.D. Research Focus: Immunology

**Start Date (CY): 1997 Est. Completion (CY): 1999 Symptoms/General Health
Environmental Toxicology**

OVERALL PROJECT OBJECTIVE: The first part of this project will examine the effect of controlled chemical exposure and psychological stress on symptoms and psychophysiologic and cognitive performance of Persian Gulf veterans previously diagnosed with fatiguing illness. Research in our Center found that relative to healthy veterans, PG veterans with fatiguing illness reported significantly more combat exposure, illness in response to chemical exposures such as diesel exhaust and greater levels of negative affect and defensiveness. Therefore, this study will test the effects of each of these variables in a controlled study. The purpose of the second part of this project will be to investigate the acute effects of exhaustive exercise on the cognitive abilities and the cardiovascular responses to autonomic stressors in Persian Gulf veterans diagnosed with Chronic Fatigue Syndrome (CFS). To date our data have indicated impaired cognitive functioning and evidence of abnormal cardiovascular reactivity to various laboratory stressors in veterans with CFS when compared to healthy veterans. Also a significant number of the fatigued veterans have indicated extreme fatigue and worsening of symptoms after even moderate physical exertion. An additional variable which will be examined will be cardiopulmonary fitness. In this area we have preliminary data from project VA-5B which indicate a reduced pulmonary function in veterans with CFS, especially those who had indicated that they became sick when exposed to smoke from oil fires in the Gulf War.

SPECIFIC AIMS: 1) To assess the effect of exposure to diesel fumes and psychological stress in the following PG veteran groups: Healthy; Healthy/Negative Affect; Fatiguing Illness/Negative Affect; Fatiguing Illness/Defensive. 2) To assess the effect of exhaustive exercise on the cognitive abilities and cardiovascular response to autonomic stressors of PG veterans with CFS and healthy PG veterans.

METHODOLOGY: Veterans who were previously evaluated at our Center will be recruited. Each veteran will undergo a complete physical and psychiatric examination. Veterans who meet criteria for one of the subject groups will participate in a single experimental session during which symptoms, psychophysiologic response (i.e., heart rate, blood pressure, respiration rate, end-tidal CO₂, finger pulse volume, and skin conductance), and performance on a computerized vigilance task will be monitored in response to a rest period, followed by a psychological stressor (Stroop Color Word test), and exposure to 5 ppm diesel fumes. Blood and breath samples will confirm body burden of exposure. For the exhaustive exercise study, PG veterans with CFS and healthy PG veterans who were previously evaluated at our Center will be recruited. The subjects will perform a cognitive test battery. Next they will perform a series of cardiovascular stressors (CVEVAL): 1) supine to standing, 2) Valsalva maneuver and 3) reaction time. In this CVEVAL session we will measure beat-by-beat blood pressure, ECG activity, respiration, and end tidal CO₂. After these sessions the subjects will perform a maximal aerobic capacity test on a bike ergometer (VO₂max). During the VO₂max test metabolic data will be measured breath-by-breath, lactic acid will be assessed from finger stick blood samples, and muscle oxygenation will be evaluated by near infrared spectroscopy. Following the VO₂max test the subjects will repeat the two pre-test sessions.

EXPECTED PRODUCTS (MILESTONES): The exposure study will determine the health effects of an acute exposure to an agent (i.e., diesel), reported by veterans with fatiguing illness, to have caused symptoms during service in the Gulf and presently. The study will also assess the differential contribution of personality variables and stress to subjective (i.e., symptoms) and objective (performance, physiology) indicators of health effects. Regarding the exercise study, research has shown that the added stress of physical exercise can in some cases expose autonomic problems when conventional test have failed. Also data in our civilian population with chronic fatigue have shown a decrease in cognitive abilities after physical exertion when compared to healthy controls. We may see similar results in this veteran population.

STATUS/RESULTS TO DATE: Recruitment of veterans who meet subject criteria is ongoing. Exposure to petroleum products has been piloted in civilians and psychophysiologic data analytic

routines are established. We expect to begin in March of 1998 when we will test two veterans per week.

PUBLICATIONS: none to date

Title: Effects of Genetics and Stress on Responses to Environmental Toxins

Project #: VA-5D Agency: VA Study Location: VAMC East Orange

Project Status: Ongoing Research Type: Basic Research

P.I.: John Ottenweller, Ph.D. Research Focus: Pyridostigmine Bromide

Start Date (CY):1994 Est. Completion (CY):1997 Interactions

OVERALL PROJECT OBJECTIVE: This project will determine whether genetic susceptibility to stress and exposure to chronic stress will result in persistent sensitization to potentially toxic effects of pyridostigmine bromide and aromatic hydrocarbons. In addition, it will examine the behavioral consequences of chronic exposure to these toxins.

SPECIFIC AIMS: 1) To expose Wistar-Kyoto (WKY) rats (stress-hyper responsive) and Sprague-Dawley (SD) rats (control) rats to chronic stress and then study the sensitivity to pyridostigmine bromide by measuring startle and neuroendocrine responses and the sensitivity to aromatic hydrocarbons by measuring the activity of hepatic enzymes induced to metabolize these compounds. 2) To treat WKY and SD rats chronically with these compounds and examine behavioral rhythms, temperature rhythms, startle responsiveness, and neuroendocrine function (both basally and in response to stress).

METHODOLOGY: SD and WKY rats will be treated with pyridostigmine bromide for 7 days and for the last 3 days will be subjected to a chronic stress regime. 24 hours after the last stressor exposure, the neuroendocrine and brain c-fos responses to pyridostigmine bromide will be evaluated, as well as the levels of specific isozymes of cytochrome P-450 in the liver. These enzymes are responsible for the metabolism of a large group of xenobiotics, including aromatic hydrocarbons. The changes in these enzyme levels will also be evaluated following stressor exposures and subsequent exposure to aromatic hydrocarbons. Similar studies will determine the behavioral and some physiological effects of chronic treatment with pyridostigmine bromide and aromatic hydrocarbons, as well as their effects on the responses to chronic stress.

EXPECTED PRODUCTS (MILESTONES): We expect that chronic stress will persistently increase the sensitivity to pyridostigmine bromide, which might explain how a drug that is used extensively in civilian populations might become toxic under the stresses of the battlefield. We also expect that this effect will be more pronounced in WKY rats, which would show that genetic predisposition might explain why some veterans developed unexplained illnesses and others did not. We expect that hepatic P-450 levels will be inhibited more in WKY rats, and that these results could explain a greater sensitivity to toxins metabolized by these enzymes. Finally, we expect that chronic exposure to pyridostigmine bromide or aromatic hydrocarbons might show long-term effects on behavior or body temperature rhythms which could be very sensitive indices of chronic toxicity.

STATUS/RESULTS TO DATE: We have been unable to validate the behavioral hyper responsiveness in WKY rats that has been reported by others. Preliminary data suggest that pyridostigmine bromide may cause a substantial decrease in voluntary running activity in rats, but it is unclear as yet whether this could be a long-term effect. However, it is clear that WKY rats are more sensitive to some effects of pyridostigmine bromide than SD rats. For example, WKY rats show an exaggerated startle response 1 to 3 weeks after ending pyridostigmine bromide treatment that is not present earlier and does not develop in SD rats. These results have led to the current studies of interactions among strain, chronic stress, and potential pyridostigmine bromide toxicity. This project was terminated in December 1997. Two manuscripts are in review and there are more being prepared. Continuation of this project has been funded under a VA Merit Review grant to Dr. Richard Servatius, that will start in April 1998.

PUBLICATIONS: Natelson BH, Ottenweller JE, Goldstein C, Beldowicz D, O'Brien WT, Tapp WN, Servatius RJ. Persisting effects of oral pyridostigmine bromide on the behavior of rats. Abstracts of the Society for Neuroscience; 22:235, 1996.

Title: Portland Environmental Hazards Research Center Program
Project #: VA-6 **Agency: VA** **Study Location: VAMC Portland**
P.I.: D. Bourdette, M.D./P. Spencer, Ph.D.

This is the parent Program for VA projects VA-6A through VA-6E.

Title: Core Program: Portland Environmental Hazards Research Center: Environment, Veterans Health and the Gulf War Syndrome. Core Project for Clinical and Epidemiology Research
Project #: VA-6Core Agency: VA Study Location: VAMC Portland
Project Status: Ongoing Research Type: Epidemiology Research
P.I.: Peter Spencer, Ph.D. Research Focus: Symptoms/General Health
Start Date (CY):1994 Est. Completion (CY):1999

OVERALL PROJECT OBJECTIVE: To illuminate the relationships between exposures (chemical, physical, stress) and unexplained illness in Gulf War veterans.

SPECIFIC AIMS: 1. To define 'caseness' of unexplained illness in Gulf War veterans; 2. To develop a questionnaire of exposures and effects based on the results of veteran interviews and other relevant data; 3. To conduct a case-control study focused on the spatial-temporal relationship between exposures and illness; 4. To provide subjects for research conducted in Projects I (6A) and II (6B).

METHODOLOGY: An epidemiological study using a population-based case-control design is being implemented. Pilot work was conducted in two phases before final determination of the research protocol. The first phase consisted of fourteen open-ended interviews with veterans with varying degrees of health and diverse military exposures. The interviews were completed in the Spring of 1995.

The second phase of the pilot work was a feasibility study in which a sample of 422 subjects, randomly chosen from a data tape of Oregon and Washington residents serving in the Persian Gulf, were mailed a survey describing the proposed plan for the large epidemiology study. Specifically, the researchers sought information on potential response rates and barriers to participation.

Modifications of the planned research protocol were made based on responses of the feasibility study.

The regional study consisted of mailing a questionnaire seeking information on exposures in the Gulf and current health status to approximately 3000 northwest PGW veterans. The data base used for the sampling frame was obtained from the DoD (Defense Manpower Data Center). The database contains the names/addresses of military personnel who were deployed to the Persian Gulf from August 1990 through July 1991, and who listed Oregon or Washington as their home state-of-record. The accuracy of the addresses in the DoD database was determined in the feasibility study. Arrangements were made with the Department of Veterans Affairs, the National Institute for Occupational Safety and Health, and the Internal Revenue Service to track individuals with invalid addresses in the DoD file. In addition, we have utilized the services of Telematch, Equifax, Transunion and numerous Internet search engines to assist in finding current addresses and telephone numbers. The questionnaire is mailed in stages based on geographic location of the potential subjects.

The survey methodology was developed with the assistance of consultants. Females and veterans in specific deployment groups (pre-combat, combat, post-combat) were over-sampled. From those who respond to the mailed questionnaire, potential cases and controls (total approximately 500) are recruited for the clinical case-control study. The mailing of the survey questionnaire began in mid-November 1995 and the case-control clinical evaluations began in January 1996.

The working case definition determined at the conclusion of the May 1995 Research Retreat (with input from our External Advisory Committee) is as follows:

Must have at least one of the following signs or symptoms to be defined as a potential case of Persian Gulf War Unexplained Illness (PGWUI). Onset must be during or after deployment to the Persian Gulf. Symptoms must have persisted for one month or longer and have occurred during the three-month period preceding the proposed physical examination.

-Muscle/joint pain

-Cognitive changes including memory loss, confusion, inability to concentrate, mood swings, and/or somnolence.

-Diarrhea

-Skin or mucous membrane lesions

-Unexplained fatigue, plus at least four associated symptoms.

Potential cases and controls receive a medical workup at the Portland VAMC. Those participating in

the clinical workup at the Portland VAMC also serve as the subjects in Projects I (VA-6A) and II (VA-6B). Multivariate analyses will be conducted to compare subjects with unexplained illness and healthy subjects with respect to reported and known exposures to specific environmental factors. EXPECTED PRODUCTS (MILESTONES): Definition of associations, and the strength of those associations, between exposures and unexplained illness.

STATUS/RESULTS TO DATE: 1. Open-ended interviews of PGW veterans have been completed, transcribed, and summarized. Information gleaned from the interviews was incorporated in the questionnaire content. 2. Feasibility study completed on 422 randomly selected Oregon veterans in the population to be studied. Changes in the design were implemented as a result of the findings:

A. Compensation of \$10 is given to all subjects who complete and return the mailed survey questionnaire. B. The first year of survey mailing focused on residents of Oregon. Initial mailing was to subjects randomly selected within a 50-mile radius of Portland. Subsequent mailings were done to different geographic areas of the State of Oregon. The final survey sampling will be representative of the geographic distribution of all potential subjects in the data base obtained from the DoD.

C. The mailing of questionnaires to potential subjects in areas outside of Portland, Oregon coincided with the negotiation of clinic space in VA facilities in outlying areas of the State. This change was needed because of the sizeable proportion of subjects in the feasibility study who reported that they would not participate in the planned study due to the traveling time to Portland VAMC.

3. Survey sampling plan for 1996-1998 is currently being implemented. Questionnaire returns are being scanned for data entry. Incomplete addresses are being tracked. As of December 1, 1997, 1982 questionnaires have been mailed to veterans who served in the PGW. Follow-up of the first two mailing waves (consisting of re-mailing questionnaires, telephone calls, and telephone-administered questionnaires) has been completed. A response rate of 58% has been achieved for the first six mailing waves. Follow-up for subsequent mailing waves is ongoing. 4. Clinical testing of subjects responding to mail survey began in January 1996. As of January, 1998, 409 subjects have been evaluated in the case-control study. Clinical testing will be completed in April 1998. 5. Mailings were made to 259 subjects living in rural areas of Oregon. In both April 1997 and June 1997, we completed four days of satellite clinical evaluation of 45 of the responders from this sample in cooperation with the Roseburg, Oregon, VAMC. In September and December 1997, we completed four days of satellite clinical evaluations of 33 of the responders from the Washington sample in cooperation with the American Lake VAMC in Tacoma, Washington. 6. Serum samples from PGWUI cases and controls are studied with an antibody-capture ELISA test which is being developed for the detection of exposure to/infection with *Leishmania tropica*. An initial batch of 200+ serum samples from cases (two-thirds) and controls contained approximately 10% that had values more than three standard deviations from the mean of negative controls. While these samples fell within the range of values obtained from sera obtained from subjects with cutaneous *L. tropica* infection (positive controls), the specificity and sensitivity of this test-under-development has yet to be established (see VA-6E). 7. Analyses have been completed in the following areas: Comparison of self-reported symptoms and symptoms reported at time of clinical evaluation; Reliability of self-reported exposure information. 8. Analyses are in progress in the following areas: Prevalence of self-reported symptoms in survey responders; Prevalence of symptoms in veterans serving in specific deployment time periods; Association between self-reported symptoms and clinical findings with time of deployment; Association between self-reported symptoms/clinical findings and specific exposures that reportedly took place while in SW Asia.

PUBLICATIONS: Ludolph AC, Spencer PS. Toxic neuropathies and their treatment. In: *Peripheral Neuropathies, Part 2, Balliere's Clinical Neurology; International Practice and Research* (ed. Hartung HP), Balliere, London, 1996.

Spencer PS, Schaumburg HH, Ludolph AC. *Experimental and Clinical Neurotoxicology*, 2nd edition, Oxford, New York, in press.

Title: Psychosocial, Neuropsychological and Neurobehavioral Assessment (Project I)
Project #: VA-6A Agency: VA Study Location: VAMC Portland
Project Status: Ongoing Research Type: Clinical Research
P.I.: Kent Anger, Ph.D. Research Focus: Brain & Nervous System
Start Date (CY):1994 Est. Completion (CY):1999 Symptoms/General Health

OVERALL PROJECT OBJECTIVE: To determine if veterans have developed adverse neurobehavioral or psychological effects from service in the Persian Gulf War. If so, to determine the triggering events, including combat stressors, life stressors, chemical exposures, personality disorders, psychological health and motivation to seek compensation.

SPECIFIC AIMS: 1. Determine if there are objective memory and attention deficits in Veterans reporting (unexplained) symptoms which developed during or since the Gulf War; 2. Determine the relationships between neurobehavioral and psychological measures and unexplained symptoms referable to the Gulf War; 3. Employ in-depth neuropsychological examinations to identify neurobehavioral, neuropsychological and psychosocial factors that may explain or more fully characterize cognitive symptoms or objective cognitive deficits.

METHODOLOGY: This project will determine if veterans of the 1991 Persian Gulf conflict have developed any of a broad range of adverse neurobehavioral or psychological health effects attributable to triggering events during their service in the 1991 Gulf War. Hypothesized triggering events include combat or life stressors and chemical exposures. Potential factors that could contribute to or influence the presence of adverse health effects will be assessed and entered into the analysis, including personality disorders, psychological health, medical conditions, and motivation to seek compensation.

Each veteran participating in the overall research program receives a screening assessment with a 4-hour battery of 18 standard, reliable and valid psychosocial, neuropsychological, and behavioral performance tests. The psychosocial and neuropsychological tests selected for the screening battery have been implemented in a user-friendly and consistent computer-administration format (Health Screening System) that does not require individual explanation for each test developed for this project. The neurobehavioral tests of attention, memory and coordination are implemented in the Behavioral Analysis and Research System (BARS), and two new tests (ODTP and Serial Digit Learning) were added to BARS for this project (Specific Aims 1 and 2).

During the course of the study, up to 50 "Cases" and 50 "Controls" (from VA-6) or referents will receive an in-depth clinical (neuropsychological) interview to more fully characterize possible disorders (Specific Aim 3). In addition, those initially demonstrating a high level of PTSD will be invited back after 1-2 years to repeat the tests to assess PTSD progression, resources permitting. Results are being analyzed, during data collection: (a) by experienced clinicians (neuropsychological exam; Specific Aim 3), (b) with multivariate techniques adjusted for confounders (neurobehavioral tests; Specific Aim 1), and (c) with principal components analysis, logistic hierarchical multiple regression, discriminant function analysis, latent growth models analysis, and structural equation modeling to characterize the nature of the psychosocial results (Specific Aim 2).

EXPECTED PRODUCTS (MILESTONES): Develop a predictive model relating the occurrence of unexplained illness to chemical exposure, psychosocial, or neuropsychological factors.

STATUS/RESULTS TO DATE: Methods Developed or Modified for Project

Twelve valid, reliable psychosocial and neuropsychological tests were selected for the screening battery in this project. They address the main psychological factors identified :
PTSD EVALUATION - Penn Inventory for PTSD; Mississippi PTSD Scale. PTSD Checklist.
PSYCHOSOCIAL FACTORS - Beck Depression Inventory (BDI); Beck Anxiety Inventory (BAI); Substance Abuse Subtle Screen Inventory (SASSI); Life Experience Scale (LES). PERSONALITY VULNERABILITY - Positive Affect Negative Affect Scale (PANAS); Minnesota Multiphasic Personality Inventory-II (MMPI-II). HEALTH SYMPTOMS - Health Status Questionnaire (SF-36); Hopkins Symptom Checklist (SCL-90R). WAR ZONE EXPERIENCE - Combat Exposure Scale, revised (CES-r).

A common presentation format was developed for the 12 diverse psychosocial and neuropsychological tests in order to improve acceptability and speed completion, using Supercard software for presentation on a Macintosh laptop computer. The new format includes a spoken test

administration option (individually selectable and deselectable at any point in testing) which has been digitally recorded for inclusion with the test presentation system and options to navigate within a test to any question or to repeat the instructions. A training program was also developed to teach veterans to complete the questionnaires, and durable external "DataSled" Response Units (developed in a separately-funded project) have been connected to the laptop computers on which the tests are being presented to up to 9 veterans simultaneously (number limited by space). The new testing format (HSS) was employed with 20 PGW veterans and 10 civilians in pilot evaluations of the prototype training program. Test subjects were very positive about the testing system, and the mean questionnaire administration time approximated the target of 3 hours in a part of that group (Kovera et al., 1996). Changes recommended by respondents were introduced into the HSS. These tests are now being administered to all veterans tested by the PEHRC Epidemiology/Medical Core unit.

The neurobehavioral tests employed in this project were selected from the Behavioral Research and Assessment System (BARS): ATTENTION, MEMORY, and LEARNING - Symbol Digit, Digit Span (forward and backward), Selective Attention Test

Serial Digit Learning; RESPONSE SPEED AND COORDINATION - Simple Reaction Time;

MOTIVATION - Oregon Dual Task Procedure (ODTP) - (modelled after the Portland Digit Recognition Test [PDRT]).

Most BARS tests were implemented under other funding to one of the project PIs (Anger), but the ODTP was prototyped and administered to pilot subjects for this project, including veterans involved in the psychosocial test development (above). The duration of testing met the target time of 60 minutes. These tests are now being administered to all Veterans tested by the PEHRC Epidemiology/Medical Core unit.

Reliability of Methods: During Summer, 1996, the 18 HSS and BARS tests used in this project were administered twice to 60 Oregonians, with one week between administrations, to evaluate their test-retest reliability. In general, reliability scores were comparable to those reported for the tests in their original format. This supports the use of the tests in the new HSS and BARS format which allows testing of larger numbers of veterans due to the increased cost-effectiveness of this system. A manuscript describing these results has been submitted for publication.

Website Describing HSS and BARS: In order to make the information about the methods used in this (and other) projects more widely available, a website has been created that describes the tests, presents test-retest reliability data, provides a simulation of training for the HSS, and depicts one screen for each test. Home Pages were thus created for both BARS and HSS. Both Home Pages are accessed through <http://home.att.net/~angerk>.

Results of Case-Control Comparisons: HSS and BARS tests have been administered to 407 veterans as of December, 1997, as part of the PEHRC clinical study (Specific Aims 1-2). The data have begun to reveal consistent and highly significant differences between cases and controls on most psychological test scales in the direction of increased distress in cases (veterans with unexplained symptoms) and slowed response latencies on several neurobehavioral tests in cases compared to controls.

Individual neuropsychological tests (Specific Aim 3) have been administered to approximately 45 people by Dr. Storzbach and, recently, Dr. Binder. These tests require scheduling a full day in the event that a diagnostic interview is required based on initial testing. This has slowed the process when last-minute schedule changes, cancellations, or no-shows effectively lose the one subject that can be scheduled for each week. Recruitment has been difficult to accomplish; most veterans are not interested in the tests and unable to absent themselves from their regular job.

PUBLICATIONS: Kovera CA, Anger K, Campbell KA, Binder LM, Storzbach D, Davis, KL, Rohlman DS. Computer-Administration of Questionnaires: A Health Screening System (HSS) Developed for Veterans. *Neurotoxicology and Teratology*; 1996, 18:511-518.

Anger WK, Kovera CA, Campbell K, Binder L. A Psychosocial and neuropsychological Testing Format: the health screening system. Presented at symposium on computerized behavioral testing of humans in neurotoxicology research; Portland, OR, 95.

Title: Clinical and Neuroendocrine Aspects of Fibromyalgia (Project II)
Project #: VA-6B **Agency: VA** **Study Location: VAMC Portland**
Project Status: Ongoing **Research Type: Clinical Research**
P.I.: Robert Bennett, M.D. **Research Focus: Immunology**
Start Date (CY):1994 **Est. Completion (CY):1999** **Symptoms/General Health**

OVERALL PROJECT OBJECTIVE: Determine the relationship between exposures during the Persian Gulf War and the development of fibromyalgia.

SPECIFIC AIMS: 1. Screen veterans complaining of fatigue and musculoskeletal symptoms with previously developed and validated questionnaires and directed physical examinations to determine if fibromyalgia is present.

2. Correlate the presence or absence of fibromyalgia with possible environmental exposures, as determined in the core part of this program, or with neuropsychological factors, as determined in Project I: Psychosocial, Neuropsychological and Neurobehavioral Assessment.

3. Evaluate a smaller group of veterans with fibromyalgia and an age and sex matched group of veterans without fibromyalgia, for the neuroendocrine abnormalities described in fibromyalgia.

4. Repeat the clinical and neuroendocrine testing on the veterans with fibromyalgia (objective 3) 24-30 months after initial evaluation.

METHODOLOGY: Utilizing the cases determined in the case-control/epidemiological study, using validated methods, screen cases complaining of musculoskeletal complaints for fibromyalgia and controls without symptoms. Perform neuroendocrine testing on both cases and controls.

EXPECTED PRODUCTS (MILESTONES): 1. Determination of the association between exposure to the Persian Gulf War and the development of fibromyalgia; 2. Determine the prevalence of neuroendocrine findings in a primarily male sample; 3. Follow the course of the clinical/neuroendocrine findings with time.

STATUS/RESULTS TO DATE: André Barkhuizen MD, Assistant Professor of Rheumatology, is the focal clinician responsible for integrating the fibromyalgia clinical study into the epidemiological case-control study. In addition to keeping abreast with national scientific work in the area of fibromyalgia and PGW veterans, preliminary work consisted of reviewing the Portland VA Persian Gulf War (PGW) Registry for cases of musculoskeletal pain. The prevalence of complaints in the Portland PGW Registry sample was 29.6% compared to the national registry prevalence of 13.9% and the DoD Comprehensive Clinical Evaluation Protocol reported prevalence of 47%. Results from a pilot population-based survey of 157 veterans eligible for our epidemiologic case-control study (see Project VA-6) indicated that the self-reported rates of musculoskeletal pain in both female and male subjects were both 38%. Responders to the mailed survey who had registered in the PGW registry were two times more likely to report musculoskeletal pain than responders who had not registered. Investigators worked closely with the epidemiological core in the development of the items to include in the survey questionnaire, telephone screening questions, and the history and physical examination to be done in the case-control study. Exclusionary diagnoses were determined. The history and physical examination was pretested on three subjects with musculoskeletal pain in January 1996. Since commencing the Persian Gulf Research Clinic in January 1996, the majority of subjects has been evaluated by a single examiner for rheumatological conditions. A blinded tender-point examination is performed followed by a full rheumatology history and physical examination. The Core laboratory data, which include an erythrocyte sedimentation rate, C-reactive protein, antinuclear antibody, rheumatoid factor, hepatitis B, C, and human immunodeficiency virus serologies, and selected joint radiographs, are reviewed at the bimonthly Clinical Case Review meeting to arrive at a final rheumatological diagnosis.

Thus far, of the 219 cases and controls (age= 32.4 + 7.9 years; 83% male) evaluated in our research clinic, 70% complained of musculoskeletal pain and were found to have (expressed as a percentage of the total clinic sample): mechanical backache 34%, myofascial pain 20%, fibromyalgia 16%, tendinitis/bursitis 12%, patellofemoral syndrome 6%, hypermobility 5%, osteoarthritis 5%, and overuse 4%. Nine percent of the subjects had musculo-skeletal pain that was unexplained and did not fall into the diagnostic categories listed above. Cases with fibromyalgia and unexplained musculoskeletal pain were much more likely than healthy controls to complain of abdominal cramps, muscle weakness, fatigue, muscle cramps, chest pain, restless legs, dry eyes,

tender skin, cold intolerance, unrefreshed sleep, headaches or post-exertional pain. Cases with fibromyalgia or unexplained muscle and joint pain scored significantly higher on the Beck Anxiety and Depression Inventory, SF 36 (sub-scales of pain, energy/fatigue, physical function) and "somatization" sub-scales of the MMPI and SCL-90 when compared to healthy controls. Cases with fibromyalgia and unexplained muscle/joint pain reported significantly more combat exposure and scored higher on post traumatic stress disorder scales (MISS, PENN, PCL C). The majority of subjects with musculoskeletal complaints was on no treatment and in full-time employment. Rheumatological laboratory investigations were normal in most subjects.

The second component entails study of the neuroendocrine axis of the Persian Gulf veterans. All subjects attending the Persian Gulf Research Clinic are screened using the serum Insulin-like Growth Factor-I (IGF-1) and Insulin-like Growth Factor Binding Protein 3 (IGFBP3) levels. This has previously been shown to be reduced in one-third of patients with fibromyalgia. In the current study, serum IGF-1 levels were not significantly different in subjects with unexplained muscle and joint pain (175.9+43.3), fibromyalgia (183.8 ng/ml+61.0), unexplained fatigue (193.4+59.9), or healthy controls i.e. (192.2+60.6). For all cases and controls, IGF-1 levels correlated with IGFBP3 ($R=0.43$; $p<0.0001$), age ($R=-0.34$; $p<0.0001$), and the neurobehavioral tests: digit span backwards ($R=0.29$; $p<0.0001$) and symbol digit latency ($R=-0.2$; $p=0.004$). There was no correlation between IGF-1 level and self-reported sleep disturbances, pain or scores for the fibromyalgia impact questionnaire. The final component involves evaluating changes in the neuroendocrine axis in greater detail. We aim to study 20 cases of fibromyalgia with low IGF-1 levels and 20 control subjects with normal IGF-1 levels over the next 2 years for an in-depth study of their hypothalamic-pituitary-growth hormone and hypothalamic-pituitary-adrenal axis. The study protocol has been approved by a peer review committee of the Clinical Research Center, a dedicated research facility at the Oregon Health Sciences University, which is funded and regularly reviewed by scientists and clinicians from the National Institutes of Health. The laboratories of the Clinical Research Center and Oregon Graduate Institute perform half-hourly cortisol and growth hormone levels, a 24-hour cortisol production rate and two growth hormone stimulation tests. This will enable the research team to objectively document an altered neuroendocrine stress response and adult growth hormone deficiency in the symptomatic veterans. When testing on all subjects is completed, the altered neuroendocrine stress response will be correlated with the neuropsychological profile and, particularly, with the stress scores collected by Project I. Finally, the stress response will be correlated with reported exposures to various environmental agents and the deployment periods of the veterans (see Project VA-6). To date, 10 cases and 4 controls have been studied, and an interim statistical analysis will be performed midway through this component of the study (summer 1998). In view of the small number of controls studied to date, we compared the results of the cases to data on healthy civilian control available from a large ongoing study of our Endocrinology Consultant. There seems to be a trend towards an elevated cortisol production rate in cases compared to healthy controls suggesting a chronic stress response (i.e. a chronically activated hypothalamic-pituitary-adrenal axis). The growth hormone stimulation tests utilizing oral clonidine and intravenous L-arginine confirms adult growth hormone deficiency (GH response < 5 ng/ml) in fibromyalgia cases with low IGF-1 levels. Because of insufficient numbers, we are unable to report on the diurnal secretory pattern of growth hormone and cortisol as measured in the half-hourly collection over 24 hours.

PUBLICATIONS: Bennett, RM, Clark, SR, Burckhardt, CS, Campbell, SM, A double blind controlled, randomized, placebo-controlled study of growth hormone therapy in patients with the fibromyalgia syndrome. *Amer. J Med*, in press 1998.

Barkhuizen A, Campbell S, Bennett R, Storzbach D, Lazarev M, Rohlman D, Anger K, Binder L, Campbell K, and the PEHRC team. Musculoskeletal syndromes, neuropsychological features and IGF-1 levels of deployed Persian Gulf War veterans. *Arthritis Rheumatol*. 40:S214, 1997; (Poster presented at 1997 ACR National Meeting).

Bennett RM, Cook D, et al. Hypothalamic-pituitary-IGF-1 axis dysfunction in fibromyalgia Patients. *J Rheumatol*, 1997, 24:1384-1389.

Title: Neurotoxicity of Environmental Pollutants and Warfare Agents (Project III)**Project #: VA-6C****Agency: VA****Study Location: VAMC Portland****Project Status: Ongoing****Research Type: Basic Research****P.I.: Rosemarie****Research Focus: Pyridostigmine Bromide****Start Date (CY): 1994 Est. Completion (CY): 1999****Environmental Toxicology**

OVERALL PROJECT OBJECTIVE: To evaluate the neurotoxic potential of a group of hydrocarbon solvents and the effects on cholinergic neurons of long-term exposure to pyridostigmine bromide (PB).
SPECIFIC AIMS: 1. To evaluate effects of pyridostigmine bromide (PB) on cholinergic function in cerebellar and spinal cord explants. 2. To identify the cellular targets of neurotoxic hydrocarbon solvents. 3. To examine the relationship between the chromogenic and neurotoxic properties of selected organic solvents.

METHODOLOGY: Spinal cord-dorsal root ganglia (DRG)-muscle cultures derived from fetal mice are used to study to effects of chronic and acute exposure to pyridostigmine on cholinergic function. Cerebellar cultures derived from newborn mice are used to study the relationship between chromogenic and neurotoxic properties of a group of monocyclic and dicyclic hydrocarbon solvents. The substances to which cultures will be chronically exposed are directly incorporated into the culture medium. The dosage and duration of exposure to pyridostigmine and each of the solvents will be experimentally determined.

Morphological Analyses - Control and treated explant cultures will be stained with thionin (for Nissl substance). This stain allows the evaluation of the various neuronal cell groups present in explant cultures by light microscopy. Central and peripheral myelination can be readily examined in the living state using bright field optics. Immunohistochemical staining will be used to identify alterations of specific neural components (neurofilaments, calbindin), neurotransmitters (choline acetyltransferase, acetylcholinesterase) and supportive glial cells (glial fibrillary acidic protein) induced by neurotoxic compounds. Synaptic changes will be elucidated by electron microscopy.
Electrophysiology - In spinal cord-DRG-muscle explants, extracellular electrodes are used to record dorsal horn, ventral horn and muscle responses evoked by extracellular stimulation of dorsal and ventral roots. In cerebellar cultures, neurons develop spontaneous activity as they mature in vitro. Extracellular recordings are used to obtain single unit discharge rates and, in combination with extracellular stimulation, are used to assess the efficacy of excitatory and inhibitory synaptic circuitry. Intracellular electrodes are used to study the active and passive properties of individual neurons (motoneurons in the spinal cord cultures and Purkinje cells in cerebellar cultures) and their synaptic responses to afferent stimulation and to record muscle activity.

EXPECTED PRODUCTS (MILESTONES): Electrophysiological and morphological characterization of the cellular neurotoxicity of selected hydrocarbon solvents and pyridostigmine bromide
STATUS/RESULTS TO DATE: We have completed an in vitro study of the effects of pyridostigmine bromide on the neuromuscular junction. Pyridostigmine bromide (PB) belongs to a group of chemicals classified as reversible inhibitors of acetylcholinesterase, the enzyme responsible for the hydrolysis of acetylcholine. During the 1991 Gulf War, troops participating in Operation Desert Storm were issued PB in blister packs containing twenty-one 30-mg tablets. One tablet was to be taken every 8 hours when under threat of attack. Previous in vivo studies have reported the neurotoxic potential associated with high-dose acute and long-term anticholinesterase therapy. Administration of anticholinesterases in high doses produced pronounced structural and functional alterations localized to the neuromuscular junction. The aim of this study was to determine whether long-term (1-2 weeks) exposure to low concentrations of PB (comparable to doses taken by Operation Desert Storm troops) affected neuromuscular function in a well characterized in vitro model.

Organotypic spinal cord-dorsal root ganglion explants co-cultured with skeletal muscle were used to evaluate the effects of long-term exposure to PB. No physiological or ultrastructural alterations were observed in dorsal root ganglia neurons or ventral horn motoneurons with exposure to 10⁻⁶M PB for periods of up to three weeks in vitro. Neuromuscular function was selectively affected by PB treatment. Acute treatment (minutes) with PB increased spontaneous muscle contraction. This effect is consistent with the inhibition of acetylcholinesterase and the consequent greater availability of acetylcholine at the neuromuscular junction. Long-term treatment (days to weeks) with PB

decreased the contractile activity of muscle fibers and their sensitivity to externally applied acetylcholine. Ultrastructural examination of neuromuscular junctions in cultures exposed to PB revealed no evident structural changes after a 1-week exposure but, after a 2-week exposure, cystic degenerative changes were evident in the nerve terminals. The postjunctional folds displayed atrophic changes and were surrounded by vacuolar spaces. These changes are indicative of both nerve terminal degeneration and a subjunctional myopathy. Exposure to PB for 1 or 2 weeks even at low dosages produced early functional and later structural alterations of the neuromuscular junction. In early stages, changes may be limited to desensitization of the acetylcholine receptors produced by the persistence of acetylcholine in the synaptic cleft, followed in later stages by degenerative changes of the neuromuscular junction. The results of this study have recently been submitted for publication.

We have also evaluated the neurological effects of the other moiety of the PB molecule, the bromide anion. The bromide anion permeates chloride channels of cell membranes and, in contrast to pyridostigmine, readily diffuses across the blood-brain barrier. The aim of this study was to examine the effect of bromide on neural activity. Cerebellar explant cultures were exposed for a period of two weeks to 10^{-6} M of sodium bromide. Neural activity was subsequently evaluated by electrophysiological recordings. Exposure to micromolar concentrations of bromide had no effect on the spontaneous activity of cerebellar neurons. Additional studies showed that a significant depression in cerebellar neural activity resulted only after substitution of sodium chloride in the nutrient media with a much higher concentration (20 mM) of sodium bromide. At this concentration, bromide has been shown to enhance gamma-aminobutyric acid (GABA)-activated currents in cultured cerebral neurons. The depressant effects of bromide on neural activity were reversed by incubation with standard saline solution.

In 1998, the neurotoxic properties of representative examples of the class of aromatic hydrocarbon solvents will be studied for neurotoxic potential and effects.

PUBLICATIONS: Drake-Baumann R, Seil FJ. Effect of long-term anticholinesterase exposure on neuromuscular function in vitro. Soc. Neuroscience (Abstract) Volume 22, 1996, pg. 273. Slide presentation at the Society for Neuroscience Annual Meeting in Washington DC, November, 1996.

Title: DNA Damage from Chemical Agents and Its Repair (Project IV)
Project #: VA-6D Agency: VA Study Location: VAMC Portland
Project Status: Ongoing Research Type: Basic Research
P.I.: Glen Kisby, Ph.D. Research Focus: Diagnosis
Start Date (CY): 1994 Est. Completion (CY): 1999 Chemical Weapons

OVERALL PROJECT OBJECTIVE: Determine the long-term consequences of genotoxin exposure on nervous system and non-nervous system tissue.

SPECIFIC AIMS: 1. Determine the type and quantity of specific DNA adducts and the capacity to repair these adducts in control and nitrogen mustard (HN2) treated genomic DNA isolated from normal human skin and brain; 2. Determine the type and quantity of specific DNA adducts and the capacity to repair these adducts in genomic DNA isolated from mouse cerebral cortex cultures treated with HN2; 3. Assess the relationship between DNA damage, DNA repair and cell degeneration in rodent neuronal cultures treated with HN2; 4. Compare and contrast DNA damage, DNA repair and cytotoxicity in primary neuronal and primary glial cultures treated with HN2.

METHODOLOGY: 1. Determine the relative sensitivity of different nervous tissue cell types to HN2. Nervous tissue cell cultures (e.g., rodent astrocytes, cerebellar granule cells, cortical explants, and SY5Y human neuroblastoma cells) will be treated for various time periods with a range of HN2 concentrations and examined for cytotoxicity by using fluorescent dyes for live (e.g., the mitochondrial fluorochrome Rhodamine 123) and dead (e.g., the nuclear DNA binding fluorochrome propidium iodide) cells. 2. Develop sensitive methods for the detection of DNA adducts (i.e., monofunctional and cross-links) in HN2 treated nervous tissue and human skin explants. The most sensitive methods for detecting HN2-induced DNA adducts are the ³²P-postlabeling/TLC (monofunctional adducts) and ethidium bromide assay (crosslinks). Therefore, the ³²P-postlabeling/TLC and ethidium bromide assay method was developed and used to detect DNA adducts in tissue treated for periods up to 7 days with HN2. 3. Develop methods for determining DNA repair levels and activity in mustard treated neuronal (e.g., cortical, cerebellar) and non-neuronal (e.g., astrocytes) cultures and human skin. The predominant DNA adduct produced by sulfur and nitrogen mustards is the alkylated guanine DNA adduct. The alkylated guanine DNA adduct is repaired by the base-excision repair (BER) pathway. The rate-limiting step in BER is the repair of an apurinic site by apurinic/apyrimidinic endonuclease (APE). APE levels and activity will be determined in nervous tissue treated with HN2. APE levels will be determined by western blotting and activity will be determined using damaged plasmid DNA. These studies will determine the relative DNA repair of HN2-induced DNA adducts in different CNS cell types. Findings from these studies will be important for establishing a possible relationship between DNA damage, genotoxin exposure and cytotoxicity of nervous tissue.

EXPECTED PRODUCTS (MILESTONES):

1. Determination of whether mustard agents can induce persistent DNA damage to nervous tissue.
2. Development of sensitive techniques for the detection and quantitation of DNA adducts induced by mustards.

STATUS/RESULTS TO DATE: 1. We began study of the response of neuronal and glial cells and human skin tissue to HN2. Human and rodent postmitotic neurons proved to be more sensitive to HN2 than glial cells.

2. We examined the acute (24 h) and long-term (7 day) cytotoxic effect of HN2 on human skin tissue (SKIN2tm). HN2 is acutely toxic to human skin tissue at high concentrations but extensive tissue damage can be induced by lower concentrations (1/10 or 1/100 of the acute dose) when tissue is exposed for long periods of time.

3. We developed assays to measure the predominant HN2-induced DNA adduct (monofunctional; GN7) and cross-links. We demonstrated that guanine-N7(GN7) levels were 2-fold higher in postmitotic rat cerebellar neurons than comparably treated dividing SY5Y cells. We showed that cross-link levels in HN2-treated human SY5Y neuroblastoma cells increased with increasing concentrations of HN2. Studies are underway to determine if these adducts persist in HN2-treated nervous tissue.

4. We developed an assay for examining the relationship between cytotoxicity and DNA damage.

5. We developed assays for measuring the effect of HN2 on neuronal and human skin tissue

DNA-repair levels and activity.

6. Nitrogen mustard (HN2) modulated the level and activity of nervous tissue DNA-repair protein APE, which specifically repairs the monofunctional HN2 DNA adduct. Apurinic apyrimidinic (APE) levels increased in HN2-treated rodent neuronal cultures (non-dividing cells) while APE levels decreased in human neuronal cultures (dividing cells). APE activity increased in HN2-treated human skin cultures (actively dividing cells). Altered levels and activity of APE may be an indication of HN2-induced DNA damage.

7. Nitrogen mustard (HN2) modulated the level and activity of APE in human skin tissue. A significant increase in immunostaining was demonstrated in human skin probed with an antibody to APE. In support, APE activity was increased in cultured human skin explants treated with HN2.

PUBLICATIONS: Kisby GE, Sweatt, Spencer PS (1995) Role of DNA repair in protecting mature nervous tissue from DNA damage. *J Cell Biochem*, 1995, 21A, 348.

Kisby GE, Springer N, Pham T, Marino, MW, Spencer PS. Determination of nitrogen mustard-induced DNA adducts by a sensitive HPLC method with electrochemical detection. In preparation.

Kisby GE, Springer N, Spencer PS. Differential sensitivity of nervous tissue cell types to nitrogen mustard-induced toxicity. In preparation.

Title: Clinical and Epidemiology Leishmania Research

Project #: VA-6E Agency: VA Study Location: VAMC Portland
Project Status: Ongoing Research Type: Clinical Research
P.I.: Dennis Bourdette, M.D. Research Focus: Diagnosis
Start Date (CY): 1994 Est. Completion (CY): 1999 Leishmaniasis Treatment

OVERALL PROJECT OBJECTIVE: Development of serological test for viscerotropic leishmaniasis and determination of infection rate.

SPECIFIC AIMS: Core Objective: To determine the possibility and rate of infection of PGW veterans with *Leishmania* spp, and to develop novel anti-leishmanial chemotherapeutics.

METHODOLOGY: Immunological screening of patient and volunteer blood. All subjects participating in the case/control study have immunological screening of their blood for evidence of *L. tropica* infection (studies performed by Corixa Corp., Seattle, WA).

Other pertinent topics being explored in the Experimental Chemotherapy laboratory of the Portland VAMC:

1. Novel chemotherapeutic agents for viscerotropic leishmaniasis.
2. Development of adjunctive therapy for use with existing medicinals used for treatment of Leishmaniasis, as well as other tropical diseases.

EXPECTED PRODUCTS (MILESTONES): Effective, non-invasive diagnosis of leishmaniasis.

STATUS/RESULTS TO DATE: Sero-diagnosis of leishmaniasis among PGW veterans

Serum samples from PGWUI cases and controls were probed with a synthetic 45 amino-acid antigen (Ltr-1) based on the known sequence of a *L. tropica* antigen which has a repeat region spanning 33 amino acids. The synthetic peptide therefore contains roughly one and one-third repeat units which Corixa has determined to be optimal for an antibody-capture ELISA test. Ltr1 is seated on the bottom of each well of a 96-well plate. The plate is blocked, diluted serum (1:50 dilution) added, and the plate washed extensively. A reporter antibody is used for detection. Serum analysis was performed on two occasions with comparable results:

Run #1: Ten of 102 samples yielded a serotest greater than three standard deviations from the mean value obtained from a control group comprising sera of military subjects (unknown exposure history) received from Walter Reed Army Institute of Research (WRAIR). Values obtained from two subjects considered positive controls (one with biopsy-proven *L. tropica* infection) fell within the range of values exhibited by the 10 seropositive samples.

Run #2: The same 10 of 102 samples yielded a serotest greater than three standard deviations from the mean value obtained from the control group which contained an additional 70 sera obtained from a commercial source in the Boston area. These sera are believed to have been donated by healthy controls but their exposure history is unknown. The 10 seropositive samples fell within the range of values obtained from additional positive control sera obtained from Turkish military subjects with the cutaneous form of *L. tropica* infection.

Subjects with seropositive samples distributed as follows: six subjects, including two with the highest antibody titer, were PGWUI cases, two were PGWUI controls, and two had been rejected as cases or controls because their symptoms had resolved at the time of clinical examination. Blood samples were screened without knowledge of the clinical status of the subjects.

In summary, approximately 10% of subjects (n=102) drawn from a random sample of NW Gulf War veterans showed reproducible evidence of seropositivity using a synthetic peptide antigen that identifies the parasite. The antibody titer is extremely low. While these results raise the possibility of a low level of active *L. tropica* infection in a proportion of subjects with/without symptoms of PGWUI, the specificity and reliability of this test-under-development are unknown. While the test apparently has high sensitivity, cross-reactivity has not been ruled out, the false-positive rate is unknown, and the control population may not be ideal. Further studies are needed to test the validity of these preliminary results. At the present time, definitive diagnosis of *L. tropica* can only be made by culturing the organism from tissue biopsies.

Chemotherapy/Drug Development:

Leishmanial protozoan pathogens cause infections ranging from cutaneous, muco-cutaneous, to visceral leishmaniasis which afflict millions worldwide primarily in the tropical and subtropical regions of the world. Chemotherapy of leishmaniasis relies heavily on the use of pentavalent antimonials

which require lengthy courses of treatment at high doses and parenteral administration. Efficacy is variable and toxic side effects occur. The standard antimonial, stibogluconate, is reported to be much less effective in treatment of leishmaniasis in patients with AIDS. Clearly, additional compounds must be identified for treatment of these opportunistic infections of immuno-compromised patients. Ideally, new drugs could be developed with fewer side effects and with broad-spectrum activity against each of these infectious agents.

Prompted by our discovery of the antimalarial potency of xanthone derivatives, we were interested in evaluating the activity of xanthenes against other protozoan parasites. For this study we chose *L. tropica* WR 1063, derived from a Persian Gulf War veteran diagnosed with viscerotropic leishmaniasis. It is well established that both malaria and leishmania parasites have a heme problem: the former has too much and has evolved a mechanism for polymerization of heme to rid itself of the accumulation of this toxic product of hemoglobin digestion; while the latter is incapable of heme biosynthesis and must scavenge for this critical nutrient from exogenous sources. For in vitro culture of *Leishmania*, heme is added directly to the medium, provided with the serum supplement, or included as part of a complex medium component such as yeast extract (e.g., Schneider's medium). Existing as amastigotes within the acidic phagolysosome of macrophages, it is unclear how the heme requirement is sustained but prior work in the leishmania field indicates that the intracellular parasites do not rely on the host cell for procurement of heme. Instead, the intracellular forms are able to acquire heme from an exogenous source which we presume to be the bloodstream. Regardless of the mechanisms involved, the parasite heme salvage process represents an ideal target for rational drug design for chemotherapy of leishmaniasis.

We have extended our knowledge of the antiparasitic nature of xanthenes to include action against the *Leishmania*. Our data are consistent with the notion that xanthenes and analogs of them may block parasite access to heme as their primary mode of action. If this is true, then the spectrum of activity of xanthenes may extend to other organisms (bacterial, fungal, or protozoal), which require access to host-derived heme for survival.

Limited testing of xanthenes presented has yielded data which point to a key structural element in common with our more extensive antimalarial structure-activity profiling, i.e., positioning of amines or amidines at the 4 and 5 positions. For example, the xanthone diamine, 4,5-DEAE-X, was the most potent xanthone included in our study. So positioned, under physiological pH, the positively charged amino groups would align themselves with the propionate side chains of heme in a net-neutral electrostatic interaction thus adding stability to the heme:drug complex. We are now directing our efforts at synthesis and evaluation of 4,5-diamidinoxanthone (so-called "Xanthamidin") and analogs of it. We predict that the amidinium cations of this compound will be in the proper geometric orientation to interact with the propoxy acid anions of heme to form a stable bidentate hydrogen-bonding pair.

Consideration of the xanthone diamidine led to our investigation of pentamidine, a well known and clinically useful diamidine, as possibly acting in similar fashion. We found that addition of one equivalent of pentamidine induced a remarkable red shift in the Soret band of the heme spectrum at neutral and mildly acidic pH. Furthermore, the in vitro antileishmanial activity of pentamidine correlated directly to the heme concentration present in the culture medium. These data demonstrate that pentamidine forms a tight association with heme and suggest a possible mechanism for its antiprotozoal effects which extend to its use in the treatment of infections due to *Trypanosoma gambiense* and *Pneumocystis carinii*. Accordingly, we propose that pentamidine, like our desired xanthone constructs, functions to restrict parasite access to heme and porphyrins key components of the energy-generating cytochrome assembly located within the parasite mitochondrion.

The absolute reliance of leishmanial parasites on an exogenous supply of heme or porphyrins had already been exposed by numerous investigators. Our preliminary results provide clues as to how this tetrapyrrole salvage process may be blocked by pharmacological agents of improved design. Taken together, and given the attendant concerns over toxicity associated with pentamidine administration and the lack of an orally available form of the drug, there is an urgent need to pursue newer and safer remedies based on biochemical principles and employing the powerful new tools of computer-aided structure-based drug design. Based on our findings of the biochemical action of pentamidine on *Leishmania* parasites and its ability to bind heme, we have employed computer-aided modeling of xanthenes complexing to heme. Our studies point to the placement of amidines at the 4 and 5 positions of the xanthone nucleus to optimize binding to the propionate side chains of free heme. We have just completed synthesis of one such diamidine, 4,5- β -amidinoethoxyxanthone, and in vitro testing will begin after complete analytical figures are obtained. 4,5-Diamidinoxanthone ("Xanthamidin")

is our next targeted synthesis.

Forthcoming procedures: To synthesize a series of 4,5-xanthone diamidines and compare their inhibitory activity against *P. carinii*, *T. gondii*, and *L. major* in vitro. This study will also include an evaluation of each drug for relative toxicity against the murine monocyte-macrophage cell line J774A.1. The inhibitory action and toxic effects of each drug will be compared to that produced by the standard agent, pentamidine.

PUBLICATIONS: Ignatushchenko M, Winter RW, Hinrichs DJ, Riscoe MK. Xanthenes as antimalarial agents: Studies of a possible mode of action. FEBS Letters, July 1997, 409:67-73.

Winter RW, Ignatushchenko M, Ogundahunsi OAT, Cornell KA, Johnson LL, Oduola AMJ, Hinrichs DJ, Riscoe MK. A radical mechanism for potentiation of antimalarial oxidant drugs. Antimicrob Agents Chemother, 1997, 1:1449-1454.

Winter RW, Cornell KA, Johnson LL, Ignatushchenko M, Hinrichs DJ, Riscoe MK. Potentiation of the antimalarial agent rufigallol. Antimicrob. Agents Chemother, 1996, 40:1408-1411.

Title: Desert Storm Reunion Survey
Project #: VA-7 Agency: VA Study Location: VAMC Boston
Project Status: Completed Research Type: Clinical Research
P.I.: Jessica Wolfe, Ph.D. Research Focus: Symptoms/General Health
Start Date (CY):1991 Est. Completion (CY):1995 Brain & Nervous System

OVERALL PROJECT OBJECTIVE: The Ft. Devens ODS Reunion Survey represents a longitudinal assessment of readjustment of Persian Gulf War veterans returning through Ft. Devens, Massachusetts following their return from the Gulf region.

SPECIFIC AIMS: 1. Describe troop demographics of the Ft. Devens cohort of Persian Gulf War veterans; 2. Examine, both cross-sectionally and longitudinally, family and unit support factors, war-zone exposure, PTSD and psychological distress symptomatology in these veterans; 3. Examine physical health status at Time 2 and explore potential risk factors important in the development of adverse physical health after return from a wartime environment.

METHODOLOGY: Subjects were recruited from a group of approximately 5000 Persian Gulf War veterans who returned home through Ft. Devens, Massachusetts after the Gulf War. The survey at Time 1 constituted a broad range of demographic questions accompanied by an assessment of Post-Traumatic Stress Disorder (PTSD) symptomatology (Mississippi Scale for Combat-related PTSD; Keane, Cadell & Taylor, 1988), general psychological well-being (Brief Symptom Inventory, BSI; Derogatis & Melisaratos, 1983), and combat exposure (Gallops, Laufer & Yager 1981; Rosenheck, 1992), along with queries of other relevant domains including change in work status, current family and social functioning, and post-deployment major life stressors (e.g., death of a loved one, separation or divorce, loss of property, physical or sexual assault) and at Time 2, additional questions were added to further assess psychological health and physical health symptoms. Individual health symptoms were assessed using the 20-item Health Symptom Checklist (Bartone et al., 1989) which asked subjects to indicate the presence and frequency of symptoms over the past several weeks using Likert-type ratings (0=none, 1=a little, 2=often, 3=very often). In addition, subjects were asked to rate their current physical and psychological health separately ('poor', 'fair', 'good', 'very good' or 'excellent') as well as to rate changes in their physical and psychological health since return from the Persian Gulf ('much worse', 'worse', 'same', 'better' or 'much better').

EXPECTED PRODUCTS (MILESTONES): Data from this investigation is expected to provide an important opportunity to examine patterns of readjustment and the factors that contribute to the changes in the years soon after return from a wartime environment. Results from analyses of Time 1 and Time 2 collected data have been published. Publications in progress include evaluation of reported health symptoms by this cohort of Persian Gulf War veterans at Time 2, examination of reported sexual harassment by the women surveyed, and a longitudinal assessment of PTSD and psychological symptomatology between Time 1 and Time 2.

STATUS/RESULTS TO DATE: Over 84 U.S. Army units (n=2949) were surveyed immediately upon their return to Ft. Devens, Massachusetts (Time 1) between April and July 1991. Persons tested at Time 1 represented approximately 60% of the troops that were deployed to the Gulf from that base, and comparisons between this sample and data available for the Ft. Devens military population at large indicate that survey respondents are generally representative of the base's military population. Examination of nonparticipants at Time 1 indicated random absences for administrative and medical purposes. The original cohort contained 2,709 men and 240 women with a mean age of 30.2 years (SD = 8.6) and 13.2 years of education (SD = 1.8). The majority of troops were Caucasian (87.4%) and reservists (52.2% National Guard; 19.9% Army reservists; 27.9% active duty). Active duty troops had more black members and were significantly younger in age compared to Guard and Reserve Troops. Overall, veterans in the original cohort do not fully reflect military assignments or ethnic and gender compositions of the total U.S. Army or Gulf force (U.S. Government Accounting Office, 1992; communication with Defense Manpower Data Center). Consistent with the Ft. Devens' mission, a higher than average proportion of the original sample had combat support and service support positions during the war, rather than active combat roles.

Approximately 18-24 months after initial testing, veterans were resurveyed (Time 2) to reevaluate their adjustment. This survey repeated all Time 1 measures and queried other relevant domains including work status, family and social functioning, health status, health service use, and intervening

life stressors. In the majority of cases, the Time 2 survey was readministered in persons during weekend unit drills. Persons in units that could not be scheduled in a timely fashion were resurveyed using an identical questionnaire sent through the mail. A total of 2,313 persons successfully completed the Time 2 survey (2,119 men, 194 women; Wolfe et al., 1993), yielding an overall response rate of 78%. Analyses of these data show no significant Time 1 differences between Time 2 respondents and non-respondents on most background or outcome measures (e.g., educational level, PTSD symptomatology). However, individuals who did not complete the Time 2 reevaluation were more likely to be younger and on active duty status. Based on veterans' verbal and written comments concerning their health status at Time 2, the Time 3 investigation (now Project #1 of the Boston Environmental Hazards Center) was designed to reexamine overall adjustment with a special emphasis on physical health and psychological status and to evaluate cognitive functioning by neuropsychological test methods on a subset of the larger cohort

PUBLICATIONS: Wolfe J. Preliminary report of reunion survey on Desert Storm returnees. In. R. Rosenheck, et al. (eds.). War-zone stress among returning Persian Gulf troops: A preliminary report. Legislative report to Congress. 1991.

Wolfe J, Kelley JM, Buscela ML, Mark WR. Ft. Devens Reunion Survey: Report of Phase I. In R. Rosenheck et al. (eds.), War-zone stress among returning Persian Gulf troops: Final Report. Legislative report to Congress. 1992.

Wolfe J, Brown PJ, Kelley JM. Reformulating war stress: Exposure and the Persian Gulf War. *Journal of Social Issues*, 1993, 49, 15-31.

Title: Psychological Test Data of Gulf War Veterans Over Time

Project #: VA-8 Agency: VA Study Location: VAMC Mountain Home
Project Status: Ongoing Research Type: Clinical Research
P.I.: Patrick Sloan, Ph.D. Research Focus: Symptoms/General Health
Start Date (CY): 1991 Est. Completion (CY): 1999 Brain & Nervous System

OVERALL PROJECT OBJECTIVE: (1) To learn more about reactions to combat exposure, stress, and Persian Gulf War-related health concerns, (2) exploration of the relationships among symptoms, demographic variables and veterans' psychological status, (3) to provide intervention, education, and support to Gulf War veterans by providing a) general information about reactions to combat/desert duty, and b) opportunities for individual discussion of experiences and current physical health, (4) administration of standardized psychological questionnaires and instruments, and (5) referral to VA and other community services available for health and other related concerns.

SPECIFIC AIMS: To learn more about psychological stress and physical symptoms experienced by Persian Gulf War veterans.

METHODOLOGY: Initially evaluated 66 Gulf War Marine reservists on a number of psychological instruments within 5 months of Operation Desert Storm using the Mississippi Scale for Desert Storm War Zone Personnel, MMPI-2, Impact of Event Scale, War Stress Inventory-Operation Desert Storm Version, Rorschach test evaluation of DSM-III-R criteria for post-traumatic stress and post-traumatic stress disorder (PTSD). At one year follow-up, the MMPI-2 and Impact of Event Scale were collected on a sub-sample of these Gulf War veterans. At 3-year follow-up, all of the original measures, plus a physical symptom questionnaire selected questions from the Persian Gulf Registry Code Sheet and The Hand Test, were administered to a sub-sample of the original group and a control group of Marine Reservists from the same unit who had never been stationed outside of the U.S. Statistical analyses employed in this study included correlation, paired and independent t-tests, ANCOVA, MANCOVA, discriminant analysis, stepwise discriminant analysis, and repeated measure design ANOVA. Currently, volunteers referred for psychological and neuropsychological evaluation will complete all the measures listed above, the Halstead-Reitan Neuropsychological Test Battery, and questions related to disability status.

EXPECTED OUTCOMES: Assess the utility of psychological instruments in the detection of war-zone stress reactions. Instrument variables associated with response to stressors are expected to be related to DSM-III-R criteria for post-traumatic stress and PTSD.

STATUS/RESULTS TO DATE: The project is currently ongoing, the sample size is increasing and data are being collected. Results to date show differences over time between the initial evaluation data and follow-up, and between initial evaluation and control group on selected variables associated acute distress, and capacity for coping. Findings are being analyzed in relation to the assessment, understanding, and treatment of post-traumatic stress symptomatology of PTSD. Continuing to collect data and have several manuscripts submitted for publication.

PUBLICATIONS: Sloan P, Arsenault L, Hilsenroth M, Harvill L, Handler L. Rorschach measures of post-traumatic stress in Persian Gulf War veterans. *Journal of Personality Assessment*, 1995, 64, 397-414.

Sloan P, Arsenault L, Hilsenroth M, Harvill L, Handler L. Rorschach measures of post-traumatic stress in Persian Gulf War veterans: A 3-year follow-up study. *Journal of Personality Assessment*, 1996, 66(1), 54-64.

Sloan P, Arsenault L, Hilsenroth M, Harvill L. Assessment of non-combat, war-related post-traumatic stress symptomatology: validity of the PK, PS, and IES Scales. *Assessment*, 1996, (3)1, 37-41.

Title: Evaluation of Cognitive Functioning in Persian Gulf War Veterans Reporting War-related Health Problems

Project #: VA-9

Agency: VA

Study Location: VAMC New Orleans

Project Status: Completed

Research Type: Clinical Research

P.I.: Patricia Sutker, Ph.D.

Research Focus: Symptoms/General Health

Start Date (CY):1994 Est. Completion (CY):1995

Brain & Nervous System

OVERALL PROJECT OBJECTIVE: Addressing a topic of timeliness and importance, this project evaluated cognitive and psychosocial functioning among Operation Desert Storm (ODS) veterans who experienced health problems and physical symptoms following return from the Persian Gulf War zone. The primary objective of the project was to explore environmental, neurotoxic, and psychological correlates of health complaints among these veterans.

SPECIFIC AIMS: The project examined potential differences between veterans reporting high levels of health concern and those reporting low levels of concern on measures of psychological well-being, psychopathology, environmental exposure to toxins and adverse environmental events in the war-zone and prior to war-zone duty, and neuropsychological functioning.

METHODOLOGY: Conducted in cooperation with a research team of psychologists working at the Boston VAMC, study design incorporated a local sample of 78 Persian Gulf returnees who were divided into high and low health concern subsets on the basis of self-reported health status.

Participants underwent a clinical diagnostic evaluation that includes administration of an environmental exposure interview; a structured psychodiagnostic interview; a battery of neuropsychological tests selected for their specificity and sensitivity to neurotoxicity and emotional and motivational factors; and self-report inventories assessing mood, emotional well-being, PTSD symptomatology, combat exposure, and physical symptoms. Statistical analysis incorporated a multivariate analysis of variance approach, comparing the two health concern subgroups from both sites on measures of environmental exposure, psychological and neuropsychological functioning, and physical symptoms.

EXPECTED PRODUCTS (MILESTONES): Given current interest in the multiple, yet inadequately diagnosed or explained health problems and symptoms reported by sizable subsets of returnees from the Persian Gulf War, this project will have direct relevance to health care delivery in the VA system nationwide. Study results will be pertinent to development of appropriate clinical diagnostic and treatment services for Persian Gulf returnees and veterans of more recent military operations and objective evaluation of veteran disability and compensation claims in the VA system.

STATUS/RESULTS TO DATE: Data collection at New Orleans site has been completed. All data have been forwarded to the Boston site for multi-site statistical analysis and dissemination of findings.

PUBLICATIONS: none to date

Title: Memory and Attention in PTSD

Project #: VA-10

Agency: VA

Study Location: VAMC New Orleans

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Jennifer Vasterling, Ph.D.

Research Focus: Brain & Nervous System

Start Date (CY): 1994 Est. Completion (CY): 1998

Symptoms/General Health

OVERALL PROJECT OBJECTIVE: Clinical reports have documented that as many as 67-100% of veterans undergoing treatment for war-related post-traumatic stress disorder (PTSD) exhibit deficits in memory and concentration. This project seeks to address the common and potentially debilitating problems of impaired learning, memory, and attention functions reported by veterans suffering PTSD. **SPECIFIC AIMS:** Incorporating two separate investigations, this research applies a comprehensive assessment protocol that includes evaluation of psychopathology and neuropsychological functioning in veterans of Vietnam and a younger cohort of Persian Gulf returnees. In particular, the project will provide in-depth description and analysis of learning, memory, and attention functioning among these veterans.

METHODOLOGY: Within each study, comparisons of neuropsychological performances are conducted between community-recruited groups of veterans with PTSD diagnoses related to war-zone duty, veterans with diagnoses of depression, and veterans found to be free of psychopathology. Psychopathology is assessed with structured clinical interview and self-report inventories. Attention, learning, memory, and other cognitive processes are assessed with standardized neuropsychological assessment instruments. Data analysis, conducted separately for the two cohorts, are accomplished using a multivariate of analysis approach.

EXPECTED PRODUCTS (MILESTONES): Because problems with attention, learning, memory, and other cognitive functions can dramatically disrupt social and occupational functioning, as well as hinder treatment efforts of war-related stress and other disorders, studies of neuropsychological sequelae to war trauma are pivotal to understanding the complex behavioral and emotional expression of PTSD, to delivery of appropriate, effective treatment interventions to these veterans, and to exploring long-term psychosocial outcomes in veterans suffering PTSD and served by the VA system.

STATUS/RESULTS TO DATE: To date, this newly funded research has been conducted on a sample of 95 Persian Gulf returnees. Findings reveal that in comparison to psychopathology-free veterans, veterans diagnosed with PTSD performed significantly more poorly on verbal intellectual tasks including those tasks thought to predict premorbid functioning. Results suggest that intellectual resources, particularly verbal skills, may buffer development of stress-related psychopathology following trauma exposure. Analysis of cognitive data revealed specific PTSD-related deficits in sustained attention, working memory, initial acquisition of new material, and resistance to retroactive interference. Collection of data with Vietnam veterans is ongoing. In addition, PTSD diagnosis was associated with errors of commission and intrusion. Results are consistent with models of PTSD that emphasize the role of hyperarousal and implicate dysfunction of frontal-subcortical systems.

PUBLICATIONS: Vasterling JJ, Brailey K, Constans JI, Borges A, Sutker PB. Assessment of intellectual resources in Gulf War veterans: relationship to PTSD. *Assessment*, 1997, 4:51-59.

Vasterling JJ, Brailey K, Constans JI, Borges A, Sutker PB. Attention and memory dysfunction in PTSD-diagnosed Persian Gulf war veterans. Poster presented at the 25th Annual Meeting of the International Neuropsychological Society, Orlando, Florida. February 1997.

Vasterling JJ, Brailey K, Constans JI, Sutker PB. Attention and memory dysfunction in posttraumatic stress disorder. *Neuropsychology*. In Press.

Title: Neuropsychological Functioning in Veterans

Project #: VA-11

Agency: VA

Study Location: VAMC New Orleans

Project Status: Completed

Research Type: Clinical Research

P.I.: Jennifer Vasterling, Ph.D.

Research Focus: Brain & Nervous System

Start Date (CY):1992 Est. Completion (CY):1995

Symptoms/General Health

OVERALL PROJECT OBJECTIVE: Complaints of impaired memory and concentration among veterans seeking treatment for post-traumatic stress disorder (PTSD) highlight the importance of systematic evaluation of these cognitive functions among PTSD-diagnosed veterans. The primary objective of this project was to collect pilot data regarding learning, memory, and attention functioning in Persian Gulf returnees reporting PTSD symptomatology.

SPECIFIC AIMS: The specific aim of this project was to compare learning, memory, and attention performances in samples of Persian Gulf returnees with and without diagnoses of PTSD.

METHODOLOGY: Subjects were 15 Persian Gulf War zone veterans diagnosed with military-related PTSD and 27 Persian Gulf War zone veterans found to be free of any PTSD symptomatology. All subjects underwent structured psychiatric interview and neuropsychological assessment using a brief screening battery designed to emphasize attention, learning, and memory functioning.

Statistical analyses included a series of univariate analyses of variance (ANOVAs) and univariate analyses of covariance (ANCOVAs) using vocabulary performance as a covariate to compare the two samples on demographic variables and dependent measures of cognitive functioning.

EXPECTED PRODUCTS (MILESTONES): Results of this project impact potentially on comprehensive assessment and treatment services provided to PTSD-diagnosed Persian Gulf War zone veterans.

STATUS/RESULTS TO DATE: Veterans in the PTSD sample were on average older, less educated, and performed more poorly on a vocabulary task used as an estimate of premorbid intellectual sophistication than veterans in the comparison sample. Comparisons of group performances on the neuropsychological tasks revealed that the PTSD sample performed more poorly than the comparison sample on measures of simple and complex attention and word retrieval. When differences in estimated native intellectual skills were controlled for statistically by the inclusion of vocabulary performance as a covariate in a series of ANCOVAs, no significant differences between groups emerged on any of the attention or memory measures. That the effects of PTSD on attention and memory functions could not be parceled out from those attributable to such factors as lower levels of education and premorbid intellectual sophistication does not negate the clinical significance of study findings. Results of this exploratory pilot project complement previous research suggesting that higher levels of education and intellectual functioning may buffer the impact of trauma on the development of subsequent stress-related psychopathology. This phase of the project is complete and has led to the development of a more refined and comprehensive neuropsychological protocol to be administered to a larger sample of Persian Gulf veterans.

PUBLICATIONS: Vasterling JJ, Root L, Brailey K, Uddo M, Sutker PB. Attention and Memory performances in post-traumatic stress disorder. Paper presented at 22nd Annual Meeting of the Internat. Neuropsychological Society, Cincinnati, OH. February 1994.

Title: Psychological Assessment of Operation Desert Storm Returnees

Project #: VA-12

Agency: VA

Study Location: VAMC New Orleans

Project Status: Completed

Research Type: Clinical Research

P.I.: Patricia Sutker, Ph.D.

Research Focus: Brain & Nervous System

Start Date (CY):1991 Est. Completion (CY):1997

OVERALL PROJECT OBJECTIVE: This research was designed to determine the psychological sequelae to war-zone exposure among troops who served in the Persian Gulf during Operation Desert Storm (ODS) compared to troops who were activated during ODS but not deployed to the Persian Gulf.

SPECIFIC AIMS: Objectives of this project were to conduct comprehensive psychological assessments and debriefings among the masses of troops mobilized in support of ODS; identify psychological, somatic, and cognitive symptoms and mental disorders, both early and persistent, that are associated with Persian Gulf war--zone exposure; and explore personal resources and environment factors that may differentiate Persian-Gulf--deployed troops exhibiting war-related psychopathology from troops found to be free of psychological disturbances.

METHODOLOGY: Comprehensive psychological assessments were conducted with non-treatment-seeking troops who served in the Persian Gulf and a comparison sample of troops from the same military units who were activated during ODS but not deployed to the Persian Gulf. Follow-up assessments were conducted to assess psychological status over time and ongoing symptomatology. The subject sample includes 1520 military personnel mobilized in support of ODS, including 517 Louisiana National Guard and 1003 Marine, Army, Air Force, and Navy Reserve troops, 194 of whom were members of Quartermaster units assigned graves registration duties. Subjects reported an average age of 29 years at time of initial assessment and completed an average of 13 years of formal education. The percentage of African- American and other minority troops was 43%, and 14% were women. An assessment battery tapping domains of personal resources, stressor characteristics, negative mood states and traits, psychiatric and physical symptoms, and symptoms of posttraumatic stress disorder (PTSD) was administered in regularly-scheduled drill exercises at 6, 8, and 12 months following ODS and at one-year follow-up intervals. An individually-administered comprehensive structured clinical diagnostic interview was conducted with a subset of graves registration troops judged to be at high risk for negative psychological sequelae because of their gruesome Persian Gulf body handling duties. Data analytic strategies for continuous variables included multivariate and univariate analyses of variance, with repeated measures where indicated, and stepwise discriminant function analyses. Chi-square analyses were conducted for categorical variables.

EXPECTED PRODUCTS (MILESTONES): By establishing prevalence estimates for negative sequelae to war--zone duty, identifying troops at high risk for development of war-zone-related psychopathology, and determining factors associated with increased psychological vulnerability to war-zone service, results have implications for development of more effective and efficient strategies and procedures for military training and preparing troops for war-zone exposure, conducting debriefing exercises to facilitate community reentry following war-zone service, and providing mental health treatment interventions specific to war-zone-related psychopathology.

STATUS/RESULTS TO DATE: The project is completed. Comparisons of data collected upon initial assessment among 876 war-zone deployed and 396 non-war-zone deployed troops showed that troops exposed to war-zone duty reported higher levels of depression, anxiety, and somatic preoccupations than did the non-war-zone deployed sample. Specifically, 23% of the war-zone deployed sample showed at least mild levels of clinical depression, and 14% met criteria for PTSD measured by paper-and-pencil instruments. War-zone troops complained more often of somatic discomfort than did non-war-zone troops, citing headaches (22% versus 13%), general aches and pains (20% versus 13%), lack of energy (18% versus 12%), sleep disturbances (17% versus 10%), and common cold or flu (16% versus 11 %).

Troops who served in the Persian Gulf described more mental health symptoms as war-zone stress severity increased, and symptom expression among 349 war-zone troops increased over a 1-year follow-up interval on measures of depression, anxiety, anger, physical distress symptoms, and PTSD. Among those who served in the Persian Gulf war-zone, ethnic minority troops were more

frequently assigned PTSD diagnoses than were their white counterparts; however, women did not evidence greater vulnerability to negative psychological sequelae to war-zone exposure than men. War-zone-related PTSD diagnoses were associated with low levels of the personality hardiness dimension of commitment, reliance on avoidance as a coping mechanism, low perceived family cohesion, and low satisfaction with available social supports, and scores on these dimensions at initial assessment predicted the presence/absence of PTSD at one-year follow-up with 77% accuracy.

Comprehensive structured clinical diagnostic interviews were administered to a subset of troops judged to be at high risk for negative psychological outcomes to war-zone trauma by virtue of particularly gruesome war-zone assignments, i.e., graves registration or body handling duties, during the Persian Gulf War. Among 24 Louisiana based troops of the 630th Quartermaster Company who performed Persian Gulf graves registration duties, prevalence of current PTSD was found to be 46%, and rates of comorbid disorders among troops assigned PTSD diagnoses included major depression (55%), alcohol abuse/dependence (27%), depressive disorder NOS (18%), and simple phobia (9%). Findings were replicated and extended by assessments conducted among the Puerto Rico-based 246th Quartermaster Battalion with subsequent comparisons of 40 troops who performed graves registration duties in the Persian Gulf to 20 similarly-trained troops who were not deployed to the Persian Gulf and who had no ODS graves registration exposure. Diagnoses of current PTSD were found in 48% of war-zone deployed troops; whereas, none of the non war zone deployed troops met PTSD criteria. Low rates of pre-ODS deployment psychiatric disorders were found in both subsets of troops. Current comorbid diagnoses among troops who met criteria for PTSD included depressive disorder (37%), alcohol dependence (21%), other anxiety disorder (5%), and polysubstance dependence (5%). One-year follow-up assessments were completed with 17 troops of the 630th Quartermaster Company who were assigned Persian Gulf graves registration duties, and 47% met criteria for current PTSD. Of the 26 troops of the 246th Quartermaster Battalion who performed war-zone graves registration duties and completed follow-up assessment, 38% evidenced current PTSD. Thus, 42% of troops assessed at follow-up were classified as positive for PTSD two years after war-zone service.

PUBLICATIONS: Vasterling JJ, Brailey K, Constans JI, Borges A, Sutker PB. Assessment of intellectual resources in Gulf War veterans. *Assessment*, 1997, 4:51-59.

Vasterling JJ, Brailey K, Constans JI, Sutker PB. Attention and memory dysfunction in posttraumatic stress disorder. *Neuropsychology* (in press).

Sutker PB, Uddo M, et al. War-zone stress, personal resources and PTSD in PG war returnees *J Ab Psych*, 1995, 104, 444-452.

Title: Neurobehavioral Aspects of Persian Gulf Experiences: A Pilot Study
Project #: VA-13 Agency: VA Study Location: VAMC Pittsburgh
Project Status: Completed Research Type: Clinical Research
P.I.: Gerald Goldstein, Ph.D. Research Focus: Brain & Nervous System
Start Date (CY):1994 Est. Completion (CY):1995

OVERALL PROJECT OBJECTIVE: To collect data on a sample of 50 Persian Gulf Veterans who have returned with physical or psychosocial complaints.

SPECIFIC AIMS: To obtain pilot data to gain objective evidence of impairment of cognitive ability and brain function, or of psychosocial disability.

METHODOLOGY: Subjects were tested in our laboratories with an extensive battery of neuropsychological tests, event-related brain potentials, measures of cardiac and pupillary reactivity, laboratory measures of attention, and various interviews, tests, and questionnaires to evaluate psychosocial status with an emphasis of PTSD. Data were analyzed descriptively and in reference to available literature and normative information. The presence of abnormal findings should provide the basis for further study with a controlled investigation.

EXPECTED PRODUCTS: To obtain neuropsychological, psychophysiological and psychosocial data from 50 Persian Gulf veterans.

STATUS RESULTS TO DATE: A paper describing the neuropsychological test results has appeared in the Journal of the International Neuropsychological Society. The findings are summarized as follows. A neuropsychological investigation of 21 Persian Gulf veterans and 38 demographically matched controls was conducted in order to make a preliminary determination concerning presence of neuropsychological deficits associated with the Persian Gulf War experience. The neuropsychological test battery consisted of measures of complex attention, memory, and motor skills, previously shown to be sensitive to exposure to environmental toxins. It was found that the Persian Gulf veterans group did not demonstrate substantial impairment, but an impairment index derived from 14 test variables was statistically significantly different from controls in the direction of poorer performance. A second study involving the psychophysiological data is currently being prepared for submission to Biological Psychiatry as a brief report. We found prolonged latencies, without reduction in amplitude, in the P300 component of the auditory event related potential (ERP) in the Persian Gulf veterans. This prolongation was significantly different from demographically matched controls when we only considered the 13 of 19 subjects who had complaints related to cognitive function, such as memory impairment. The remaining 6 subjects had normal latencies. Prior research has shown that increased latencies are associated with decline in cognitive efficiency. We therefore concluded that reported cognitive deficits may not be associated with psychological distress alone. A paper reporting these results is under review by the Journal of the International Neuropsychological Society.

PUBLICATIONS: Goldstein G, Beers SR, Morrow L A, Shemansky WJ, Steinhauer SR. A preliminary neuropsychological study of Persian Gulf veterans. Journal of the International Neuropsychological Society, 1996, 2:368-371.

Steinhauer SR, Beers SR, Goldstein G, Shemansky WJ, Morrow LA.
Neurophysiological and neuropsychological characteristics of Gulf War veterans. J Intern Neurop Soc (abstract), 1996, 2:68.

Title: Vaccine-Mediated Immunity Against Leishmaniasis

Project #: VA-15 Agency: VA Study Location: VAMC Cleveland

Project Status: Ongoing Research Type: Basic Research

P.I.: Frederick Heinzl, M.D. Research Focus: Leishmaniasis

Start Date (CY):1993 Est. Completion (CY):1999 Prevention

OVERALL PROJECT OBJECTIVE: To determine the expression and function of T cell costimulatory molecules during vaccination of mice with *Leishmania* major antigen and cytokine adjuvants. We hypothesize that distinct costimulatory interactions can be manipulated *in vivo* to specifically induce or enhance unipolar Th1 CD4+ responses that produce interferon-gamma and thereby protect against disease.

SPECIFIC AIMS: 1. Characterize differences in the expression of B7-1, B7-2, and CTLA4 regulatory molecules during infection or vaccination of susceptible and resistance mice with *Leishmania major*; 2. Determine the function of B7-1, B7-2, and CTLA4 using bulk cultures of T cells; 3. Redirect vaccine- and infection-induced CD4+ T cell responses by *in vivo* manipulation of B7 or CTLA4.

METHODOLOGY: Mice are infected or vaccinated subcutaneously with *Leishmania major* and the draining lymph nodes analyzed by polymerase chain reaction or flow cytometry to characterize the expression of CTLA4, B7-1, B7-2 on T cells and accessory cells. Comparisons are made between naturally resistant and susceptible mice (C57BL/6 and BALB/c, respectively). The function uninfected or infected mice and stimulated by antibody-mediated cross-linking of CD3 in conjunction with CD28 or CTLA4. Treatment of infected or vaccinated mice with antibodies against these costimulatory molecules will be used to determine *in vivo* function.

EXPECTED PRODUCTS (MILESTONES): Dysregulated Th1 and Th2 development mediate common human diseases, such as asthma and autoimmune diabetes mellitus and multiple sclerosis. Since blocking B7-2 function *in vivo* protects susceptible strains of mice against progressive leishmaniasis, existing humanized CTLA4-Ig antagonists of B7-1 and B7-2 might be useful as immunotherapy in leishmaniasis or other diseases resulting from inappropriate polarizations of T cell phenotype.

STATUS/RESULTS TO DATE: B7-2 is increasingly expressed on T cells developing the memory cell phenotype, suggesting either a role in costimulating other T cells or in mediating unique signals when T cell B7-2 is cross-linked by other T cells bearing CD28 or CTLA4. B7-2 positive cells may constitute a functionally distinct subset of CD4+ T cells. Treatment with anti-B7-2 cures leishmaniasis in susceptible BALB/c mice. Blockade of CTLA4 *in vivo* exacerbates disease by promoting earlier Th2 cell development, whereas B7-2 blockade slows T cell development and favors Th1 predominance.

PUBLICATIONS: Heinzl FP, Rerko RM, Ahmed F, Pearlman E. Endogenous interleukin-12 (IL-12) is required for control of Th2 cytokine responses capable of exacerbating leishmaniasis in normally resistant C57BL/6 mice. *J Immunol*, 1995,155:730-739.

Heinzl FP, Hujer AM, Ahmed F, Rerko RM. Interferon-gamma independent production of interleukin-12 during murine endotoxemia. *J Immunol*, 1996;157:4521-8.

Heinzl FP, Ahmed F, Hujer AM, Rerko RM. Immunoregulation of murine leishmaniasis by interleukin-12. *Research in Immunology*, 1995, 146:153-60.

Title: Protective Immunity in Experimental Visceral Leishmaniasis

Project #: VA-16 Agency: VA Study Location: VAMC San Antonio

Project Status: Completed Research Type: Basic Research

P.I.: Peter Melby, M.D. Research Focus: Leishmaniasis

Start Date (CY):1994 Est. Completion (CY):1997

OVERALL PROJECT OBJECTIVE: Characterization of the protective immune mechanisms in experimental visceral leishmania, and identification of the parasite antigens which elicit such protective responses.

SPECIFIC AIMS: Studies of the murine model of visceral leishmaniasis will provide a means to characterize the operative protective immune mechanisms as well as identify parasite antigens which have potential use for human vaccination. Characterization of the protective immune mechanisms in experimental visceral leishmaniasis will be accomplished by studies of the in situ cytokine response to primary infection in susceptible mice, as well as identification of responses associated with the acquisition of immunity. Once the mechanisms associated with protective immunity have been defined the parasite antigens relevant to immunity can be identified. Purified and recombinant *Leishmania donovani* antigens which elicit an in vitro cytokine response which correlates with in vivo immunity will be identified and characterized.

METHODOLOGY: The murine model of visceral leishmaniasis will be used to characterize the mechanisms involved in protective immunity at the tissue level. These studies were performed using a reverse transcriptase polymerase chain reaction methodology for measuring cytokine (IL 4, IL 6, IL 10, IL12, IFN - γ) gene expression in skin, Lymph node, and spleen. Comparisons were made between mice infected locally (skin) and systematically (intravenous). Expression of these cytokines in the spleen was also studied at the protein level using immunohistochemical techniques. Because infection of mice with *L. donovani* does not result in a progressive lethal infection, we have also begun to study the immunopathogenesis of VL in the Syrian hamster model which very closely mimics the progressive, fatal disease seen in humans. Because there were no reagents available to study this model, we cloned and sequenced a number of the hamster cytokine genes (IL-2, IL-4, IL-10, IL-12, IFN- γ , TNF alpha, and TGF-beta). These molecular probes were then used to characterize the expression of cytokine mRNAs in active VL in the hamster model. In our work to identify vaccine candidates for this disease, we have cloned recombinant *L. donovani* antigens into a eukaryotic vector for use in DNA immunization studies. Because of the prominent role IL-12 plays in the local containment of *L. donovani* infection through induction of a Th1 response, we have constructed an IL-12 expression plasmid for use as an adjuvant in the DNA immunization studies. Once the in situ mechanisms are better defined, in vitro correlates of immunity (e.g. a certain cytokine profile) can be identified. Semipurified or purified soluble antigens will then be tested in vitro for their capacity to elicit a proliferative response and protective cytokine profile in stimulate splenocytes or Lymph nodes. A recombinant library will also be directly screened using spleen cells from immune animals. This will enable the identification of vaccine candidates which can subsequently tested in the animal model.

EXPECTED PRODUCTS (MILESTONES): 1. Identification of cytokines associated with control of infection; 2. Identification of cytokines associated with resistance to reinfection; 3. Identification of antigens having the potential to stimulate protective immunity.

STATUS/RESULTS TO DATE: Mice infected with *L. donovani* amastigotes by the intradermal route develop minimal cutaneous swelling, regional lymphadenopathy, but no detectable visceral (hepatic) parasite burden. In contrast, mice infected by the intravenous route develop progressive hepatosplenomegaly and a visceral parasite burden that increases up to 4-6 weeks after infection. To understand the mechanisms associated with the local control of infection and active visceral disease, we have characterized the in situ expression of cytokines (IFN- γ , IL-4, IL-10, and IL-12) in the spleen, draining the lymph node, and cutaneous site of inoculation compared to the spleen following systemic inoculation. IL-10 and TGF- β mRNA and protein expression were prominent in the spleens of systemically infected animals. There was significant expression of both IL-10 and IL-12 at the cutaneous site of inoculation starting 7 days after infection. Thus there seems to be a mixed Th1/Th2 type response to primary infection in this model, but local control (skin and draining LN) appears to be associated with markedly increased levels of IFN- γ and IL-12 RNA. These differences

may be due to more efficient antigen presentation at the LN level following cutaneous infection (possibly from migration of cutaneous cells), or the induction of immunological tolerance following intravenous infection.

Additional studies were performed using a similar methodology to characterize the immunopathogenesis of VL in the highly susceptible hamster model. In order to study the model, multiple hamster cytokine genes were cloned, sequenced and used as molecular probes to determine the levels of mRNA expression in response to active visceral disease. There was a strong Th1 cytokine expression in the spleen in spite of the progressive nature of disease in this model of lethal infection. IL-10, but not IL-4 expression was markedly increased in response to infection and is likely to play a role in the progressive nature of this disease.

A number of *L. donovani* antigens previously identified as a target of immune T cells have been cloned for study of vaccine candidates. The open reading frames have been cloned into a eukaryotic expression vector, expressed in mouse fibroblasts and macrophages, and the nature of the T cell response directed toward it is being characterized. Mice have been immunized with these constructs and studies to determine their protective efficacy are in progress. The IL-12 expression vector we have constructed will be used as an adjuvant in these immunization studies.

PUBLICATIONS: Melby PC, Yang YZ, Cheng J, Zhao W. Regional differences in the cellular immune response to experimental cutaneous or visceral infection with *Leishmania donovani*. *Infect Immun* 66:18-27, 1998.

Melby PC, Tryon VV, Chandrasekar B, Freeman GL. Cloning of Syrian hamster (*Mesocricetus auratus*) cytokine cDNAs and analysis of cytokine mRNA expression in experimental visceral leishmaniasis. 1998 (manuscript submitted).

Title: Immunological Evaluation of Persian Gulf Veterans

Project #: VA-17 Agency: VA Study Location: VAMC Birmingham

Project Status: Completed Research Type: Clinical Research

P.I.: Michael P. Everson Research Focus: Immunology

Start Date (CY):1994 Est. Completion (CY):1995

OVERALL PROJECT OBJECTIVE: To understand possible contributions of immunological abnormalities to disease manifestations in Persian Gulf War veterans. The chief complaints of these veterans include arthralgias and central nervous system dysfunctions, i.e., disorders which may have a common immunological basis. The intent of this proposal was to expand upon ongoing medical and neurobehavioral analyses of veterans from the Birmingham VA Medical Center to include an immunologic component in the evaluation of these veterans.

SPECIFIC AIMS: Evaluate potential changes in the immunological status of Persian Gulf War veterans using sensitive biological assays of immune cellular function such as those previously described for use in evaluating chemical sensitivities.

METHODOLOGY: Functional immune integrity of peripheral blood mononuclear cells was tested in subjects (approximately 40) and age- and sex-matched controls (approximately 10). Longitudinal samples were obtained from 2 subjects. Antigenic and mitogenic responses were determined using the recall antigen tetanus toxoid and the T-cell mitogen phytohemagglutinin. Proliferative responses were measured by tritiated thymidine uptake and compared with cultures of unstimulated subject or control cells. Data were reduced and statistically compared using a two-sample, unpaired t test.

STATUS/RESULTS TO DATE: The final number of samples analyzed for immune function in mitogen-induced and antigen-induced proliferative assays included approximately 40 Persian Gulf veterans and 7 age- and sex-matched controls. When these two groups were compared for mitogenic responses, no significant difference was noted ($p > 0.06$). This finding suggested that the subjects and controls had similar T cell function potential since the phytohemagglutinin mitogen is a polyclonal stimulant used to assess potential for T cell responses. However, when these two groups were compared for antigenic response using the tetanus toxoid recall antigen, a significant difference was noted ($p = 0.027$). With less than 50 subjects and less than 10 controls, these data are to be viewed and interpreted only as preliminary findings suggestive of a difference in recall response to antigenic stimulation. More subjects are needed prior to publication or dissemination of these preliminary findings.

PUBLICATIONS: none to date

Title: Chronic Gastrointestinal Illness in Persian Gulf Veterans
Project #: VA-18 Agency: VA Study Location: VAMC Boston
Project Status: Completed Research Type: Clinical Research
P.I.: Mark Sostek, M.D. Research Focus: Symptoms/General Health
Start Date (CY):1994 Est. Completion (CY):1996

OVERALL PROJECT OBJECTIVE: Clearly define the most prevalent chronic gastrointestinal symptoms in a unit of Persian Gulf veterans.

SPECIFIC AIMS: 1. Determine and define symptom complex. 2. Look for underlying pathophysiology.

METHODOLOGY: Survey of a single unit of Persian Gulf veterans. Hydrogen breath tests on affected individuals.

EXPECTED PRODUCTS (MILESTONES): Ultimately to identify pathophysiology of the gastrointestinal symptoms.

STATUS/RESULTS TO DATE: Survey completed. Have identified most prevalent symptoms.

Note: Following is quoted from poster accepted for Poster Presentation at Digestive Disease week in San Diego, May 1995, Chronic Gastrointestinal Symptoms In Persian Gulf Veterans; MB Sostek, S Jackson, JK Linevsky, EM Schimmel, BG Fincke; Departments of Medicine and Social Services, Boston Veterans Affairs Medical Center and Boston University School of Medicine, Boston, MA.

Background: Persian Gulf Syndrome is characterized by a constellation of chronic symptoms postdating deployment to the Gulf Region in 1991. Intermittent diarrhea is one of the eight most commonly reported symptoms. The prevalence of non-diarrheal gastrointestinal (GI) symptoms is not well documented. Over the past year, we have received increasing referrals for evaluation of Persian Gulf veterans (PGV) with various GI complaints. The aims of this study were I) to determine the prevalence and spectrum of GI complaints in a representative sample from this population and II) to compare this data to a control group of soldiers not deployed to the gulf region.

Methods: A 4-page questionnaire was mailed to the 92 members of a National Guard Unit deployed to the Persian Gulf Region in 1991 and distributed to 44 members (controls) of the same unit who were not deployed to the Gulf Region in 1991. The questionnaire asked the veterans to grade current severity of 26 GI and 10 non-GI symptoms. The survey also asked veterans to recall occurrence of 5 GI symptoms either during or before the Dessert Storm mission.

Results: 57/92 Persian Gulf veterans (62%) responded to the survey. All 44 of the controls returned the survey. The table below summarized the reported frequency of several GI symptoms in this population:

GI Symptom	Before ODS	After ODS	Controls (Current)
Loose Stool	2/57 (3%)	39/57 (68%)*	4/44(9%)
Abdominal Pain	2/57 (3%)	32/57 (56)*	3/44 (7%)
Excessive Gas	5/57 (9%)	42/57 (74%)*	10/44 (23%)
Nausea/Vomiting	2/57(3%)	13/57 (23%)	1/44 (2%)
Hematochezia	no data	4/57 (7%)	0/44 (0%)

*p<.0001 compared to symptom frequency before desert storm.

Additional frequently reported GI symptoms among PGV's in this survey include: sensation of incomplete rectal evacuation post defecation 34/57 (60%), and watery bowel movements following episodes of abdominal pain 30/57 (53%). The most frequent non-GI symptoms were: fatigue 46/57 (81%), joint pains 42/57 (74%) and headache 38/57 (67%).

Conclusion: A significant number of veterans from a single National Guard unit, deployed to the Persian Gulf, developed GI symptoms while in that region. The majority of these veterans currently continue to report persistent loose stools, lower abdominal pain, excessive gas and tenesmus. Veterans in the same unit, who were not deployed to the Gulf region, report significantly fewer chronic GI symptoms at the present time. While these symptoms are suggestive of the development of irritable bowel syndrome following Persian Gulf deployment, further studies are needed to better understand the pathophysiology of this combination of chronic GI symptoms.

PUBLICATIONS: Abstract accepted for Poster Presentation at Digestive Disease week in San Diego, May 1995.

Sostek M. High prevalence of chronic gastrointestinal symptoms in a national guard unit of Persian Gulf veterans. *Am J of Gastroent.* December 1996, Vol 91, (12)2494-2497.

Title: Psychological Adjustment in Operation Desert Shield/Storm Veterans
Project #: VA-20 Agency: VA Study Location: VAMC Gainesville
Project Status: Completed Research Type: Clinical Research
P.I.: Patricia Sohler Research Focus: Brain & Nervous System
Start Date (CY):1991 Est. Completion (CY):1993

OVERALL PROJECT OBJECTIVE: The study examined the psychological effects of military service in the Persian Gulf during Operation Desert Shield/Storm.

SPECIFIC AIMS: Noncombat reserve and national guard troops who were in the Persian Gulf were compared to activated and nonactivated troops who remained in the United States during the Persian Gulf War. The study determined the difference between the three groups in terms of psychological adjustment and impact on cognitions and feelings about the war.

METHODOLOGY: Subjects were 507 members of National Guard and Reserve units in Northcentral Florida. Data was collected over a two-month time period from July 29 to September 23, 1991. Thus, the data was collected approximately 6 months after the ground war ended, and usually from one to two months after the troops had returned to the United States. Data was collected during the customary monthly weekend drills, and with the endorsement of the units' commanders. For units that had been deployed to the Persian Gulf, troops were also briefed about the potential emotional and family problems associated with post-war adjustment, were provided with information about veterans benefits, and were advised of the availability of readjustment counseling in our VAMC and other facilities in their communities. The order of presentation of these elements of necessity varied from site to site, and sometimes within a site, as it was imperative to oblige the needs of the units' and those of others involved (e.g. Vet Center counselors, veterans benefits counselors).

Subjects were assured of the anonymity of their responses, and were instructed to hand their packets of questionnaires directly to the study's investigators, in an attempt to assuage subjects' concerns that their careers might be jeopardized by their disclosures. However, it is evident that doubts lingered, as some subjects commented on their concerns about how the results might be used, and many failed to provide their names (requested in order to conduct follow-up). Six troops omitted information about their gender as well, and were thus excluded from analysis.

The subjects, tested at different sites, consisted entirely of combat support troops, including engineering units, mobile medical units, transportation units, military police, a quartermaster unit, and a demolition unit. Of the 507 troops included as subjects, 397 were male and 110 were female. Although a minority in the other units, females made up about 50% of the mobile medical units. For the purpose of analysis, subjects are categorized as deployed (i.e. activated and sent to the Persian Gulf during Operation Desert Storm/Shield), activated/not deployed (activated for duty during Operation Desert Storm/Shield but serving in the United States) and not activated (remaining on non-active status throughout Operation Desert Storm/Shield).

	Males	Females
Deployed	213	75
Not Activated	173	26
Activated/Not Deployed	11	9

Due to the low numbers currently available in the Activated/Not Deployed category, the analysis is restricted to Deployed and Not Activated.

Unit officers provided strong encouragement to their troops to participate in the study, and although it is not possible to know exactly what percentage did actually participate, 90% would be a reasonable estimate. Of those subjects that did return their questionnaire packets, many did not complete the entire protocol, which normally required about one hour to complete. The questionnaires were presented in the following order: 1) demographic questionnaire, 2) combat exposure scale from the War Stress Interview, 3) SCL-90 plus 15 additional items from the Cincinnati Stress Reaction Scales, 4) Gainesville Readjustment Questionnaire, 5) Family Environment Scale, 6) Impact of Events Scale (IES), 7) Work Environment Scale.

RESULTS: Analysis of the data indicated that individuals deployed to the Persian Gulf differed significantly from individuals in reserve and guard units who were not activated during ODS. Specifically, they reported a greater degree of intrusion and avoidance symptoms related to the war, as measured by the IES. ODS veterans who were also veterans of prior wars reported fewer

intrusion and avoidance symptoms. Age was not found to be a significant factor. The scores on the Gainesville Questionnaire, postulated to indicate enhanced coping strategies, did not correlate significantly with evidence of superior mental health on the SCL-90-R or the IES. A lack of significant difference between ODS veterans and control group in terms of SCL-90-R scores suggests that the overall psychological health of most of the ODS veterans does not appear to have been affected by the war. However, it is apparent that some subjects had lingering doubts about the possible repercussions of admitting problems, and this is likely to have suppressed some of the SCL-90-R scores, which showed less pathology than non-patient norms in the general population. Additionally, the units available to this investigation were all combat support units, and combat exposure was consequently more limited and less severe than for combat units. Only 156 subjects returned follow-up data, with only 27 completing all 3 follow-ups. An ANOVA conducted to determine if the follow-up group was representative of the larger population resulted in a significant interaction effect, in which ODS veterans who cooperated with the follow-up admitting to higher intrusion and avoidance symptoms on their initial testing. Thus, the follow-up data was concluded to not represent a random sample of the desired population. One subject found the mailed follow-up questionnaires caused discomfort and indicated that she did not want to be contacted further. We abided by her request. No other adverse effects were noted.

PUBLICATIONS: Returning Persian Gulf Troops: First Year Findings, published by Northeast Program Evaluation Center (NEPEC), VAMC, West Haven, Connecticut, 1992.

Title: A Comparison of PTSD Symptomatology among Three Army Medical Units Involved in ODS

Project #: VA-21 Agency: VA Study Location: VAMC Phoenix
Project Status: Completed Research Type: Clinical Research
P.I.: Nancy Errebo, Psy.D. Research Focus: Brain & Nervous System
Start Date (CY):1992 Est. Completion (CY):1994

OVERALL PROJECT OBJECTIVE: The objective of the study was to compare three Arizona Reserve Medical Units involved in Operation Desert Storm on PTSD symptomatology one year after the war utilizing the revised Mississippi Scale for Combat-Related PTSD (Keane, Caddell, and Taylor, 1988). One of the groups was deployed to Saudi Arabia(N=42), one to England (N=37), and one to Arizona (N=17).

SPECIFIC AIMS: The hypothesis was that the group deployed to Saudi Arabia would have significantly higher scores than the other two groups.

METHODOLOGY: Subjects consisted of three Arizona Military Reserve Medical Units, one deployed to Saudi Arabia, one deployed to England and one deployed locally. Members of all units included physicians, nurses and other medical specialists.

The group deployed to Arizona (AZ) consisted of 17 subjects, 10 females (58.8%) and 7 males (41.2%). Of the 13 subjects in AZ who provided demographic data, 2 were age 20-29 (15.4%), 5 were 30 to 39 (38.5%), and 6 were 40 to 50 (46.2%). Ages ranged from 20 to 54. The mean age was 38.9. Mean age for females was 34.7. Mean age for males was 43.1.

The group deployed to England (ENG) consisted of 37 subjects, 21 females (56.8%) and 16 males (43.2%). Eleven were age 20-29 (29.7%), 14 were 30 to 39 (37.8%), 9 were 40 to 49 (24.3%), and 1 was 50 to 59 (2.7%). Ages ranged from 21 to 56. The mean age was 33.7. Mean age for females was 34.4. Mean age for males was 33.

The group deployed to Saudi Arabia (SA) consisted of 42 subjects, 20 females (47.6%) and 22 males (52.4%). Twelve subjects were age 20-29 (28.6%), 10 were 30-39 (23.8%), 15 were 40-50 (35.7%) and 5 were 50-59 (11.9%). Ages ranged from 21 to 56. The mean age was 37. Mean age for females was 35.8. Mean age for males was 38.1. Design, Procedures, and Instruments. These data were collected in the first half of 1992, approximately one year after the end of the Persian Gulf War. At a regular monthly unit meeting, consenting subjects were administered the current revision of the Mississippi Scale for Combat-Related PTSD (Keane, Caddell and Taylor, 1988) and a questionnaire on demographics, substance abuse and effects on family. The validity and reliability of the Mississippi Scale have been established by Keane, Caddell and Taylor (1988). Subjects were invited to write additional comments about the impact of the war and were given an opportunity to volunteer to be interviewed.

The purpose and procedures of the study were described to subjects, and they were informed that they could withdraw from the study at any time prior to publication. They were also informed that participation or refusal to participate will not affect their military careers or subsequent benefits. All responses remained confidential. Data were identified by codes rather than names.

Mississippi Scale means of the three groups were analyzed by a one-way Analysis of Variance. The Scheffe Test of Multiple Comparisons was performed post hoc. Subjects' written comments, as well as responses regarding drug and alcohol use, were compiled and summarized but not statistically analyzed. The Semi-Structured Initial Interview for Desert Storm War-Zone Personnel (Litz, Knight, Wolfe, Kaloupeck, Quinn, Krinsley, Fisher, Weathers, & Keane, 1991) was administered to ten members of SA by one of the authors. The authors analyzed the data for common themes. Interviewees' descriptions of war-zone stressors are reported in the results section of the paper.

Table 1

Demographics of Interviewees (N=10)

Variable	Males (N=3)	Females (N=7)
Mean age	48.6	39.9
Age range	45-52	22-50
Marital status		
Married	3	4
Single	--	3

Military rank		
Officer	2	5
Enlisted	1	2
Education		
Doctorate	1	--
Masters	--	2
Bachelors	--	2
Some college	2	3
Race		
Caucasian	3	7

RESULTS: Analysis of Mississippi Scale Scores

A one-way Analysis of Variance revealed a significant difference in Mississippi Scale scores among the three groups ($p < .001$). Post hoc analyses revealed that the mean score of the unit deployed to Saudi Arabia (SA) ($M = 83.98$) was significantly higher than that of the unit deployed to England (ENG) ($M = 73.61$) and the unit deployed to Arizona (AZ) ($M = 73.61$), $F(2, 103) = 9.83$, $p < .001$. Also, ENG obtained significantly higher scores than AZ. Means, standard deviations and test results are presented in Tables 3, 4 and 5.

Table 3: Means and Standard Deviations

	Mean	SD	N
SA	83.97	19.58	43
ENG	73.6	15.88	38
AZ	66.00	11.43	25

Table 4: Analysis of Variance

Effect	SS	df	MS	F	p
VAR1	5453.91	2	2726.954	9.83	.001
Within	28570.06	103	277.379	--	--

Table 5: Post hoc Comparison: Scheffe Test: p-levels marginal means for indep. var.

	AZ	ENG	SA
mean=66.00 level code:	AZ X	0.178	0.00
mean=73.61 level code:	ENG 0.178	X	0.04
mean=83.98 level code:	SA 0.00	0.04	X

Six SA subjects (14%) scored greater than 107, the cutoff score for the diagnosis of PTSD. One of those subjects wrote on the questionnaire that he believed that his distress was caused by his Vietnam experience rather than his ODS experience. One subject in ENG scored over 107 (2.7%).

War-Zone Stressors of SA as Described by Interviewees. The unit arrived in Dhahran, Saudi Arabia, on January 4, 1991 and was evacuated on April 4, 1991. Initially billeted in Al Khobar, near the Dhahran Air Base, they were in Al Khobar when the SCUD missile hit. As medical personnel, they were not allowed to carry weapons according to the Geneva Convention, but they were required to pull guard duty both in Dhahran and in the field. They were responsible for setting up their own combat support hospital 10 miles north of the Iraqi border. The closest support hospital to the front lines, they were married with a helicopter unit and received 32% of the war's casualties from both Allied and Iraqi forces. Specific casualties were seen as especially stressful: The first female casualty, abandoned Iraqi children for whom they had no pediatric instruments, and a nurse and physician who were killed when the Claymore mines they had gathered as souvenirs exploded. Nine subjects feared for their lives. Seven said the SCUD missile landing was highly stressful. Two women found the casualties, particularly women and children, extremely stressful. Others said the casualties were not particularly stressful because they saw severe injuries in their daily work.

Noncombat War-Zone Stressors. All subjects said that they were anxious and/or bored waiting for war to begin. Worries included fear of dying, chemical warfare, the morality of the war, and welfare of family members at home or serving in Saudi Arabia. Six subjects mentioned cultural or environmental stressors. A Jewish man feared torture if taken prisoner. Two women said Saudi men touched them and/or made sexual comments. A woman feared "crazy" Saudi drivers. Two women and two men spoke of heat and the "endless" and "dark" desert. Other stressors were relationships with other unit members, distrust of leaders, poor sanitation, and physical labor. Deployment and Homecoming Stressors. Deployment stressors included anxiety about leaving family members and disruption of careers. Three women mentioned leaving children as the hardest thing they had ever done. One woman worried about her aging mother. Homecoming stressors included

physical problems and changes in relationships and careers. Three women said that readjustment was more difficult than they had anticipated. A man divorced after the war. A woman had to start over in school. One woman attributed symptoms of diarrhea, fatigue, and irritability to a "parasite". Another had carpal tunnel. All subjects denied exacerbation of previous traumas.

Coping Strategies. Subjects coped with stress by talking to friends, relying on religious faith, reading, calling or writing home, self-talk (e.g., "Calm down." "It will be all right."), playing cards, keeping busy/exercise/physical labor, shopping, joking, eating, writing a journal, remembering home, and romance.

Several subjects compared coping strategies of men and women. One woman said that women were supportive of one another and thus had fewer emotional problems than men. Another woman said women talked easily and could cry. She said, " Maybe it seems like you're hysterical, but then you feel better. You get hugged when you cry." She said women negotiated assignments if they didn't get along with tentmates; men accepted assignments they didn't like. She noted that both soldiers who were evacuated were men. One man stated that men "adjusted better" while women complained about "access to phones, the filth, and not getting mail".

Some interviewees compared coping strategies of younger soldiers and older soldiers. Three women in their forties and a 49 year old man stated that younger soldiers had more trouble coping than older ones. Older soldiers "laughed a lot, read a lot, played bridge, [and] didn't dwell on" troubles. The man and two of the women said the younger members engaged in sex to cope with stress. One woman noticed that younger soldiers who coped best kept active. Two women noted that some unit members drank alcohol to cope.

Lasting Psychological Impact of the War. Positive effects of the war included greater faith in self, lasting friends, pride in accomplishments, adventure, and a greater sense of purpose.

Only one subject approached full PTSD. She had a flashback while working in the emergency room and still reacted to loud noises. One man said he had not slept through the night since the war. One woman was emotionally numb. One man was avoidant.

Several interviewees thought that members most affected by the war had quit the Reserves. Several thought that people who had served in Vietnam were more distressed by ODS than other members. A nurse who served in both wars said that better facilities made Vietnam "easier"; "primitive" conditions made ODS very stressful.

Common Themes of Structured Interviews (N=10)

Stressor	Number of Subjects	Women	Men
Afraid of dying	10 (100%)	7	3
SCUD landing	7 (70%)	5	2
Seeing severe casualties	2 (20%)	2	0
Waiting for war to begin	10 (100%)	7	3
Cultural/Environmental factors	6 (60%)	3	3
Interpersonal relations	3 (30%)	1	2
Distrust of leaders	3 (30%)	2	1
Hard physical labor	2 (20%)	1	1
Poor sanitation	3 (30%)	3	1
Leaving children	3 (30%)	3	0
Leaving other family members	1 (10%)	1	0
Relationship and/or career disruption	5 (50%)	4	1
War-related physical problems	2 (20%)	2	0
Coping Strategies			
Talking to friends	6 (60%)	4	2
Religion	4 (40%)	3	1
Reading	4 (40%)	2	2
Calling/Writing home	3 (30%)	2	1
Exercise/Physical Labor	3 (30%)	2	1
Self-talk (e.g., "calm down", "I'm all right")	3 (30%)	3	0
Shopping	2 (20%)	1	1
Joking	2 (20%)	1	1
Eating	1 (20%)	1	1
Writing a journal	1 (10%)	1	0

Remembering home	1 (10%)	0	1
Romance	1 (10%)	0	1
Lasting Psychological Impact (At the time of the study)			
Positive effects	6 (60%)	5	1
Confidence/Pride/Purpose	4 (40%)	3	1
Lasting friends	3 (30%)	2	1
Adventure	2 (20%)	1	1
PTSD Symptoms	4 (40%)	2	2
Intrusive thoughts	1 (10%)	1	--
Startle response	1 (10%)	1	--
Sleep disturbance	1 (10%)	--	1
Emotional numbing	1 (10%)	1	--
Flashbacks	1 (10%)	1	--
Avoidance	1 (10%)	--	1

PUBLICATIONS: none to date

Title: Stress Symptoms and Their Causal Attribution in Desert Storm Veterans
Project #: VA-36 Agency: VA Study Location: VAMC Clarksburg
Project Status: Completed Research Type: Epidemiology Research
P.I.: Ralph E. van Atta, Ph.D. Research Focus: Brain & Nervous System
Start Date (CY):1995 Est. Completion (CY):1997

OVERALL PROJECT OBJECTIVE: Describe motivational aspects of stress in relationship to war experience.

SPECIFIC AIMS: Determine stress symptoms and their causal attribution in Desert Storm veterans.

METHODOLOGY: Survey Methodology: Life stress questionnaire, multiple regression analysis.

EXPECTED PRODUCTS (MILESTONES): Pilot study - may lead to further studies.

STATUS/RESULTS TO DATE: An inquiry was launched into the stress levels of a sample of Desert Storm veterans and the causes to which they attributed their stress levels. The instrument that we used to assess stress levels and the causes to which they were attributed is the life stress questionnaire (LSQ). This device assesses stress level (SL) as the total number of 52 psychiatric symptoms (expressed in layman's language) that are endorsed by the respondent. It assesses attributive causes of this stress level by means of ratings by the respondent of 14 domains of causation. For the purpose of this research, attributive ratings were requested for an additional domain of causation, "after effects of my military experience".

200 veterans on a VAMC Desert Storm registry were randomly selected as respondents.

Questionnaires were mailed to them. The follow-up process was complicated since many subjects had moved and left no forwarding address. When this occurred, new subjects were randomly drawn from the registry file. After 8 follow-ups over a 4-month period, 119 questionnaires (59.5 percent return rate) had been accumulated and data analysis was undertaken. These questionnaires were alternately assigned as they had been received to either a trial group (Group T) or a replication group (Group R). Stress symptoms endorsed by a majority of the subjects in both groups included muscular stiffness and pain, fatigue, restlessness, forgetfulness, and irritability. SL for Group T was 13.25 with an SD of 1.25. For Group R, SL was 15.84, SD 1.43. The leading attributive cause in both groups was After Effects of Military Experience (AEM). For the Trial Group, the mean for this item was 3.41 (on a 5-point scale); for the R Group, the mean for AEM was 3.73. Multiple regression analyses were run to determine the pattern of attributive causes first in the Group T and then in the Replication Group. This analysis yielded large and highly significant multiple Rs in both groups. Since Rs approach the range that is more typical of reliability coefficients than validity coefficients, it should be mentioned that past experiences with the LSQ suggest that the strength of the coefficients reflects the high consistency of the instrument and, perhaps, in this case, the motivation of the subjects and the meaningfulness of the assignment. In the Group T, $R=.94$, $p<.0001$ with the Social Conflict (SC). In the Group R, $R=.89$, $p<.0001$ with attributive causes including SC, SI, Health and Self, Health and Dependent, and Demands of School or Class work. The LSQ has been used in several other studies and it is of interest to remark that the stress levels reported by these Desert Storm veterans are higher than in any of our previous studies. It is also important to consider that although the predominant attribution of the subjects was to AEM, this variable appeared as a predictor only in the Group T. The study was exploratory in nature and therefore the size of our trial and replications groups was smaller than would generally be acceptable when multiple regression methodology is employed. The inductive nature the multiple regression may have resulted in the identification of unique features of either or both Groups. Whether the findings that characterize either group would generalize to a larger registry sample or to Desert Storm veterans as a group is unknown. Had we found more commonality between the predictors for Group T and those for Group R we might have ventured a hypothesis about attributive causation in Desert Storm veterans. But lacking such a commonality, we will point only to the appearance of Social Isolation and Social Conflict in the equations for both groups. Finally, it should be mentioned that the predominant or most popular attributive cause in both group was the AEM; the ratings of this variable by the Group R were even higher than for Group T. The failure of the AEM variable to survive in the multiple regression analysis may reflect the greater convergence of opinion of veterans about the impact of their military experience in their present lives. In variance may have resulted in exclusion of these variables in the statistical analysis.

PUBLICATIONS: none to date

Title: Musculoskeletal Symptoms in Gulf War Syndrome

Project #: VA-40

Agency: VA

Study Location: VAMC Long Beach

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Pamela E. Prete, M.D.

Research Focus: Symptoms/General Health

Start Date (CY):1994 Est. Completion (CY):1999

OVERALL PROJECT OBJECTIVE: The Gulf War Syndrome is most likely a heterologous group of disorders resulting from the interaction of each veteran's host defense against the variety of environmental, viral, bacterial and stress toxins unique to the Persian Gulf War. One of the more debilitating symptoms of the GWS has been the arthralgia and arthritis. These complaints are relatively common to foreign deployments. A question arises as to whether musculoskeletal complaints are indeed a manifestation unique to Gulf War Syndrome. The project will address a fundamental question about the Gulf War Syndrome: Do the musculoskeletal symptoms in the GWS reflect those of long foreign deployment or are there unique aspects of these symptoms that identify these symptoms as part of Gulf War Syndrome.

SPECIFIC AIMS: 1. Survey deployments for musculoskeletal symptoms via questionnaire; 2. Compare comparative peace-time vs. Gulf War deployments for musculoskeletal symptoms.

METHODOLOGY: Veterans of the Persian Gulf War with and without symptoms of the Gulf War Syndrome and healthy veterans of the same period who did not serve in the Persian Gulf but have been deployed for peaceful missions will receive a questionnaire which, if positive, will be followed by physician interview in the Department of Veterans Affairs Medical Center, Long Beach (VAMCLB) and March Air Force Base, California. Statistical analysis of the comparative features of those examinations will be evaluated for appearance of features that could be related to the other than foreign deployment, i.e., GWS.

EXPECTED PRODUCTS (MILESTONES): 1. Survey and range of musculoskeletal symptoms secondary to foreign deployments; 2. Comparison of symptoms between foreign deployments and those to GW theater; 3. Statistical analysis based on chi square and regression analysis of independent variables. Review of available data of deployed reservists/veterans with persistent arthritis/arthralgia complaints from GWS.

STATUS/RESULTS TO DATE: 200+ initial questionnaires; 25 comprehensive examinations. Analysis suspended due to insufficient resources.

PUBLICATIONS: none to date

Title: Diarrhea in Persian Gulf Veterans: An Irritable Bowel-Like Disorder
Project #: VA-46 Agency: VA Study Location: VAMC Gainesville
Project Status: Ongoing Research Type: Epidemiology Research
P.I.: Charles A. Sninsky, MD Research Focus: Symptoms/General Health
Start Date (CY): 1995 Est. Completion (CY): 1996

OVERALL PROJECT OBJECTIVE: To compare visceral sensation in normal subjects, patients with irritable bowel syndrome and patients with diarrhea that abruptly began while serving in the Persian Gulf.

SPECIFIC AIMS: a) To determine if Persian Gulf veterans with abdominal pain, diarrhea and bloating with a negative investigative work-up have an underlying visceral hypersensitivity or a generalized decreased pain threshold. b) To examine the heightened visceral sensitivity present in patients with irritable bowel syndrome and compare the data to those obtained for Persian Gulf veterans with diarrhea. c) To investigate whether an acute treatment with octreotide will increase the tolerance to balloon distention of the rectum and extremity immersion in ice water in Persian Gulf veterans, patients, with the irritable bowel syndrome, and controls. d) To determine the effect of octreotide on oral-cecal transit as measured by the lactulose hydrogen breath test.

METHODOLOGY: Study Design: Informed consent is being obtained prior to the start of the trial. A complete medical history and physical exam will be performed. Blood samples will be collected for a pregnancy test when appropriate. Participants will be asked to discontinue all medications for at least 24 hours before the study, to fast for 12 hours, and to take a Fleet's enema 2 hours before the test procedure. Both the ice water tolerance test and the balloon distention tolerance test will be performed in controls, patients with irritable bowel syndrome, and in Persian Gulf veterans both at baseline and after the administration of octreotide 100 mg subcutaneously. Oral-cecal Transit: The effect of octreotide on small bowel transit will be assessed by the hydrogen breath test. Lactulose (10 g) will serve as the carbohydrate substrate for the breath test. Breath hydrogen concentration is measured by gas chromatography every 15 minutes until peak hydrogen excretion occurs. Peak hydrogen excretion is defined as the highest hydrogen concentration before two successive lower concentrations. Peak hydrogen excretion is the point in time at which the lactulose substrate has reach the cecum. Two points on the hydrogen curve will be used to calculate small intestinal transit. The first is the time to an initial sustained increase of hydrogen concentration of $^{3}10$ ppm over baseline. The second point is the time to peak hydrogen excretion. Ice Water Tolerance: The ice water tolerance test is performed to determine if patients have a generalized nonspecific lowered pain threshold from a central pathway. All subjects will be asked to insert their right hand into a mixture of ice and water to the wrist point and to hold it there for as long as possible. The time before withdrawing the hand from the water will be measured with a stop watch for each subject. If the hand is not withdrawn before 4 min, the subject will be instructed to withdraw the hand and a value of 240s will be assigned. Balloon Distention Tolerance: The balloon distention test evaluates a specific visceral afferent pathway as a cause of symptoms in patients with diarrhea. After each subject withdraws their hand from the ice water, a 5-min recovery period is observed. A standard anorectal manometry catheter or barostat (modified manometry balloon that measures rectal compliance) will then be inserted into the rectum of a depth of 10-15 cm. In normal subjects, progressive rectal distention initially leads to the perception of pressure, followed by fecal urgency, and then pain. The subjects' response to progressive rectal distention will be quantitated as: 1) first rectal sensation of the balloon, 2) urge to defecate from balloon distention, 3) rectal discomfort from balloon distention and, 4) rectal pain from balloon distention. The rectal balloon will then be distended in a stepwise fashion by adding 20 ml of air every 3 minutes. Unless this represents the subject's maximum tolerable volume, additional 20-ml increments of air will be added at 3-min intervals up to a maximum of 300 ml. The maximum tolerable volume will be defined as the volume at which the subject requests termination of the procedure because of discomfort. If the subject does not report discomfort, a value of 200 ml will be assigned. After baseline measurements of ice water and balloon distention tolerance, all subjects are being brought back another day for octreotide testing. Octreotide 100 mg will be subcutaneously administered. Thirty minutes following the injection, the ice water and balloon distention tolerance will be repeated in exactly the same manner as above and compared to baseline values.

EXPECTED PRODUCTS (MILESTONES): Expected results are that the patients with Persian Gulf diarrhea, as well as irritable bowel syndrome, will have similar rectal sensation, as well as similar rectal urge to defecate, as well as similar discomfort relative to distention. Second, it is not expected that there should be a difference in ice water tolerance among either of the three groups.

STATUS/RESULTS TO DATE: At this time, 18 patients have been studied (4 normals, 5 irritable bowel syndrome patients, and 9 patients with Persian Gulf diarrhea). Patients who developed chronic diarrhea while in the Persian Gulf have a significantly lower threshold for symptoms of initial rectal sensation and the perception of discomfort with rectal balloon distension. This visceral hypersensitivity in Persian Gulf Veterans is similar to that observed in patients with Irritable Bowel Syndrome. The hydrogen breath test and octreotide protocols have not yet been completed. Additional testing was added to the protocol to include psychological testing. They include the State Anxiety Index, the Beck Depression Index, and the NEO. These non-invasive tests will be done by the subjects on a volunteer basis. The results will be compared among the three groups: Persian Gulf war veterans who had abrupt onset of chronic diarrhea; controls; and subjects with irritable bowel syndrome.

PUBLICATIONS: Gordon JM, Verne GN, Davis RH, Sninsky CA, Gainesville VAMC, Dept of Med Univ of FL, Gainesville, and Dept of Med, MUSC, Charleston SC. Is the diarrhea in Persian Gulf war veterans caused by an underlying visceral hypersensitivity? Biomedicine, May 1996.

Title: Retrospective Verification of Mustard Gas Exposure
Project #: VA-47 Agency: VA Study Location: VAMC Louisville
Project Status: Ongoing Research Type: Clinical Research
P.I.: Jerald L. Hoffman, Ph.D. Research Focus: Chemical Weapons
Start Date (CY): 1997 Est. Completion (CY): 2000 Diagnosis

OVERALL PROJECT OBJECTIVE: Determine if there is a correlation between mustard gas exposure and human reproductive and developmental problems.

SPECIFIC AIMS: 1. To establish a GC/MS method for analysis of the mustard gas adduct with the N-terminal valine of hemoglobin. 2. To analyze blood samples from Persian Gulf War veterans collected within eight months of potential exposure to chemical weapons. 3. To analyze blood samples from military personnel at the Bluegrass Army Depot collected during routine health surveillance. 4. To survey Persian Gulf War veterans and military personnel working with chemical agents for reproductive difficulties and for developmental problems in their children. 5. To search for a correlation of verified exposure to mustard gas with reproductive and developmental problems. 6. To examine cross-linked hemoglobin as a more sensitive marker of mustard gas exposure. 7. To further characterize the endogenous protection against mustard gas afforded by S-methylation by the human enzyme, thioether methyltransferase.

METHODOLOGY: 1) to establish a GC/MS method for analysis of the mustard gas adduct with the N-terminal valine of hemoglobin; 2) to analyze blood samples from Persian Gulf War veterans collected within eight months of potential exposure to chemical weapons; 3) to analyze blood samples from military personnel at the Bluegrass Army Depot collected during routine health surveillance; 4) to survey Persian Gulf War veterans and military personnel working with chemical agents for reproductive difficulties and for developmental problems in their children; and 5) to search for a correlation of verified exposure to mustard gas with reproductive and developmental problems.

EXPECTED PRODUCTS (MILESTONES): This research will be helpful in defining the role of mustard gas exposure in the Persian Gulf War 'syndrome' of which many VA patients complain.

STATUS/RESULTS TO DATE: Progress to date has involved the chemical synthesis of valine hemoglobin mustard adducts derivatized for gas chromatography/mass spectroscopic analysis (GC-MS). These include deuterium-labeled forms which will be used as internal standards for GC-MS analysis of hemoglobin samples. We are presently beginning to standardize the analysis of these reagents in the GC-MS.

A second approach to determining exposure to mustard gas has been initiated. Since mustard gas is bifunctional it has the potential to cross-link the various pairs of subunits of hemoglobin. Such cross-linked dimers could be detected by the appearance of a 32 kDa band (2x16 kDa subunits) in western blots of hemoglobin probed with anti-Hb antibody. This method potentially has much higher sensitivity than adduct analysis since chemiluminescent detection can be used.

We have also expanded the project to include studies of the endogenous protection against mustard gas provided by the enzyme, thioether methyltransferase. This enzyme methylates the S atom in thioethers including mustard gas analogs which renders them nonreactive as alkylating agents. We originally discovered this enzyme in mice, and cloned and sequenced the cDNA from that organism. Using the mouse cDNA as a probe the human thioether methyltransferase cDNA has been cloned from a liver library. Base sequence analysis revealed only one amino acid difference from the mouse. This indicates that our rabbit antiserum against the mouse enzyme can also be used for studies of the tissue distribution of human thioether methyltransferase by western blotting, and such experiments are in progress.

PUBLICATIONS: none to date

Title: Cross-Sensitization as a CNS Model for Gulf War Chemical Intolerance
Project #: VA-48 Agency: VA Study Location: VAMC Tucson
Project Status: Ongoing Research Type: Clinical Research
P.I.: Iris R. Bell, M.D. Research Focus: Environmental Toxicology
Start Date (CY): 1998 Est. Completion (CY): 2001 Brain & Nervous System Interactions

OVERALL PROJECT OBJECTIVE: To test the neural sensitization/cross-sensitization hypothesis for acquired chemical intolerance (ACI) in Persian Gulf veterans and appropriate controls and to determine individual differences in vulnerability to this process. Neural sensitization is the progressive amplification of a given response over the course of repeated, intermittent exposures to a particular stimulus. As many different classes of stimuli (e.g. chemicals, stress, drugs, microbial toxins) can initiate and elicit sensitized responding, cross-sensitization between multiple pre-War, War, and post-War factors could account for the clinical pictures in certain PGW veterans.

SPECIFIC AIMS: 1. Evaluate patterns of responsibility over time of four groups of veterans to repeated jet fuel or sham laboratory exposures, using blood pressure, heart rate, eye blink (spontaneous and acoustic-startle induced) reactions as outcome measures. The four groups will include three groups of PGW veterans i) ill with increased chemical intolerance attributed to military service; ii) ill without increased chemical intolerance; iii) healthy, without increased chemical intolerance; and an additional group of healthy Gulf War era veterans. 2. Assess for cross-sensitization between jet fuel or sham exposures and non-War related chemical exposures (i.e., perfume). 3. Compare veterans by groups and by degree of laboratory sensitization for premorbid individual difference characteristics (host and environment) that would enhance vulnerability to sensitization.

METHODOLOGY: The primary between-groups, six-session study of veterans (Ntotal = 200) will compare ill PGW veterans who report increases in acquired chemical intolerance since the Gulf War with three age-, sex-, and education-matched control groups (ill PGW veterans without increased ACI, healthy PGW veterans, healthy era veterans) for current baseline sensitization and laboratory-induced sensitizability and cross-sensitization of neurobehavioral responses (e.g., spontaneous eyeblink, acoustic startle, reaction times, vigilance task performance, blood pressure, heart rate) to chemical and nonchemical (e.g., noise) stimuli.

The second study will compare premorbid individual difference variables drawn from previous animal and human research that may contribute to susceptibility to neural sensitization in the four groups and in subjects who did versus those who did not show consistent objective evidence of physiological sensitization and cross-sensitization. Data analyses will utilize (1) repeated measure, multivariate analyses of variance and covariance and (2) discriminant analysis and logistic regression involving the individual difference measures. Women and Hispanics will be oversampled because of evidence of being over represented, respectively, on the national Persian Gulf War Registry and among those on the Tucson VAMC PGW Registry reporting wartime environmental contaminant exposures.

EXPECTED PRODUCTS (MILESTONES): Completion of 50 participants between 4/98 to 3/99; 90 participants between 4/99 to 3/00; 60 participants and final data analysis, report, and publications in 4/00 to 3/01. Data from this sensitization/cross-sensitization proposal will provide an empirical approach to testing for objective abnormalities in PGW veterans not seen in studies designed from a classical toxicology perspective using similar substances. This work could point to a multifactorial pathway by which sensitization-related disorders initiated by chemicals, stress, drugs, microbial toxins, or a combination of these factors during the Gulf War might contribute to the longitudinal development of nonspecific somatic complaints as well as of certain medical conditions common in veterans, notably hypertension and its vascular sequelae. Findings will provide a rationale for systematic pharmacological interventions.

STATUS/RESULTS TO DATE: Ongoing.

PUBLICATIONS: none to date

Title: Sensitivity to Pyridostigmine Bromide: Persistent Neural Dysfunction
Project #: VA-49 Agency: VA Study Location: VAMC East Orange
Project Status: Ongoing Research Type: Basic Research
P.I.: Richard J. Servatius, Research Focus: Pyridostigmine Bromide
Start Date (CY): 1998 Est. Completion (CY): 2002 Brain & Nervous System

OVERALL PROJECT OBJECTIVE: During the Persian Gulf War, troops were given access to the experimental agent pyridostigmine bromide as a prophylactic against nerve gas exposure. Usage of pyridostigmine bromide was recommended based on two assumptions: 1) prophylactic levels of pyridostigmine bromide did not alter central nervous system activity and 2) its peripheral side effects were transient. Our recent research questions these two assumptions. Rats given PB exhibit a persistently exaggerated startle response which has a delayed appearance. These findings are without precedent.

SPECIFIC AIMS: STUDY A will determine the pharmacokinetics of PB in SD and WKY rats. We will determine if there are strain differences in the rate of clearance from portal and jugular vein administration of C14-labeled PB. We will also determine if there are strain differences in the degree and duration of inhibition of BuChE activity.

STUDY B will compare the effects of two cholinesterase inhibitors, edrophonium (EDRO) and neostigmine (NEO) to PB on measures of erythrocyte (E-) cholinesterase activity, BuChE activity, and startle responding. Unlike NEO and PB, EDRO does not affect BuChE. We will determine: a) whether the effects of PB are mediated through BuChE activity, and b) whether the appearance of delayed-onset startle sensitization is specifically related to PB.

STUDY C will provide evidence that persistent startle sensitization after exposure to prophylactic levels of PB in rats with abnormal BuChE activity is mediated by central, as opposed to peripheral, nervous system dysfunction. To determine whether central cholinergic activity mediates the delayed-onset startle sensitization observed after prophylactic PB treatment in WKY rats, we will perform two similar experiments. In Experiment 1, we will address muscarinic receptor mediated cholinergic activity. WKY rats will be given PB, PB and atropine (central and peripheral activity), PB and methylatropine (peripheral activity), the antagonists alone, or tap water. In Experiment 2, we will address nicotinic neurotransmission. WKY rats will be given PB, PB and mecamylamine (MEC, centrally active), PB and hexamethonium (HEX, peripherally active), the antagonists alone, or tap water. For both experiments, startle responses will be measured 1, 8, and 15 days after the end of treatment. Blood samples for BuChE determination will be obtained the day before PB treatment, 4 days after the onset of PB treatment, and the day after the end of PB treatment. In Experiment 3, SD and WKY rats will be given PB treatment or water for 7 consecutive days. Rats will be sacrificed either on the last day of treatment or 8 days after the end of treatment. We will determine AChE activity in the cervical spinal chord, hippocampus, cortex, and brain stem, as well as blood BuChE activity.

STUDY D will determine how long enhanced startle responsivity lasts after appearance and the nature of the enhanced responsivity in rats with abnormal BuChE activity. In Experiment 1, WKY and SD rats will be treated with either PB or tap water for 7 consecutive days. The acoustic startle response will be measured beginning 1 day after the end of treatment and continuing every week until normal startle amplitudes are detected in two consecutive weeks. Separate groups of SD and WKY rats will be tested only on the 15th day after the end of treatment to determine whether enhanced responsivity is related to repeated testing. In Experiment 2 WKY and SD rats will be treated as in Experiment 1 except that the single-intensity protocol will be used for behavioral testing. In both experiments blood samples for BuChE determination will be obtained the day before PB treatment, 4 days after the onset of PB treatment, and the day after the end of PB treatment.

STUDY E will attempt to produce delayed-onset startle sensitization in rats with otherwise normal BuChE activity. In Experiment 1, half of each strain, WKY and SD rats, will be given a single session of tailshock stress. Following stress, rats will be given either 7 consecutive days of PB or tap water and vehicle. Acoustic startle responses will be measured 1 day after the end of PB treatment and every week thereafter for 4 weeks.

STUDY F will directly evaluate the scavenger hypothesis as a possible mechanism for the persistent startle sensitization in WKY rats. In Experiment 1, WKY rats will be given 7 consecutive days of

either PB, PB with FSB AChE, FSB AChE or tap water. Startle responses will be measured 1, 8, and 15 days after the end of treatment. Status: This project is slated to start in 4/98.

METHODOLOGY: see above.

EXPECTED PRODUCTS (MILESTONES): Studies A and B will be completed in Year 1, Studies C in Year 2, Studies D and E in Year 3, and Study F in Year 4.

STATUS/RESULTS TO DATE: Ongoing.

Title: Neuropsychological findings in a sample of Operation Desert Storm veterans
Project #: VA-50 Agency: VA Study Location: VAMC Detroit
Project Status: Completed Research Type: Clinical Research
P.I.: Bradley Axelrod, Ph.D. Research Focus: Brain & Nervous System
Start Date (CY): Est. Completion (CY): 1997

OVERALL PROJECT OBJECTIVE: Objectively evaluate the subjective reports of changes in the cognitive functioning of returning Gulf War veterans.

METHODOLOGY: Volunteers for a single Army reserve unit were given a comprehensive neuropsychological and psychological evaluation using clinical tools according to each measures' standardized instructions. In response to ongoing complaints of memory, attention, and problem-solving difficulties among veterans of Operation Desert Storm and Shield (ODSS), a sample of 44 male veterans of ODSS underwent a comprehensive neuropsychological evaluation.

EXPECTED PRODUCTS (MILESTONES):

STATUS/RESULTS TO DATE: Deficits relative to normative data were observed only on finger dexterity (Grooved Pegboard, bilaterally) and the Stroop Color and Word Test. Those with impaired Pegboard performance had lower performance on other tasks requiring psychomotor speed. Those with impaired Stroop had significantly lower motor and set-shifting performance. Scores of both impaired groups were higher on many clinical and supplemental scales of the MMPI. Despite subjective cognitive complaints reported in 39% of the overall sample, veterans with cognitive complaints differed from their peers primarily in greater psychological distress as depicted on the MMPI. The data represented as preliminary clinical findings. This evaluation was exploratory in nature and two major weaknesses included the small sample size and that the veterans were volunteers. The data should not be misinterpreted as generalizable to all ODSS veterans. The most difficult aspect of interpreting the neuropsychological and psychological data relates to lack of other external data, including possible causes of the symptoms. The prospective study with objective neuropsychological data of individuals who have had a known exposure to specific toxins is an important next step in clinical research.

PUBLICATIONS: Axelrod BA, Milner IB. Neuropsychological findings in a sample of Operation Desert Storm veterans. *J of Neuropsychiatry and Clinical Neurosciences*, 1997, 9:23-28.

Title: Psychobiological Assessment of Desert Storm Veterans

Project #: VA-51

Agency: VA

Study Location: VAMC Cincinnati

Project Status: Ongoing

Research Type: Clinical Research

P.I.: Dewleen Baker, M.D.

Research Focus: Brain & Nervous System

Start Date (CY): 1992 Est. Completion (CY): 2002

Symptoms/General Health

OVERALL PROJECT OBJECTIVE: While prior studies show that combat veterans with posttraumatic stress disorder (PTSD) report more physical symptoms than veterans without PTSD, the link between PTSD and somatic complaints in Persian Gulf War veterans (PGWVs) is yet to be evaluated.

SPECIFIC AIMS: We examine the relationship of PTSD and somatic complaints in PGWVs, specifically to determine if PGWVs exhibit PTSD effects similar to those observed in veterans of other conflicts and to delineate the specific somatic complaints accompanying the PTSD symptoms in the Gulf War group.

METHODOLOGY: A questionnaire booklet was completed by 188 PGWVs, of whom half were patients in a veterans health screening clinic and half were non-treatment-seeking volunteers on active duty. The booklet included the Combat Exposure Scale, the Mississippi Post-Traumatic Stress Disorder Scale (MPTSD), and a subjective symptom-based health questionnaire.

EXPECTED PRODUCTS (MILESTONES):

STATUS/RESULTS TO DATE: The 24 PGWVs (12.8%) with PTSD (MPTSD score >116) reported more combat exposure ($P=.02$) and a greater number of physical symptoms ($P=.001$) than other PGWVs. Fatigue, nausea, muscle aches, dizziness, back pain, stomach ache, and numbness were much more likely to be reported by those with PTSD (MPTSD score >116) than by those without PTSD (MPTSD score <95). Physicians examining PGWVs should be alert to the possibility of PTSD in this group and that those with PTSD are more likely to report physical symptoms that may overlap with those in Persian Gulf syndrome. Consequently, mental health screening is essential, since for those veterans with PTSD diagnosis of other coexisting conditions may be confounded and early effective treatment of their PTSD may be delayed. Also, given the increased reporting of certain symptoms by those with PTSD, those seeking the cause of Persian Gulf syndrome should control for PTSD when determining the symptom cluster that may constitute this condition.

PUBLICATIONS: Baker DG, Mendenhall CL, Simbartl LA, Magan LK, Steinberg JL. Relationship between posttraumatic stress disorder and self-reported physical symptoms in Persian Gulf war veterans. Arch Intern Med, 1997, 157:2076-78.

Kellner M, Baker DG, Yehuda R. Salivary Cortisol in Operation Desert Storm Returnees. Bio Psych, 1997, 849-850.

Title: Spouses and Children Program

Project #: VA-53

Agency: VA

Study Location: VAMC Denver

Project Status: Ongoing

Under Public Law 103-446, Section 107 the VA was authorized to provide medical examinations to any individual; who:

a. Is the spouse or child of a veteran, is listed in the Persian Gulf War Veterans Registry established under Public Law 102-585, Section 702; and is suffering from illness or disorder.

b. Is suffering from, or may have suffered from, an illness or disorder (including a birth defect, miscarriage, or stillbirth) which cannot be disassociated from the veteran's service in the Southwest Asia theater of operations.

c. Has granted VA permission to include in the Registry relevant medical data from the evaluation.

Under this program, a eligible Gulf War veterans would receive examinations at the local VA Medical Center, and the examinations of spouses and children would be conducted under contract at the affiliated university medical center. This program will continue until September 30, 1998 or a maximum of \$2.0 m has been expended.

PUBLICATIONS: none to date

Title: VA/DoD Multi-site treatment trial for Chronic Fatigue Syndrome and Fibromyalgia in Gulf War Veterans

Project #: VA/DoD-1D* Agency: DoD

Study Location:

Project Status: Ongoing

Research Type: Clinical Research

Start Date (CY): 1998

Research Focus: Symptoms/General Health

Est. Completion (CY): 1999

Treatment

*Same as Project VA/DoD-1V

This Project is currently being planned as a part of the VA Cooperative Studies Program.

PUBLICATIONS: none to date

Title: VA/DoD Multi-site treatment trial for Chronic Fatigue Syndrome and Fibromyalgia in Gulf War Veterans

Project #: VA/DoD-1V* Agency: VA

Study Location:

Project Status: Ongoing

Research Type: Clinical Research

Start Date (CY): 1998

Research Focus: Symptoms/General Health

Est. Completion (CY): 1999

Treatment

*Same as Project VA/DoD-1D

This Project is currently being planned as a part of the VA Cooperative Studies Program.

PUBLICATIONS: none to date

Title: Follow-Up investigation of troops exposed to nerve agents at Aberdeen Proving Ground
Project #: VA/DoD-2DA* **Agency:** DoD **Study Location:** Aberdeen Proving Ground
Project Status: Ongoing **Research Type:** Epidemiology Research
P.I.: William Page, Ph.D. **Research Focus:** Chemical Weapons
Start Date (CY):1996 **Est. Completion (CY):**1999

*Same as Project VA/DoD-2VA

OVERALL PROJECT OBJECTIVE: Between 1955 and 1975, the U.S. Army enrolled 6,720 soldiers in an experimental exposure program of chemical warfare and other agents at the Edgewood Arsenal, Maryland. In 1980 the Army asked the National Research Council (NRC) to study the possible long term health effects of these exposures. A three-volume report was issued, the last volume dealing with the current health status of test subjects, including 1,581 men exposed to anticholinesterase compounds such as GA (tabun), GB (sarin), GD (soman), GF, and VX. The report indicated that "the limited information available from the follow-up on these soldiers does not permit definitive conclusions regarding the nature and extent of possible long-term problems resulting from chemical exposure at Edgewood." A pilot study began to determine whether follow-up of these test subjects is feasible and whether it would provide useful information.

Taking the suggestion of the NRC committee, we propose to examine the Edgewood subjects who were exposed to anticholinesterase agents for the more common OP exposure-associated outcomes; neurological deficits, particularly peripheral nerve disease, and neuropsychological impairment, including sleep disorders, anxiety, and depression.

SPECIFIC AIMS:

METHODOLOGY: The Edgewood subjects who were unexposed to chemical agents will serve as the first control group. However, this is not an ideal control group because the original Edgewood protocol did not include random assignment of subjects to treatment and control groups. In particular, it is thought that healthier men were more likely to have been assigned to chemical exposure groups and less healthy men to the control group. To counteract the effect of this putative assignment bias, a second control group consisting of men who were exposed to chemical agents other than anticholinesterases will also be included in the follow-up.

We are proposing a two-phase study that will first screen by telephone questionnaire all exposed subjects and controls for neurological deficit and neuropsychological impairment, including sleep disorders, anxiety, and depression. In the second phase, all individuals and controls who tested positive on any of the neurological deficit or neuropsychological impairment scales, as well as a sample of those who tested negative in each group, will be called in for a clinical examination. The screening would make use of existing data collection instruments, such as the Neuropsychological Impairment Scale.

EXPECTED PRODUCTS (MILESTONES):

STATUS/RESULTS TO DATE: MFUA has discussed the results of this pilot study with the Neurology Dept and the Washington DC VA Medical Center and the Minneapolis MN VA Medical Center. They have agreed to work together if a full-scale study is funded. MFUA will also consult with the staff of the Board on Environmental Studies and Toxicology, National Research Council, with regard to toxicological matters.

PUBLICATIONS: none to date

Title: Follow-Up investigation of troops exposed to nerve agents at Aberdeen Proving Ground
Project #: VA/DoD-2VA* Agency: VA Study Location:
Project Status: Ongoing Research Type: Epidemiology Research
P.I.: William Page, Ph.D. Research Focus: Chemical Weapons
Start Date (CY):1996 Est. Completion (CY):1999

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We are proposing a two-phase study that will first screen by telephone questionnaire all exposed subjects and controls for neurological deficit and neuropsychological impairment, including sleep disorders, anxiety, and depression. In the second phase, all individuals and controls who tested positive on any of the neurological deficit or neuropsychological impairment scales, as well as a sample of those who tested negative in each group, will be called in for a clinical examination. The screening would make use of existing data collection instruments, such as the Neuropsychological Impairment Scale.

EXPECTED PRODUCTS (MILESTONES):

STATUS/RESULTS TO DATE: MFUA has discussed the results of this pilot study with the Neurology Dept and the Washington DC VA Medical Center and the Minneapolis MN VA Medical Center. They have agreed to work together if a full-scale study is funded. MFUA will also consult with the staff of the Board on Environmental Studies and Toxicology, National Research Council, with regard to toxicological matters.

PUBLICATIONS: none to date

Title: Patterns of Pre-Persian Gulf War Illness and Health Care Seeking
Project #: VA/DoD-2DB* Agency: DoD Study Location:
Project Status: Ongoing Research Type: Epidemiology Research
P.I.: Richard Miller, M.D. Research Focus: Symptoms/General Health
Start Date (CY):1996 Est. Completion (CY):1998

*Same as Project VA/DoD-2VB

OVERALL PROJECT OBJECTIVE: To answer the question whether there are patterns of illness and the patterns of use of the Department of Defense (DoD) outpatient care during the year prior to deployment to the Persian Gulf War (PGW) in personnel who subsequently developed poorly defined illnesses different from the patterns of controls?

METHODOLOGY: Conducting extensive searches of relevant literature and researching various methods of obtaining medical records; Data abstraction and categorization.

STATUS/RESULTS TO DATE: Approvals have been obtained from the NRC Governing Board and from the NRC Institutional Review Board. Permission to access records has been received from: VA BIRLS, the Army Personnel Center, and the Army Surgeon General's Office (SGO). We have developed a short study protocol, produced case definitions, and created an algorithm for identifying cases from the PGHR and the CCEP as well as for controls. A database containing a record of all cases and controls has been created in Paradox and SAS. An algorithm for acquiring medical records has also been developed. A second request for claims folders has also been mailed. A registry match of records to determine those that are located at the National Personnel Records Center (NPRC) has been completed.

We have obtained over 1,100 medical records and have begun the abstracting process.

It is predicted that the pilot will be completed and the data analyzed by the end of the year. No plan for a full study can be constructed until the completeness of record ascertainment by PGHR or CCEP/control status is known.

PUBLICATIONS: none to date

Title: Patterns of Pre-Persian Gulf War Illness and Health Care Seeking
Project #: VA/DoD-2VB* Agency: VA Study Location:
Project Status: Ongoing Research Type: Epidemiology Research
P.I.: Richard Miller, M.D. Research Focus: Symptoms/General Health
Start Date (CY):1996 Est. Completion (CY):1998

*Same as Project VA/DoD-2DB

OVERALL PROJECT OBJECTIVE: To answer the question whether there are patterns of illness and the patterns of use of the Department of Defense (DoD) outpatient care during the year prior to deployment to the Persian Gulf War (PGW) in personnel who subsequently developed poorly defined illnesses different from the patterns of controls?

SPECIFIC AIMS:

METHODOLOGY: Conducting extensive searches of relevant literature and researching various methods of obtaining medical records; Data abstraction and categorization.

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It is predicted that the pilot will be completed and the data analyzed by the end of the year. No plan for a full study can be constructed until the completeness of record ascertainment by PGHR or CCEP/control status is known.

PUBLICATIONS: none to date

Title: VA/DoD Core funding of the Medical Follow-up Agency

Project #: VA/DoD-2D* Agency: DoD

Project Status: Ongoing

P.I.: Richard Miller, M.D.

*Same as Project VA/DoD-2V

This is the parent program for VA/DoD-2DA&2VA and VA/DoD-2DB&2VB.

This Program is a joint program funded by VA and DoD to provide the Medical Follow-up Agency with core funding to maintain its operational capabilities to perform epidemiological research on veterans. The MFUA maintains a number of important veterans databases. VA and DoD consider this Program a vital adjunct to its Gulf War programs. It provides VA and DoD with a vehicle by which outside expert epidemiological skills can be quickly brought to bear to conduct small scale pilot projects to test the feasibility of new ideas, and to provide recommendations and proposals to VA and DoD for the conduct of more extensive projects. This Program was authorized under P.L. 102-585, and initially served to fund the IOM/MFUA study panel "The Health Consequences of Military Service during the Persian Gulf War", which produced its final report in September 1996.

PUBLICATIONS: none to date

Title: VA/DoD Core funding of the Medical Follow-up Agency

Project #: VA/DoD-2V* Agency: VA

Project Status: Ongoing

P.I.: Richard Miller, M.D.

*Same as Project VA/DoD-2D

This is the parent program for VA/DoD-2DA&2VA and VA/DoD-2DB&2VB.

This Program is a joint program funded by VA and DoD to provide the Medical Follow-up Agency with core funding to maintain its operational capabilities to perform epidemiological research on veterans. The MFUA maintains a number of important veterans databases. VA and DoD consider this Program a vital adjunct to its Gulf War programs. It provides VA and DoD with a vehicle by which outside expert epidemiological skills can be quickly brought to bear to conduct small scale pilot projects to test the feasibility of new ideas, and to provide recommendations and proposals to VA and DoD for the conduct of more extensive projects. This Program was authorized under P.L. 102-585, and initially served to fund the IOM/MFUA study panel "The Health Consequences of Military Service during the Persian Gulf War", which produced its final report in September 1996.

PUBLICATIONS: none to date