

UNCLASSIFIED

AD NUMBER: AD0261618

LIMITATION CHANGES

TO:

Approved for public release; distribution is unlimited.

FROM:

Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational Use; 30 Jun 1961. Other requests shall be referred to the Walter Reed Army Institute of Research, 503 Robert Grant Avenue, Washington, DC 20307-5100.

AUTHORITY

USAMRDC LTR, 19 JUL 1976

THIS REPORT HAS BEEN DELIMITED
AND CLEARED FOR PUBLIC RELEASE
UNDER DOD DIRECTIVE 5200.20 AND
NO RESTRICTIONS ARE IMPOSED UPON
ITS USE AND DISCLOSURE.

DISTRIBUTION STATEMENT A

APPROVED FOR PUBLIC RELEASE;
DISTRIBUTION UNLIMITED.

UNCLASSIFIED

AD **261 618**

*Reproduced
by the*

ARMED SERVICES TECHNICAL INFORMATION AGENCY
ARLINGTON HALL STATION
ARLINGTON 12, VIRGINIA



UNCLASSIFIED

NOTICE: When government or other drawings, specifications or other data are used for any purpose other than in connection with a definitely related government procurement operation, the U. S. Government thereby incurs no responsibility, nor any obligation whatsoever; and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data is not to be regarded by implication or otherwise as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use or sell any patented invention that may in any way be related thereto.

Reference. R. J.

WALTER REED ARMY INSTITUTE OF RESEARCH

WALTER REED ARMY MEDICAL CENTER

WASHINGTON 12, D.C.

CATALOGED BY ASTMA 261618
AS AD NO. _____

FOR REFERENCE AT ASTIA HEADQUARTERS
ONLY. THIS REPORT CANNOT BE SATIS-
FACTORILY REPRODUCED; ASTIA DOES NOT
FURNISH COPIES.

ANNUAL PROGRESS REPORT



Reports Control Symbol MEDDH -288

1 July 1960 - 30 June 1961



Volume I

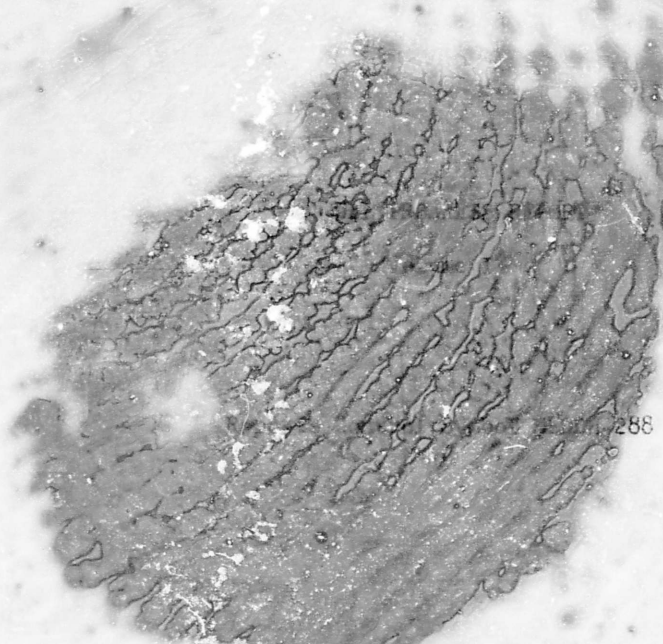
926600

Qualified requestors may obtain copies of this report from ASTIA

WALTER REED ARMY INSTITUTE OF RESEARCH

Walter Reed Army Medical Center

Washington 12, D. C.



Qualified requestors may obtain copies of this report from ASTIA.

TABLE OF CONTENTS

Volume I

Page

✓ 6X59-01-001 Traumatic Surgery and Shock	
1. Etiologic Factors in Wound Shock	1
2. Infections and Antibiotics	3
3. Role of Bacteria and Endotoxins in Shock	7
4. Gas Gangrene	12
17. Acute Renal Injury and Failure	19
23. Organ Repair and Replacement	29
24. Experimental Orthopedics	40
27. Resistance to Shock	46
33. Experimental Anesthesia	53
34. Nursing Measures for Oral and Nasal Hygiene	59
35. Intracapillary Thrombi in the Etiology of Shock, Renal Failure, and Other Conditions	64
✓ 6X59-05-001 Blood, Blood Derivatives and Artificial Expanders	
1. Immunohematology	73
8. Revision of Field Transfusion Service	80
9. Functions and Disorders of the Spleen	83
12. The Bone Marrow's Function and Its Reaction to Injury	87
13. Blood and Blood Disorders	90
✓ 6X59-06-001 Radiation and Thermal Burns	
4. Immune Mechanisms in Thermal Burns	102
✓ 6X60-01-001 Internal Medicine	
4. Experimental Arterial and Heart Disease	110
22. Instrument Development for Cardiac Research	121
27. Effects of Physical Agents on Skin and Its Permeability	124
28. Capillary and Lymphatic Circulation of the Skin	127
30. Identification of the Principles of Nursing Care Underlying Taking Oral Temperatures	132
31. Gastrointestinal Physiology	141
32. Influence of Bed Bath Procedures on Skin Conditions	145
✓ 6X60-09-001 Metabolism	
1. Pressor Substances	150
2. Bio-Assay of Biologically Active Substances by Tissue Culture	155
6. Metabolic and Nutritional Problems Associated with Injury	161

~~6X60-09-001~~ Metabolism (continued)

8. Interrelationships of Parathyroid Gland Activity and Calcium and Phosphorus Metabolism	177
14. Wound Healing	182
15. Metabolic Effects of Radiation and Combined Radiation and Traumatic Injury	189
19. Physiology of Cell Growth and Regeneration	195
23. Methods for the Chemical and Radiochemical Analysis of Foods	205
24. Investigation and Analytical Determination of Drugs and Compounds of Toxicological Importance	211

~~6X60-10-001~~ Neuropsychiatry and Stress

1. Analysis of Therapeutic Methods	217
3. Psychological, Physiological, Metabolic, and Endocrinological Homeostatic Mechanisms in Health and Disease	226
4. Anatomical and Physiological Substrata of Behavior	240
5. Electrophysiological Studies of the Nervous System	245
6. Measurement and Analysis of Psychological Mechanisms and of Their Modification by Drugs, Sleep Deprivation, Brain Injury, and Other Agents	260
7. The Blood-brain Barrier and the Responses of Cerebral Tissues to Injury	270

Volume II

~~6X61-03-001~~ Communicable Diseases

1. Arthropod-borne Infections	273
3. Rickettsial Diseases	294
7. Mycotic Diseases	309
8. Protozoan Diseases	320
10. Acute Gastroenteritis	325
11. Enteric Virus Infections	333
13. Metabolic Patterns of Pathogenic Bacteria	340
14. Schistosomiasis and Other Parasitic Diseases	344
15. Serodiagnosis of Parasitic Diseases	363
17. Significance of Pleuropneumonia-like Organisms in Human Disease	376
19. Development and Maintenance of Specific Pathogen-free Animal Colonies	383
22. Laboratory Diagnostic Procedures for Microbial Diseases	392
24. Characterization of Leptospire	413
25. Leptospirosis	421

6X61-03-001 Communicable Diseases (continued)

28. Growth and Metabolic Activity of Viral and Rickettsial Agents	427
29. Plague	431
30. Virus Diseases of Animals Transmissible to Man	442
31. Bacterial Infections	447
32. New Drugs and Antibiotics in the Treatment and Control of Communicable Diseases	454
33. Mode of Action of Antimicrobial Agents	463
✓ <u>6X61-04-001</u> Acute Respiratory Diseases	
1. Acute Virus Infections of the Respiratory Tract	470
✓ <u>6X61-08-001</u> Germfree Animal Studies	480

Volume III

✓ <u>6X61-09-001</u> Studies in Immunization	
1. Multiple Jet Injection Apparatus for Mass Immunization	495
2. Immunization Against Smallpox and Other Viral Diseases	499
3. Mechanism, Pattern, and Specificity of the Immune Response	508
4. Comparison and Evaluation of Domestic and Foreign Vaccines	524
5. Responses to Immunization	532
6. Pathogenesis and Immunity in Typhoid Fever	538
7. Pathogenesis and Immunity in Enteric Diseases	548
10. Action of Bacterial Viruses on Antigenic and Metabolic Characteristics of Enterobacteriaceae	558
11. Pathogenesis and Immunity in Cholera	565
12. Development and Modification of Biological Products	575
13. Characterization, Identification, and Purification of Antigens and Antibodies	590
15. Mechanisms of Immune Hemolysis and Anaphylaxis	594
16. Rift Valley Fever--Immunization of Man	602
✓ <u>6X61-13-001</u> Ecology and Control of Disease Vectors and Reservoirs	
2. Arthropods of Medical Importance in Overseas Areas	610
7. Diagnosis of Canine Filariasis	618

✓ 6X63-01-001 Oral Diseases

3.	Dental Caries	623
4.	Effect of Irradiation on Oral Tissues	634
6.	Periodontal Disease	639
9.	Clinical Practice	643
12.	Nursing Therapy and Procedures in the Treatment of Induced Oral Lesions and Manifestations	658
13.	Epidemiology of Oral Disease	663
14.	Biologic Effects of the Use of High Speed Rotary Instruments on the Hard and Soft Tissues of the Mouth	669
15.	Experimental Pathology as Related to Oral Tissues and the Jaws	682
16.	Natural History of Neoplastic and Non-neoplastic Lesions of the Oral Cavity and Jaws	698

✓ 6X64-14-001 Biological and Medical Aspects of Ionizing
Radiation

1.	Metabolism in Radiation Injury	721
3.	Clinical Use of Radioisotopes	732
10.	Biological Dosimetry	736
12.	Effects of Whole Body Radiation in Man	741
14.	Immunological Response Following Total Body Radiation	746
15.	Methods for Casualty Assessment in Nuclear Warfare	755
18.	Whole Body Counting Facility	759
19.	Effect of X-irradiation on Animals Maintained in a Germfree Environment	766
21.	Nursing Measures Which Contribute to Development, Prevention, Care and Treatment of Pressure Areas and Decubiti	769
22.	Chemical Protection Against Total Body Radiation	775
24.	Mechanisms of Protection and Recovery in Cellular Radiation Injury	790

	Distribution	797
--	--------------	-----

ANNUAL PROGRESS REPORT

Project 6X59-01-001, Traumatic Surgery and Shock

Task 1, Etiologic Factors in Wound Shock

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Department of Bacteriology
Division of Communicable Disease
and Immunology

Period Covered by Report: 1 July 1960 through 30 June 1961

Principal Investigator: Howard E. Noyes, Ph.D.

Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

ABSTRACT

Project 6X59-01-001

Title: Traumatic Surgery and Shock

Task No. 1

Title: Etiologic Factors in Wound Shock

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Period Covered by Report: 1 July 1960 through 30 June 1961

Author: Howard E. Noyes, Ph.D.

Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

Death of the "Edgewood Goat" has been related to clostridia in the wound. In the future these studies will be reported under Task No. 4 - Gas Gangrene.

ANNUAL PROGRESS REPORT

Project 6X59-01-001, Traumatic Surgery and Shock

Task No. 2, Infections and Antibiotics

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Department of Bacteriology
Division of Communicable Disease
and Immunology

and

Department of Experimental Surgery
Division of Surgery

Period Covered by Report: 1 July 1960 through 30 June 1961

Principal Investigator: Howard E. Noyes, Ph.D.

Assistants: Jimmy R. Evans, M.S.
Capt Austin D. Potenza, MC
Ruth G. Wittler, Ph.D.
SP4 Alfred A. Serritella, B.S.

Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

ABSTRACT

Project 6X59-01-001

Title: Traumatic Surgery and Shock

Task No. 2

Title: Infections and Antibiotics

Reporting Installation:

Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Period Covered by Report: 1 July 1960 through 30 June 1961

Authors:

Howard E. Noyes, Ph.D.
Jimmy R. Evans, M.S.
Capt Austin D. Potenza, MC
Ruth G. Wittler, Ph.D.
SP4 Alfred A. Serritella

Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

1. Acute sub-lethal total body X-irradiation increased susceptibility of rats to experimental peritonitis. This increased susceptibility was not decreased by a single administration of penicillin, chloramphenicol, or oxytetracycline. Chronic total body X-irradiation did not increase susceptibility to this infection.

2. Investigation of non-surgical management of a grossly contaminated, standard wound in the rabbit has been continued. Topical therapy has been emphasized. Aerosol preparations of penicillin, two different mixtures of peptide antibiotics and non-antibiotic substances such as iodine are currently being evaluated.

3. A patient with chronic osteomyelitis due to Staphylococcus aureus phage type 80/81 was treated by intramuscular administration of 6 gms/day of dimethoxyphenyl penicillin for 24 days. During this period an original nonunion of the radius began to heal. Blood taken during antibiotic therapy yielded transitional forms of a bacterium which reverted to a nontypable S. aureus. A second blood culture yielded transitional cell forms which did not revert to a bacterial form. Forty-three conventional cultures of wound exudate yielded cultures of S. aureus 80/81 all of them sensitive only to chloramphenicol and dimethoxyphenyl penicillin.

BODY OF REPORT

Project 6X59-01-001

Title: Traumatic Surgery and Shock

Task No. 2

Title: Infections and Antibiotics

Description: The object of this task is evaluation of therapeutic regimens in wound infections. Experimental models include peritonitis in the rat and a standardized soft tissue wound in rabbits. When laboratory evaluation of a new drug is promising, clinical studies are carried out. This report covers the use of antibiotics in the management of endogenous peritonitis in the rat, evaluation of topical antibiotics in the therapy of soft tissue wounds and the use of dimethoxyphenyl penicillin in a case of osteomyelitis.

Progress:

1. The influence of sub-lethal chronic and acute total body X-irradiation on the course of experimental peritonitis in rats has been determined. Mortality rates of rats receiving 200 r of acute total body X-irradiation were increased when peritonitis was induced 96 or 168 hours after irradiation, but there was no increase when peritonitis was induced 2 hours after irradiation. Mortality rates of rats receiving 400 r or 600 r were increased when peritonitis was induced 2, 96 or 168 hours after irradiation. The use of a single dose of penicillin, oxytetracycline or chloramphenicol failed to prevent death or to modify appreciably the bacterial flora of rats receiving 400 r of total body X-irradiation 96 hours prior to induction of peritonitis. Simulated chronic irradiation, i.e., 25 r/day for 5 weeks did not increase susceptibility of rats to experimental peritonitis.

2. Little progress has been made on evaluation of therapeutic regimens in soft tissue wounds of rabbits within the last year because of emphasis on other studies. Three aerosol preparations of antibiotics for topical use have been obtained and are being compared with other antimicrobial agents. It should be emphasized that antibiotic therapy is not to provide a substitute for surgical intervention but rather to delay the time between initial infection and necessary surgical intervention.

3. A 22-year-old white male with fractures of the ulna and radius sustained in July 1960 had an original nonunion of the radius with an infectious process diagnosed as osteomyelitis. Bacteriologic cultures revealed that pus from the opening on the dorsal and lateral surfaces of the arm contained S. aureus, phage type 80/81, which was resistant in vitro to antibiotic

concentrations greater than 10 mcg/ml of the tetracyclines, erythromycin, and penicillin G but sensitive to 5 mcg/ml of chloramphenicol and 1.6 mcg/ml dimethoxyphenyl penicillin. The latter antibiotic was administered intramuscularly in a dosage of 1 g every four hours daily from the 17th of January through 10 February. No antibiotics have been administered since 10 February. Cultures taken on 10 February and 13 February were sterile but on 17 February the same organism was again cultured. This organism continued to be present on all 25 subsequent cultures in numbers ranging from 10^3 to 10^5 /ml of pus. Blood cultures taken 30 January were positive for transitional forms of an organism which after repeated subculture reverted to a nontypable staphylococcus. Another blood culture taken on 28 February again yielded transitional forms but these did not revert to a bacterial form. The radius started to heal during the period of antibiotic therapy and union has progressed. Dimethoxyphenyl penicillin therapy will be initiated again just prior to surgical intervention for removal of intramedullary rods. At this time cultures of sequestra and blood will be prepared and examined for coccal and transitional forms of bacteria.

Summary: Acute, but not chronic, total body X-irradiation increased mortality rates of rats with experimental peritonitis. This increase in susceptibility was not reversed by administration of penicillin, oxytetracycline or chloramphenicol. Topical administration of aerosol preparations of antibiotics are being evaluated in soft tissue wounds of rabbits but not enough work has been completed on this problem to justify conclusions. One patient with chronic osteomyelitis was treated with dimethoxyphenyl penicillin with clinical and bacteriological improvement.

Publication:

Noyes, H. E. and Evans, J. R. Antibiotics in the Management of Endogenous Peritonitis in Irradiated Rats. *Bact. Proc.*, 105, 1961.

ANNUAL PROGRESS REPORT

Project 6X59-01-001 Traumatic Surgery and Shock

Task 3 Role of bacteria and endotoxins in shock

**Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.**

**Department of Surgical Metabolism and Physiology,
Division of Surgery**

and

**Department of Germfree Research,
Special Activity**

Period Covered by Report: 1 Jul 60 - 30 Jun 61

**Principal Investigators: Albert Einheber, Ph. D., Stanley M. Levenson, M. D.
and Bud C. Tennant, 1st Lt, VC**

Assistants: PFC Robert E. Wren

Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

ABSTRACT

Project No. 6X59-01-001 Traumatic Surgery and Shock

Task No. 3 Role of bacteria and endotoxins in shock

**Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.**

Period Covered by Report: 1 Jul 60 - 30 Jun 61

**Authors: A. Einheber, Ph. D., Stanley M. Levenson, M. D., and Bud C. Tennant,
1st Lt, VC, and PFC R. E. Wren**

Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

Intracorporeal bacteria and their products have been ascribed a crucial role in determining irreversibility of animals to profound shock despite treatment. Experiments with a variety of germfree animals subjected to a variety of shock procedures have revealed that fatal shock may be induced in the absence of viable bacteria. We have also shown that normal germfree mice are not more susceptible to injected bacterial endotoxin than normal conventional mice. However, we are now conducting experiments to determine whether this relationship holds true after injury. In the course of standardizing suitable combinations of tourniquet injury and endotoxin, we have found that an otherwise low-lethal tourniquet injury of conventional mice is converted to a highly lethal injury by the injection (3 hours after tourniquet removal) of a dose of endotoxin which alone caused no deaths. These experiments are being extended to germfree mice and mice with controlled bacterial flora. Development of chemically defined, Seitz filtered, water soluble diets composed of simple nutrients is underway.

BODY OF REPORT

Project No. 6X59-01-001 Traumatic Surgery and Shock

Task No. 3 Role of bacteria and endotoxins in shock

Description: Intracorporeal bacteria and their products have been ascribed a crucial role in determining irreversibility of animals to profound shock despite treatment. Experiments with a variety of germfree animals subjected to a variety of shock procedures have revealed that fatal shock may be induced in the absence of viable bacteria. We have also shown that normal germfree mice are not more susceptible to injected bacterial endotoxin than normal conventional mice. However, we are now conducting experiments to determine whether this relationship holds true after injury. In the course of standardizing suitable combinations of tourniquet injury and endotoxin, we have found that an otherwise low-lethal tourniquet injury of conventional mice is converted to a highly lethal injury by the injection (3 hours after tourniquet removal) of a dose of endotoxin which alone caused no deaths. These experiments are being extended to germfree mice and mice with controlled bacterial flora. Development of chemically defined, Seitz filtered, water-soluble diets composed of simple nutrients is underway.

Progress: A large controversial literature has accumulated in the past decade which points to the participation of bacteria and their products in the responses of the animal to injury and shock. Some investigators have placed much emphasis on the possible crucial role of altered host-resistance to bacterial endotoxins of intestinal origin in the failure of experimental animals to survive following treatment for prolonged, severe shock ('irreversibility'). We have thus far not been able to corroborate the presence of a circulating lethal factor, in the blood of rabbits and dogs dying either of irreversible hemorrhagic shock or of fatal bowel ischemia induced by temporary or permanent occlusion of the superior mesenteric artery. Furthermore, we have found that germfree rats, mice, and chicks die following their subjection to a variety of shock procedures, and in so doing, behave in a manner seemingly no different than that of their concurrently shocked bacteria-laden counterparts. The latter findings indicate that in the absence or presence of living bacteria, lethal processes are evoked by standard shock procedures. Are these lethal shock processes the same for both the germfree and conventional animal? May we exclude the participation of bacteria from the responses to injury and shock of the conventional animal? These important questions remain to be answered. A comprehensive series of investigations entailing the use of germfree, control-contaminated (formerly germfree), and conventional animals is underway.

While viable bacteria are excluded as participants in the responses of the germfree animal to injury and shock, we have considered the possibility that the well known thermostable properties of the endotoxic portions (somatic antigen) of dead gram-negative bacteria may allow endotoxic activity to persist in the present autoclaved diet fed to the germfree animal. Presumably because of the less well stimulated and hence less well developed antibacterial and anti-endotoxic mechanisms of the germfree animal, the small amounts of endotoxin ingested by them in their autoclaved diet might play a functional role comparable to that proposed for the presumably larger amounts of endotoxin ordinarily present or readily available to the conventional animal in its gastrointestinal tract.

However, we have shown that normal germfree mice are not more susceptible to administered endotoxin than normal conventional bacteria-laden mice, and, in fact, the germfree mice may be more resistant. Nevertheless, this same relationship may not necessarily hold true after injury. Preliminary to investigating this problem with germfree and conventional mice in Reyniers tanks (germ-free isolators) we have been conducting experiments to standardize suitable combinations of endotoxin dosage and tourniquet injury with laboratory room conventional mice. In the course of these studies, we have found that tourniquet injury renders mice highly susceptible to administered endotoxin. This observation confirms those of Greisman (S. E. Greisman: On the collapse of bacterial endotoxin resistance following hemorrhage. *J. Exp. Med.*, 112: 257, 1960) with tourniqueted rabbits, and Oldstone (Oldstone, M.B.A., Altered Reactivity to *Escherichia coli* Endotoxin of mice subjected to sub-lethal tourniquet injury, *Proc. Soc. Exp. Biol. Med.*, 102: 256, 1959) with tourniqueted mice.

Walter Reed "pathogen-free" mice (250 males, 30 females) weighing 22-36 grams) were employed. Tourniquet injury was produced by the Rosenthal technic. Bilateral hind-limb ischemia was induced for 1 or 1.25 hours. In each experiment, three groups of mice were used: Group A received tourniquets and 3 hours after their removal an intraperitoneal injection of 0.1 ml isotonic saline; Group B received a sham tourniquet application and removal, and a dose of endotoxin 3 hours after the latter; and Group C received tourniquets and a dose of endotoxin 3 hours after their removal; in some experiments additional subgroups of Group C included mice that received lesser amounts of endotoxin. Endotoxin was administered 3 hours after tourniquet release because from what is known of this insult in mice, maximal fluid loss into the injured limbs would have occurred by this time and the animals would have been reacting accordingly. The body weights of the mice in the several groups were matched as closely as possible; and group manipulations were rotated. Mice were allowed food and water to the time of tourniqueting but none thereafter. The *E. coli* endotoxin stock solution was diluted in isotonic saline (supplied by Dr. Howard Noyes) and was always given in a total volume of 0.1 ml intraperitoneally. The results of 8 experiments done over a 3 month period are summarized in Table 1.

Table 1 -- % Mortality

No. of Expt	B.W.	Tourniquets + Saline, i.p. Group A	Sham Tourniquets + Endotoxin* i.p. Group B	Tourniquet + Endotoxin, i.p. Group C		
				0.01** ml	0.001** ml	0.0001** ml
One-Hour Tourniquets						
2	22-34	3	0	45	-	20
One-Hr. & 15 Min. Tourniquets						
6	22-36	17	0	85	63	50
8	22-36	12	0	72	63	35

*Max. dose of endotoxin used in an experiment always given.

**Vol. of stock endotoxin sol. contained in the saline-diluted std. 0.1 ml vol. injected.

It is apparent that an otherwise low lethal tourniquet injury (12% mortality at 24 hours) was converted to a highly lethal injury (72% mortality at 24 hours) by the injection of a dose of endotoxin which alone caused no deaths.

These experiments are being extended to germfree mice and mice with rigidly controlled, defined bacterial flora. In addition, chemically defined, water soluble, Seitz filtered diets, composed of low molecular weight nutrients (amino acids, simple sugars, etc.) are being developed to reduce the antigenic and endotoxic properties of the sterile diets presently fed germ-free animals and their conventional controls.

Summary and Conclusions: Intracorporeal bacteria and their products have been ascribed a crucial role in determining irreversibility of animals to profound shock despite treatment. Experiments with a variety of germfree animals subjected to a variety of shock procedures have revealed that fatal shock may be induced in the absence of viable bacteria. We have also shown that normal germfree mice are not more susceptible to injected bacterial endotoxin than normal conventional mice. However, we are now conducting experiments to determine whether this relationship holds true after injury. In the course of standardizing suitable combinations of tourniquet injury and endotoxin, we have found that an otherwise low-lethal tourniquet injury of conventional mice is converted to a highly lethal injury by the injection (3 hours after tourniquet removal) of a dose of endotoxin which alone caused no deaths. These experiments are being extended to germfree mice and mice with controlled bacterial flora. Development of chemically defined, Seitz filtered, water soluble diets composed of simple nutrients is underway.

List of Publications: A. Einheber, Discussion of Dr. Fine's Paper - Shock Symposium, Fed. Proc. (in press).

ANNUAL PROGRESS REPORT

Project 6X59-01-001, Traumatic Surgery and Shock

Task 4, Gas Gangrene

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Department of Bacteriology
Department of Applied Immunology
Division of Communicable Disease
and Immunology

Period Covered by Report: 1 July 1960 through 30 June 1961

Principal Investigator: Howard E. Noyes, Ph.D.

Assistants: Eugene H. LaBrec, Ph.D.
Susan E. Chapman, B.A.
William C. Branche, Jr., M.S.

Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

ABSTRACT

Project 6X59-01-001

Title: Traumatic Surgery and Shock

Task No. 4

Title: Gas Gangrene

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Period Covered by Report: 1 July 1960 through 30 June 1961

Authors: Howard E. Noyes, Ph.D.
Eugene H. LaBrec, Ph.D.
Susan E. Chapman, B.A.
William C. Branche, Jr., M.S.

Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

1. An attempt was made to use the fluorescent antibody technique to demonstrate in vivo distribution of clostridial toxins. Commercial typing sera, well characterized in terms of their antitoxin content have been conjugated with fluorescein isothiocyanate. When sections of muscle infected with Clostridium perfringens were treated with these antisera, cell walls of organisms identified as C. perfringens fluoresced with heterologous as well as homologous clostridial antitoxins. These conjugated antitoxins will be absorbed with washed cells of appropriate clostridial species in an effort to remove antibodies to somatic antigens.

2. At present the only technique for typing Clostridium perfringens strains is in laboratory animals. The use of tissue culture techniques offers a more convenient and less expensive method for quantitation and identification of toxic components in culture filtrates or wound exudates. It was found that wound exudates were toxic for monkey kidney cells in direct proportion to mouse lethality. However, neutralization of toxins with specific antisera was unsuccessful, presumably because of preservative in the typing antisera.

3. Earlier studies have indicated that the death of the wounded "Edgewood Goat" is related to the presence of Clostridium perfringens Type A. Wound exudates were shown to be lethal for laboratory animals and lethality was reversed by the prior or concurrent administration of commercial trivalent gas gangrene antitoxin. Assays of wound exudates contained alpha, beta, and

mu toxins of C. perfringens in increasing amounts in succeeding samples. Failure to obtain complete neutralization of the in vitro assay results with antitoxin indicated that either (1) the assay techniques used were not specific enough or (2) the toxins were unavailable to the antitoxins. Currently toxins in the exudates are being measured in terms of specific antitoxins.

BODY OF REPORT

Project 6X59-01-001

Title: Traumatic Surgery and Shock

Task No. 4.

Title: Gas Gangrene

Description:

1. Objectives of this task are to develop or modify techniques used in the identification of those organisms involved in gas gangrene, and to determine the lethal component of this infection with an ultimate objective of developing a satisfactory toxoid.

2. The demonstration of the presence or absence of a toxigenic species of clostridia has little meaning to the clinician. The demonstration of a specific toxin in the wound, however, could be of great value if the prevalent theory that death from gas gangrene is assumed to be the result of toxin formed in the wound and transported via the blood stream to some vital center(s). Some understanding of this disease could be gained by measuring in vivo distribution of toxins in terms of vital area(s) involved. A study using fluorescent antibody techniques for this purpose is underway. Conventional techniques for typing clostridial species involve laboratory animals with an end point of death. The use of tissue culture techniques for this purpose offers the advantages of convenience, less space requirements, lower cost, and possibly characterization of toxins in terms of intermediate cytopathogenic effects rather than a single end point of death. The lethal factor in gas gangrene infections is not known. A standardized blast wound in goats has been related to its C. perfringens content and lethality of the wound exudate can be prevented by commercial trivalent gas gangrene antitoxin. Studies are being carried out to determine lethal factors of this exudate in the hope that such knowledge will form a basis for the formulation of an effective toxoid for the prevention of gas gangrene. Studies of clinical gas gangrene in the earthquake zone of Chile were unsuccessful because no cases were available.

Progress:

1. Commercial antitoxin typing sera for fluorescent antibody studies supplied by the Wellcome Research Laboratories, Beckenham, England, were conjugated with fluorescein isothiocyanate. Sera to toxins of the following organisms were used: Clostridium perfringens A-F, Clostridium novyi A and B, Clostridium septicum and Clostridium chauvoei. A control of horse serum processed similarly to typing sera was also conjugated. Sections of muscle infected with C. perfringens were reacted with these sera. It was found that all of the conjugated sera and the conjugated horse serum reacted with the organism, imparting a yellow-green

fluorescence around each bacterium, indicating an antigen antibody reaction at the cell surface. These antisera were prepared by injection of unfiltered cultures and it will be necessary to remove antibodies against somatic antigens before further studies can be done.

2. A standardized wounding procedure, using a tetryl pellet as the explosive, results in a massive unilateral open wound in a hindquarter of the goat. Exudate from these wounds is obtained by placing sterile 4X4 cotton sponges in the wound. Two to 3 hours later the sponges are wrung out by hand, the collected exudates are immediately frozen at -60C and stored. Immediately prior to use they are centrifuged at 5C for one hour at 15,000 rpm and then filtered through Seitz pads. Wound exudates from this wound preparation were lethal for monkey kidney cells in direct ratios to the number of viable C. perfringens present before filtration and lethality for mice. Attempts to neutralize the cytopathogenic effect with commercial antitoxin preparations were unsuccessful because the antitoxins were lethal for monkey kidney cells. This toxicity is presumed to be due to 0.35 per cent cresol, the preservative in the antitoxin preparations. Attempts will be made to remove the preservative by dialysis, after which the study will be continued.

3. (a) Serial samples of filtered wound exudates from tetryl blasted goats were incubated with sterile egg-yolk solution in attempts to demonstrate alpha toxin (lecithinase). Results on 29 goats showed a positive correlation between the number of C. perfringens present, positive lecithovittelin (egg yolk) tests, survival time of the goats and lethality of sterile exudates for mice. The positive lecithovittelin tests were inhibited either not at all or just slightly by commercial trivalent gas gangrene antitoxin.

(b) Theta toxin is an oxygen labile hemolysin. In this study it was assayed by its ability to lyse sheep erythrocytes. All twenty-five goats tested had theta toxin present in the exudates in increasing amounts as the goats neared death. Trivalent antitoxin inhibited the lysis of sheep erythrocytes. It must be pointed out, however, that addition of reducing agents did not increase activity of the erythrocyte-lysing material in wound exudate. An increase should have occurred if this were free theta toxin.

(c) Mu toxin is a hyaluronidase. Commercial sodium hyaluronate was used as the substrate in the determination of hyaluronidase present in goat wound exudates. Results paralleled those obtained in the determination of alpha and theta toxins; this test was only partially inhibited by trivalent antitoxin.

(d) Attempts were made to relate pH of the wound exudate to death or the presence of specific toxins. pH values ranged from 6.8 to 8.2 but no relationship could be found between pH values and any of the parameters mentioned above.

(e) Fractionation studies of wound exudates have been initiated in an attempt to concentrate the lethal factors. Practically every purification technique used reduced toxicity in a direct ratio to the amount of nitrogen removed. The possibility that alpha toxin is irreversibly bound to blood constituents as it is formed "in vivo" is one theory of why it has never been detected in the circulation of wounded animals. An experiment was carried out in which partially purified alpha toxin was added to goat wound exudate which had been tested previously and found to have no lecithovittelin titer. After addition of alpha toxin, the lecithovittelin test was positive to the exact dilution that was positive for the alpha toxin alone. This reaction was inhibited by antitoxin. The same titer and the same inhibition were obtained when this specimen was tested 6 weeks later. Obviously in this instance wound exudate (Seitz filtered) did not combine with the alpha toxin in such a way as to prevent the action of antitoxin in the lecithovittelin test.

(f) Current studies are being carried out in an attempt to quantitate toxins in wound exudates in terms of characterized antitoxin preparations. Other parameters to be studied are the effects of various physical and chemical agents on the stability of these exudates. It is planned that fractionation procedures will enable the separation and identification of component toxins.

4. The principal investigator was assigned to the 7th Field Hospital in Valdivia, Chile in May and June 1960 to study traumatic wounds, particularly the diagnosis and antitoxin therapy of gangrene. Reports of injuries in the earthquake area were incorrect and the few trauma cases that had occurred were evacuated prior to the arrival of the 7th Field Hospital.

Summary: New techniques are being evaluated for in vitro, and in vivo characterization of clostridia and their toxins. The use of fluorescent antibody techniques is not suitable for identification of specific toxins but it is felt absorption techniques may solve this problem. The use of tissue culture techniques holds promise for typing C. perfringens. Goat wound exudate contains lethal factors which are being characterized. Several known toxins have been found but lack of complete neutralization by antitoxin preparations suggests that heretofore undescribed toxins may be present. Quantitative studies of the toxic components of wound exudate are in progress.

Publication:

Noyes, H. E., Evans, J. R. and Baker, H. J. Techniques for Bacteriological Studies of Field Specimens. Armed Forces Med. J. 11, No. 12, 1516-1519, Dec 1960.

ANNUAL PROGRESS REPORT

Project 6X59-01-001, Traumatic Surgery and Shock

Task 17, Acute Renal Injury and Failure

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Department of Metabolism
Division of Medicine

Period Covered by Report: 1 July 1960 through 30 June 1961

Principal Investigator: Lt. Colonel Jacques L. Sherman, Jr., MC

Assistants: Major Kevin G. Barry, MC
Captain Thomas E. Davis, MC

Reports Control Symbol: MEDDH-288

Security Classification: Unclassified

ABSTRACT

Project 6X59-01-001, Traumatic Surgery and Shock

Task 17, Acute Renal Injury and Failure

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Period Covered by Report: 1 July 1960 through 30 June 1961

Authors: Lt. Colonel Jacques L. Sherman, Jr., MC
Major Kevin G. Barry, MC
Captain Thomas E. Davis, MC

Reports Control Symbol: MEDDH-288

Security Classification: Unclassified

Clinical research on the prevention and treatment of acute renal failure is being continued. Peritoneal dialysis is being studied as an adjunct to the artificial kidney in management of renal failure. Resin therapy is so successful in preventing potassium intoxication that dialysis for this is not necessary. The effect of 20% mannitol on renal function is being studied.

BODY OF REPORT

Project 6X59-01-001, Traumatic Surgery and Shock

Task 17, Acute Renal Injury and Failure

Description: The overall problems of the prevention, diagnosis and therapy of acute renal failure are being evaluated in order to provide measures to reduce the morbidity and mortality from this complication of shock and trauma.

Progress:

1. Mechanism of Acute Renal Failure. This department continues to have responsibility for the clinical care of all WRGH patients with acute renal failure (ARF). As in the past, careful daily measurements are made of intake and output, biochemical alterations, and studies of blood and urine osmolarity. Continued observations on all patients with acute renal failure are being carried out to test the concepts of the mechanism of renal failure. Animal studies are also being performed.

2. Infection Complicating Acute Renal Failure. Systemic infection is an important consideration in the management of any patient in acute renal failure. Several aspects of this problem have been considered by this department previously and are listed in earlier reports. Findings here have led to employing the following in the management of our patients: (1) the use of intermittent catheterization instead of the indwelling catheter in attempt to eliminate one source of infection, (2) the use of specific antibiotic therapy when the need arises in the individual patient rather than the prophylactic use of antibiotics in all patients, and (3) the alteration of dosage of administered antibiotics to compensate for the inability of the kidney in failure to excrete, thereby preventing the rise in concentration to toxic levels.

3. Influence of Aortic Clamping on Renal Function. Studies have been continued in animals and patients on the effect of cross clamping the aorta in renal function. Mannitol infusion is being used to prevent the oliguria which normally follows this clamping and has been successful.

4. Diagnostic Aids in Acute Renal Failure. Over 20 patients tentatively diagnosed as acute organic shutdown have received mannitol infusion. In 14 of 22 patients with detailed data, mannitol produced clinically effective diuresis. These studies will continue.

5. Resin Therapy in Acute Renal Failure. Kayexalate, a polystyrene resin administered by mouth or as an enema, has shown itself to be of considerable therapeutic value during the past year. This resin lowers the serum potassium, and has been quite effective in preventing the development of hyperkalemic cardiotoxicity. This drug represents a major improvement in the treatment of acute renal failure, as it has decreased the frequency of hemodialysis procedures in many patients.

6. Methods of Dialysis. Patients with acute renal failure requiring dialysis have been treated by means of the Twin-Coil apparatus and also by intermittent peritoneal dialysis. Both methods are being evaluated and both are very valuable. The Kolff rotating drum apparatus is not being used at the present time, since the Twin-Coil unit is definitely superior.

7. Influence of Mannitol in Renal Function. Studies in dogs have demonstrated that even when animals were maintained in shock with blood pressure of 50 mm Hg. mannitol restored P.A.H and creatinine clearances to normal. The implications of this are great in that it demonstrates that renal function may be maintained in the shock state with appropriate therapy.

Summary and Conclusions:

Clinical research on the prevention and treatment of acute renal failure is being continued. Peritoneal dialysis is being studied as an adjunct to the artificial kidney in management of renal failure. Resin therapy is so successful in preventing potassium intoxication that dialysis for this is not necessary. The effect of 20% mannitol on renal function is being studied.

Publications:

1. Benyajati, C., Keoplug, M., Beisel, W. R., Gangerosa, E. J., Sprinz, H., and Sitprijja, V.: Acute Renal Failure in Asiatic Cholera: Clinicopathologic Correlations with Acute Tubular Necrosis and Hypokalemia Nephropathy. *Ann. Int. Med.* 52: 960, May, 1960.

2. Barry, K. G., Cohen, A., Knochel, J. F., and LeBlanc, P. C.: Prevention of Oliguria Attending Cross Clamping of the Abdominal Aorta Below the Renal Arteries (CCA.) in Humans by Mannitolization (M). Clinical Research VIII: 364, 1960 (Abstract).
3. Knochel, J. P., Beisel, W. R., Herndon, E. G. Jr., Gerard, E. S., and Barry, K. G.: The Renal, Cardiovascular, Hematologic and Serum Electrolyte Abnormalities of Heat Stroke. Amer. J. Med. 30: 299, February, 1961.
4. Barry, K. G., Cohen, A., and LeBlanc, P.: A Preliminary Report - Mannitolization: I. The Prevention and Therapy of Oliguria Associated with Cross-Clamping of the Abdominal Aorta. Accepted for publication in Surgery.
5. Barry, K. G., Cohen, A., Knochel, J. P., Whelan, T. J. Jr., Beisel, W. R., Vargas, C., and LeBlanc, P. C.: Mannitol Infusion: II. The Prevention of Acute Functional Renal Failure During Abdominal Aortic Aneurysmectomy. New Engl. J. Med. 264: 967, May 11, 1961.
6. Barry, K. G. and Berman, A. R.: Mannitol Infusion: III. The Acute Effect of the Intravenous Infusion of Mannitol on Blood and Plasma Volumes. Accepted for publication in New Engl. J. Med.

ANNUAL PROGRESS REPORT

Project 6X59-01-001 Traumatic Surgery and Shock

Task 17 Acute renal injury and failure

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12 D. C.

Department of Surgical Metabolism and Physiology,
Division of Surgery

Period Covered by Report: 1 Jul 60 - 30 Jun 61

Principal Investigator: Robert W. Clarke, Ph. D.

Assistants: G. James Cerilli, Capt, MC Albert Einheber Ph. D., John A.
Gagnon, M.S., Erving Geever, M. D., and Paul E. Teschan, Maj, MC

Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

ABSTRACT

Project No. 6X59-01-001 Traumatic Surgery and Shock

Task No. 17 Acute renal injury and failure

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Period Covered by Report: 1 Jul 60 - 30 Jun 61

Authors: T. James Cerilli, Capt, MC, Robert W. Clarke, Ph. D. Albert
Einheber, Ph. D., John A. Gagnon, M. S., Erving F. Geever, M. D.,
and Paul E. Teschan, Major, MC

Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

Renal blood flow, its reliable measurement and its significance in relation to post-surgical, hypotensive and experimental acute renal failure have been given critical attention. To determine the constancy of renal functions in dogs over considerable periods of time, clearances have been measured in conscious and anesthetized dogs and significant time-related changes have been revealed which point to the need for increased caution in the interpretation of results on the unselected animals which we and most other investigators have been using. Because clearance methods are inapplicable in anuric states and are meaningless when a steady state of renal activity is not present, we have just begun to use the electromagnetic flowmeter. With this device we are attempting to settle the question: is the apparent prophylactic action of mannitol against the renal failure of arterial hypotension related to its properties as an osmotic diuretic or to its ability to improve renal circulation? Studies of experimental acute renal failure in a primate have continued using the African green monkey. A supra-renal aortic clamp applied for 8 hours, thus greatly reducing renal arterial pressure, gives only transient renal effects in normally hydrated animals but is fatal in those dehydrated 24 hours beforehand. Eight monkeys kept hypotensive (35 to 50 mm Hg mean systemic arterial pressure) for 5 to 9 hours showed no renal failure.

It has been shown that whole-body hypothermia of properly chosen intensity and duration may have a very significant therapeutic effect in dogs subjected to renal insult (intra-arterial adrenalin). This finding may have useful clinical application.

BODY OF REPORT

Project No. 6X59-01-001 Traumatic Surgery and Shock

Task No. 17 Acute renal injury and failure

Description: Renal blood flow is the primary point of interest in the current studies of acute renal failure. This derives largely from our finding that in the dog a renal arterial pressure of about 25 mm Hg for ten hours produces only minor transient renal functional deficits and no lasting renal pathology. Similar experiments in monkeys showed them to have at least an equally high resistance to this renal insult. The effect of infused mannitol on renal blood flow is of prime interest.

Hypothermia is being tested as a means of reducing the renal tissue derangement due to ischemia or other action of infused adrenalin. In the dog it appears that there may be an optimum combination of intensity and duration of hypothermia best suited to cut short the damaging effects of the renal insult and to aid the restorative processes.

Progress: The use of mannitol as an effective prophylactic measure against the acute renal failure which we have produced in dogs has been extended to its use in patients undergoing surgery, where acute renal failure sometimes also occurs (see preceding section of report). In each situation the PAH clearance increased and it was concluded that this apparent effect of mannitol on renal blood flow is closely related to the favorable outcome. PAH clearance is not a measure of renal blood flow unless its renal extraction is known and allowed for and unless steady states of blood flow and PAH movements are known to exist. To observe the constancy of steady state conditions in our dogs we have made clearance measurements at intervals of 7 to 30 days, each time collecting data over from 2 to 7 hours. In 20 of 22 such tests on 8 of 9 dogs there was a significant rise of CPAH during the course of each experiment. One dog of this series showed a decline of up to 40% in CPAH in 4 out of 5 experiments. The state of hydration appeared not to be a factor nor did pentobarbital anesthesia if kept reasonably constant throughout. When anesthesia was induced midway in an experiment three dogs showed no changes but one (the exception noted above) showed an abrupt 40% fall. Additional data were collected, e.g. creatinine clearance, plasma total solids, hematocrit, arterial blood pressure, etc., but will not be discussed here. We conclude that in experiments where prolonged periods of observation are used it may be necessary to pre-test each dog to determine its control pattern.

As an extension of our previous similar work in the dog, African green monkeys were subjected to varying periods of total suprarenal aortic occlusion. Periods up to 8 hours in normally hydrated animals produce only a transient rise in BUN, a temporary proteinuria and in some animals a 24-hour sodium diuresis. Twenty-four hours of pre- or postoperative dehydration has a marked effect on the result, producing a high operative mortality and no survivors beyond 5 days. The terminal BUN values were high and there were obvious renal infarcts seen at autopsy but the cause of death is unknown.

In 19 experiments mannitol has been infused by vein as a 20% solution at 2 ml/min for 4-5 hours or rapidly injected into the femoral artery or jugular vein in the same animals as mentioned above. Because of the dynamic state of the clearances of Cr and PAH which we observed during the control studies it was difficult to assess the role of mannitol in any individual experiment. However, by comparing the clearances of a mannitol infusion experiment with the clearance data obtained during several control experiments on the same animal, the effect of mannitol has been determined. Over a period of time similar to that of the control experiments, mannitol was infused and the C_{PAH} increased as much as 30% in those animals which had shown this trend during the control experiments. A decrease of 25% was observed in the only animal which quite consistently had a declining C_{PAH} . The C_{Cr} usually paralleled such changes.

When 50 ml of a 25% mannitol solution was rapidly injected into the blood stream but with no significant change in arterial blood pressure, there was a significant increase in the C_{PAH} AND C_{Cr} during the first 10-20 min. This was usually followed by a fall to or below the pre-injection rates within the next 10-20 min. The hematocrit and plasma total solids usually declined during the first post-injection period and then quickly returned to normal.

From these preliminary results it would appear as though mannitol, when infused at a rate sufficient to cause a 10-20 fold increase in urine flow, does not produce any significant changes in C_{PAH} or C_{Cr} in the healthy mongrel dog. The transient changes observed following the rapid injection of mannitol may be due to a decrease in the viscosity of the blood perfusing the renal bed causing a real increase in blood flow. It may also be due to a rapid washout of PAH from the tubule which one could falsely interpret as an increase in renal blood flow. In hemorrhagic shock (before resuscitation) and in aortic surgery, both tending to produce acute renal failure, clearance methods cannot be applied. We have just turned to the electromagnetic flowmeter with the technical cooperation of Department of Cardiovascular Diseases to obtain reliable information on renal blood flow during hemorrhagic hypotension in the dog and the influence of mannitol upon it. Technical difficulties have not been entirely overcome in the few trials done, but it already appears that valid and highly significant data can be obtained.

Acute renal injury was produced in 62 normothermic, uninephrectomized, normally hydrated, mongrel dogs by infusing epinephrine for 1½ hours directly into the renal artery at the rate of 5 micrograms/min. (in 0.25 ml of 5% dextrose solution). Thirty to forty minutes following infusion, 19 animals were returned directly to their cages (control group #1) and allowed food ad lib; 7 animals were kept anesthetized, normothermic and placed on a respirator for 26-28 hours (control group #2); 9 animals while on a respirator were cooled to 28-30° C for 6 hours (experimental group #1); 27 animals were placed on a respirator and cooled to 28-30° C for 26-28 hours (experimental group #2). All survivors were sacrificed at 21 days.

In control group #1, one animal died without regaining consciousness, 13 (72%) subsequently died of renal failure (average 7-8 days). In control group #2 one animal died while anesthetized and 5 (83%) subsequently died of

renal failure. Six dogs (66%) in experimental group #1 died of renal failure with no deaths during hypothermia. In experimental group #2, fourteen animals (52%) died during hypothermia or during the rewarming phase; of the surviving 13 animals only one died (7.7%) of renal failure. These survivors were in good condition at sacrifice. The rise of BUN and proteinuria were higher in the control groups. At autopsy or sacrifice the control groups were found to have the more severe cortico-medullary congestion and necrosis, patchy medullary necrosis and cortical degeneration. To determine if the 7.7% renal death rate in the prolonged hypothermic group is based on a selective death during hypothermia, a control series of infusions without epinephrine and followed by 28 hours of hypothermia is now in progress.

Summary and Conclusions: From the particular attention we have given to renal blood flow we have concluded that it must be given further intensive study. The limitations of clearance methods and the availability of the electromagnetic flowmeter have led us to start work with the latter which already gives promise of future value in this program.

The use of hypothermia as a post-insult, therapeutic measure against acute renal failure, in contrast to the pre-insult prophylactic use of mannitol has been suggested by experiments in dogs. Verification and extension of this finding may lead to broad and useful laboratory and clinical applications.

List of Publications: None.

ANNUAL PROGRESS REPORT

Project 6X59-01-001, Traumatic Surgery and Shock

Task 23, Organ Repair and Replacement

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Department of Experimental Surgery
Division of Surgery

Period Covered by Report: 1 July 1960 through 30 June 1961

Principal Investigators: Lt Col Thomas J. Whelan, Jr., MC*
Capt S. Hedberg, MC**

Reports Control Symbol: MEDDH-288

Security Classification: Unclassified

*Dept. of Surgery, Walter Reed General Hospital

**Now Surgical Resident, Massachusetts General Hospital, Boston, Mass.

Project No. 6X59-01-001

Title: Traumatic Surgery and Shock

Task No. 23

Title: Organ Repair and Replacement

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Period Covered by Report: 1 July 1960 through 30 June 1961

Authors: Lt Col Thomas J. Whelan, Jr., MC; Capt S. Hedberg, MC

Reports Control Symbol: MEDDH-288

Security Classification: Unclassified

Arterial Prosthesis. The abdominal aortic grafts were subjected to a more stressing test from the hemodynamic point of view with two end-to-end suture lines within 5-7 cm of each other and a tube of 8 mm inner diameter. This resulted in failures of the composite graft of 37 C.P.I., a graft which, when placed in the thoraco-abdominal aorta with proximal end-to-end anastomosis and a distal end-to-side anastomosis and with a 12 mm inner diameter tube, yielded excellent results.

Arterial Dilatation to Further Small Artery Anastomosis. Traumatic arterial spasm is a serious sequel of arterial dissection. This is particularly troublesome in surgical repair of small arteries. A method has been devised in the dog by means of which the diameter of small spastic arteries can be greatly increased, facilitating their surgical repair.

Common Bile Duct Prosthesis. Foamed acrylic amide tubes have been used successfully as arterial prostheses but the application of this material for the replacement of the common bile duct has met with difficulty. Encouraging results have followed the use of (1) prostheses implanted near the bile ducts prior to anastomosis, to allow ingrowth of fibrous tissue before exposure to bile, and (2) prostheses of foam construction bonded to a thin "intima" of acrylic amide film combined with temporary splinting of each hepatic duct, the common bile duct, and the prosthesis over a forked polyethylene tube.

Radioisotopic Delineation of Third Degree vs. Non-Third Degree Burns. A search has been made for a method that would permit prediction of the depth of a cutaneous burn or at least enable differentiation between full-thickness and lesser degrees of burn. Using a graduated thermal burn in pigs, it was found that radioactive phosphate (as $\text{Na}_3\text{P}^{32}\text{O}_4$) localizes in areas which grossly on section appear only partially burned, while uptake in full-thickness areas is markedly depressed. Histologic verification of the exact depth of burning has thus far not been possible with accuracy. It has not been possible to histologically pinpoint the interface between full and partial thickness burns.

20.0

Splenic Homotransplant after whole Body Radiation. Homotransplantation of the intact spleen in dogs was studied with the following objectives: (1) to evaluate the possible radioprotective effect of splenic homotransplantation, and (2) to study the effects of lethal whole body irradiation and splenic homotransplantation on the immunologic functions of surviving animals.

Transplantation of the spleen by vascular anastomosis has been technically possible in approximately 50 percent of cases. Spleen transplantation following irradiation to the host has not provided radioprotection. The transplanted spleen, however, is apparently not rejected by the heavily irradiated host.

BODY OF REPORT

Project No. 6X59-01-001

Title: Traumatic Surgery and Shock

Task No. 23

Title: Organ Repair and Replacement

Description:

Arterial Prosthesis. Continuation of a search for an ideal prosthesis has prompted the following protocol: Grafts of 37 C.P.I. (courses per inch) knitted dacron coated with acrylate amide terpolymer foam (butyl acrylate, methyl methacrylate, and methacrilamide) were placed in the abdominal aorta, femoral artery, and in the thoracoabdominal aorta of dogs. Fifteen (15) abdominal grafts were placed; eleven thoracoabdominal grafts were placed, and six paired femoral grafts were placed. Harvesting of these grafts has been carried out after performing arteriography. Photographs of the specimens and microscopic slides have been obtained.

Arterial Dilatation to Further Small Artery Anastomosis. This is the final report on this phase, covering the period 3 May 1960 through 31 June 1961. Superficial femoral arteries of 46 mongrel dogs ranging in weight from 5.4 to 26 kg. with a mean of 13.6 kg. were studied. The left femoral artery was chosen as the experimental one, the right served as control. Under anesthesia each artery was exposed by careful dissection and the outer diameter of each vessel was carefully measured. In 18 dogs a segment of the experimental artery in the area in which it was to be divided was occluded gently with bull-dog clamps and the occluded segment was dilated by careful injection of saline solution under pressure. In 22 dogs the dissected experimental vessel was simply grasped with the fingertips to occlude it first distally and then proximally and the pressure of blood thus entrapped was slowly elevated by a gentle rolling action of the fingers. In all cases this also achieved dilatation of the experimental segment of artery. The outside diameter of each vessel was measured following dilatation. All vessels were then stripped of adventitia over the midportion of dissected areas and divided cleanly between surgical coarctation clamps. Ends of vessels were then sutured together carefully with 6-0 arterial silk using a standard Carrel technique. All wounds were closed in layers. After surgery patency of anastomoses was determined by palpation through skin, by aortography, via carotid artery catheterization, and ultimately by gross and histologic examination of excised vessels.

Common Bile Duct Prosthesis. This is the final report on this phase, covering the period 5 January 1960 through 31 June 1961. There is clinical need for a consistently successful method of reconstructing or replacing the injured, divided or strictured common bile duct. Acrylate amide foam tubes have been used successfully as arterial prostheses. It has been observed that the interstices of the foam structure become occupied by in-growing connective tissue, while an endothelium soon lines the lumina of such tubes. It was hoped that a similar reaction might occur if these terpolymer tubes were used to bridge defects in the common bile duct.

Radioisotopic Delineation of Third Degree vs. Non-Third Degree Burns.

This is the final report on this phase, covering the period 3 March 1959 through 31 June 1961. Early excision has generally been accepted as a desirable objective in the treatment of full thickness burns. As a practical matter, however, it is too frequently difficult to determine with precision the amount of skin to be excised, so that more conservative methods, with all their disadvantages, are still widely practiced. The objective of this phase was to develop a reliable and practical method for estimating the depth of cutaneous burns.

Splenic Homotransplant after Whole Body Radiation. This is the final report on this phase, covering the period 10 August 1959 through 31 June 1961. The radioprotective effects of organ shielding and postirradiation administration of marrow and spleen cells in rodents are now well recognized. Furthermore, animals protected against otherwise lethal doses of radiation by infusion of homologous cells have been found in some cases to be tolerant not only of the cells given immediately after radiation, but also of other tissues from the same donor transplanted later. The objective of this phase was to evaluate the possible radioprotective and immunologic potential of the transplanted canine spleen.

Progress:

Arterial Prosthesis. Results of the abdominal aortic grafts reveal that with the exception of one 37 C.P.I. graft, all underwent dilatation and late hematoma formation. Previous studies revealed that four of five abdominal aortic grafts with grafts of 56-76 C.P.I. were well incorporated in tissue without dilatation or thrombosis. Results of thoraco-abdominal grafts of 37 C.P.I. dacron-supported tubes were excellent with ten well intimized, patent, non-dilated grafts. Three of six femoral grafts were well intimized and patent but follow-up studies of successful grafts were only two and one-half months. A six month follow-up revealed thrombosed grafts bilaterally.

Arterial Dilatation to Further Small Artery Anastomosis. Pressures necessary to cause dilatation of spastic vessels ranged from 180 to 240 mm. of mercury. For each vessel there was a specific pressure at which dilatation occurred. When this "dilatation pressure" was reached dilatation proceeded to completion in 3 or 4 seconds without further increase in pressure. Pressures much higher than dilatation pressure, that is up to 350 mm. of mercury, had no apparent effect: further dilatation was not produced and rupture did not occur. Dilatation was most effective in those vessels where it should be of most help to the surgeon; the smallest arteries were doubled in their outside diameter which averaged 3.11 mm. before dilatation. After dilatation the arteries on the experimental side averaged 4.29 mm. This figure represents a significant ($P = .02$) increase in outer diameters. Anastomotic patency as determined by palpation was confirmed in every case by aortography. Furthermore, no vessel found patent by palpation or aortography was later found occluded on excision. All thromboses in this series occurred in the first 8 cases operated upon, in spite of the fact that small animals became available only later in the series. None of the last 64 vessels studied, dilated or not, became thrombosed after suture.

Histologic examination of specimens removed at time of sacrifice revealed intimal thickening in a few vessels but its presence was related neither to previous dilatation nor to length of time since operation. Adventitial stripping of the canine artery led to a moderate degree of medial degeneration, with early loss of muscle and later fragmentation of medial elastic fibers. Medial degeneration appeared more extensive in dilated vessels. Repair of the medial lesion by collagen deposition occurred before 26 weeks. At that time approximately one-third of the media appeared to consist of collagen while the remainder was muscle and elastic fibers. Intima and internal elastic lamella appeared undamaged immediately following dilatation and did not undergo degeneration in the stripped vessels, dilated or not. Dilated vessels excised 26 to 37 weeks postoperatively usually were larger in diameter than non-dilated control vessels.

Common Bile Duct Prosthesis. A foam tube 5 mm O.D. has been used in 14 dogs. The prosthesis was carefully preclotted with the dog's own blood prior to insertion. In 9 cases the prosthesis was interposed between the ends of the divided duct, anastomosis being accomplished with 5-0 silk or catgut. In 5 cases the tube was sutured only to the proximal duct, the distal duct being ligated, while the distal end of the tube was simply inserted into the duodenum.

Five dogs died from 4 to 35 days postoperatively of biliary tract obstruction, infection or perforation. In no case was the prosthesis incorporated by fibrous tissue. Four dogs died from 58 to 104 days, with essentially the same findings at autopsy. Of four dogs sacrificed from 90 to 114 days, three appeared to have an excellent functional and anatomical result. One dog remains well at 134 days. In the three dogs classified as good results, the prosthesis was found firmly fixed in -- but not permeated by -- scar tissue. The presence of the prosthesis maintained patency of the duct system, while firmness of the cicatrice prevented displacement of the prosthesis as seen in most other dogs.

Because of the poor results, modifications in technic were made. The prosthesis in seven cases was preliminarily implanted near the common bile duct and cholecystectomy performed. As a second stage, after ingrowth of fibrous tissue had taken place, the proximal end of the duct was anastomosed to the tube and its surrounding scar, and the distal end of the tube opened into the duodenum. Survivals were longer, but the results were essentially the same. Gross and histological examination of the prostheses showed that even when fibrous tissue had completely grown into them, subsequent exposure to bile resulted in dissolution of the fibrous bonds.

The original idea of depending on host fibrous tissue to line the prosthesis was therefore abandoned. Subsequent experiments were carried out using foamed acrylate amide tubes made impervious to bile by the addition of a very thin inner lining of the terpolymer in sheet form.

One stage operations using the new prosthesis were performed in 9 dogs inserting the distal end of the prosthesis into the duodenum. Normal serum bilirubins were maintained for 2 to 12 weeks, but chemical or clinical jaundice eventually developed in all animals. They died or were sacrificed from 80 to 160 days after operation, and in every case the

prosthesis had been passed through the intestinal tract. In most cases the tunnel occupied by the prosthesis was still partially or widely patent, but severe cholangitis was always present in spite of the fact that cholecystectomies had been performed in these animals.

Two stage procedures were carried out in eight dogs. At the first stage the prosthesis was laid in a subserosal tunnel that extended from the porta hepatis down the duodenum, and the gallbladder was removed. Two months later the proximal duct was anastomosed to the end of the prosthesis lying beside it. The duodenum was opened opposite the other end of the prosthesis, and mucosa overlying the tube was trimmed away until a good flow of bile was obtained through it. Six dogs died from 2 to 5 months after operation, with findings essentially the same as before. Two dogs were still alive, with normal bilirubins 5 and 6 months after operation.

In a final series of seven animals cholecystectomies were performed two to three months before insertion of the prosthesis. At re-exploration the common bile ducts had enlarged sufficiently to allow insertion of the prosthesis into each end of the divided duct. A forked polyethylene catheter was led from each hepatic duct through the common bile duct, the prosthesis, the sphincter of Oddi, and out through the duodenal and abdominal walls. The catheter was fixed in the terpolymer tube and the tube in the bile duct by means of a simple ligature around each end of the duct. Thus a perfectly tight anastomosis was obtained. The polyethylene tubes were removed three weeks after operation. All dogs had normal serum bilirubins up to three months after operation, and are being retained for long follow-up.

In all animals sacrificed or autopsied, careful dissections were made of duodenum and biliary tract, photographs were obtained, and important tissues preserved.

While it is still too early for final evaluation of the last experiment, it may be concluded that acrylate amide prostheses of the pore size used are not suitable where the foam structure may be exposed to bile or intestinal juice. Film-lined prostheses may have some usefulness when both ends of the divided duct can be located.

Radioisotopic Delineation of Third Degree vs. Non-Third Degree Burns.
Using rabbits, a device has been developed which permits the infliction of a graduated thermal burn — full thickness at one end, partial thickness at the other. At some intermediate point lies a more or less well-defined interface between full and partial thickness burn. This burn, which represents the clinical problem in miniature, was studied as follows: Color and infrared photographs were taken; the burns were tested with pin-prick; radioactive elements were injected intravenously and their uptake in various areas of the burn measured by means of a modified Geiger probe.

Silver, sodium and arsenic, though having a predilection for skin and hair, did not produce measurably different uptakes even between the two extremities of the burn. It was concluded that their gamma emissions are too penetrating to permit accurate localization without elaborate

collimation of the scanning probe. Radioactive thallium 204, which has a marked predilection for hair follicles and yields excellent autoradiographs of these structures, has such a soft beta ray that its emission even over normal skin could not be detected by the scanning probe. Radioactive phosphorus yielded some differentiation, and was selected for use in pigs, whose skin more closely resembles human skin. Infrared photography was disappointing in the majority of cases, in spite of the very thin epithelium of rabbits.

In 14 pigs 91 burns were studied. Radioactive phosphorus 32 is taken up by partial thickness burn in greater concentration than by normal skin, while deeply burned skin shows less radioactivity than normal. A sharp point of demarcation is found in the graduated burn, but precise evaluation of the true depth of burn at this point has not yet been determined histologically: The problem has proved insurmountable by recommended histological methods. The modified Van Gieson method of Hinshaw and Pierce, for example, has on occasion showed viable epidermis overlying dead dermis in burns caused by externally applied heat.

Splenic Homotransplant after Whole Body Radiation. Transplantation of the intact spleen by vascular anastomosis has never been attempted as a means of radiation protection, and has apparently not been considered as a prelude to the transplantation of other tissues and organs.

In order to demonstrate various phenomena, experiments were carried out as follows:

a) The radioprotective effect of normal spleen compared with that of spleen containing autologous bone marrow. After 800 r total body irradiation, twelve normal dogs died within five to nine days. Nine animals whose spleens were exteriorized and shielded by lead during total body irradiation did not survive longer. Twelve animals whose spleens contained their own marrow survived indefinitely (up to 240 days) in 80% of cases. In these, the dogs were prepared by introduction into their spleens of splinters of their own ribs. Six weeks after this procedure the rib sections had "taken" in the spleen, and their cancellous bone contained active marrow. At intervals after total body radiation splenic biopsies were performed and complete blood counts obtained. It was found in most cases that the hemograms of marrow dogs showed the same depression of white cell, red cell, reticulocyte and platelet counts as the controls. At seven to fourteen days after radiation (when the controls had already died), the peripheral blood counts of marrow dogs began a rapid return to normal. From these data one may postulate the liberation by intact marrow of some factor that permits survival of an irradiated animal without affecting its peripheral blood cell counts in the critical period.

b) The feasibility of autotransplantation of the spleen. In thirteen animals the spleen was resected and replaced by vascular anastomosis, with failure in all but two. Technics of small vessel anastomosis, including a method of dilating spastic vessels, were gradually developed, and in ten additional animals success was obtained in all but two. Surviving spleens appeared grossly normal from one to 51 days after replantation.

c) Histological changes occurring in normal spleen after homotransplantation to a normal host dog. Transplantation in ten pairs of dogs was successful in eight cases as evidenced by arterial bleeding at the time of biopsy one to six days later. Preliminary review of the histological material indicates marked follicular activity from two to four days after operation, followed by disseminated round cell infiltration up to the time of rejection six to ten days later. It is felt that the follicular reaction represents a graft versus host response, while round cell infiltration is the usual expression of rejection of foreign tissue by an homologous host.

d) Histological changes occurring in spleen during rejection by a normal animal when the spleen has been rendered incapable of reacting against its host. Hume et al. showed absence of rejection phenomena in dogs after doses of total body radiation between 1000 r and 1500 r. In this experiment, twelve dogs were given 1500 r from a 2.2 Mev source, and their spleens then transplanted into normal dogs. In eight animals the spleen was dead when first inspected. In the remaining four, biopsies were performed one to eight days after operation. Grossly, follicular hyperplasia was absent. Rejection was accompanied by swelling of the spleen without various congestion, loss of architectural features, and decreased vigor of arterial bleeding on cut section despite abundant blood flow through the splenic arteries. One wonders if the high incidence of early rejection in this series might not connote destruction by irradiation of some defense mechanism normally present in spleen.

e) Histological changes occurring in normal spleen after transplantation into a dog incapable of reacting against the graft. Thirteen dogs were given 1200 to 1500 r and on the following day underwent splenectomy and implantation of a normal spleen. At sacrifice one to three days later spleens were viable in four of five animals. The remaining eight animals failed to survive beyond four days. Grossly, their transplanted spleens appeared normal at autopsy, but because of postmortem autolysis tissue specimens were unsatisfactory.

f) The radioprotective effect of normal spleen transplanted to an irradiated host. In three dogs normal spleens were transplanted to irradiated dogs that had received 500, 775 and 1000 r total body x-irradiation. In no case was survival of a recipient animal more prolonged than that of a control animal irradiated simultaneously. At autopsy twelve, nine and seven days after transplantation the spleens and anastomoses appeared in excellent condition grossly, although microscopic autolysis prevented histologic interpretation. Hematologic follow-up on these dogs showed no advantage of the transplanted dogs over their controls.

g) The radioprotective effect of marrow-containing spleen transplanted into an irradiated host. The previous experiments demonstrate (1) the protective effect of shielded marrow-containing spleen after high doses of total body radiation, (2) the technical feasibility of spleen transplantation and (3) the failure of irradiated animals to reject homologous spleens.

In anticipation of eventual success, donor and recipient animals were so chosen and prepared as to permit demonstration of the transfer to the

recipient of immunological properties and blood cell characteristics of the donor. In collaboration with the Dept. of Immunology and Immunohematology donor and recipient animals were screened for blood type so that in each case the erythrocytes of the recipient contained the weak D-antigen, while those of the donor lacked it. This difference is not attended by incompatibility even by indirect Coombs test, but permits identification by Ashby counts of D negative cells should they appear in the recipient's blood. Female dogs were used as donors and males as recipients so that donor neutrophils might be distinguished from those of the recipient. Donor and recipient animals were immunized against tetanus toxoid and a diphtheria-typhoid mixture respectively and their antibody titers followed at intervals. The appearance in a recipient of tetanus antibodies, or an anamnestic response to a first injection of tetanus toxoid would have been of great interest, particularly if the response to diphtheria-typhoid were found impaired following irradiation.

In seven pairs of animals so prepared splenic vein thrombosis occurred in all but one. The one animal whose splenic vessels were patent at the time of death (nine days after transplantation) was subjected to splenic biopsy at five and seven days. Histological sections showed marked hyperplasia of transplanted marrow at five days, and at seven days splenic pulp also had become the site of active hematopoiesis. Peripheral blood examination showed doubling of white cell and platelet counts between the fifth and eighth days. It is felt that the trauma of repeated general anesthesia, laparotomy, and blood loss may well have contributed to the demise of this animal. One may conjecture that the active production of marrow factors by the donor spleen might account for the high rate of venous thrombosis in the ribbed spleens compared with transplanted normal spleens.

In an attempt to minimize the effect of this factor, five more pairs of animals were prepared. In these cases the transplants were performed using 6/0 silk for the arteries, as before, but the veins were joined by means of carefully machined, very thin stainless steel cuffs. No thromboses occurred in this group but the animals died at 5, 6, 7, 9 and 9 days post-operatively. Platelet, reticulocyte and/or WBC counts had risen from their low points in two of the animals before death. Biopsies were not performed, and postmortem material was unsatisfactory for histological preparations, Ashby counts showed no increase in D negative erythrocytes in the short follow-up possible, and no attempt at re-immunization was made before death of the animals.

It is not known how the shielded marrow-containing spleen was able to protect the totally irradiated dog: peripheral blood cell depression was unaffected during the period when control animals were dying a presumably "hematologic" death with equally depressed blood counts. Nor is it yet understood why the marrow-spleen was unable to protect an irradiated homologous recipient after technical obstacles were overcome.

Summary and Conclusions:

Arterial Prosthesis. (a) The abdominal aortic grafts were subjected to a more stressing test from the hemodynamic point of view with two end-to-end suture lines within 5-7 cm. of each other and a tube of 8 mm. inner

diameter. This resulted in failures of the composite graft of 37 C.P.I., a graft which, when placed in the thoraco-abdominal aorta with proximal end-to-end anastomosis and a distal end-to-side anastomosis and with a 12 mm inner diameter tube, yielded excellent results.

Only abdominal aortic grafts will be tested.

(b) A tighter knit of dacron will be used than the 37 C.P.I. Since 56-76 C.P.I. were successful in pilot experiments and since we feel that the loosest knit dacron consistent with sustained strength of graft is desirable, a series of 60 C.P.I. has been initiated.

(c) Longer term animals with 4 mm inner diameter femoral grafts will follow after decision as to the most ideal knit of the supporting dacron tube.

Arterial Dilatation to Further Small Artery Anastomosis. Up to 100 percent increase in size of spastically contracted arteries can be produced by a simple manipulation resulting in arterial dilatation. This dilatation affords increased ease and accuracy of arterial suture. Long-term patency rates in control non-dilated vessels compare favorably with those in the dilated group. However, dilatation of divided arteries does facilitate their repair and make the sutural exercise easier. It would seem that meticulous technique is of primary importance in determining patency rates after anastomoses of canine femoral arteries, since dilatation did not improve or compromise the results of suture anastomosis when compared with an undilated control group.

Common Bile Duct Prosthesis. Acrylate amide tubes of foam construction, with and without inner linings of filmed terpolymer, have been used to replace segments of the common bile duct in dogs. Encouraging results have been obtained using a 2-stage operation with film-lined tubes, combined with splinting of the hepatic ducts, common bile ducts, and prostheses over a forked polyethylene tube which is led to the exterior through the duodenum. The polyethylene tube is removed after 21 days.

Radioisotopic Delineation of Third Degree vs. Non-Third Degree Burns. Effort has been directed toward a reliable and practical method for estimating the depth of cutaneous burns. Of the substances studied, the radioactive phosphorus by virtue of its differential uptake has provided the sharpest distinction between full-thickness and partial-thickness burns. Histologic proof of the validity of this method has, however, not been possible.

Splenic Homotransplant after whole Body Radiation. A study has been conducted aimed at evaluating the possible radioprotective and immunologic potential of the transplanted canine spleen. Results have been exceedingly interesting from an immunologic point of view; however, long-term survival of irradiated recipients has not been achieved.

List of Publications:

1. Hedberg, S. E.: Suture Anastomosis of Small Vessels Following Relief of Spasm by Hydrostatic Pressure Dilatation. To be published.

APR 10 1958
39

ANNUAL PROGRESS REPORT

Project 6X59-01-001, Traumatic Surgery and Shock

Task 24, Experimental Orthopedics

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Department of Experimental Surgery
Division of Surgery

Period Covered by Report: 1 July 1960 through 30 June 1961

Principal Investigator: Capt Austin D. Potenza, MC

Reports Control Symbol: MEDDH-288

Security Classification: Unclassified

ABSTRACT

Project No. 6X59-01-001 Title: Traumatic Surgery and Shock

Task No. 24 Title: Experimental Orthopedics

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Period Covered by Report: 1 July 1960 through 30 June 1961

Author: Capt Austin D. Potenza, MC

Reports Control Symbol: MEDDH-288

Security Classification: Unclassified

Tendon Study. Poor functional results often follow repair of long flexor tendons within flexor digital sheaths. No comprehensive study of the processes of tendon healing within flexor digital sheaths has been reported; it was essential, therefore, to undertake such a study in order to evaluate the factors that influence functional results after tendon surgery. Dogs were used, and a standard tendon wound was designed. The results have been uniformly consistent, and reproducible: tendon is united by granulation tissue produced by the sheath and surrounding tissues; tendon takes no active role in this effort. Adhesions form at the repair line and at each point at which the surface of the tendon is disturbed. The adhesions are filmy and loose after 28 days of immobilization.

Plastic Fixation of Bone. Evaluation of polyurethane foam for the internal fixation of fractures. During the past year two phases of this experiment were performed:

In the first phase 14 dogs have been operated upon, segments of ribs on either side of the chest were resected and the defect filled with polyurethane foam to determine if PUF allows new bone to grow into the plastic and replace the plastic.

In the second phase radial defects were created in 15 dogs. Polyurethane wafers were inserted into the right radial defects. Nothing was inserted into the left radial defects. The dogs were sacrificed at varying periods up to 9 months.

These experiments showed that polyurethane foam acts as a barrier to osteogenic cells and actually delays or inhibits bony union. Furthermore, it does not form a true bond to bone and is ultimately surrounded by a dense fibrous tissue wall in which late metaplastic changes have been noted.

BODY OF REPORT

Project No. 6X59-01-001

Title: Traumatic Surgery and Shock

Task No. 24

Title: Experimental Orthopedics

Description:

Tendon Study. Many of the casualties of World War II and the Korean War who required prolonged hospitalization had serious hand injuries, necessitating multiple surgical procedures. This problem became so serious that hand centers were established at various Army hospitals; as a result of this program, hand surgery has evolved as a specialty in its own right. However, clinical results following injury to the flexor digital tendons in the fingers are far from ideal; this has stimulated research concerned with the use of blocking membranes and tubes to prevent adhesions to tendons as well as several more basic studies. In spite of many new studies reported in recent years, controversy as to the exact nature of tendon healing persists. Clinically, satisfactory functional results are most difficult to achieve after injury to the flexor digital tendons within the fingers. Here the flexor tendons are unique in that they are inclosed in a lubricated synovial sheath and are held close to the underlying bone by inelastic condensations of the sheath. In spite of this, however, most previous investigations of tendon healing have been conducted on canine carpal flexor or extensor tendons, and rabbit tendo achillis. These do not present the anatomic peculiarities seen in the fingers.

Plastic Fixation of Bone. Polyurethane foam has been used clinically to treat certain fractures of long bones by various civilian practitioners. Its use in human patients has been highly controversial because little was known about the reaction of polyurethane foam in the body. It was hoped that polyurethane foam would be of use in reestablishing bony continuity in the shafts of long bones where large bony defects existed and that the plastic might act as an absorbable trellis through which host cells might grow, laying down new bone and leading to the eventual reestablishment of true bony continuity.

Progress:

Tendon Study. If we are ever to improve the functional results following tendon surgery on the hand we must first have a basic knowledge of the processes of tendon healing and the factors which affect and influence them. Accordingly, this study was undertaken. After designing a standard tendon wound which permitted atraumatic repair of the divided tendon, 100 canine flexor digitorum profundus tendons were divided and atraumatically repaired within the flexor digital sheath of the forepaw. In this location the flexor tendon mechanisms of the canine digits are comparable to those of human fingers. Tendons were repaired by means of a standard Bunnel pull-out suture using No. 36 stainless steel wire. After surgery sterile fluff compression dressings and long-leg reinforced casts were applied to the operated extremities. Animals were sacrificed at varying times from 1 through 128 days postoperatively. In a second and third phase

of this study the area of repair of the divided tendon was covered in one series with polyethylene tubing and in a second series with nylon-reinforced Millipore tubing.

Examination of histologic material as well as gross examination at time of sacrifice revealed that a digital flexor tendon which has been divided within the flexor digital sheath heals by means of the cellular activity of the sheath and the surrounding tissues. The cells of the tendon proper play no active role in repair of the tendon. Furthermore, examination of numerous control tendons reveals that there is no epitendinous layer of cells on the tendon that takes part in the healing process. Early anatomic studies failed to reveal the presence of such a layer on normal untraumatized tendons; this series of experiments further substantiated those earlier findings.

In the healing process, the synovial layer of the sheath disrupts and allows the formation of granulation tissue with its subsequent growth between ends and into suture tracts in the tendon. Disruption of the synovial layer of the sheath in the vicinity of the tendon wound is complete by the 4th or 5th postoperative day. Later, with advanced formation of granulation tissue from the sheath, the synovial layer is gradually restored; this begins at approximately the 14th postoperative day and is complete by the 21st postoperative day except for persistent filmy adhesions at the line of division.

Granulation tissue (capillary buds and fibroblasts) begins to form in the disrupted digital sheath on the 4th postoperative day; this is preceded by proliferation and then disruption of the synovial cell layer. This granulation tissue ultimately grows between tendon ends and into suture tracts in the tendon. Fine, young collagen fibers appear in this granulation tissue on the 7th postoperative day, and by the 10th postoperative day the granulation tissue has pronounced fibroblastic character. The fibroblasts grow between tendon stumps in a plane perpendicular to the longitudinal axis of the tendon, and the collagen fibers they lay down are similarly oriented. In contrast, collagen fibers laid down in granulation tissue on the surface of the tendon are parallel to that surface. By the 21st postoperative day the fibroblasts in the scar between tendon ends begin to orient themselves in line with the longitudinal axis of the tendon; by the 28th postoperative day this process is complete so that all fibroblasts and new collagen fibers are aligned with the long axis of the tendon.

New collagen fibers in the tendon scars remain as separate, though parallel fibers until the 90th postoperative day when there is evidence of early collagen bundle formation; this progresses so that, by the 112th postoperative day, collagen in the former scar between tendon ends is in the form of bundles, identical microscopically with those of the original tendon. Although there ultimately is complete maturation of collagen in the tissue between tendon ends, the collagen and the persistent filmy adhesions attached to the line of repair never become prominent; they remain sparse, delicate and loose and do not interfere with the functional integrity of the tendon.

Thus, tendon is quite passive in the process of its healing; the sheath and sheath tissues are the active healing agents.

These results were further confirmed in the studies in which the area of tendon repair was surrounded by first, polyethylene tubing, and secondly, nylon-reinforced Millipore tubing. It was shown conclusively that in order for the tendons to heal, granulation tissue from the sheath first had to grow beneath either edge of the tubing and along the surface of the tendon to the line of division. Only then did repair of the tendon by the granulation tissue begin. Thus the use of blocking membranes about an area of tendon repair is contraindicated and is, moreover, a procedure based upon ignorance of the true processes of tendon healing. Such techniques only delay tendon healing and result in thickening of the tendon surface by the proliferating granulation tissue beneath the tubing. Moreover, at the conclusion of tendon repair, instead of having the delicate filmy adhesions directly at the repair site which one would normally expect, one finds instead thick dense adhesions at either end of the tubing.

This study of normal tendon healing is continuing and we are now evaluating the role of the peritendinous tissues in tendon healing, the role of the vincula, and the role of sublimis excision upon healing of the profundus tendon. In addition we are attempting to quantitate trauma and its effects upon the physiology and healing of tendons. Only after these studies are complete can we design a critical method of evaluation of present techniques used in tendon and hand surgery in the hope of improving clinical functional results.

Plastic Fixation of Bone. The experiments conducted consisted of two parts: In the first part 14 dogs were operated upon. Four ribs on either side of the chest were exposed and a 3/8 inch segment was removed from each rib and treated as follows: (1) In the first rib the segment was replaced and the periosteum was closed. (2) In the second rib the periosteum was closed. (3) In the third rib a 3/8 inch segment of polyurethane foam was placed in the defect and the periosteum was closed. (4) In the fourth rib an osteoperiosteal segment was removed. All ribs were removed according to schedule at periods ranging from 5 days through 24 months. All material collected was to be examined microscopically to determine if true bone grows into and replaces the polyurethane foam and if so the role of the periosteum in the process.

In the second phase of this experiment 15 dogs were used to determine whether or not polyurethane foam acts as a barrier to the normal healing of fractured bones. A complete 1/8 inch transverse segment was removed from both radii. A one-inch thick polyurethane wafer was then placed in the defects in the right radii. The left radial defects were untreated. The dogs were sacrificed at periods ranging from 4 days to 24 months post-operatively.

The results of these studies indicate that polyurethane foam in living bone acts as a barrier to osteogenic cells and both inhibits and delays bone union. The polyurethane foam is eventually walled off from the host bed

by a dense wall of collagenized fibrous tissue. Moreover, in one of the 9 month specimens definite metaplastic and proliferative cellular changes were seen in this fibrous wall so that nests of large bizarre cartilage cells, many with double nuclei were produced. This would tend to strengthen the suspicion that polyurethane foam is carcinogenic. Thus, it would appear that polyurethane foam has no useful purpose in human surgery since it acts as a barrier to osteogenic cells, delays bony union, and leads to possible dangerous metaplastic changes in the surrounding tissue.

Summary and Conclusions:

Tendon Study. A detailed analysis of the processes of tendon healing within the canine flexor digital sheath following aseptic division and atraumatic repair has been performed. Only the sheath tissues provide the active healing elements in the healing of a flexor digital tendon within its sheath. Tendon is united by scar produced by fibroblasts derived from the sheath. These fibroblasts lay down collagen which is indistinguishable from that of the tendon proper by 128 days. Microscopically, wounds before 28 days appear too immature for active mobilization. By 35 days they look as though they would offer satisfactory functional results. Nutrition of the fibroblasts from the sheath and the scar they produce may be supported by the persistent loose filmy adhesions between the sheath and tendon scar. There is no evidence that these interfere with the function of the healed tendon. The use of artificial blocking membranes around a tendon repair site in the hope of reducing adhesions is contraindicated because this idea is based upon an erroneous concept of tendon healing.

Plastic Fixation of Bone. Polyurethane foam when foamed in living bone does not form a bond with the bone. The ultimate fate of polyurethane in the body -- its metabolic pathways or its storage sites, if any -- is unknown. When placed in bony defects, the plastic interferes with the normal healing process. This results in non-union. The plastic initially induces a fibroangiomatic reaction in the tissue around it and ultimately is encased by a dense fibrous wall in which metaplastic changes are apparent. Accordingly, it cannot be said that polyurethane foam has any favorable effect on fracture healing.

List of Publications:

1. Potenza, A. D.: An Experimental Model and Technique for the Study of Tendon Repair. To be published.
2. Potenza, A. D.: Tendon Healing Within the Flexor Digital Sheath: An Experimental Study. I. Normal Healing Processes. To be published. Presented at the Meeting of the American Orthopaedic Association, Yosemite, California, 22-25 May 1961.
3. Potenza, A. D.: Progress Report on the Use of Polyurethane Foam in the Treatment of Fractures. Presented at the Regional Meeting of the American College of Surgeons, Philadelphia, Pa., 6 March 1961.

ANNUAL PROGRESS REPORT

Project 6X59-01-001 Traumatic Surgery and Shock

Task 27 Resistance to shock

**Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.**

**Department of Surgical Metabolism and Physiology,
Division of Surgery**

Period Covered by Report: 1 Jul 60 - 30 Jun 61

Principal Investigators: Albert Einheber, Ph. D., G. James Cerilli, Capt, MC,

Assistant: PFC Robert E. Wren

Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

ABSTRACT

Project No. 6X59-01-001 Traumatic Surgery and Shock

Task No. 27 Resistance to shock

**Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.**

Period Covered by Report: 1 Jul 60 - 30 Jun 61

Authors: A. Einheber, Ph. D., G. J. Cerilli, Capt, MC, and PFC R. E. Wren

Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

Studies to clarify the pathogenesis of irreversible shock and the mechanisms underlying natural and acquired resistance to shock are continuing. The course of hemorrhagic shock of African green monkeys is under study because this animal is more closely related phylogenetically to man than the usual experimental animals (rats, dogs, mice, guinea pigs, rabbits) used. We are also investigating the effects of prior adaptation to water intoxication or potassium toxicity on the resistance of such animals to shock. The rationale of these studies is detailed in the report. Also under investigation is the physiological significance of some of the biochemical changes which occur after injury and the possible effect of Circadian rhythms on the responses of animals to food deprivation and injury.

BODY OF REPORT

Project No. 6X59-01-001 Traumatic Surgery and Shock.

Task No. 27 Resistance to shock

Description: Studies to clarify the pathogenesis of irreversible shock and the mechanisms underlying natural and acquired resistance to shock are continuing. The course of hemorrhagic shock of African green monkeys is under study because this animal is more closely related phylogenetically to man than the usual experimental animals (rats, dogs, mice, guinea pigs, and rabbits) used. We are also investigating the effects of prior adaptation to water intoxication or potassium toxicity on the resistance of such animals to shock. The rationale of these studies is detailed in the report. Also under investigation is the physiological significance of some of the biochemical changes which occur after injury and the possible effect of Circadian rhythms on the responses of animals to food deprivation and injury.

Progress: Hemorrhagic Shock of African green monkeys: The pathogenesis of irreversible shock continues unsolved. Because the African green monkey is closely related phylogenetically to man, study of hemorrhagic shock in this primate was undertaken. No antibiotics were given prior to experimentation. Typical *E. coli* organisms were present in the stools. All were on a weighed diet of bananas and oranges. The femoral artery was isolated and cannulated under light intravenous sodium pentothal and local procaine. Twelve successful experiments using our modification of the Lamson elevated reservoir technic have been performed. Hypotension at 35 mm Hg for periods exceeding 7 hours led to irreversible shock in 2 monkeys, while 3 out of 4 monkeys hypotensive for 7 hours at 35 recovered. None of the 6 animals subjected to shorter periods at 35 mm Hg or higher died. There were no essential differences in pre-operative blood pressure (avg. 120), pulse (range 90-200), respiration (range 14-40) among the survivors and the irreversibly shocked monkeys. The irreversibly shocked monkeys had an average maximum bleed-out volume of 3.9% of the body weight and had taken up 21 % of the shed blood at reinfusion. The reversibly shocked animals shed 3.1% of their body weight and had taken up 17% of the shed blood at reinfusion. A progressive decline in rectal temperature of 5-6° C, a maximum fall of 34% in hematocrit and a marked rise in serum lactic acid dehydrogenase accompanied the hypotension with the values in the survivors and the irreversible animals being similar in magnitude. Most animals were remarkably alert during these prolonged periods of oligemic hypotension and have exhibited a high degree of resistance to low blood pressure. It was not possible to correlate the clinical condition of the monkeys during shock or their response to reinfusion with the subsequent outcome. No animals exhibited bloody diarrhea. Autopsy of the 2 animals dying of shock revealed only scattered pin-head petechiae in the colon and subendocardial hemorrhage.

Water Intoxication and Resistance to Shock. It is well established that animals can develop a tolerance for ordinarily lethal amounts of trauma, endotoxins, oxygen lack, drugs, etc., by being subjected in advance to repeated sublethal amounts of such noxae; tolerance for one damaging stimulus may also

confer a cross-tolerance for others. This has recently been extensively studied in relation to shock induced by several means (Zweifach).

In the search for the biological mechanisms which influence the fate of the injured animal and the convalescence of its damaged tissues and organs, many diverse endogenous agencies such as vasoactive peptides and amines, hormones, proteolytic enzymes, blood coagulation factors, fluid and electrolyte shifts, and pH changes, have been considered. It occurred to us that one of the most ubiquitous, chemically simple and abundant constituents of living matter, water, is toxic when present in the body in excessive amounts. This well known phenomenon of water intoxication has been studied experimentally, but the mechanism of death is incompletely known. Of special interest to us is the fact that resistance to the toxic and lethal effects of excess water given orally can be rapidly induced in rats by subjecting them in advance to repeated, progressively increasing doses of water (Liling and Gaunt). While the induced water tolerance is importantly related to an augmented diuresis, tolerant rats may retain amounts of water ordinarily toxic for non-conditioned normal animals (Liling and Gaunt).

One of our chief research aims is to explore and devise ways of increasing "native" resistance of animals to otherwise lethal episodes of shock from hemorrhage and other forms of physical injury such as burns, muscle and intestinal ischemia, x-irradiation, etc., and to determine what the inherent protective modalities are. With these objectives in mind, we have begun an extensive program of studies to evaluate the effect of oral and parenteral doses of water and hypertonic solutions on the local and systemic responses of animals injured by burns, hemorrhage, tourniquets and endotoxin. We are especially interested in determining whether we can increase the local and systemic resistance of animals to injury by single or repeated doses of water or hypertonic solutions, or by other fluid regimens we may empirically establish. When we delineate clearly what the favorable or adverse effects of the various treatments are, we would like to learn what these are due to.

Hypotonia from overhydration may cause many changes, among which we recognize the following as noteworthy: 1) cytolysis, e.g., of mast cells, with release of heparin, serotonin, histamine, proteases, peptidases, etc., 2) redistribution or depletion of electrolytes, 3) non-specific augmentation of hormone release, characteristic of stress situations, 4) renal functional changes, 5) alteration of central nervous functions, 6) hemoglobinemia and 7) hypothermia. All of these are recognizable as possible sequelae of trauma.

The first experiments involved the intraperitoneal or intramuscular injection of water (volumes equal to 5% to 30% body weight) into mice and rats followed by time-variable or dose-variable tests of resistance by further doses of water or by an experimental burn. We gave water intraperitoneally rather than orally because from what is known of such treatment (Karady, et al.) it seemed to us that there was likely to be more direct cell "damage", e. g., lysis and/or degranulation of mesenteric mast cells, and a more rapid absorption of the water. Furthermore, that increased resistance to ordinarily toxic doses of water administered intraperitoneally can be induced in mice or rats has not to our knowledge been demonstrated.

We found that the majority of deaths of mice injected with water occurred within, and usually before, 3 hours after injection. Survival for 24 hours usually meant permanent survival. The severity of the illness of the water-treated mice generally correlated with the volume of water given. In contrast, mice that concurrently received up to 30% of their body weight as isotonic saline showed virtually no ill effects, remained active, and did not die.

There were large quantities of straw colored fluid in the peritoneal cavity of mice that died after the injection of water. Neither the amount or composition has yet been studied. We intend to do further tests in which its amount is measured, its chemical make-up determined and its biological activity tested in suitable subjects.

Seven experiments in mice and three experiments in rats were performed to attempt to induce either increased water tolerance (mice and rats) or tolerance for a standardized hot water burn (mice). The water-priming regimens so far tested have failed to provide clear-cut evidence of protection.

Potassium, Shock and Endotoxin: Tissue injury, prolonged oligemic hypotension, and anoxic states result in the release of potassium from the tissues into the blood; renal shut-down incident to the traumatic shock state magnifies the blood picture. Hyperkalemia consequent to the tissue injury and erythrocytolysis of burns is also a common finding. Invariably accompanying post-traumatic potassium liberation are fluid and sodium losses, which combination of effects may alone prove fatal if uncorrected. We have shown that a non-mammal, the 10-day old chick, has a plasma potassium level twice the control value 1-3 hours after release of 3-hour bilateral leg tourniquets. It has been demonstrated that tourniquet-injured mice and rabbits are highly susceptible to the lethal effects of administered potassium, e.g., in orange juice per os. We and others have also shown that tourniquet-injured mice and rabbits are also highly susceptible to administered Gram-negative bacterial endotoxin; this is also true for the rabbit after reversible hemorrhagic shock. Some investigators have even suggested a causative role of elevated potassium levels in death from shock.

The latter observations have suggested to us that while there may be a nonspecific enhancement of sensitivity to certain toxic agents following shock, a special relationship between the expected post-traumatic potassium shifts and enhanced sensitivity to administered endotoxin may exist, i.e., that the oft demonstrated increase in sensitivity to endotoxin after shock is somehow related to the concomitant potassium alterations. The increased sensitivity to endotoxin after injury may not be related to the actions of potassium per se alone, but possibly also to other metabolic responses of the animal to injury as might be reflected in that fraction of post-injury potassium change resulting from altered carbohydrate metabolism, as well as to that fraction liberated by direct tissue injury.

While it is known that endotoxin stimulates the pituitary-adrenocortical and sympatho-adrenomedullary systems, both of which influence the rate of carbohydrate metabolism, it also markedly lowers serum calcium (rabbits) which might influence capillary permeability, vascular smooth muscle and cardiac

excitatory activity; the latter could be especially important because the plasma calcium/potassium ratio which may already be depressed after injury might be further critically reduced by administered endotoxin. We have accordingly begun a study of the possible interaction between potassium shifts after injury and administered endotoxin. Our first objective was to establish toxicity levels of KCl in mice which would allow us to study the effects, e.g., of combined administration of sublethal amounts of potassium and endotoxin to see if a potentiation or synergism of toxic effects will be demonstrable. We also plan to study the possibility of inducing tolerance in mice for otherwise lethal doses of KCl by repeated sublethal injections and, if successful, to see if this provides a cross-tolerance for otherwise lethal amounts of tourniquet injury, scalds and endotoxin. Injecting 0.01 ml/g body weight of 7.5% KCl intraperitoneally resulted in a 94% 24 hour mortality; the same dosage of 5.5% KCl or of lesser concentrations resulted in no deaths. The estimated LD₅₀ is 0.65 mg/g body weight intraperitoneally or 0.01 ml/g body weight of a 6.5% KCl solution. This is similar to that reported earlier by Tabor and Rosenthal.

Effect of Circadian Rhythms on Resistance to Starvation and Shock: That periodic changes (diurnal variations, Circadian rhythms, etc.) in the physiologic state of the animal may influence the organism's tolerance for damaging agents, e.g. endotoxin, has been extensively studied and reviewed by Halberg (Halberg, F. The 24-Hour Scale: A time dimension of adaptive functional organization, Perspectives in Biol. and Med., III: 491, 1960). This work has emphasized the need for the standardization of light-dark periods to which animals are exposed for reliable routine experimentation and also that its controlled manipulation may be useful. Starvation increases the susceptibility of guinea pigs to fatal enteric infection with Shigella flexneri organisms and to the lethal effects of endotoxin (Formal, S.B., Noyes, H. E., and Schneider, H.) Experimental Shigella Infections III. Sensitivity of Normal, Starved and Carbon Tetrachloride Treated Guinea Pigs to Endotoxin, Proc. Soc. Exp. Biol. Med., 103, 415, 1960). Furthermore, the reticulo-endothelial system phagocytic function of rabbits is depressed by 4 days of starvation; this suggests that the decreased resistance to infection of starved animals may be related to this defect (Juhlin, L. The effect of starvation on the phagocytic activity of the RES, Acta physiol. Scand., 43: 262, 1958). Unfortunately, the experimental dark-light schedule was not specified nor was it stated whether it was or was not rigidly controlled. It would seem that this is important since "... rabbits starved in the dark survived longer than those starved in the day-light." (Mawatari, T. An experimental study of starvation, Igaku Kekyu, 28: 4566-4592, 1958, Abstract from Biol. Abstracts, 36: 841, 1961). We have begun studies on the effect of continuous darkness versus continuous light on the survival of conventional mice allowed no food or water. Since motor activity and feeding of mice and rats is greatest at night, we hypothesized that the presumably greater activity of dark-exposed animals should predispose them to shorter survival than the light-exposed mice.

Two preliminary experiments thus far show that, as expected, mice continuously exposed to darkness die sooner of water and food deprivation than their concurrent partner mice continuously exposed to light.

These observations have been undertaken in part because of the fact that the food and water intake of most animals subjected to experimental shock declines sharply. In fact, for small animals what we see are the combined effects of the circulatory failure and the accompanying acute food deprivation.

Biochemical Changes following Hemorrhagic Shock of Rats. It is recognized that serious biochemical changes may follow severe shock. Our projected studies of the tyrosine oxidizing system of the liver with McElroy and the plasma free amino acids of rats in reversible and irreversible hemorrhagic shock with Levenson and Rosen require standardized procedures for consistently producing these states of shock in rats. We have, therefore, devised and assembled a simple automatic blood pressure stabilizing device for reproducibly accomplishing hemorrhage and the maintenance of any desired level of post-hemorrhagic hypotension; this apparatus has definite advantages over previous types described. Our experience to date shows that the adult rat bled to 35 mm Hg mean arterial pressure survives if the shed blood is reinfused after 2 hours while death from shock results if reinfusion is delayed until 5 hours. The biochemical studies are just getting underway.

Summary and Conclusions: Studies to clarify the pathogenesis of irreversible shock and the mechanisms underlying natural and acquired resistance to shock are continuing. The course of hemorrhagic shock of African green monkeys is under study because this animal is more closely related phylogenetically to man than the usual experimental animals (rats, dogs, mice, guinea pigs, rabbits) used. We are also investigating the effects of prior adaptation to water intoxication or potassium toxicity on the resistance of such animals to shock. The rationale of these studies is detailed in the report. Also under investigation is the physiological significance of some of the biochemical changes which occur after injury and the possible effect of Circadian rhythms on the responses of animals to food deprivation and injury.

ANNUAL PROGRESS REPORT

Project No. 6X59-01-001

Title Traumatic Surgery and Shock

Task No. 33

Title Experimental Anesthesia

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Department of Resuscitation
Division of Surgery

Period Covered by Report: 1 July 60 - 30 June 61

Principal Investigator: Major Timothy G. Barila, MC

Assistants: Capt. Richard R. Ritter, MC*, Capt. James A. Moyer, MC*,
Capt. Berly E. Bridges, Jr., MC* and Capt. John T. Whitley, USAF(MC)*

Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

* Resident in Anesthesiology, Walter Reed General Hospital

ABSTRACT

Project No. 6X59-01-001

Title Traumatic Surgery and Shock

Task No. 33

Title Experimental Anesthesia

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Period Covered by Report: 1 July 60 - 30 June 61

Authors: Major Timothy G. Barila, MC, Capt. Richard R. Ritter, MC, Capt. James A. Meyer, MC, Capt. Berly E. Bridges, Jr., MC and Capt. John T. Whitley, USAF (MC)

Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

Blood pump. A pump capable of propelling blood for extended periods without excessive damage to blood is described. A unique feature, simplifying existing pump designs, is the power system which utilizes interacting fluid techniques, eliminating moving parts, except for the ventricle and the valves. This minimized motive power permits a servo action to be utilized to equalize volumetric flows between two pumps operating in series.

Valve design and fabrication is reported and a valve testing device has been made to observe functional characteristics during different volumetric flows and resistive pressures. A test circuit for evaluating blood-damaging properties of the pump is used in these studies.

Closed chest cardiac massage. A successful method of resuscitation after cardiac arrest has recently been described which permits cardiac massage through an intact chest. The advantages of this method in an area far removed from the asepsis of the operating room are obvious and, if further studies indicate that consistent results can be obtained, this method of closed chest cardiac massage will become a useful adjunct in our field resuscitation armamentarium. The present study is designed to observe the effectiveness of closed chest cardiac massage in maintaining adequate circulation to some of the vital organs of the dog, as well as to show a very significant survival rate with very minimal sequelae after the heart has been arrested.

BODY OF REPORT

Project No. 6X59-01-001

Title Traumatic Surgery and Shock

Task No. 33

Title Experimental Anesthesia

Description:

Blood pump. A phase of this task is to develop a pump, powered and controlled by interacting fluid techniques, that can propel blood in human circulatory systems for sustained periods without excessive amounts of hemolysis. The pump will have pulsatile flow and will have pumping characteristics closely resembling those of the human heart. It will be unique in that it will have no moving parts with the exception of artificial ventricles and valves.

The immediate objective of the program is to develop a pump that will improve the design of existing heart-lung machines by (1) simplification of design and (2) a significant reduction in the production of hemolysis to permit extended pumping operations. The opportunity for these low hemolysis rates is afforded by the fact the pump minimizes the amount of motive energy required to establish a particular volumetric flow rate, consequently the amount of turbulence generated in the blood stream is minimized. Intermediate objectives include investigations as to the feasibility of using this type of pump for left heart by-pass with autogenous oxygenation, for mechanical support of the circulation with diminished cardiac output and potentially reversible cardiac lesions, and for similar augmenting use in renal failure patients during hemodialysis. It is an ultimate future objective of the program to develop an artificial heart that can permanently replace the irreparable sick human heart.

Closed chest cardiac massage. This is a study of the effectiveness of closed chest cardiac massage. This method of cardiac resuscitation has far-reaching application for it is generally accepted that cardiac arrest due to ventricular fibrillation or asystolic standstill is not necessarily associated with severe disease in coronary arteries and muscle. If an essentially healthy heart needs only a second chance to surmount a temporary difficulty, adequate ventilation and rhythmic compression of the heart should increase the frequency of resuscitation of patients whose hearts are structurally sound. The present study is designed to observe the effectiveness of closed chest cardiac massage in maintaining adequate circulation to some of the vital organs after cardiac arrest. For this purpose the following observations were felt necessary: (1) EKG, (2) EEG, (3) Arterial blood pressure and (4) central venous pressure. The immediate objective of the study is to demonstrate the effectiveness of this form of cardiac resuscitation by showing a very significant survival rate with very minimal sequelae after the heart has been arrested.

The next step is to show by the use of more refined physiological measuring techniques that a sufficient amount of oxygenated blood is flowing to vital areas during the application of closed chest cardiac massage.

The future objective of this plan is to carry the study into a clinical environment, realizing the obvious limits of its control and application.

Progress:

Blood pump. Two pump designs have been built and initial tests have begun. It has been tentatively established that flow rates (using water) to at least 7 liters per minute (objective - 10 liters per minute) can be achieved. It has also been demonstrated that the newer design can pump against pressure resistances of at least 340 mm. Hg. (objective - 220 mm. Hg. exclusive of cannular resistances.) In both cases the measuring apparatus limited higher readings. Both designs have a variable pumping frequency and variable ventricle displacement with displacement increasing with increases in pumping frequency. The newer prototype is designed to equalize the volumetric flows between two pumps operating in series. This servo action has been demonstrated for low flows (about 2 liters per minute) but much effort is required to improve the servo action over the range of flows and resistances anticipated. The action itself is based upon the principle used by the human heart that the auricular filling pressure determines the output flow from the corresponding ventricle.

The effort on pump design to date has, therefore, demonstrated the ability of interacting fluid techniques to power and control satisfactorily a pump with no moving parts and which can be made to duplicate closely the pumping character of the heart. More effort is required to tailor the design to the requirements.

The hemolytic character of the first pump design was estimated by the Cleveland Clinic, Cleveland, Ohio, using 500 cc. of heparinized human blood. Test results showed a hemolysis index of 0.001 as compared to 0.004 for the best pump previously tested by the Clinic. Also the pump produced 1.9% blood damage of its standard as compared to 3.4% for the best previously tested pump.

A technique for making semi-lunar tri-cuspid heart valves has been developed which strives for reproducibility and lowered fabrication costs and which provides the ability to use dissimilar cusp and mount materials. The metal mandrels for mitral and aortic valves have been completed, and several sample valves have been made. Additional effort is required in this area before satisfactory operational valves are realized.

A valve testing device has been made which permits the visual observation of the functioning valve while subjected to different volumetric flows and resistive pressures.

A standard blood test circuit has been fabricated for the hemolytic evaluation of the pump. This circuit is operational and the hemolytic evaluation of the first pump design is underway. Flow and frequency characteristics were established with water, and iso-osmotic fluids and blood. Blood is being studied for rate of free hemoglobin in plasma (Crosby modified benzedine method).

Closed chest cardiac massage. With dogs used as the experimental animal, cardiac arrest in the form of ventricular fibrillation was induced. In the early experiments, dogs of variable sizes and breeds were used and closed chest cardiac massage was applied with the animal supported between sandbags. It was noted that with the onset of ventricular fibrillation the arterial pressure

rapidly fell to almost zero levels and the central venous pressure rose to a very significant level. Within a few minutes there was a marked change in the EEG pattern characterized by diminished electrical activity. Closed chest cardiac massage was then applied for variable periods of time and it was observed that 75 to 100 mm. Hg. pressure could be attained with relative ease. Because of technical problems the EEG activity could not be evaluated during the period of closed chest cardiac massage. Following defibrillation, the EKG pattern reverted to a normal rhythm and the arterial pressure rose quickly, usually to above pre-test levels. The venous pressure fell simultaneously to normal levels. In most instances the activity of the EEG resembled that of the pre-test pattern. The experiment was carried out under pentobarbital anesthesia and ventilation was supported by O₂ and, when necessary, N₂O + O₂, delivered through a standard anesthetic machine. The most favorable rhythm during application of closed chest cardiac massage seemed to be five strokes followed by a pause during which the lungs were ventilated with O₂.

Studies of cardiac arrest after anesthetic overdose and arrest due solely to anoxia were also done to a more limited extent. It was the impression that the ease of cardiac resuscitation was directly related to the speed with which closed chest cardiac massage was applied after the complete cessation of electrical activity of the heart and that re-institution of normal blood pressure was almost instantaneous when closed chest cardiac massage was applied before cessation of electrical activity of the heart even though the blood pressure had fallen down to near zero levels.

An inspection of several sacrificed dogs failed to reveal any significant damage to the bony structure or internal organs of these animals after closed chest cardiac massage. There is routinely noted some flattening of the ^ shaped chest of these animals but this has always been in our experience at the anterior costo-chondral junction and has resulted in no obvious detriment to their recovery and subsequent activity. This problem would likely be of very little significance in the human because of the favorable anatomic configuration of the human chest.

Summary and Conclusions:

Blood pump. The objectives for next year's effort are:

1. The improvement of the prototype pump design (including valve design) to encompass all of the appropriate requirements
2. The development of mathematical models of the system from which optimum design parameters can be deduced for the engineering of advanced pump designs.
3. A continued evaluation of the hemolytic characteristics of the pumps and related systems, in vitro and in vivo. Cr⁵¹ tagging of red blood cells is planned. Activity in the plasma will be an index of cell destruction.

After testing, the blood will be returned to the animal to determine influence on red cell survival. A final laboratory test will be left and right heart by-pass studies (vena cava to pulmonary artery and left auricle or ventricle to systemic artery).

4. An evaluation of the life and reliability characteristics of the ventricles and valves.

Closed chest cardiac massage is effective in cases of cardiac arrest and would appear to provide circulation adequate to maintain the heart and the central nervous system.

The intended plan for further studies involving the measurement of arterial blood flow in various vessels during closed chest cardiac massage will afford valuable information about the true hemodynamics occurring at the time. This work will be accomplished in the near future by the use of electromagnetic flow meters applied directly to the coronary, pulmonary, or carotid arteries. This will be done in a joint program with the Dept of Cardio-Respiratory Disease at the Forest Glen Laboratory.

With the recent development of mechanical equipment for applying rhythmic pressure to the chest, further comparative studies will be done to assess its value in the overall problem of cardiac resuscitation.

This program has afforded an opportunity for many personnel in the allied medical fields to learn about this technique and in some instances to become familiar with the application of closed chest cardiac massage during these experimental conditions.

NOTE: Investigators participating under this task number also materially contributed to research under the following tasks and progress is summarized under such tasks:

6X59-01-001 Traumatic Surgery and Shock
Task 17 Acute renal injury and failure (Dept. Metabolism)
Task 35 Intracápillary thrombi in the etiology of shock,
renal failure and other conditions

6X60-10-001 Neuropsychiatry and Stress
Task 5 Electrophysiological studies of the nervous
system (Dept. of Neurophysiology)

6X64-14-001 Biological and Medical Aspects of Ionizing Radiation
Task 22 Chemical protection against total body radiation
(Pharmacology Section, Dept. Radiobiology)

Reference:

Hardaway, R. M., Barila, T. G., Burns, J. W. and Mock, H. P.: Studies on pH Changes in Endotoxin and Hemorrhagic Shock. Submitted for clearance 31 May 61 for consideration for publication in The J. Surg. Research,

ANNUAL PROGRESS REPORT

Project No. 6X59-01-001 Traumatic Surgery and Shock

Task No. 34, Nursing Measures for Oral and Nasal Hygiene

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Department of Nursing

Period Covered by Report: 1 July 1960 through 30 June 1961

Principal Investigators: Major Gertrude J. Bossler, ANC
Captain Miriam K. Ginsberg, ANC
(Reassigned August 1960)

Reports Control Symbol: MEDDH-288

Security Classification: Unclassified

ABSTRACT

Project No. 6X59-01-001 Title: Traumatic Surgery and Shock
Task No. 34 Title: Nursing Measures for Oral and
 Nasal Hygiene

Reporting Installation: Walter Reed Army Institute of Research
 Walter Reed Army Medical Center
 Washington 12, D. C.

Period Covered by Report: 1 July 1960 through 30 June 1961

Author: Major Gertrude J. Bossler, ANC

Reports Control Symbol: MEDDH-228

A systematic evaluation of the use of a comfort measure to combat thirst of patients on restricted fluids is being conducted. Patients' opinions have been collected concerning the effectiveness of a small, internally cooled, metal disc which is placed in the mouth ad libitum to alleviate thirst. Mechanical improvements have been made on the comfort disc apparatus during the report period. Following each improvement opinions of patients and personnel are obtained.

BODY OF REPORT

Project No. 6X59-01-001 Title: Traumatic Surgery and Shock

Task No. 34 Title: Nursing Measures for Oral and Nasal Hygiene

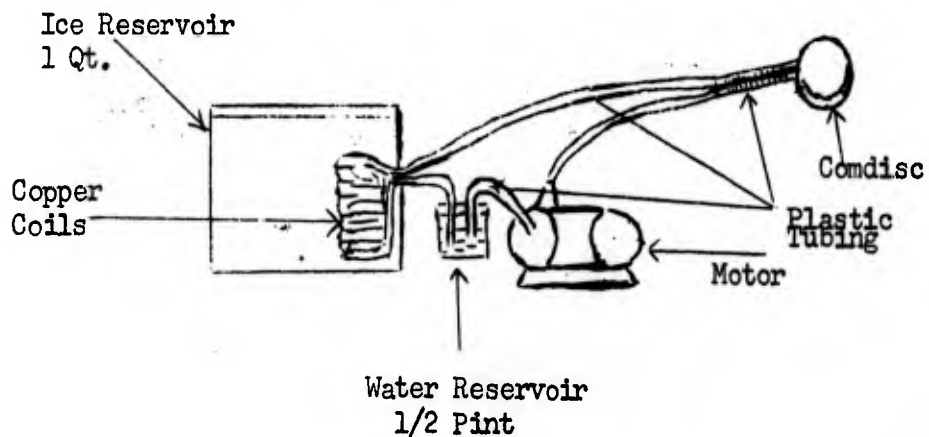
Description:

It has been observed that a frequent complaint of patients on restricted oral fluid intake is excessive thirst. In order to enhance patient comfort and to relieve thirst a comfort disc (Comdisc) was developed and tested. The theory underlying the use of the Comdisc is that, like the pebble in the mouth, it relieves thirst and stimulates salivation.

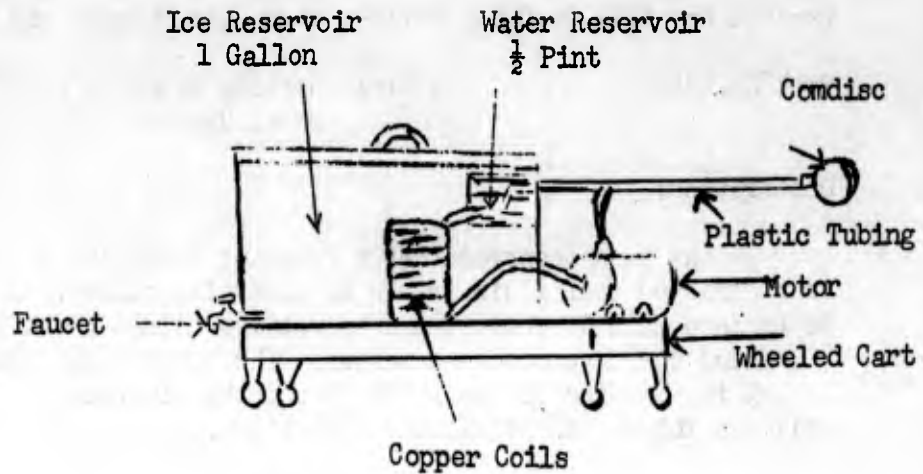
The Comdisc is a small, stainless steel, internally cooled disc approximately three centimeters in diameter, and one-half centimeter thick. The disc is connected to a closed system of circulating ice-cooled liquid which keeps it cool. The patients may place the disc in their mouths ad libitum to relieve thirst.

Progress:

Several improvements were made on the Comdisc apparatus during the report period. The unit was consolidated by mounting the motor, ice container and liquid reservoir on a mobile stand which may be readily placed within the patient unit. The consolidated apparatus occupies a minimal space at the bedside. The improvements for consolidating the Comdisc unit were based on the evaluation of its use by six patients and the nursing personnel caring for them. The original Comdisc unit and the remodeled consolidated Comdisc unit are illustrated in Figures 1 and 2 below:



Original Comdisc
Figure 1



Revised Comdisc
Figure 2

The remodeled compact Comdisc unit has been tested by ten patients on restricted fluids, with the diagnosis of acute renal failure. The opinions of patients obtained by a questionnaire revealed the following:

1. The patients liked the Comdisc.
2. The Comdisc alleviated thirst and oral discomfort.
3. A metallic taste developed in the patients' mouths with frequent and prolonged use.

The results of a series of unstructured interviews with nursing personnel indicate that the remodeled compact unit is more operable, easy to handle, and saves time and space.

Plans are being made to cover the Comdisc with a thin coat of plastic to prevent the metallic taste which may occur after prolonged use. The plastic-covered disc will be tested on a series of patients on restricted oral fluid intake.

Implications for wider use of the Comdisc unit may be studied in relation to maintaining the heat or cold of topical applications to other areas of the body.

Summary and Conclusions:

Thirst and dryness of the mouth are common subjective complaints of patients with orders for restricted oral fluids. An attempt has been made to gain information concerning patient acceptance of the Comdisc to relieve thirst discomfort.

The Comdisc unit has been remodeled into a compact apparatus which has been tested on ten patients on restricted oral fluid intake. The patients' appraisal of the Comdisc unit indicates that it relieves thirst and oral discomfort. Patients complained of a metallic taste after prolonged use. Plans are being developed to alleviate this complaint by covering the metal disc with a thin coat of plastic. Nursing personnel have found that the remodeled compact Comdisc unit is effective, easy to use and saves time and space.

List of Publications: - - -

Ginsberg, Miriam K. "Oral Hygiene Nursing Care." To be published in The American Journal of Nursing.

ANNUAL PROGRESS REPORT

Project 6X59-01-001 Traumatic Surgery and Shock

Task No. 35 Intracapillary Thrombi in the Etiology of Shock, Renal Failure,
and Other Conditions.

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Division of Surgery

Period Covered by Report: 11 Oct 60 through 15 May 1961

Principal Investigator: Colonel Robert M. Hardaway, MC

Assistants: Major E. A. Husni*, Major W. H. Brune**, Capt. R. E. Neimes*** and
Dr. H. E. Noyes.

Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

- * Now attending Surgeon, Huron Road Hospital, East Cleveland, Ohio
- ** Member Military Med. and Allied Sciences Course
- *** Surgical Resident, WRGH

ABSTRACT

Project No. 6X59-01-001

Title: Traumatic Surgery and Shock

Task No. 35

Title: Intracapillary Thrombi in the
Etiology of Shock, Renal Failure
and Other Conditions.

Period Covered by Report: 11 October 1960 through 15 May 1961.

Authors: Colonel R. M. Hardaway, Major E. A. Husni, Major W. H. Brune, Capt. R. E. Neimes and Dr. H. E. Noyes.

Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

Endotoxin and hemorrhagic shock was investigated in dogs with special attention to the blood coagulation mechanism. It was found that both endotoxin and hemorrhage caused an episode of intravascular coagulation in the capillaries and small vessels. These thrombi temporarily and partially blocked circulation in the lungs, liver and other organs, producing a decreased cardiac return and shock. The thrombi stimulated the endogenous activation of heparin and fibrinolysin which washed away most of them. However, under certain conditions they persisted long enough to cause focal necrosis in the liver, kidneys and pancreas and a hemorrhagic necrosis of the gastrointestinal mucosa which resulted in death. The formation of the thrombi (and both temporary and irreversible shock) could be prevented in large part by preheparinization.

BODY OF REPORT

Project No. 6X59-01-001

Title: Traumatic Surgery and Shock

Task No. 35

Title: Intracapillary Thrombi in the Etiology of Shock, Renal Failure and Other Conditions.

Description:

Endotoxin shock. In previous experiments it was demonstrated that the intra-aortic injection of incompatible blood, amniotic fluid, or thrombin in the dog resulted in an immediate drop in the arterial blood pressure.* This drop was reversible, and the pressure usually returned to normal spontaneously in 15 to 45 minutes. It was also observed that under certain conditions a secondary fall in the blood pressure occurred a short time later with ultimate death of the animal. Autopsy regularly showed hemorrhagic gastrointestinal lesions similar to those occurring subsequent to the administration of endotoxin. Because the clinical and pathological picture of endotoxin shock seemed identical to that produced by the injection of incompatible blood, amniotic fluid, and thrombin and since the latter substances were shown to cause intravascular coagulation with resulting changes in the clotting mechanism, it seemed desirable to investigate endotoxin shock utilizing the same methods which had been used for the study of incompatible blood and thrombin shock.

Hemorrhagic shock. It has been known for several years that the gastrointestinal lesions of dogs dying in irreversible hemorrhagic shock resembled those in dogs dying of endotoxin shock. Evidence has recently been presented to show that the mechanism of action of endotoxin shock was an episode of intravascular coagulation in visceral capillaries. Previous evidence has been presented to show that these same lesions could be produced by intra-aortic injection of incompatible blood and thrombin and that these substances produced intravascular coagulation.* The following experiments were set up to study the possibility that the irreversibility of irreversible hemorrhagic shock was due to a similar episode of intravascular coagulation.

Progress:

Endotoxin shock. Ninety dogs were anesthetized with sodium pentobarbital. Records of the arterial blood pressure were made on a Sanborn Recorder from a large polyethylene catheter inserted into the right femoral artery. The vena caval pressure was also measured. A small-sized catheter inserted into the left femoral artery up to the level of the thoracic aorta was used to inject endotoxin and to withdraw blood samples. Each dog was given 4 mg. of Escherichia coli endotoxin per kilogram of body weight diluted to 60 ml. with saline. The injection was given over a five-minute period using a mechanical syringe in order to insure a constant, even injection. The endotoxin was prepared by the Department of Bacteriology at Walter Reed Army Institute of Research. The dogs were divided into two groups: Group A received endotoxin alone. Group B received heparin in addition to endotoxin. The experiments were carried out on paired animal preparations, one dog receiving the endotoxin alone, the other receiving 12 mg. of 1-1000 heparin per kg., intravenously, ten minutes before the administration of endotoxin. The toss of a coin immediately before the experiment decided which dog was to receive heparin. Some heparinized dogs were given a lower dosage of heparin consisting of 6 mg./kg. before endotoxin and 3 mg./kg. one hour after endotoxin.

Blood samples were taken before injection of any material, and at 20 and 60 minutes after the administration of endotoxin. In Group B, an additional blood sample was taken ten minutes after the administration of heparin just preceding the endotoxin injection. The following determinations were done on the blood samples: 1. pH, 2. pCO₂, 3. fibrinogen, 4. platelet count, 5. prothrombin time, 6. thromboelastogram, 7. clotting time, 8. antithrombin titration, 9. protamine titration, 10. thromboplastin generation, 11. labile factor, 12. lactic acid level, and 13. hematocrit. Autopsies and histologic studies were carried out on all cases of fatality.

In these animals the aortic blood pressure, after an initial brief rise, always plummeted from a mean of about 150 mm. Hg. to depths ranging from 25 to 60 mm. Hg. thirty to forty-five seconds after the intra-aortic injection of endotoxin. At the same time, the pulse pressure narrowed down from a control level of about 50 mm. Hg. to ranges of 10 - 15 mm. The drop in the blood pressure lasted for several minutes and was then followed by a slow climb. The narrowed pulse pressure likewise persisted for a similar interval and then began to slowly widen toward normal. Both pressures approached their control levels within 20 to 40 minutes in most animals. Concomitant with the precipitous fall of the arterial blood pressure, the inferior vena caval pressure dropped from preinjection levels of 4.5 cm. H₂O to almost zero. Hyperpnea ensued and persisted throughout the duration of the experiment.

A secondary drop in the arterial pressure was observed to occur in most dogs starting about 40 to 50 minutes after the injection of the endotoxin. This was slow and gradual as compared to the dramatic earlier drop. Furthermore, it displayed no signs of reversibility and eventually culminated in the animals' demise. Many dogs remained deeply asleep and all succumbed within two to 24 hours of the injection of endotoxin.

Autopsy consistently showed a marked hemorrhagic necrosis of the gastrointestinal mucosa, usually most pronounced in the upper small bowel, but involving most or all of the alimentary tract. Sloughing off of the superficial mucosal layers was frequently encountered in the terminal ileum leaving a hemorrhagic mucosa beneath. In several animals numerous acute ulcerations were seen in various parts of the gastrointestinal tract but mostly in the duodenal mucosa. These appeared similar to the stress ulcers often seen in human autopsy material. There was moderate congestion of the liver, spleen and kidneys in all dogs, and in one animal hemorrhage in the pancreas was also noted. The lungs were heavy, moderately congested and contained numerous areas of hemorrhage. The right side of the heart was usually collapsed but otherwise normal. Histologic examinations disclosed numerous plugs consisting of fibrin and an amorphous collection of agglutinated red cells and platelets in the capillaries and small vessels of the lungs, liver, gastrointestinal mucosa, kidneys, pancreas and spleen. These were associated with focal necrosis in the liver, infarction in the spleen, superficial necrosis, and hemorrhage and ulceration in the gastrointestinal mucosa. Heart tissue appeared normal.

The blood coagulation mechanism was markedly altered. Blood clotted normally prior to the injection of endotoxin but frequently failed to clot for hours in the specimens taken 20 and 60 minutes after the injection. Average silicone clotting time for pre-endotoxin blood was 10'20". Average

post-injection clotting time was several hours. There was a significant drop in the fibrinogen level. This finding did not account for the failure of the blood to clot in most cases. This abnormality was apparently due to endogenous heparin. The decrease in levels of clotting elements is probably due to their being used up in a clotting episode. In addition, there was activation of fibrinolysin in some cases and in most cases there was evidence of activation of antithrombins. There was a marked increase in lactic acid level of the blood after endotoxin and a swift production of a metabolic acidosis.

In Group B (preheparinized) 50 per cent survived. Of the dogs given the reduced and divided heparin dosage, 75 per cent survived. The initial blood pressure responses were variable. In some animals there was no significant drop. In some, the drop in the mean pressure was slight, 10 - 30 mm. Hg. In the remaining animals a marked fall occurred. This was similar to, but not as marked as, that observed in Group A dogs. The recovery started sooner and proceeded faster than in Group A. The diastolic pressure returned to 75 per cent of the control levels in an average time of 4.9 minutes in these animals, as compared to a mean of 29.1 minutes in Group A dogs. Statistical analysis of these readings shows a "t" value of 3.25, significant at the one per cent level. The systolic and pulse pressure readings of both groups were also subjected to statistical analysis and the differences were found to be significant at the one per cent level. Additional significance is provided by the fact that all the mean pressure readings of the heparinized dogs in the post-endotoxin period were significantly higher than any of those in the nonheparinized group. The secondary fall starting after 40 or 50 minutes, which was prominent in Group A, was only slight in Group B and intimated that these dogs had a better chance of survival. The respiratory disturbances (apnea followed by hyperpnea) were not nearly so prominent as in Group A.

Autopsy findings were generally different from those of Group A. The lesions in the gastrointestinal mucosa were usually extremely mild in comparison to those in Group A. Petechial hemorrhages were encountered in the heart and other organs in 10 animals. This may have been related to possible overdosage of heparin. In the lungs, only a few very small petechiae were seen as opposed to the large areas of discoloration and hemorrhage in Group A. Microscopic sections showed none of the obstructing intravascular thrombi noted in the previous group of animals and there was no hemorrhagic necrosis evident in the liver.

Blood coagulation studies were rendered impossible since heparin was used. However, determination of fibrinogen showed that, while heparin itself caused a moderate drop in fibrinogen, there was no further drop in this element after the injection of endotoxin.

Hemorrhagic shock. One hundred and eight dogs of both sexes were sedated by subcutaneous morphine. A large polyethylene catheter was inserted in the right femoral artery under procaine anesthesia up to the level of the abdominal aorta. This was filled with saline and connected to a Sanborn Recorder to record aortic blood pressure. A similar catheter was inserted into the left femoral artery and used to bleed the dog, reinject blood and take blood samples. The animals were bled as rapidly as the blood would flow through an ion exchange resin for decalcification. Three hundred to 400 ml. of blood was collected in this manner and kept in a plastic bag for retransfusion into the animal. The remaining blood was collected into 100 ml. beakers and heparinized with 1 ml. heparin in each beaker. (The reason that only 300 to 400 ml. of blood was bled through the ion

exchange resin was that the blood became so hypercoagulable in later stages of bleeding that it would clot in the column). No anticlotting agent was given to the animal. Blood was collected until the recorded systolic blood pressure was 50 mm. Hg. At this time enough heparin-free blood was retransfused to raise the systolic blood pressure to 100 mm. Hg. This usually required about 20 per cent of the total bled volume. This pressure was maintained over a two-hour period. The mean pressure was 65 to 70 Hg. When necessary small amounts of nonheparinized blood (5-10 ml.) were added or withdrawn. At the end of two hours, the entire bled volume of blood was returned resulting in a return to normal blood pressure. The catheters were removed and the animal returned to his cage where food and water was available but no special treatment given. Blood samples were taken before start of bleeding, at the end of bleeding, and after two hours just before return of the entire volume of blood. These samples were subjected to the following test procedures 1. Lee White clotting time, using siliconized tubes, 2. thromboelastogram, 3. fibrinogen level, 4. prothrombin level, 5. heparin titration with protamine, 6. lactic acid level, 7. pH, 8. HCO_3 and 9. hematocrit. Clotting times were done on a sample of each 100 ml. of bled blood and every 30 minutes throughout the two-hour shock period. Two variations of the experiment were done. Group A had no additional procedure other than described above. Group B were given 2 mg. per kilogram of heparin just before start of bleeding and 1 mg. per kilogram one hour later. All dogs were paired, one from Group A and one from Group B. The pair was done simultaneously on one table under identical circumstances in so far as possible. Dogs that died were autopsied immediately and tissue sections taken of abdominal and thoracic organs.

GROUP A Of the nonheparinized dogs, 96 per cent were dead within 24 hours in spite of the fact that the blood pressure had returned to normal level after retransfusion of all blood. Autopsy showed a consistent hemorrhagic necrosis of the gastrointestinal mucosa involving all organs from the esophagus to the colon. There were frequent subendocardial hemorrhages. Lungs were always hemorrhagic. The kidneys showed marked medullary congestion and hemorrhage with juxta-medullary congestion and pale, thickened cortex with some areas of hemorrhage. There were occasional infarcts. There was occasional pancreatic hemorrhage. The liver was congested and mottled. Microscopic examination revealed plugging of numerous capillaries with eosinophilic staining material. This was associated with hemorrhagic necrosis of the bowel mucosa, focal necrosis of the liver, focal necrosis and infarction of the kidney. There were similar hemorrhagic lesions in the pancreas.

Silicone clotting times fell from an average of 18 minutes before start of bleeding to an average of five minutes at the end of hemorrhage about 14 minutes later. This further declined to one-half or one minute in the subsequent hour or so. Occurring usually about 90 to 120 minutes after hemorrhage, the blood rather suddenly became almost incoagulable requiring more than two hours to clot. These changes are dramatically recorded by a thromboelastogram. This incoagulability was largely due to the liberation of autogenous heparin by the animal. This was demonstrated by return to normal clotting time by titration with protamine. The autogenous heparin produced a clearing in serum lipids. This heparin was evident by the end of hemorrhage (before the two-hour shock period) as evidenced by heparin titration and lipid clearing in spite of the short coagulation time at this period. No fibrinolysin was noted. During the hemorrhage there was a marked drop in fibrinogen from an average of 313 mg. per cent to 241 mg. per cent. This fall continued to 172 mg. per cent during the period of shock. However, the fibrinogen level never descended below the level required to

clot blood. Prothrombin also fell markedly. The readings on the post-shock samples were affected by endogenous heparin. If this heparin was neutralized with protamine before prothrombin determination, the average post-shock reading was 38 per cent, still a considerable fall from the 100 per cent initial reading. A severe metabolic acidosis occurred with a fall in both blood and plasma pH, a rise in lactic acid and a fall in HCO_3 . The hematocrit fell precipitously during hemorrhage but remained stable during the two hours of shock.

GROUP B. Of the preheparinized dogs 63% lived. Autopsy of those which died showed only very mild lesions of the gastrointestinal tract as compared with GROUP A. Microscopic examination showed an absence of the masses of eosinophilic staining plugs occluding the capillaries and small vessels and an absence of focal necrosis. Of course, the preheparinization prevented study of clotting times. However, the fall in fibrinogen while occurring both as a result of the heparin (due to heparin-fibrinogen precipitation) and hemorrhage, was much less than with GROUP A dogs and fibrinogen levels remained unchanged during the two-hour shock period. This difference between Group A and Group B was found to be significant with a P value of less than .03. If fibrinogen readings are calculated to reflect dilution of serum with fibrinogen-free fluid as denoted by the hematocrit, there was no change at all in fibrinogen level in the GROUP B dogs. Of course, this diluting fluid probably contains fibrinogen but most likely in quantities less than in the serum. Changes in prothrombin were distorted by the presence of heparin in all specimens. However, if heparin was neutralized with protamine, the fall in prothrombin was still small. Changes in lactic acid, pH and related readings were almost identical with those in GROUP A as were hematocrit changes. Miscellaneous data on the two groups are as follows: (all are averages)

	<u>GROUP A</u> (normal)	<u>GROUP B</u> (heparinized)
Duration of hemorrhage	14.0 min.	13.2 min. (purposely slowed to be equal to GROUP A)
Total amount bled	646 ml.	654 ml.
Weight of dog	12.9 Kg.	13.1 Kg.
Amount bled per Kg.	50.0 ml.	50.0 ml.
Blood retransfused to bring systolic pressure to 100 mm. Hg.	164 ml.	135 ml.
Hours lived (permanent survival counted as 24 hours)	5.8	20.7

Summary and Conclusions:

Endotoxin shock. Intra-aortic injection of E. coli endotoxin produces intravascular coagulation as evidenced by:

1. Decrease (due to using up) of blood clotting factors including:
 - a. Fibrinogen
 - b. Other clotting elements
2. Finding of thrombi in tissue section.
3. Finding of focal necrosis and infarcts.
4. Prevention of these findings by preheparinization.

There is evidence that the immediate (reversible) fall in blood pressure after endotoxin injection is caused by a decreased cardiac output secondary to decreased venous return to the left heart because of acute cor pulmonale due to blockage of pulmonary capillaries by thrombi and associated vasospasm. There is decreased venous return to the right heart due to damming of blood in the portal system by thrombi in the liver, and associated vascular spasm contributing to a low inferior vena caval pressure, which is also partially due to increased peripheral resistance.

This immediate blood pressure drop can be prevented in part by heparin. Recovery from it may be associated with the appearance of fibrinolysin in the blood and lysis and washing out of the clots and plugs in the capillaries and small vessels. There is evidence that the secondary (irreversible) fall in blood pressure and death is caused by necrosis (secondary to temporary thrombi) of the intestinal mucosa causing loss of blood and serum into the bowel lumen, and focal necrosis of the liver, kidneys, pancreas and other organs.

Hemorrhagic shock. This procedure resulted in a 96 per cent mortality using a shock period of only two hours at a blood pressure of 100 mm. Hg. systolic or 65 mm. Hg. mean pressure. Hemorrhage resulted in the rapid development of hypercoagulability of the blood with clotting time in siliconized tubes falling from an average of 18 minutes to one minute. The hypercoagulability plus a stagnant circulation as produced by a low blood pressure and low cardiac output produced an episode of intravascular clotting in capillaries and small vessels. Clotting was indicated by a fall in blood clotting elements (fibrinogen and prothrombin) due to their being used up, by the finding of intracapillary thrombi in tissue sections, and by protection afforded by heparin. These intravascular clots caused tissue necrosis and infarction particularly in the liver, kidney, gastrointestinal tract and other organs. The body responded to this episode of intravascular coagulation by the liberation of heparin (and in some cases fibrinolysin) as a protective mechanism. However, this reaction was too late to save the animal in the present experiments.

Both endotoxin and hemorrhagic shock resulted in the rapid development of a metabolic acidosis characterized by marked increase in lactic acid, fall in HCO_3 and in pH. Preheparinization protects against these changes significantly when it is allowed to protect against blood pressure fall, as in endotoxin shock, but not if the blood pressure is kept constant, as in hemorrhagic shock. The metabolic acidosis resulted from stagnation of circulation and accumulation of metabolites as a result of low blood pressure. Therefore acidosis is probably not the lethal factor in shock and correction of it would probably not prevent irreversible shock.

List of Publications:

1. Hardaway, R. M., Husni, E. A., Geever, E. F., Noyes, H. E. and Burns, J. W.: Endotoxin Shock, A Manifestation of Intravascular Coagulation. Ann. Surg. In press.
2. Hardaway, R. M., Husni, E. A., Geever, E. F., Noyes, H. E. and Burns, J. W.: Studies on the Relationship of Bacterial Toxins and Intravascular Coagulation to Pseudomembranous Enterocolitis. The J. Surg. Research. In press
3. Hardaway, R. M., Brune, W. H., Geever, E. F., Burns, J. W. and Mock, H. P.: Studies on the Role of Intravascular Coagulation in Irreversible Hemorrhagic Shock. Submitted for clearance 17 May 61 for consideration for publication in Ann. Surg.

4. Hardaway, R. M.: A Unified Theory of Shock. Submitted for clearance 29 May 61 for consideration for publication in Ann. Surg.
5. Hardaway, R. M., Barila, T. G., Burns, J. W. and Mock, H. P.: Studies on pH Changes in Endotoxin and Hemorrhagic Shock. Submitted for clearance 31 May 61 for consideration for publication in The J. Surg. Research.

*NOTE Previous work on this task was done under Project No.6-60-01-001.

ANNUAL PROGRESS REPORT

Project 6X59-05-001 Blood, Blood Derivatives and Artificial Expanders

Task 1, Immunohematology

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Department of Immunohematology
Division of Communicable Disease and Immunology

Period Covered by Report: 1 July 1960 through 30 June 1961

Principal Investigator: Mary B. Gibbs, M. S.

Assistant: Frank Camp, Capt., MSC *

Reports Control Symbol: METDH-288

Security Classification: UNCLASSIFIED

*Fellow in Blood Banking.

ABSTRACT

Project No.: 6X59-05-001

Title: Blood, Blood Derivatives and
Artificial Expanders

Task No.: 1

Title: Immunohematology

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Department of Immunohematology
Division of Communicable Disease and Immunology

Period Covered by Report: 1 July 1960 through 30 June 1961

Authors: Mary B. Gibbs, M. S., Frank Camp, Capt., MSC

Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

1. Quantitative hemagglutination inhibition studies have shown that the specificity of natural anti-A isoantibodies from group B individuals for hog A substance varies greatly in specificity and avidity. Blood group A substances from human A1 and A2 secretors have been purified and used to immunize volunteers for comparative studies with sera from hog A substance immunized individuals.
2. Three quantitative subgroups of the B antigen have been demonstrated by quantitative hemagglutination procedures to differ among Caucasoid, negroid and mongoloid peoples.
3. A and B agglutinability of red cells was unaltered by long-term storage in the frozen state in liquid nitrogen or glycerol, while at refrigerator temperatures in ACD or modified Alsever's solutions a two-stage decrease occurred.
4. Studies of the free-cell phenomenon in the ABO and Rh systems indicated that inagglutinable A or B cells are due to antibody excess and not somatic mutation.
5. In general, the rate of binding of agglutinin to red cell increases as temperature increases; naturally occurring isoantibodies are bound more rapidly than immune isoantibodies; anti-A more rapidly than anti-B. The rate of agglutination (2nd stage) increases with increase in temperature and avidity but is prolonged by the presence of non-agglutinating (incomplete) isoantibodies.
6. Quantitative hemagglutination studies of the thermal amplitude of ABO isoantibodies have shown that the reactivity of natural isoantibodies increases with decrease in temperature while the immune isoagglutinins are either equally reactive at all temperatures or equally reactive

in the 4C - 25C temperature range but less active at 37C or increase in reactivity as temperature decreases.

7. Quantitative hemagglutination studies of the anti-A and anti-B activity of serum fractions eluted from DEAE-cellulose have shown that anti-B and immune anti-A activities of group O sera predominate in the 7S antibody fraction.

8. Anti-D(Rho) sera evoked by immunization with CDe (R₁) cells have been found by the quantitative hemagglutination technic to possess a greater reactivity with homozygous and heterozygous {CDe(R₁) cells than with the homozygous cDE(R₂) cells. The cDE(R₂) cell is generally attributed with greater reactivity with all anti-D sera.

BODY OF REPORT

Project No.: 6X59-05-001

Title: Blood, Blood Derivatives and Artificial Expanders

Task No.: 1

Title: Immunohematology

Description: A study of the phenomena involved in the agglutination reactions, normal and abnormal, of the blood group system.

Progress:

1. The work previously reported with the quantitative hemagglutination inhibition method on the change in character of anti-A isoantibodies following immunization with blood group A substance from hog gastric mucosa was continued. The previous finding: that anti-A isoagglutinins from 11 volunteers immunized with the same hog A substance were identical in character in that all required the same amount of hog A substance to inhibit a standard HD_{50} unit of anti-A activity and all produced the same log-probit regression line slope (-1.80), was followed up by a study of naturally occurring anti-A isoagglutinins from 13 group B individuals. The natural anti-A sera showed great variation in that the inhibitory dose of hog A substance ranged from 5 to 500 micrograms and the slopes of log probit regression lines ranged from -0.60 to -1.14. These variations have been attributed to differences in the specificity and avidity of the naturally occurring isoagglutinins for the hog A substance. To confirm this, human A substances were purified from A_1 and A_2 secretor salivas. These substances were used to immunize volunteers of blood groups B and O. Antisera from these individuals were collected before immunization and at 3-day intervals following immunization up to 20 days; monthly samples have been collected thereafter. These samples will be compared with those from individuals immunized with hog A substance.

2. Work was continued on differentiation of the three quantitative subgroups of the B antigen which was previously reported. No additional subgroups were identified in a survey of 148 group B cells of three racial groups. Based on the relative amounts of anti-B serum required to agglutinate 50% of a constant number of red cells, these three subgroups have been assigned values of 100% for the most reactive group, 60% and 40% for the two groups of lesser reactivity hence they have been termed $B^{1.0}$, $B^{0.6}$ and $B^{0.4}$, respectively. The results of the survey are summarized in the table:

<u>Racial group</u>	<u>Type of B Antigen</u>		
	<u>$B^{1.0}$</u>	<u>$B^{0.6}$</u>	<u>$B^{0.4}$</u>
Caucasoid	8	88	9
Negroid	12	13	0
Mongoloid	0	15	3

From these results it appears that the frequency of these subgroups differs among racial groups and that the $B^{1.0}$ antigen is a character of the Negro race.

3. The previous report of the preservation of agglutinability of erythrocytes of the ABO groups in liquid nitrogen was extended. A comparative study of the agglutinability of these cells stored frozen in liquid nitrogen for 2 years and in glycerol at -20C for 9 months showed that the agglutinability of the cells was not altered in this interim. Samples of these same cells stored at refrigerator temperatures (10 -14C) in ACD or modified Alsever's solution, however, gradually lost up to approximately 40% their original agglutinability during the first 5 days of storage. The reactivity of the cell stabilized from the 5th to approximately the 15th storage days when a second period of decrease in activity was noted between the 15th and 23rd storage day. The reactivity of the cells restabilized with only 70% their original activity from approximately 23 days to the end of the test period at 33 days.

4. A study was performed of the free-cell phenomenon in the ABO and Rh systems. It was found that the inagglutinable cell count does not differ markedly for A₁, A₁B and A₂ cells and the change in slope of the log probit assay curve is not affected by the inagglutinable count. The similarity of behavior of A-anti-A and B-anti-B agglutination in the area of the free-cell phenomenon to that of the Rh cell in the area of prozone lends support to the contention that inagglutinable A or B cells are the results of antibody excess and not somatic mutations as proposed by Atwood and Scheinberg. This work has not been completely evaluated and follow-up experiments may be necessary before publication. The Ashley free-cell counting technic for study of the survival of transfused red cells was modified to assure enumeration of inagglutinable cells at the optimal ratio of agglutination to cellular antigen in a stable system.

5. Quantitative hemagglutination studies were devised to study the rates of the first and second stages of the reactions of the ABO system. The rate of sensitization of the red cell antigen was determined by quantitative hemagglutination assays of the antibody activity remaining in solution after exposure to a constant number of test cells for varying periods of time at 4C, 25C and 37C. In general, it was found that the rate of sensitization increases as temperature increases and that the sensitization of A cells by anti-A is considerably faster than that of the B cell by anti-B. Furthermore, the naturally occurring isoagglutinins are bound more rapidly than immune agglutinins. The rate of the second stage of the reaction was established by determination of the percentages of cells agglutinated at varying intervals of time until the system reaches equilibrium. The rate of this stage of the hemagglutination reaction is independent of the rate of sensitization and is determined by the avidity and temperature of the reaction, as well as the presence of non-agglutinating antibody. From preliminary studies, it appears that the Coulter electronic counter can be used in both sensitization-time and equilibrium-time studies.

6. Quantitative hemagglutination studies were also initiated on the thermal amplitude of the antibodies of the ABO system. In general, greater amounts of naturally occurring anti-A and anti-B are absorbed by a given number of test cells and higher agglutination is noted as

temperature decreases. Three patterns of behavior have been seen with immune antibodies; (1) equal amounts of antiserum absorbed and the same degree of agglutination at 4C as at 37C (most common); (2) equal amounts absorbed and the same degree of agglutination at 4C and 25C but less absorption and agglutination at 37C, and (3) the same as with naturally occurring antisera; i.e. less absorption and agglutination as temperature rises across the entire temperature range.

7. Studies were initiated on the anti-A and anti-B agglutinins obtained in fractions from DEAE-cellulose column fractionation of natural and immune antisera. It was found that the majority of the anti-A activity of immune group B sera is associated with the fraction containing 19S antibodies while with natural anti-B from group O and an immune anti-A from a group O, the majority of activity resides in the fraction containing 7S antibodies.

8. Work continued on the study of saline anti-D(Rh₀) sera. Of 12 anti-D sera studied, three were found to react considerably stronger with homozygous and heterozygous CDe(R₁) cells than with the standard homozygous cDe(R₂) cell. The slope of the assay curves was steeper and the region of linearity far exceeded that of the cDe(R₂) cell which is reported to be the most reactive of all Rh types with all anti-D sera. Two of these sera are of known CDe(R₁) stimulation; therefore the increased activity with CDe(R₁) cells over cDe(R₂) cells is probably due to their greater specificity for the CDe(R₁) cell. Differentiation of anti-D sera according to Rh type of the immunizing cell has heretofore been impossible because of the relatively crude methods in general use. Additional anti-D sera of known origin are to be obtained in order to confirm these observations.

Summary and Conclusions:

1. Quantitative hemagglutination inhibition studies have shown that the specificity of natural anti-A isoantibodies from group B individuals for hog A substance varies greatly in specificity and avidity. Blood group A substances from human A₁ and A₂ secretors have been purified and used to immunize volunteers for comparative studies with sera from hog A substance immunized individuals.

2. Three quantitative subgroups of the B antigen have been demonstrated by quantitative hemagglutination procedures to differ among Caucasoid, negroid and mongoloid peoples.

3. A and B agglutinability of red cells was unaltered by long-term storage in the frozen state in liquid nitrogen or glycerol, while at refrigerator temperatures in ACD or modified Alsever's solutions a two-stage decrease occurred.

4. Studies of the free-cell phenomenon in the ABO and Rh systems indicated that inagglutinable A or B cells are due to antibody excess and not somatic mutation.

5. In general, the rate of binding of agglutinin to red cell increases as temperature increases; naturally occurring isoantibodies are bound more rapidly than immune isoantibodies; anti-A more rapidly than anti-B. The rate of agglutination (2nd stage) increases with increase in temperature and avidity but is prolonged by the presence of non-agglutinating (incomplete) isoantibodies.

6. Quantitative hemagglutination studies of the thermal amplitude of ABO isoantibodies have shown that the reactivity of natural isoantibodies increases with decrease in temperature while the immune isoagglutinins are either equally reactive at all temperatures or equally reactive in the 4C - 25C temperature range but less active at 37C or increase in reactivity as temperature decreases.

7. Quantitative hemagglutination studies of the anti-A and anti-B activity of serum fractions eluted from DEAE-cellulose have shown that anti-B and immune anti-A activities of group O sera predominate in the 7S antibody fraction.

8. Anti-D(Rho) sera evoked by immunization with CDe (R₁) cells have been found by the quantitative hemagglutination technic to possess a greater reactivity with homozygous and heterozygous CDe(R₁) cells than with the homozygous cDE(R₂) cells. The cDE(R₂) cell is generally attributed with greater reactivity with all anti-D sera.

Publications:

1. R. Silber, M. B. Gibbs, E. F. Jahn and J. H. Akeroyd, "Quantitative hemagglutination studies in the Rh blood group system. I The assay of the anti-D(Rh₀) agglutinin", Blood, 17:282, 1961.
2. R. Silber, M. B. Gibbs, E. F. Jahn and J. H. Akeroyd, "Quantitative hemagglutination studies in the Rh blood group system. II A study of the D(Rh₀) agglutinin", Blood, 17:291, 1961.
3. M. B. Gibbs and J. H. Akeroyd, "Quantitative hemagglutination inhibition studies of blood group substances. I The assay of the activity of blood group A substances", J. Immunol., (in press).
4. M. B. Gibbs, N. F. Laffer, C. J. Dunne and J. H. Akeroyd, "Quantitative hemagglutination inhibition studies of blood group substances. II Characterization of anti-A isohemagglutinins by their behavior with blood group A substances", J. Immunol., (in press).
5. M. B. Gibbs, E. D. Zapf and J. H. Akeroyd, "Quantitative subgroups of the B antigen in man", submitted for publication in Nature. .

ANNUAL PROGRESS REPORT

Project No. 6X59-05-001 Blood, Blood Derivatives and
Artificial Expanders

Task No. 8 Revision of field transfusion service

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Department of Hematology
Division of Medicine

Period Covered by Report: 1 July 1960 through 30 June 1961

Principal Investigator: Colonel William H. Crosby, MC

Assistants: Captain Munsey Wheby, MC
Captain O'Neill Barrett, MC
Captain Wilmer C. Hewitt, Jr., MC*

Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

* WRGH

ABSTRACT

Project No. 6X59-05-001

Blood, Blood Derivatives
and Artificial Expanders

Task No. 8

Revision of field
transfusion service

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Period Covered by Report: 1 July 1960 through 30 June 1961

Authors: Colonel William H. Crosby, MC
Captain Munsey Wheby, MC
Captain O'Neill Barrett, MC
Captain Wilmer C. Hewitt, Jr., MC

Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

1. It has been established that corticosteroid does not prevent hemolytic transfusion reaction resulting from incompatibility of donor and recipient.
2. Means are sought to tag blood typing antibodies with a radioactive isotope. This may provide a means of automating blood typing and crossmatching.

BODY OF REPORT

Project No. 6X59-05-001

Blood, Blood Derivatives
and Artificial Expanders

Task No. 8

Revision of field transfusion
service

Description:

A continuing study to improve quality and safety of the Army's blood transfusion service.

Progress:

In control experiments small volumes of incompatible, chromium⁵¹ tagged red cells were given to recipients. Several days later the studies were repeated 30 minutes after the intravenous administration of prednisolone. Prednisolone was found completely ineffective in prolonging the survival of small amounts of red cells in the presence of incompatible antibodies of the ABO and Rh systems.

An attempt is being made to tag anti-A and anti-B antibodies with Cr⁵¹. If this is possible, such tagged anti sera could be incubated with red cells, the red cells then washed and the positive antigen-antibody reaction identified by a Geiger tube. In this way blood typing could be automated.

Summary and Conclusions:

1. It has been established that corticosteroid does not prevent hemolytic transfusion reaction resulting from incompatibility of donor and recipient.

2. Means are sought to tag blood typing antibodies with a radioactive isotope. This may provide a means of automating blood typing and crossmatching.

List of Publications:

1. Hewitt, W. C. Jr., Wheby, M. and Crosby, W. H.:
Effect of prednisolone on incompatible blood transfusions.
Transfusion 1: 184, 1961.

ANNUAL PROGRESS REPORT

Project 6X59-05-001 Blood, Blood Derivatives and Artificial
Expanders

Task 9. Functions and disorders of the spleen

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Department of Hematology
Division of Medicine

Period Covered by Report: 1 July 1960 through 30 June 1961

Principal Investigator: Colonel William H. Crosby, MC

Assistant: Lt Colonel Donald L. Howie, MC

Reports Control Symbol: MEDDH-288

Security Classification: Unclassified

ABSTRACT

Project No. 6X59-05-001 Blood, Blood Derivatives and Artificial Expanders

Task No. 9. Functions and disorders of the spleen.

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Period Covered by Report: 1 July 1960 through 30 June 1961

Authors: Colonel William H. Crosby, MC
Lt Colonel Donald L. Howie, MC

Reports Control Symbol: MEDDH-288

Security Classification: Unclassified

Animal studies have demonstrated that splenectomy increases the tolerance to chemotherapeutic agents. There are also clinical indications of a similar improvement of therapeutic index. This hypothesis is being tested in two series of patients with malignant disease, one series with disseminated lymphoma, the other with inoperable testicular or intra-abdominal cancers.

BODY OF REPORT

Project No. 6X59-05-001

Blood, Blood Derivatives and
Artificial Expanders

Task No. 9

Functions and disorders of
the spleen

Description: The effect of splenectomy on the therapeutic
index of chemotherapeutic agents

Progress:

A long-term investigation concerning the effect of splenectomy on malignancy has been instituted. This study was prompted by observations indicating that patients with various lymphomas and leukemias who had undergone splenectomy could tolerate considerably more chemical and irradiation therapy before developing signs of clinically important bone marrow toxicity than they could prior to splenectomy. A protocol has been established calling for splenectomy in selected patients with systemic lymphomatous malignancies, including chronic lymphocytic leukemia and Hodgkin's disease early, rather than late, in the course of their diseases. Local clinical experience justifies this departure from established systems of therapy and data derived from animal experimentation supports the belief that the spleen in some manner is synergistic to the toxic, but not the therapeutic effects of treatment modalities.

The hypothesis that the spleen in some manner multiplies the toxic effect of chemotherapeutic agents on the bone marrow is being tested further in a cooperative study by the Department of Hematology, the Cancer Chemotherapy Service and the Department of Surgery, Walter Reed General Hospital. Patients with testicular malignancies and inoperable malignancies who are laparotomized are candidates for the study if it is anticipated that they will receive chemotherapy. An unbiased pairing system has been put into effect whereby half of these patients will have their spleens removed at the time of surgery. In this manner the therapeutic index of a given drug can be established in patients with intact spleens versus those with no spleens.

Summary and Conclusions:

Animal studies have demonstrated that splenectomy increases the tolerance to chemotherapeutic agents. There are also clinical indications of a similar improvement of therapeutic index. This hypothesis is being tested in two series

of patients with malignant disease, one series with disseminated lymphoma, the other with inoperable testicular or intra-abdominal cancers.

List of Publications:

1. Crosby, W. H. and Benjamin, N. R.: Frozen spleen reimplanted and challenged with Bartonella. Amer. J. Path. (In Press)
2. Crosby, W. H. and Conrad, M. E.: Hereditary spherocytosis: Observations on hemolytic mechanisms and iron metabolism. Blood 15: 662, 1960.

ANNUAL PROGRESS REPORT

Project 6X59-05-001 Blood, Blood Derivatives and
Artificial Expanders

Task 12 The bone marrow's function and its reaction to injury

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Department of Hematology
Division of Medicine

Period Covered by Report: 1 July 1960 through 30 June 1961

Principal Investigator: Colonel William H. Crosby, MC

Assistants: Captain Marcel E. Conrad, Jr., MC
Captain O'Neill Barrett, MC

Reports Control Symbol: MEDDH-288

Security Classification: Unclassified

BODY OF REPORT

Project No. 6X59-05-001

Blood, Blood Derivatives
and Artificial Expanders

Task No. 12

The bone marrow's function
and its reaction to injury

Description:

An investigation of the rate and pattern of recovery of bone marrow after chemotherapeutic or irradiation injury.

Progress:

The reaction of bone marrow to massive doses of nitrogen mustard has been observed in an additional four patients with advanced Hodgkin's disease. Pretreatment of such patients with heparin appeared to increase their tolerance for mustard, but it may also have impaired the drug's effectiveness against the neoplasm. In a series of rabbits it was found that animals pretreated with whole-body X-irradiation were more susceptible to injury by mustard even though they had completely recovered from the hematologic effects of irradiation.

Summary and Conclusions:

Further observations have been made on the recovery of bone marrow after injury by nitrogen mustard or irradiation.

List of Publications:

1. Daniell, H. W. and Crosby, W. H.: Effect of homologous bone marrow - spleen cell suspension on survival of swine exposed to radiation from a nuclear weapon. Blood 15: 856, 1960.
2. Conrad, M. E. and Crosby, W. H.: Massive nitrogen mustard therapy in Hodgkin's disease with protection of bone marrow by tourniquets. Blood 16: 1089, 1960.
3. Barrett, O'N.: Effect of nitrogen mustard in rabbits following exposure to X-irradiation. Proc. Soc. Exper. Biol. Med. 105: 445, 1960.
4. Conrad, M. E. and Crosby, W. H.: Bone marrow biopsy: Modification of the Vim-Silverman needle. J. Lab. Clin. Med. 57: 642, 1961.

ANNUAL PROGRESS REPORT

Project No. 6X59-05-001 Blood, Blood Derivatives and
Artificial Expanders

Task No. 13. Blood and blood disorders

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Department of Hematology
Division of Medicine

Period Covered by Report: 1 July 1960 through 30 June 1961

Principal Investigator: Col William H. Crosby, MC

Assistants:

Dr. Kichiro Amamoto ¹	James Eichelberger
Pearl Anderson, Ph.D.	Capt E. J. Gangarosa, MC
Capt Stanley Balcerzak, MC	Thelma Johnson
Capt O'Neill Barrett, Jr., MC	Capt John G. Maier, MC ⁴
Dr. Raul Baco ²	Capt Donald B. Sodee, MC ⁵
Naomi Benjamin	Capt Robert van Hoek, MC
Dr. Luciano Bruzzese ³	Capt Harvey Weiss, MC
Ann Berman ⁴	Capt Munsey Wheby, MC
Capt Marcel E. Conrad, Jr., MC	Maj P. Thirayothin ⁶

Reports Control Symbol: MEDDH-288

Security Classification: Unclassified

1. ABCC Fellow
2. PHS Research Fellowship
3. Fulbright Fellow
4. Radioisotope Section, WRGH
5. WRGH
6. Med. Corps, Royal Thai Army

Project No. 6X59-05-001 Blood, Blood Derivatives and Artificial Expanders

Task No. 13. Blood and blood disorders

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Period Covered by Report: 1 July 1960 through 30 June 1961

Authors:

- | | |
|--------------------------------|--------------------------|
| Col William H. Crosby, MC | James Eichelberger |
| Dr. Kichiro Amamoto | Capt E. J. Gangarosa, MC |
| Pearl Anderson, Ph.D. | Thelma Johnson |
| Capt Stanley Balcerzak, MC | Capt John G. Maier, MC |
| Capt O'Neill Barrett, Jr., MC | Capt Donald B. Sodee, MC |
| Dr. Raul Baco | Capt Robert van Hoek, MC |
| Naomi Benjamin | Capt Harvey Weiss, MC |
| Dr. Luciano Bruzzese | Capt Munsey Wheby, MC |
| Ann Berman | Maj P. Thirayothin |
| Capt Marcel E. Conrad, Jr., MC | |

Reports Control Symbol: MEDDH-288

Security Classification: Unclassified

1. A modification of the thromboplastin generation test has permitted the demonstration of platelet abnormality in patients with hitherto undiagnosed bleeding disorders.
2. Injection of bacterial endotoxin produces multiple lesions in the clotting mechanism.
3. Clot structure in granulocytic leukemia is disturbed in a manner which can be corrected by addition of glutathione.
4. An albumin inhibitor of thromboplastin is found not to be affected by administration of coumarin drugs.
5. Iron (Fe^{59}) absorption is studied in man and animals using whole-body scintillation counters.
6. The distribution of iron after absorption has been studied in small animals following injection and ingestion of iron.

7. Iron-protein complexes such as ferritin and transferrin are being investigated to develop methods for their identification and purification.

8. Absorption of vitamin B₁₂ is studied by means of models employing rat intestine. Complementary studies are carried out in vivo.

9. A survey of plasma and red cell copper is being completed, using a method developed last year.

10. Plasma hemoglobin continues to be investigated to ascertain the areas other than kidney at which the hemoglobin is cleared from the plasma.

11. A method for performing erythrocyte sedimentation rate using capillary tubes is being studied.

BODY OF REPORT

Project No. 6X59-05-001

Blood, Blood Derivatives and
Artificial Expanders

Task No. 13

Blood and blood disorders

Description: This task encompasses the study of the function and disorders of the blood plasma factors and formed elements.

Progress:

1. Thrombopathia. A semi-quantitative method for preparing platelets for the thromboplastin generation test (TGT) was used to study patients with mild undiagnosed bleeding disorders. By all routine coagulation studies no defect could be demonstrated. When very low concentrations of platelets were used in the modified TGT, all patients were found to be abnormal. Higher concentration of platelets gave normal results. The defect is probably an inability to release platelet factor 3, since disruption of platelets by sonic oscillation produced normal activity.

2. Coagulation defects induced by endotoxin. The effect of bacterial endotoxin on the coagulation system of dogs was studied. The injection of purified *E. coli* endotoxin results in an initial thrombocytopenia and marked prolongation of the clotting time. Protamine titrations showed the latter to be due to the release of endogenous heparin. After a variable period of time, the platelets return to normal level, indicating prior sequestration rather than utilization. The clotting times return toward normal, probably reflecting heparin clearing and neutralization by platelets. Decreases in fibrinogen are moderate. Initial changes in the prothrombin time are probably due to heparin as are transient defects of serum in the TGT. There was no evidence of an initial hypercoagulability. Unlike thrombin injections, there were no changes in factor V. Further studies on the effects of endotoxin are continuing.

3. Clot structure in chronic granulocytic leukemia. Further studies have demonstrated that the abnormal thromboelastogram in CGL can be corrected in all cases by reduced glutathione (GSH). It was also observed that the clots in CGL may be dissolved in 5M urea. These two observations suggested that they were lacking in L-L factor (fibrin stabilizing factor). However, further proof for this was lacking since GSH does not prevent dissolution by urea and the

clot does not reappear after dialysis of the urea. The urea probably acts by potentiating proteolytic activity in the leukocytes. Such potentiation by urea derivatives has been described for other proteolytic systems.

4. Effect of anti-coagulants on albumin inhibitor of thromboplastin. It was found that in patients receiving coumarin drugs, the prothrombin time is more prolonged with lung thromboplastin (Tpl) than with brain Tpl. This could be attributed to an inhibitor in the albumin fraction of the plasma to which the lung Tpl is more sensitive. The effects of the inhibitor apparently become pronounced when factor VII is reduced during coumarin therapy. An assay for the inhibitor was devised and the plasmas of patients receiving anticoagulants were studied to see if the inhibitor itself changed during treatment. A comparison of 50 patients with an equal number of normals revealed no difference. Further studies demonstrated that the protein inhibitor is probably a co-factor to a lipid component in the Tpl. Because of the importance of tissue Tpl in initiating certain kinds of thrombosis, further studies on possible abnormalities of the plasma co-factor in other conditions are in progress.

5. Iron absorption from food stuffs. Fe⁵⁹ was incorporated into chicken eggs by intramuscular injection, 15 - 20 μ c. The radioactive yolk was fed to rats by esophageal tube and per cent retention was studied by liquid scintillation animal body counter. The anemic rats, induced by bleeding, absorbed more iron than the normal rats. Oral administration of ascorbic acid significantly increased the iron absorption from egg yolk both in the anemic and normal rats. The normal rats absorbed more iron when ascorbic acid was given compared with the anemic rats fed only with the egg yolk. Comparison with the absorption of inorganic iron is carried on.

Whole milk tagged with Fe⁵⁹ was fed to rats by esophageal tube. Anemic rats absorbed more iron than the normals. The normal rats seem to absorb less iron in presence of the milk than when the iron was fed alone.

The effect of inflammation on iron absorption. The radioactive iron absorption of untreated patients with Hodgkin's disease is being determined using the whole body counter. To date eight such individuals have been studied. Seven have shown absorptions within the normal range. The one patient whose absorption was above normal had donated 500 ml. of blood 3 months previously.

The effect of chronic hypoxia on iron absorption in humans is also being investigated using the whole body counter. At present five patients have been studied. All have oxygen desaturation at rest. All absorb radioactive iron above the normal limit of 10 per cent.

Role of bile in regulating iron absorption. By placing biliary catheters into a normal and an iron-deficient dog and establishing cross-flow of bile, we have found that bile does not appear to have a role in the physiological control of iron absorption. Bile did have an effect in facilitating iron absorption in the iron-deficient dog as absorption decreased when the biliary flow was excluded from the duodenum. Radioactivity was detected in bile 30 minutes after oral administration of Fe⁵⁹ to the dogs.

Effect of pancreatic duct ligation on iron absorption. In order to determine the possible role of pancreatic juice in regulating iron absorption and to evaluate the reported finding of iron loading following pancreatic duct ligation, the following experiments were done. Two dogs had their pancreatic ducts ligated. Iron absorption was determined in one preoperatively and in both at varying intervals postoperatively. We found decreased iron absorption in each study done post operatively.

Erythropoiesis and iron absorption. We are studying the relationship of marrow erythroid activity to iron absorption, specifically to the enhanced absorption of iron deficiency. Results thus far suggest that depressing erythroid activity by acute hypertransfusion depresses markedly iron absorption in the iron deficient animal. Iron absorption studies using the human whole body counter are being done in patients with hemolytic anemia. One patient with hereditary spherocytosis was found to have normal absorption.

Role of the small intestinal mucosal cell in regulating iron absorption. Until recently no evidence existed for determining whether the regulation of iron absorption is accomplished by factors preventing iron uptake by the mucosal cells or by limiting iron release from the mucosal cells into plasma, or whether both were important. We have been studying this in rats by administering Fe⁵⁹ either intragastrically or intraduodenally and then determining at various intervals thereafter the uptake of Fe⁵⁹ by the small intestine and the amount of Fe⁵⁹ actually absorbed into the blood. Results thus far suggest that the regulation of absorption or "block" to iron absorption is related to uptake of iron by the mucosal cells. There is a significant difference in the amount of iron gaining access into the iron deficient intestine as compared to the iron replete intestine. Further studies are underway and will include use of an intact intestinal loop for perfusion studies using Fe⁵⁹.

Function of mucus in iron absorption. Preliminary results indicate that rat gastric and duodenal mucus bind iron. The possible function of this phenomenon in iron absorption is now being evaluated and extended to humans. Human duodenal mucus is being tested for iron binding.

6. The distribution of iron injected intraperitoneally. Three days after the intraperitoneal injection of 50 mg. of iron as iron-dextran much iron had accumulated as ferritin in the liver and spleen. (The animals were 200 gm. albino rats of the WRCF strain. The method of estimating ferritin was a micro modification of Granick's $CdSO_4$ technique for crystallization of ferritin from tissue homogenates.) Ferritin could also be recovered from the intestine, especially the duodenum and upper jejunum. There was none in the stomach and colon. Ten days after injection ferritin could not be recovered from the gut, though it was present in heavy concentration in liver and spleen. When the iron was injected intravenously or subcutaneously in heavier doses (150 mg.) it went to the liver and spleen without preliminary accumulation in the small intestine. In the significant experiment a loop of small intestine at the duodenojejunal junction was brought out of the peritoneum and tacked under the skin. Two weeks were allowed for healing of the wound. Iron-dextran was then injected intraperitoneally and after three days the animals were sacrificed. Ferritin was found in the small intestine adjacent to the extraperitoneal loop, but no ferritin was found in that loop. It is concluded that the ferritin which is present in the small intestine following intraperitoneal injection of iron-dextran is derived from iron or iron-dextran which migrates through the serosal surface of the intestine.

After ingestion of radioactive iron, significant amounts of radioactivity were found in blood, liver and marrow. There was little or none in the spleen.

7. Magnetic separation of iron-containing biological materials. Attempts to concentrate a commercial sample of iron-saturated transferrin by electromagnetism technique were unsuccessful, however the equipment was not ideally constructed and further study is necessary to learn whether this technique can be used. In order to test the equipment, crude samples of hemosiderin and ferritin have been isolated and passed through the magnetic separator, the ferritin in the form of the ammonium sulfate precipitate suspended in an ammonium sulfate solution. In both cases particles were attracted to the magnetic poles and fractions rich in iron-containing material were obtained.

Study of methods for the determination of micro quantities of iron in biological materials. A study of procedures for the determination of micro quantities of iron has continued. One hundred serum samples analyzed by an α, α -dipyridyl method of Ramsay, 1957, and by a sulfonated bathophenanthroline method of Trinder, 1956, gave the same average value by the two methods. Seventy-five per cent of the samples, analyzing over 60 micrograms iron per 100 ml., by the Ramsay technique, gave values by the Trinder technique which came within + 20 per cent of the Ramsay value; 21 per cent were within + 20 per cent, 23 per cent within -20 per cent, and 31 per cent very close to perfect agreement. A procedure involving wet digestion to eliminate all organic material, followed by color development with α, α -dipyridyl, was developed for analysis of 0.5 to 1 ml. of serum.

Ferritin, a storage protein for iron, plays a role in iron absorption. Work is in progress on a technique for the histologic demonstration of this protein using a fluorescent ferritin antibody as a tag for locating the tissue ferritin. Although tagging occurs with the antibody serum, the specificity of the reaction is not yet certain. At present experiments are under way to determine whether or not what is seen to fluoresce is actually ferritin.

Transferrin, the serum iron carrier, has been shown to be important in the regulation of iron absorption at least under certain experimental conditions. When incubated *in vitro* with Fe^{59} labelled transferrin, reticulocytes can be demonstrated to incorporate the labelled iron. Using this technique an attempt was made to show a difference in the incorporation of iron bound to hemochromatotic transferrin compared to that bound to normal transferrin. In preliminary experiments no difference was apparent.

8. Vitamin B_{12} absorption studies. Using Herbert's method of the everted gut sac, the effect of tryptophan and some of its derivatives on vitamin B_{12} absorption have been studied. It was our purpose to study the relationship of vitamin B_{12} malabsorption and increased excretion of urinary indole acetic acid noted in some patients with various small intestinal diseases, assuming bacterial products or metabolites in the gut might interfere in some way with B_{12} absorption. So far, using various concentrations in the incubation mixtures, tryptophan, indole and indole acetic acid do not seem to have any significant effect on the *in vitro* uptake of B_{12} by the rat intestine. The experiments are being repeated for definite results and other amino acids having metabolic pathways similar to tryptophan's will be studied.

In vivo studies. Animal whole-body counter. Vitamin B₁₂ absorption and turnover rates are being studied in normal rats using various concentrations of ⁵⁷Cu-labelled vitamin B₁₂ given orally or intraperitoneally. Curves for the oral doses available show three components: 1) an initial, rapid one from day 1 - 4, in which the decrease in body radioactivity and T_{1/2} vary according to the dose given; 2) a slow component from day 5 - 25 with T_{1/2} of approximately 35 days; and 3) a final, slow component from day 28 on, probably representing a "steady state" and whose T_{1/2} might represent the biological half-life for vitamin B₁₂. The results agree with published results determined from urine and fecal radioactivity, calculating remaining body radioactivity. The results for the intraperitoneal doses are not completed. Intestinal loops have been surgically created in rats to simulate the condition seen in humans and study vitamin B₁₂ absorption in them. After a period of observation, orally administered vitamin B₁₂ absorption is being studied using the whole-body counter. The material accumulated in the loops will be studied bacteriologically and chemically. Some of it will be used in the in vitro experiments. The effect of chronic iron deficiency on vitamin B₁₂ absorption is being studied in rats made deficient by repeated bleedings and low-iron diet over a period of four months.

9. Copper determinations in human plasma and RBC have been performed by a method developed during last year. Since the determinations have been mainly done in a blood-donor population, therefore it is necessary to collect more samples from more randomly distributed subjects in order to obtain normally representative figures. Copper determinations have been started in pathological conditions. Preliminary experiments have been performed to study by paper chromatography the phospholipid distribution in the erythrocytes from animals made anemic by a copper deficient diet. Further studies are in progress. At the present time studies on the effect of copper on iron absorption in normal rats have been started and they will be carried out also in iron-deficient rats and in copper-loaded rats. The copper distribution in red blood cells and the incorporation of copper in bone marrow will be studied with ⁶⁷Cu if technical difficulties, mainly due to the short half life of the isotope, can be overcome.

10. Studies on the metabolism of plasma hemoglobin. Normal removal curves of plasma bound hemoglobin have been established. Preliminary results have been obtained regarding sites of removal. Further studies are underway to determine the effect of reticulo-endothelial blockade with Thorotrast on the removal curves and site.

11. The use of capillary tubes in determination of erythrocyte sedimentation rate. The capillary tubes and capillary blood were planned to be used in erythrocyte sedimentation rate determination. The capillary tubes were filled with 3.8 per cent sodium citrate and blood in ratio of 1:4 or 1:3, gently mixed and poked vertically into putty and let stand for an hour at room temperature. The results correlate well with those from Wintrobe's method, although the number tested was small. The method is easy in handling, eliminates venous puncture special tubes and washings. It will be useful in children.

Summary and Conclusions:

1. A modification of the thromboplastin generation test has permitted the demonstration of platelet abnormality in patients with hitherto undiagnosed bleeding disorders.

2. Injection of bacterial endotoxin produces multiple lesions in the clotting mechanism.

3. Clot structure in granulocytic leukemia is disturbed in a manner which can be corrected by addition of glutathione.

4. An albumin inhibitor of thromboplastin is found not to be affected by administration of coumarin drugs.

5. Iron (Fe^{59}) absorption is studied in man and animals using whole-body scintillation counters.

6. The distribution of iron after absorption has been studied in small animals following injection and ingestion of iron.

7. Iron-protein complexes such as ferritin and transferrin are being investigated to develop methods for their identification and purification.

8. Absorption of vitamin B_{12} is studied by means of models employing rat intestine. Complementary studies are carried out in vivo.

9. A survey of plasma and red cell copper is being completed, using a method developed last year.

10. Plasma hemoglobin continues to be investigated to ascertain the areas other than kidney at which the hemoglobin is cleared from the plasma.

11. A method for performing erythrocyte sedimentation rate using capillary tubes is being studied.

List of Publications:

1. Barrett, O'N., Berman, A. and Maier, J. G.: Uptake of I¹³¹-l-triiodothyronine in various erythrocyte abnormalities. J. Clin. Endocr. 20: 1467, 1960.
2. Barrett, O'N., Conrad, M. E. and Crosby, W. H.: Chronic granulocytic leukemia in childhood. Amer. J. Med. Sci. 240: 587, 1960.
3. Conrad, M. E., Crosby, W. H. and Howie, D. L.: Hereditary nonspherocytic hemolytic disease. Amer. J. Med. 29: 811, 1960.
4. Conrad, M. E., Knochel, J. P. and Crosby, W. H.: Novobiocin jaundice: Demonstration of a hemolytic state. Antibiot. Med. 7: 382, 1960.
5. Crosby, W. H.: Die nachtlliche paroxysmale Hamoglobinurie. Handbuch der gesamten Hamatologie. Vol. III. Urban & Schwarzenberg, Berlin, 1960.
6. Crosby, W. H.: Hemorrhagic Complications in Surgery. Chap. 6. Complications in Surgery and Their Management. Artz and Hardy, Editors. W. B. Saunders Company, Publishers, Philadelphia, 1960.
7. Salvidio, E. and Crosby, W. H.: Thrombocytopenia after intravenous injection of India ink. J. Lab. Clin. Med. 56: 711, 1960.
8. Sheehy, T. W. and Berman, A.: The anemia of cirrhosis. J. Lab. Clin. Med. 56: 72, 1960.
9. Sheehy, T. W. and Ramos, H. S.: Hemostasis. U. S. Armed Forces Med. J. 11: 1133, 1960.
10. Sodee, D. B., Conrad, M. E. and Zone, R. N.: Cyclophosphamide in the treatment of leukemia and solid tumors: (Abst.) Clin. Res. 8: 18, 1960.
11. Wheby, M. S., Barrett, O'N. and Crosby, W. H.: Serum protein binding of myoglobin, hemoglobin and hematin. Blood 16: 1579, 1960.
12. Crosby, W. H.: The daily dose of folic acid. J. Chron. Dis. 12: 583, 1960.

13. Weiss, H. J., Eichelberger, J. W. and Crosby, W. H.: Studies on the differences in activity of lung and brain thromboplastin. *J. Clin. Invest.* 40: 205, 1961.
14. Adelson, E., Rheingold, J. J., Parker, O., Buenaventura, A. and Crosby, W. H.: Platelet and fibrinogen survival in normal and abnormal states of coagulation. *Blood* 17: 267, 1961.
15. Crosby, W. H., Vullo, C. and Garriga, S.: Hemolysis in vitro in acute leukemia and the anemia of leukemia. Blood. In press.
16. van Hoek, R. and Conrad, M. E.: Iron absorption. Measurement of ingested iron⁵⁹ using a human whole-body liquid scintillation counter. *J. Clin. Invest.* In press.
17. Eisen, B., Demis, D. J. and Crosby, W. H.: Preliminary observations on 6-Thioguanine therapy of "auto-immune" syndromes.
18. Johnson, T. and Barrett, O'N.: A rapid quantitative paper electrophoretic technique for determining A₂ hemoglobin. *J. Lab. Clin. Med.* In press.
19. Munn, J. L. and Crosby, W. H.: Red cell lipids in various abnormalities in the human red cell. *Brit. J. Haemat.* In press.
20. Adelson, E., Rheingold, J. J. and Crosby, W. H.: The platelet as a sponge. A review. *Blood*. In press.
21. Thirayothin, P.: The distribution of iron injected intraperitoneally. Evidence of serosal absorption by the small intestine. (Abst.) *J. Clin. Invest.*
22. Wheby, M. S., Conrad, M. E., Hedberg, S. E. and Crosby, W. H.: The role of bile and pancreatic juice in the control of iron absorption. (Abst.) *Clin. Res.* 9: 168, 1961.

FINAL REPORT

Project 6X59-06-001, Radiation and Thermal Burns

Task 4, Immune Mechanisms in Thermal Burns

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Department of Surgical Metabolism and Physiology
Department of Experimental Surgery
Division of Surgery

and

Department of Germfree Research, Special Activity

Period Covered by Report: 1 July 1958 through 30 June 1961

Principal Investigator: Ole J. Malm, M.D., Dr.Med.(Oslo)*

Assistants: Capt George J. M. Slawikowski, MC
Capt Richard E. Horowitz, MC**
Stanley M. Levenson, M.D.

Reports Control Symbol: MEDDH-288

Security Classification: Unclassified

*Now Scientific Director, Inst. of Exper. Medicine, Univ. of Oslo,
Ullevaal Hospital, Oslo, Norway.
**Now Dept. of Pathology, Mt. Sinai Hospital, New York, N. Y.

ABSTRACT

Project No. 6X59-06-001 Title: Radiation and Thermal Burns

Task No. 4 Title: Immune Mechanisms in Thermal Burns

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Period Covered by Report: 1 July 1958 through 30 June 1961

Authors: Ole J. Malm, M.D.; Capt George J. M. Slawikowski, MC; Capt Richard E. Horowitz, MC; Stanley M. Levenson, M.D.

Reports Control Symbol: MEDDH-288

Security Classification: Unclassified

Effects of different types of sterile convalescent sera on mortality of severely burned rats were evaluated. A hot plate burning technic yielding a constant percentage of full-thickness skin burns was perfected. Double-blind methods were used.

Sera collected from rats convalescing from a single burn were consistently found to diminish mortality of rats with burns of more than 50 percent skin area, but not of those with smaller burns. The lowering of mortality occurred primarily between the third and fifth days after burn; differences in cumulative mortality rates remained significant up to 28 days. Sera obtained from rats convalescing from excised skin wounds or sera obtained from rats after repeated burns had no prolonged effect on cumulative mortality; rats treated with open wound convalescent serum showed higher mortality during the first 48 hours, on the third day the mortality was diminished, and thereafter there was no effect. Mortality was unaffected by sera of rats convalescing from closed fractures. Chloromycetin alone was found to reduce mortality of burned rats; chloromycetin supplemented by normal or burn convalescent serum was not more effective than chloromycetin given with saline.

BODY OF REPORT

Project No. 6X59-06-001

Title: Radiation and Thermal Burns

Task No. 4

Title: Immune Mechanisms in Thermal Burns

Description:

The experiments reported here were undertaken (1) to evaluate the effectiveness of sterile burn convalescent serum in reducing mortality from severe burns of rats and (2) to compare this serum with other types of convalescent sera, and thus to determine if such protective action is unique for the burn convalescent serum.

Progress:

The progress reports of 30 June 1959 and 30 June 1960, and recent publications by Malm, and by Malm and Slawikowski in the Proceedings of the First International Congress on Research in Burns, 1961, have surveyed in detail the background of this task and have given a description of the methodology and the first three experiments using approximately 250 animals. Briefly, burn convalescent serum obtained three months after a single burn involving 25 percent skin area produced a consistent, though small, lowering of the mortality of rats after burns involving 55 to 65 percent skin area (e.g. at 7 days, cumulative mortality was 27.5 percent as compared to 41.0 percent). Serum obtained ten days after five repeated burns five days apart totalling 25 percent of skin area was not effective. The experiments reported in detail here used approximately 500 burned and treated rats, and were designed to extend and confirm these findings.

In experiments 4-A, 5 and 6 the burns involved 55 to 65 percent of postburn skin area; in experiment 4-B, 40 to 45 percent of skin area. All rats received subcutaneous injections of 7 percent body weight of 0.9 percent sodium chloride solutions immediately and 3 hours after burns. Sera were given intraperitoneally, 0.5 percent body weight, immediately after burn; thereafter intramuscularly: 0.4 percent after 24 hours, 0.3 percent after 48 hours, and 0.2 percent body weight on days 5, 9, 12 and 16 after burns. In experiment 6, chloromycetin succinate, 20 mg per kg body weight, was administered by intramuscular injections daily for 14 days after the burns, with or without sera.

EXPERIMENT 4

Burn Convalescent Serum Compared With Open Wound Convalescent Serum and Normal Serum

a. Burns of 55-60% skin area (Day-to-Day Mortality %)			b. Burns of 40-45% skin area (Day-to-Day Mortality %)			
A (n=70)	B (n=70)	C (n=69)	A (n=45)	B (n=45)	C (n=45)	D (n=44)
17.1	14.2	11.5	0	6.2	4.4	4.5
47.1	40.0	50.8	31.1	26.6	44.4	36.4
10.0	27.2	11.6	22.2	22.3	15.6	22.7
2.9	10.0	7.2	11.1	11.1	15.6	13.6

A (n=70)	B (n=70)	C (n=69)	A (n=45)	B (n=45)	C (n=45)	D (n=44)
(Cont'd)						
0	0	0	0	0	0	2.3
0	0	0	0	0	0	0
1.4	0	0	0	4.5	2.2	0
2.9	2.8	0	2.3	0	2.2	4.5
1.4	0	0	4.4	4.4	2.3	6.9
0	0	0	2.2	6.7	2.2	0

- Group A: Treated with burn convalescent serum, obtained 3 months after a single burn of 25 percent skin area.
- Group B: Treated with normal serum (from unburned rats).
- Group C: Treated with open wound convalescent serum, obtained 3 months after excision of 25 percent skin area.
- Group D: Treated with saline only.

EXPERIMENT 5

Test-Burn Survivor Serum Compared With Closed Fracture Convalescent Serum and Normal Serum

Days After Burn	Burns of 55-65% Skin Area		
	(Day-to-Day Mortality %)		
	A (n=43)	B (n=43)	C (n=42)
1	20.9	16.2	7.1
2	58.8	70.1	73.9
3	9.3	2.4	7.1
4	2.3	0	7.1
5	2.4	2.3	2.4
6	0	2.3	0
7	0	0	0
14	2.3	2.4	0
21	0	0	0
28	0	0	0

- Group A: Treated with burn convalescent serum obtained from rats surviving 3 months in Experiment 3.
- Group B: Treated with normal serum.
- Group C: Treated with convalescent serum obtained 3 months after closed bilateral femoral fractures.

Effect of Chloromycetin, With or Without Burn
Convalescent Serum

Days After Burn	Burns of 55-60% Skin Area			
	(Day-to-Day Mortality %)			
	A (n=55)	B (n=54)	C (n=53)	D (n=54)
1	0	0	0	0
2	18.2	18.5	15.0	24.1
3	9.1	3.7	9.4	25.9
4	14.5	16.6	11.3	14.7
5	0	0	5.7	1.9
6	7.3	5.5	1.9	3.7
7	0	1.9	1.9	1.9
14	20.0	22.2	17.0	7.4
21	9.1	11.1	3.8	1.8
28	0	7.4	0	1.9

Group A: Chloromycetin plus 3-month burn convalescent serum.

Group B: Chloromycetin plus normal serum.

Group C: Chloromycetin plus saline only.

Group D: No chloromycetin, saline only.

Appropriate data from the above experiments and experiments previously reported were pooled for statistical comparison.

No effect of burn convalescent serum (BCS) was seen in rats receiving burns of less than 50 percent skin area (experiment 4-B). Following test burns of 50 to 65 percent of skin area (experiments 1, 2, 3 and 4-A) BCS obtained 2 to 5 months after 25 percent burns was consistently protective as compared to normal serum (NS). Mortality on the third day was 7.5 percent of 158 BCS-treated rats vs. 14.9 percent of 161 NS-treated rats ($P = 0.02$) and on the fourth day 2.5 percent and 9.3 percent respectively ($P = 0.007$). Accordingly, cumulative mortality differences reached the highest significance ($P = 0.002$) on the fifth day; they were seen as late as 28 days after burn ($P = 0.02$). The mortality during the first two days after burn was identical in the two test groups, justifying an evaluation of cumulative mortality of rats surviving the first 48 hours. Such evaluation showed an even higher significance of the differences between BCS- and NS-treated rats. (P is less than 0.001 on days 4, 5, 6 and 7); these differences persisted until 28 days following burn ($P = 0.02$). These data indicate that the protective effect of BCS is primarily exerted on the third and fourth days after burns and that this protection is not offset by a rebound mortality within the first month.

By contrast, the open wound convalescent serum (experiments 1 and 4), gave an increased mortality on the second day (43.4 percent vs. 34.0 percent, $P = 0.03$), so that lowered mortality on the third day (12.4 percent of 114 vs. 22.2 percent of 115 rats, $P = 0.01$) may have been due to accelerated deaths. Cumulative mortality figures revealed no net

difference. It is of interest that in experiment 3, treatment with serum obtained 10 days after repeated small burns had also increased the mortality on day 2 (11.5 percent of 78 vs. 24.1 percent of 120 rats, $P = 0.01$).

No protective effect was observed following treatment with fracture convalescent serum or test burn survivor serum, but high mortality in the first 48 hours may have obscured such an effect.

Chloromycetin alone reduced mortality on the second, third, and to a lesser extent, on the fourth day after burns so that cumulative mortality on the fourth day was 35.9 percent of 53 treated vs. 64.8 percent of 54 untreated rats ($P = 0.002$). This difference persisted in cumulative mortality until treatment was terminated 14 days after burn ($P = 0.02$), indicating an absence of early rebound mortality. Chloromycetin alone was equally effective as when given with BCS or NS. The difference in mortality rates in rats treated with BCS as compared to those treated with NS was not seen when Chloromycetin was also given.

Ancillary Studies (experiments 7-A and 7-B).

EXPERIMENT 7-A. Estimation of surface area of the rat and the percentage of skin area burned.

Sixty burned and unburned rats were studied. The skin area was estimated by tracing it out on aluminum foil of constant area per unit weight. Undue stretching and distortion of the skin was prevented by measuring the length of the rat and the circumference at three different levels along the body. A weight range from 276 to 325 grams was studied. Plotting log weight against log skin area the formula for skin area based on body weight was established as $5.83 \times \text{body weight}^{0.667}$. The corresponding area of stretched skin was $29.67 \times \text{body weight}^{0.48}$, with the stretch factor $5 \times \text{body weight}^{-0.2}$.

It was confirmed that when 45 percent of the calculated preburn surface area is burned the resultant burn involves approximately 65 percent of the post-burn skin area, 35 percent of preburn area corresponds to approximately 55 percent of post-burn area, and 30 percent of preburn area corresponds to approximately 45 percent of post-burn area. The percentage of skin, burned by the technic employed throughout these experiments by the authors, was shown to be remarkably consistent in the various weight ranges studied.

EXPERIMENT 7-B. Bacteriology of burns, and antibacterial titres of therapeutic sera.

Only pilot studies were performed. Random samples of burn sites yielded cultures of E. coli and Proteus. Three strains of Proteus were tested by Major Muschel (Serology, WRAIR) against all the sera used in the various experiments, 10⁷ organisms being subjected to the action of 0.1 ml of each serum. Antibacterial effect was estimated by the photometric method. The Proteus under these conditions showed no susceptibility to any of the sera. This was particularly significant since as little as

10^{-2} gram (millimicrogram) of antibody has been known to produce antibacterial effect with this technic. Some antibacterial activity of burn convalescent serum was observed on *Shigella* in two trials, but not in a third one.

Summary and Conclusions:

The primary objectives of this task have been achieved: burn convalescent sera collected under sterile precautions 2 to 5 months after burns, were shown to cause consistent though small lowering of the mortality after severe burns in rats (greater than 50 percent skin area), the greatest effect being seen on the third and fourth days after burns, and persisting in cumulative mortality figures as late as three and four weeks after burn.

Sera from rats repeatedly burned, or from rats whose skin was excised and the wound was left open, or from rats suffering closed femoral fractures, were not shown to exert this protective effect. The open wound convalescent serum showed greater mortality on the second day so that the small protective effect on the third day after burn may have been due to accelerated deaths; cumulative mortality was essentially the same as that of rats treated with normal serum.

The marked effect of chloromycetin in reducing burn mortality, and the observation that burn convalescent serum was not more protective than normal serum when these sera were administered together with chloromycetin, suggests indirectly that the burn convalescent serum might have exerted some of its protective activity through antibacterial action; however, the finding that open wound convalescent serum has not been equally protective suggests that some other factors also contribute to the lowering of mortality by burn convalescent serum.

Estimations of surface area of the rat on the basis of weight, and the estimation of percentage of skin area burned by the standard hot plate technic were confirmed by special studies.

Antibacterial titres of sera were studied against Proteus cultured from burned skin.

A detailed account of these experiments will appear at a later date in a monograph.

List of Publications:

1. Malm, O. J.: Research on "Burn-Toxemia," Past, Present and Future. Presented at the First International Congress on Research in Burns, Washington, D. C., 20 September 1960. To be published in the Proceedings of this Congress by the American Institute of Pathological Sciences in July, 1961.
2. Malm, O. J. and Slawikowski, G. J. M.: Evaluation of Different Types of Convalescent Burn Sera in the Rat. Ibid.

3. Malm, O. J. and Slawikowski, G. J. M.: Mortality after Severe Experimental Burns of Rats Treated with Chloromycetin, and with Sera of Rats Convalescing from Burns or from Open Wounds. To be presented at the Surgical Forum of the American College of Surgeons, Chicago, Illinois, October 1961. To be published in Surgical Forum, Vol. 12, 1961.
4. Malm, O. J. and Slawikowski, G. J. M.: Lethal Factors in Burns — An Evaluation of the "Burn-Toxin" Theory and Convalescent Serum Therapy, with Emphasis on Experimental Methods. A Monograph in preparation for publication.

ANNUAL PROGRESS REPORT

Project 6X60-01-001, Internal Medicine

Task 4, Experimental Arterial and Heart Disease

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Department of Cardiorespiratory Diseases
Division of Medicine

Period Covered by Report: 1 July 1960 through 30 June 1961

Principal Investigator: Donald E. Gregg, Ph.D., M.D.

Assistants: LtCol Robert J. Hall, MC*
Capt Jay D. Coffman, MC
Capt Lloyd C. Fisher, MC
Capt Andrew Huvos, MC
Capt Ray A. Olsson, MC
Claudia R. Rayford, Ph.D.
Nicholas Cambosos, Ph.D.
Edward M. Khouri
Klaus Thurau, M.D.**
Lino Granata, M.D.#
Takeo Tsunekawa, M.D.##

Reports Control Symbol: MEDDH-288

Security Classification: Unclassified

- * Walter Reed General Hospital
- ** U. S. Public Health Service Foreign Research Fellow, Goettingen, Germany
- # Fulbright Fellow from the University of Parma, Italy
- ## Foreign Trainee from Japan

ABSTRACT

Project No. GX60-01-001

Title: Internal Medicine

Task No. 4

Title: Experimental Arterial and Heart Disease

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Period Covered by Report: 1 July 1960 through 30 June 1961

Authors: Donald E. Gregg, Ph.D., M.D. Claudia R. Rayford, Ph.D.
Lt Col R. J. Hall, MC Nicholas Cambosos, Ph.D.
Capt Jay D. Coffman, MC Edward M. Khouri
Capt Lloyd C. Fisher, MC Klaus Thurau, M.D.
Capt Andrew Huvos, MC Lino Granata, M.D.
Capt Ray A. Olsson, MC Takeo Tsunekawa, M.D.

Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

The central and peripheral regulation of regional blood flow and metabolism has been studied by means of an improved electromagnetic flow meter chronically implanted in the active unanesthetized dog. A circumflex coronary-pulmonary artery anastomosis in the chronic dog has been developed and is being studied for its future value in investigating determinants of coronary collateral flow. In the normal dog, coronary flow per minute increases in excitement but not during exercise. Cardiac sympathetic nerve stimulation increases the stroke coronary flow as in excitement. Exercise and excitement increase external iliac (skeletal muscle) flow, while passive change from the standing to the squatting position or to the upright position, decreases the flow. Splanchnic nerve stimulation, exercise, excitement and certain postural changes all decrease mesenteric flow. Renal blood flow is not significantly changed by carotid occlusion, large arterial oxygen saturation changes, reasonable blood pressure changes, postural changes, exercise and excitement. A fixed relationship exists in the kidney between sodium reabsorption and oxygen consumption at high and low levels of renal metabolic activity. In micro-puncture studies of the renal papillae of the hamster, plasma albumin concentrations progressively increase to the papilla tip, while the hematocrit progressively decreases. Pressure-flow curves for the aorta, superior mesenteric, renal and external iliac arteries in unanesthetized dogs are not significantly different during hemorrhage at the onset of irreversible shock as compared to those during simple hemorrhagic hypotension.

BODY OF REPORT

Project No. 6X60-01-001, Internal Medicine

Task No. 4, Experimental Arterial and Heart Disease

Description:

The general plan of study is to obtain a broad perspective of the central nervous and local controls of the circulation of the unanesthetized dog exposed to normal and abnormal stresses of every day life. This is made possible without the associated insults of anesthesia, trauma, and surgery through the development of the electromagnetic flow meter for chronic implantation on the aorta and various regional arteries. The early experimental results are reported.

Progress:

Improvements in the electromagnetic flow meter have permitted its implantation for long term studies on chronic dogs. The general plan is to study the central and peripheral regulation of the regional blood flow and metabolism in dogs exposed to a variety of stresses including those that naturally occur, such as exercise and excitement. In each dog, present facilities permit simultaneous measurements of blood pressure through an indwelling polyvinyl coated nylon tube in the aorta, of aortic or pulmonary flow by a flow transducer on the ascending aorta or pulmonary artery, and of flows through one or two arterial beds such as the renal and mesenteric arteries.

1. Improvement and evaluation of an electromagnetic flow meter. The electromagnetic flow meter amplifier now has improved gain and signal to noise ratio. Three amplifiers have been built. Transducers have been constructed in various sizes and these have been applied to the aorta, the common carotid, vertebral, iliac, mesenteric and renal arteries, and to the coronary arteries. Techniques have been developed for splicing sturdier cables to the transducers without sacrifice of size or flexibility, thus reducing the incidence of failure due to cable breakage. New plastics are being investigated to minimize or eliminate failure due to tissue fluid seepage.

2. Development of a method to study collateral blood flow in the unanesthetized dog. Retrograde circumflex-coronary blood flow in the intact dog, shunted into the pulmonary artery via an end to side anastomosis is being studied for its value in establishing some determinants of the intercoronary collateral circulation. Anastomotic flow in the unanesthetized animal will be determined by the electromagnetic flow meter and mean systemic and pulmonary pressures monitored by the use of chronic, indwelling, polyvinyl catheters. It is hoped that some insight into the basic pathophysiology of the aberrant left coronary artery will be obtained.

3. Left coronary blood flow during rest, exercise and excitement. Studies have been made in the intact dog of the total left coronary artery and systemic cardiac output, at rest, during exercise, and following various stimuli. The stroke coronary flow decreases during exercise while the coronary flow per minute increases considerably. The cardiac output increases by a factor of two to three while the stroke volume does not increase sensibly. Increase in heart rate seems primarily responsible for the elevated cardiac output and coronary flow. During excitement, the stroke coronary flow temporarily decreases mildly, then rapidly rises to a peak of about three times the control values. The systemic stroke volume temporarily decreases and then increases moderately, while the cardiac output becomes quite large.

4. The effect of cardiac sympathetic nerve stimulation on left coronary blood flow in the chronic dog. Stimulation of cardiac sympathetic fibers in chronic unanesthetized dogs is accomplished by means of bipolar platinum electrodes mounted in an acrylic plastic holder and placed around the stellate ganglion or the anterior root of the ansa subclavia. The effects of sympathetic stimulation are compared with those of excitement and exercise. Early experiments show that following nerve stimulation, there is an initial reduction in coronary flow and then an increase in flow. This response is similar to that obtained during excitement.

5. Chronic measurements of coronary flow and myocardial metabolism at rest. Left ventricular work (cardiac output x arterial blood pressure) and left ventricular metabolism are being studied in chronic dogs by means of electromagnetic flow meters implanted around the main left coronary artery or one of its major branches, and by means of chemical analyses of arterial and coronary venous blood obtained from the aorta and the coronary sinus, respectively, through indwelling polyvinyl catheters. Preliminary measurements have been made in three dogs.

6. Determinants of skeletal muscle flow in the chronic dog. The flow in the external iliac artery is being studied under various experimental conditions. An increase in flow, accompanied by an increase in arterial pressure and cardiac output is observed in response to exercise and to excitement. Change from the standing to squatting position decreases iliac and aortic flow, and similar effects are noted as the result of change from the standing position to that which simulates the upright position of man. The results on tilting experiments are equivocal.

7. Determinants of mesenteric blood flow in the chronic dog. An electromagnetic flow meter is implanted on the inferior mesenteric artery, and bipolar platinum electrodes are placed around the splanchnic nerve. Preliminary experiments indicate that the mesenteric flow decreases during the stimulation of the splanchnic nerve and, to an even greater extent, immediately after the cessation of the stimulation. These effects are accompanied by a rise in aortic blood pressure. Reactive hyperemia is moderate. A reduction in mesenteric flow together with an increase in arterial blood pressure and cardiac output has been observed in response to excitement, to exercise, and to some postural changes such as head down tilt.

8. Autoregulation of renal blood flow in the chronic dog. Experiments have been performed regarding the autoregulation of renal blood flow in the normal unanesthetized dog, using the electromagnetic flow meter. Reactive hyperemia following artery occlusion is very small. There is essentially no change in renal blood flow with the following variables: bilateral carotid occlusion (anesthetized dogs), lowering of the arterial saturation by breathing a low oxygen mixture, excitement, large reductions of arterial blood pressure by hemorrhage, marked postural changes, light and heavy treadmill exercise of short and long duration. The basic mechanism of such control is being investigated.

9. Renal sodium reabsorption and oxygen uptake in dogs during hypoxia and hydrochlorothiazide infusion. It is well established that sodium reabsorption in the kidney is an active process and that this process requires the bulk of the oxygen consumed by the kidney. In the present study, to test this at low levels of kidney activity, the active reabsorption of sodium and the oxygen consumption of the kidney are decreased by having the dogs breathe a 7.2% oxygen mixture, or by intravenous administration of hydrochlorothiazide. The renal blood flow is measured by an electromagnetic flow meter. In this severe hypoxia, the renal blood flow is essentially unchanged while it decreases mildly with hydrochlorothiazide infusion. Active sodium reabsorption and net oxygen consumption are lowered proportionately during the induction of both variables, thus indicating the persistence of a fixed relation between sodium reabsorption and oxygen consumption at this low level of renal metabolic activity. The basal oxygen consumption of the kidney is about 1 μ ml/gm/kidney/min.

10. Micropuncture studies of albumin concentration and hematocrit in renal medullary blood of hydropenic hamsters. Renal medullary vasa recta blood samples are obtained by micropuncture techniques in golden hamsters following the intravenous injection of I¹³¹ labeled serum albumin. Analysis of vasa recta blood reveals a progressive increase in plasma albumin concentrations to the tip of the papilla. Ratios of vasa recta to peripheral plasma albumin concentrations near the tip of the papilla range from 2.1 to 2.9. Hematocrits of vasa recta blood seem to fall from base to tip of papilla ranging from 12 to 30 proximally, and from 8 to 18 at the tip. The progressive increase in concentration of albumin in the vasa recta blood must be due to water loss into the hypertonic environment. This is interpreted to indicate that the permeability of these vessels is similar to that of other capillaries. The progressive decrease in hematocrit in the vasa recta blood probably results from short-circuiting of red cells into the medullary vessels.

11. Aortic and regional pressure flow relationships during hemorrhagic shock in unanesthetized dogs. The effects of hemorrhagic shock on the pressure-flow relationship in various vascular beds of unanesthetized dogs are being studied by means of electromagnetic flow meters chronically placed around the aorta, the superior mesenteric, renal and external iliac arteries. Blood pressure is monitored through an indwelling polyvinyl catheter in the aorta. Graded hemorrhage is induced by removal of blood

from the aorta through a femoral arterial catheter connected to a blood reservoir. An arterial pressure of 30-40 mm.Hg is maintained for approximately 90 minutes, following which reinfusion occurs through the femoral vein. Then, after an additional 2-4 hours when blood pressure may start to decline toward shock levels, hemorrhage is again reinstated. With the simple initial hemorrhage, aortic resistance is generally mildly increased, but the effects of this hemorrhage appear to be widely different in the three peripheral vascular beds thus far studied; the resistance to flow changes little or decreases in the renal circuit until quite low pressure levels are reached when it rises, whereas the mesenteric and, to an even greater extent, the external iliac beds respond to hemorrhage with an increase in resistance. When irreversible shock is imminent as evidenced after reinfusion by the onset of declining arterial blood pressure, the pressure-flow relationship in these vascular beds is not significantly changed from that observed earlier in the same experiment with simple hemorrhagic hypotension.

Summary and Conclusions:

The central and peripheral regulation of regional blood flow and metabolism has been studied by means of an improved electromagnetic flow meter chronically implanted in the active unanesthetized dog. A circumflex coronary-pulmonary artery anastomosis in the chronic dog has been developed and is being studied for its future value in investigating determinants of coronary collateral flow. In the normal dog, coronary flow per minute increases in both exercise and excitement; the flow per heart beat increases in excitement but not during exercise. Cardiac sympathetic nerve stimulation increases the stroke coronary flow as in excitement. Exercise and excitement increase external iliac (skeletal muscle) flow, while passive change from the standing to the squatting position or to the upright position decreases the flow. Splanchnic nerve stimulation, exercise, excitement and certain postural changes all decrease mesenteric flow. Renal blood flow is not significantly changed by carotid occlusion, large arterial oxygen saturation changes, reasonable blood pressure changes, postural changes, exercise and excitement. A fixed relationship exists in the kidney between sodium reabsorption and oxygen consumption at high and low levels of renal metabolic activity. In micropuncture studies of the renal papillae of the hamster, plasma albumin concentrations progressively increase to the papilla tip, while the hematocrit progressively decreases. Pressure-flow curves for the aorta, superior mesenteric, renal and external iliac arteries in unanesthetized dogs are not significantly different during hemorrhage at the onset of irreversible shock as compared to those during simple hemorrhagic hypotension.

List of Publications:

1. Gregg, D. E., J. D. Coffman, A. Huvos and K. Thurau: Best & Taylor, Physiological Basis of Medical Practice. Revision of Chapters 14-28 dealing with cardiovascular system. Williams & Wilkins Co., Balto., 1961.

2. Gregg, D. E.: Physiology of the Coronary Circulation. *Annals of New York Academy of Sciences* 90:145, 1960.
3. Coffman, J. D. and D. E. Gregg: Ventricular fibrillation during uniform myocardial anoxia due to asphyxia. *American Journal of Physiol.* 198:955, 1960.
4. Coffman, J. D., F. B. Lewis and D. E. Gregg: Effect of prolonged periods of anoxia on atrioventricular conduction and cardiac muscle. *Circulation Research* 8:649, 1960.
5. Khouri, E., Gregg, D. E., Hall, R. and Rayford, C. R.: (Abstract) Regulation of coronary flow during treadmill exercise in the dog. *The Physiologist* 3:93, 1960.
6. Coffman, J. D. and D. E. Gregg: Reactive hyperemia characteristics of the myocardium. *American Journal of Physiology* 199:1143, 1960.
7. Lewis, F. Bruce, J. D. Coffman and D. E. Gregg: Effect of heart rate and intracoronary isoproterenol, levarterenol and epinephrine on coronary flow and resistance. *Circulation Research* 9:89, 1961.
8. Gregg, D. E.: Collateral circulation of the heart and the pathophysiology of different surgical approaches to human atherosclerosis. *Third International Convivium, Ganassini Foundation, Milan, 1960.*
9. Gregg, D. E. and J. D. Coffman: Physiology of the Coronary Circulation. *Monograph on Blood and Lymph Vessels. In press. Academic Press.*
10. Coffman, J. D. and D. E. Gregg: Pharmacology of the Coronary Circulation. *Monograph on Blood and Lymph Vessels. In press. Academic Press.*
11. Hall, R. J., E. M. Khouri and D. E. Gregg: Coronary-internal mammary artery anastomosis in dogs. *In press. Surgery.*
12. Thurau, K.: Renal Na-reabsorption and O₂ uptake in dogs during hypoxia and hydrochlorothiazide infusion. *Proceedings of Society for Experimental Biol. & Medicine* 106:714, 1961.
13. Coffman, J. D. and D. E. Gregg: Oxygen metabolism and oxygen debt repayment following myocardial anoxia. *In press. American Journal of Physiology.*
14. Gregg, D. E.: Collateral circulation of the heart and pathophysiology of the different surgical approaches to human atherosclerosis. *Atherosclerosis and Enfermedad Coronaria, Editorial Interamericana, S. A., Mexico, 1960.*
15. Thurau, K., T. Sugiura, and L. S. Lilienfield: Albumin concentration and hematocrit in renal medullary blood of hypopenic hamsters: Results of a micropuncture study. *Submitted for publication.*

ANNUAL PROGRESS REPORT

Project 6X60-01-001, Internal Medicine

Task 4, Experimental Arterial and Heart Disease

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Department of Experimental Surgery
Division of Surgery

Period Covered by Report: 21 December 1959 through 30 June 1961

Principal Investigator: Maj Elias A. Husni, MC*

Reports Control Symbol: MEDDH-288

Security Classification: Unclassified

*Now Attending Surgeon, Huron Road Hospital, East Cleveland, Ohio.

ABSTRACT

Project No. 6X60-01-001

Title: Internal Medicine

Task No. 4

Title: Experimental Arterial and Heart Disease

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Period Covered by Report: 21 December 1959 through 30 June 1961

Author: Maj Elias A. Husni, MC

Reports Control Symbol: MEDDH-288

Security Classification: Unclassified

Evaluation of Post-thrombotic Arterial Atrophy. The physiologic and anatomic responses of muscular arteries to chronic reduction of the local hydrostatic pressure were investigated in adult mongrel dogs. Thrombosis of the common femoral arteries was produced by intraluminal implantation of a coil of thrombogenic wire (98 percent magnesium and 2 percent aluminum). Thrombosis or patency of the arteries was determined by arteriography and/or surgical exploration. After hemodynamic studies, animals were sacrificed at varying periods from 2 to 7 months postoperatively.

BODY OF REPORT

Project No. 6X60-01-001 Title: Internal Medicine

Task No. 4

Title: Experimental Arterial and Heart Disease

Description:

Evaluation of Post-thrombotic Arterial Atrophy. To study the physiologic properties and anatomic changes, if any, of the media of muscular arteries in response to chronic reduction in local blood pressure.

Progress:

Evaluation of Post-thrombotic Arterial Atrophy. In a previous study concerned with the laboratory evaluation of the peripheral circulation in patients with occlusive arterial disease, it was disclosed that a remarkable state of hyperemia of the extremity ensues upon surgical reestablishment of arterial continuity. The data strongly suggested that the physiologic mechanism primarily responsible for this phenomenon was a reduction in the tone of arterial and arteriolar smooth muscle incident to the "chronic diminution of the normal stimulus, stretch, for the contraction of smooth muscle." This diminution was presumed to be a reduction in intraluminal blood pressure resulting from arterial occlusion. Since medial hypertrophy has been observed to occur in vessels subjected to a chronically elevated intravascular pressure, and since there is evidence that the process is reversible, it was contended that the reverse should also be possible, i.e., a chronically reduced intravascular pressure should condition medial atrophy and loss of tone. The present study was therefore undertaken in an effort to validate or refute this contention experimentally.

Controlled experiments were carried out upon 55 adult mongrel dogs. Gradual thrombosis (10 to 30 days for complete occlusion) was conditioned in the common femoral artery on one side by the intraluminal implantation of a coil of thrombogenic wire (98% Mg and 2% Al). The contralateral side served as control for future studies.

Two to seven months later, when occlusion was complete and collateral circulation fully developed, hemodynamic studies were undertaken in an air-conditioned room maintained at approximately 21 degrees Centigrade. Animals were anesthetized with a mixture of Dial Urethane and Sodium Pentobarbital. Skin temperature, volume of pulse, and rate of blood flow in the paws were measured. Blood pressure in communicating small arteries and venules in the paw was determined by microcatheterization utilizing a stainless steel canula (O.D. 0.5 mm). The popliteal and common femoral arteries of both sides were then exposed and careful measurements of their outside diameters (O.D.) were carried out. Intraluminal pressure was determined in both right and left external iliac, and popliteal arteries and veins. Finally, the entire arterial tree was exposed. Equal segments were taken from the right and the left arteries at the same levels. These specimens were used for determination of dry weight, total nitrogen and histologic sections.

Summary and Conclusions:

Evaluation of Post-thrombotic Arterial Atrophy. 1. The extent and the rapidity of induced thrombosis could be predetermined fairly accurately on the basis of the size of the individual coil and the number of coils per centimeter of thrombogenic wire. This technique was developed by trial and error, and through serial arteriographic and histologic studies. The average thrombus involved both the common femoral and the superficial femoral arteries for a total length of 7.0 cm. Several bridging collaterals joining the terminal branches of the internal iliac and the popliteal arteries were always demonstrated by arteriography. The mean blood pressure in arterial segments distal to the thrombus was reduced by about 22 mm. Hg as compared to the normal contralateral side. Pressure in communicating foot pad arteries and venules was reduced by 17 and 25 percent, respectively. The O. D. of the popliteal arteries on the experimental side was 25 percent less than on the opposite control side.

The volume of pulse in the paw was much smaller in the thrombosed side than in the normal contralateral side. The rate of blood flow, however, was approximately the same in both paws under the condition of these experiments. The peripheral resistance in the paw was therefore significantly lower on the thrombosed side as compared with the control side.

2. Upon histologic examination a remarkable thinning of the media was demonstrable in the arterial tree distal to the thrombotic occlusion when compared to the control side. The dry weight and total nitrogen determinations of arterial segments (1.0 cm. long) obtained from both sides at similar levels in the arterial tree yielded mean values of 4.5 mgm and 0.83 mgm for the experimental side and 6.3 mgm and 1.2 mgm for the control side.

In conclusion, a technique was standardized for production of predictable thrombosis in the femoral artery of the dog. These thrombotic occlusions effected a chronic reduction in the hydrostatic blood pressure in the vascular tree distal to the thrombus from the level of the largest artery to the venule in the paw. This reduction in blood pressure was associated with medial atrophy and relative loss of tone in these arteries, as evidenced by a significant decrease in the O. D. of these vessels, a remarkable thinning of the media, diminished dry weight and total nitrogen content and a lower peripheral resistance on that side.

This phase is concluded.

List of Publications:

1. Husni, E. A.: Experimental Thrombosis of the Canine Femoral Artery: Hemodynamic Studies. To be published.

ANNUAL PROGRESS REPORT

Project 6X60-01-001, Internal Medicine

Task 22, Instrument Development for Cardiac Research

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Department of Cardiorespiratory Diseases
Division of Medicine

Period Covered by Report: 1 July 1960 through 30 June 1961

Principal Investigator: Merlin Davis*

Assistants: Donald E. Gregg, Ph.D., M.D.
Edward M. Khouri

Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

* National Bureau of Standards

ABSTRACT

Project No. 6X60-01-001

Title: Internal Medicine

Task No. 22

Title: Instrument Development
for Cardiac Research

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Period Covered by Report: 1 July 1960 through 30 June 1961

Authors: Merlin Davis (National Bureau of Standards)
Donald E. Gregg, Ph.D., M.D.
Edward M. Khouri

Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

Final design of a new micropressure manometer with improved characteristics is being evaluated.

BODY OF REPORT

Project No. 6X60-01-001, Internal Medicine

Task No. 22, Instrument Development for Cardiac Research

Description and Progress:

The National Bureau of Standards believes that the following characteristics and/or requirements have been attained and/or met in the development of a micropressure manometer for use in arteries and heart cavities: (1) adequate sensitivity and stability, (2) ruggedness and durability which permit its disconnection from wornout catheters for salvage and reuse, (3) elimination of zero pressure drift, (4) simplification of construction details and layout to permit manometer construction by personnel of average training and skill. A construction manual with adequate detail has been supplied. Five finished micro-manometers are being tested from the standpoint of final physical characteristics and of functional characteristics in use in the cardiovascular system.

Summary:

Final design of a new micropressure manometer with improved characteristics is being evaluated.

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001 Internal Medicine

Task No. 27 Effects of physical agents on skin and its permeability

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Department of Dermatology
Division of Medicine

Period Covered by Report: 1 July 1960 through 30 June 1961

Principal Investigator: D. Joseph Demis, Captain, MC

Assistant: James Lawler, Captain, MC

Reports Control Symbol: MEDDH-288

Security Classification: Unclassified

ABSTRACT

Project No. 6X60-01-001

Internal Medicine

Task No. 27

Effects of physical agents on
skin and its permeability

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D, C.

Period Covered by Report: 1 July 1960 through 30 June 1961

Authors: D. Joseph Demis, Captain, MC
James Lawler, Captain, MC

Reports Control Symbol: MEDDH-288

Security Classification: Unclassified

Impedance to passage of alternating current has been found to be significantly lower in the skin lesions of patients with atopic dermatitis.

BODY OF REPORT

Project No. 6X60-01-001

Internal Medicine

Task No. 27

Effects of physical agents
on skin and its permeability

Description:

These studies are intended to establish base line values for the electrical characteristics of human skin in both normal and disease states in order to evaluate the usefulness of the technique in diagnosis and management of cutaneous disease.

Progress:

The electrical impedance of human skin to the passage of an alternating current has been measured by constructing an equivalent circuit against which the skin could be balanced. A range of normal of 946 ohms to 2,818 ohms at 4,000 cycles per second has been established in 104 normals. Impedance and phase angle were found to be low in 23 patients with atopic dermatitis at sites of the lesions but no significant deviation from normal values was found in the uninvolved skin.

Summary and Conclusions:

Impedance to passage of alternating current has been found to be significantly lower in the skin lesions of patients with atopic dermatitis.

List of Publications:

1. Lawler, J. C., Davis, M. J. and Griffith, E. C.:
Electrical characteristics of the skin. J. Invest.
Derm. 34: 301, 1960.

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001 Internal Medicine

Task No. 28. Capillary and lymphatic circulation of the skin

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
• Washington 12, D. C.

Department of Dermatology
Division of Medicine

Period Covered by Report: 1 July 1960 through 30 June 1961

Principal Investigator: D. Joseph Demis, Captain, MC

Assistant: James Lawler, Captain, MC

Reports Control Symbol: MEDDH-288

Security Classification: Unclassified

ABSTRACT

Project No. 6X60-01-001

Internal Medicine

Task No. 28

Capillary and lymphatic
circulation of the skin

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Period Covered by Report: 1 July 1960 through 30 June 1961

Authors: D. Joseph Demis, Captain, MC
James Lawler, Captain, MC

Reports Control Symbol: MEDDH-288

Security Classification: Unclassified

Large amounts of free histamine and normal amounts of 5-hydroxyindoleacetic acid (5-HIAA) were excreted by 13 patients with urticaria pigmentosa (mastocytosis cutis). This excretion was not influenced by conventional therapy but could be correlated with symptoms attributable to histamine. A review of mastocytosis cases reported during the past ten years reveals these symptoms to occur with sufficient regularity to justify recognition of a syndrome associated with mastocytosis. Findings in the mastocytosis syndrome include skin lesions, hepatosplenomegaly and bone lesions; symptoms include flushing, pruritus and gastrointestinal complaints. Headache, malaise, weight loss and other symptoms occur with lesser frequency.

Histidine metabolism in patients with urticaria pigmentosa has been investigated using in vitro and in vivo techniques. The skin lesions of these patients were shown to have approximately ten times as much histidine decarboxylase activity as uninvolved skin. The metabolism of orally administered histidine -C¹⁴ has been evaluated in eleven controls, two patients with urticaria pigmentosa, and one with chronic urticaria. Despite increased urinary excretion of free histamine by these patients, essentially no radioactivity could be demonstrated within the first five hours after administration in histamine or its metabolites.

BODY OF REPORT

Project No. 6X60-01-001

Internal Medicine

Task No. 28

Capillary and lymphatic
circulation of the skin

Description:

These studies of the metabolic abnormalities in urticaria pigmentosa have been pursued with the expectation that they may serve as useful models for elucidation of the pathophysiologic processes involved in the urticarias in general.

Progress:

An increased urinary excretion of free histamine was found in all cases of urticaria pigmentosa studied. The amount detected in the urine, while consistently greater than control values, varied often in an unpredictable fashion.

Increased urinary excretion of free histamine could be correlated with symptoms in some patients. One of the patients had prominent erythematous flushing of her face and moderately severe frontal headaches. Another patient experienced bright erythematous flushes (each lasting approximately 15 to 20 minutes), diffuse headache, weakness, anorexia and vague midepigastric pain. Another patient was briefly hospitalized because of marked oliguria, headache, bright erythematous flushing of the face and neck, nausea, vomiting, midepigastric pain and generalized weakness associated with moderate transient hypotension (80/50).

An evaluation of the effects of antihistaminic and reserpine therapy on histamine excretion by patients with urticaria pigmentosa has been undertaken. Following two weeks or more of therapy, these agents had no apparent effect on the urinary excretion of free histamine. In addition, all urine specimens were analyzed for 5-hydroxyindoleacetic acid. Every sample gave a value within normal limits despite symptoms, grossly elevated histamine excretion, or therapy. Analysis of vesicular fluid revealed only trace amounts of serotonin and 5HIAA.

A tendency for patients with urticaria pigmentosa to have relatively prolonged periods of oliguria with occasional episodes of diuresis has been observed. For example, the urinary output of one patient, a male weighing 215 pounds ranged from 360 ml. to 1,570 ml. but averaged only 620 ml. per 24 hours.

The daily urinary volumes of other patients also tended to be smaller than the generally accepted norm and in one case oliguria was one of the chief causes for hospitalization.

A trace amount of radioactive histidine was administered to two patients with urticaria pigmentosa, one patient with chronic urticaria, and to eleven volunteer male control subjects. All medications were withheld for three weeks, and neither food nor liquids were given for twelve hours immediately prior to the experiment. Subjects received orally 10 microcuries of chromatographically pure, uniformly labelled L-histidine- C^{14} (approximately 14 mg.) dissolved in orange juice. Urine was collected for five hours, desalted, filtered, and fractionated using two-step ion exchange column chromatographic procedures in order to separate the histidine metabolites. The following metabolites were separated and their amounts measured: histamine, 1,4-methylhistamine, 1,4-methylimidazole acetic acid, imidazole-acetic acid, ribozyl imidazoleacetic acid, urocanic acid, formimino-glutamic acid, glutamic acid, hydantoin propionic acid, uric acid and unknowns. Repeated recrystallizations and isotope dilution determinations allowed final quantitative analysis of radioactive metabolites. Of the radioactivity initially administered orally, about three per cent was present in the urine; of this approximately one-third was histidine, apparently excreted unchanged, and one-half was present as unidentified metabolites.

There were no significant differences between eleven controls and the patients studied. The detailed fractionation procedure did not reveal the presence of any peaks of radioactivity not found in controls, nor were any of the normal metabolites absent in urticaria pigmentosa or chronic urticaria. It is especially noteworthy that despite the increased urinary excretion of free histamine by these patients, there was found, as with controls, essentially no radioactivity in histamine or any of its known metabolites.

Homogenates of skin lesions of urticaria pigmentosa were incubated with C^{14} labelled histidine. Significant amounts of radioactive histamine were formed by homogenates prepared from the skin lesions of urticaria pigmentosa. Radioactive histamine was recovered by chromatographic techniques. Small amounts (constant specific activity less than 2-3 times background) of radioactive histamine were also formed during incubation of histidine with uninvolved skin. Heating of homogenates to boiling prior to histidine incubation prevented the formation of histamine, indicating this to be an enzymatic process.

The enzymatic ability of homogenates of the lesion to synthesize histamine is approximately ten times that of uninvolved skin. The most active preparation formed 0.68 micromoles of histamine per gram of involved skin per hour. Smaller amounts of histamine were formed by the pooled biopsies from children and this may reflect less histidine decarboxylase activity in childhood mastocytosis.

Summary and Conclusions:

Large amounts of free histamine and normal amounts of 5-hydroxyindoleacetic acid (5-HIAA) were excreted by 13 patients with urticaria pigmentosa (mastocytosis cutis). This excretion was not influenced by conventional therapy but could be correlated with symptoms attributable to histamine. A review of mastocytosis cases reported during the past ten years reveals these symptoms to occur with sufficient regularity to justify recognition of a syndrome associated with mastocytosis. Findings in the mastocytosis syndrome include skin lesions, hepatosplenomegaly and bone lesions; symptoms include flushing, pruritus and gastrointestinal complaints. Headache, malaise, weight loss and other symptoms occur with lesser frequency.

Histidine metabolism in patients with urticaria pigmentosa has been investigated using in vitro and in vivo techniques. The skin lesions of these patients were shown to have approximately ten times as much histidine decarboxylase activity as uninvolved skin. The metabolism of orally administered histidine- C^{14} has been evaluated in eleven controls, two patients with urticaria pigmentosa, and one with chronic urticaria. Despite increased urinary excretion of free histamine by these patients, essentially no radioactivity could be demonstrated within the first five hours after administration in histamine or its metabolites.

List of Publications:

1. Demis, E. J., Walton, M. D. and Higdon, R. S.: Histaminuria in urticaria pigmentosa. Arch. Derm. 83: 127, 1961.
2. Demis, D. J. and Brown, D. D.: Histidine metabolism in urticaria pigmentosa. J. Invest. Derm. 36: 253, 1961
3. Williams, H. E., Demis, D. J. and Higdon, R. S.: Ataxis-Telangiectasia. Arch. Derm. 82: 937, 1960.

FINAL REPORT

Project No. 6X60-01-001 Internal Medicine

Task No. 30, Identification of the Principles of Nursing Care
Underlying Taking Oral Temperatures

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Department of Nursing

Period Covered by Report: 15 October 1958 through 30 June 1961

Principal Investigator: Major Phyllis J. Verhonick, ANC

Assistant: 1/Lt. Ann E. Yoder, ANC

Reports Control Symbol: MEDDH-288

Security Classification: Unclassified

ABSTRACT

Project No. 6X60-01-001

Title: Internal Medicine

Task No. 30

Title: Identification of the Principles of Nursing Care Underlying Taking Oral Temperatures

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Period Covered by Report: 31 October 1958 through 30 June 1961

Author: Major Phyllis J. Verhonick, ANC

Reports Control Symbol: MEDDH-288

Security Classification: Unclassified

An experimental study has been completed to test the variables which may influence oral temperature readings. The hypotheses tested were that cigarette smoking, gum chewing and ingestion of hot or cold liquids do not make a significant difference in the degree of oral temperatures in afebrile and in febrile patients. Analysis of variance tests of significance shows that cigarette smoking and gum chewing do not make a significant difference in the degree of oral temperature in afebrile patients, but ingestion of hot and cold liquids by afebrile patients does make a difference in the degree of oral temperature at the .05 level of significance. The findings in regard to cigarette smoking and gum chewing contradict published results of previous investigators. The findings in this research may be attributed to the experimental design in which experimental and control groups were used. In studies previously done, subjects were used as their own controls.

Significant differences were shown in the oral temperature readings of febrile patients, which may be attributed to the alteration of the experimental design. Febrile patients were used as their own controls because the existence of fever had different causes and control groups could not be designated.

A series of unstructured interviews were completed to gain knowledge concerning the consideration given to variables prior to taking oral temperatures.

On the basis of the experimental findings four principles were formulated as guides for the nurse practitioner when taking oral temperatures of afebrile patients.

BODY OF REPORT

Project No. 6X60-01-001 Title: Internal Medicine

Task No. 30 Title: Identification of the Principles of Nursing Care Underlying Taking Oral Temperatures

Description:

An experimental study was designed to identify principles of nursing to serve as a guide in taking oral temperatures. Data were collected during the winter months of the past two and one-half years. The basic premise is concerned with the influence of selected variables on oral temperature.

• Reports in the nursing literature make reference to the effect of variables on oral temperature but these discussions are not ordinarily documented by findings from research, and particularly experimental research. Observations of the cardinal signs made by nursing practitioners tend to have an empirical basis as opposed to a scientific basis which relies on the findings of experimental investigation.

The three hypotheses tested were:

1. Cigarette smoking does not make a significant difference in the degree of oral temperatures in afebrile and in febrile patients.
2. Chewing gum does not make a significant difference in the degree of oral temperature in afebrile and in febrile patients.
3. Hot and cold liquids do not make a significant difference in the degree of oral temperature in afebrile and in febrile patients.

Requirements for selection of the afebrile sample were male patients, age range of 18-30 years, voluntary participation, hospitalized for the period of four days during data collection, on a regular hospital diet, same ward environment, not receiving prescribed anti-pyretic drugs during the study, "average" smokers consuming 20 to 40 cigarettes per day, and confined to the ward on complete or partial bed rest.

The total sample was divided into experimental and control groups. Temperatures were taken twice; then the subjects of the experimental group smoked a popular brand, unfiltered cigarette.

Temperatures of patients in both groups were then taken immediately after members of the experimental group completed smoking, ten minutes after smoking and twenty minutes after smoking. For each replication, on each day, members of the groups were reversed, or those who were in the experimental group were assigned to the control group and vice versa.

Progress:

To test the first two hypotheses concerning smoking cigarettes and chewing gum, twelve and eight replications were done respectively for afebrile patients. Because the results were consistent in each replication only two replications were accomplished for testing the effects of ingesting hot and cold liquids. Findings obtained for the hypotheses on cigarette smoking and gum chewing contradicted previous findings cited in the nursing literature, (Brim and Chandler 1950).^{*} This difference may be attributed to the differences in the experimental design. In previous investigations "normal subjects" were used as their own control. Variations in temperature were shown following smoking and chewing gum. The results shown in this research revealed that there was as much variation in control as there was in experimental groups, therefore the differences in the degree of oral temperature were not statistically or observably significant. The numbers of replications and reversal of group assignment permit challenge of previous findings.

The data were analyzed statistically by analysis of variance. Examples of the findings of two replications of two different hypotheses tested for afebrile patients are shown in Tables I and II.

^{*}Brim, Katharine and Chandler, Betty A. "Changes in Oral Temperature." The American Journal of Nursing. 48:772 (December 1948).

Table I. Test for Differences in Oral Temperatures
Following Cigarette Smoking by Afebrile Patients

Analysis of Variance			
Source of Variation	D.F.	Sum of Squares	Mean Square
(a) Smoking - Yes vs No	1	0.13	0.13
(b) Readings* 1 + 4 vs 2 + 3	1	0.08	0.08
(c) Readings 1 vs 4	1	0.06	0.06
(d) Readings 2 vs 3	1	0.006	0.006
(e). (a) x (b)	1	0.57	0.57
(f) (a) x (c)	1	0.44	0.44
(g) (a) x (d)	1	0.41	0.41
Totals	7	1.69	1.69

*Readings 1 - Pre-smoking reading
 2 - Immediately after smoking
 3 - 10 minutes after smoking.
 4 - 20 minutes after smoking

The F ratio for the foregoing analysis of variance was not statistically significant. The observable differences in the degree of oral temperature readings varied from + 0.2° to + 0.4°F in most instances obviating the insignificance of change.

Table II. Test for Differences in Oral Temperature Following the Ingestion of a Cold Liquid 5°C

Analysis of Variance			
Source of Variation	D.F.	Sum of Squares	Mean Square
(a) Liquid - Yes - No	1	7.29	7.29
(b) Readings* 1 + 4 vs 2 + 3	1	4.41	4.41
(c) Readings 1 vs 4	1	1.06	1.06
(d) Readings 2 vs 3	1	0.24	0.24
(e) (a) x (b)	1	1.28	1.28
(f) (a) x (c)	1	0.76	0.76
(g) (a) x (d)	1	0.28	0.28
Totals	7	15.32	15.32

*Readings 1 - Pre-ingestion of cold liquid
 2 - Immediately after ingestion of cold liquid
 3 - 10 minutes after ingestion of cold liquid
 4 - 20 minutes after ingestion of cold liquid

The F ratio for the analysis of variance concerning the ingestion of cold liquids is significant at the .05 level.

It was necessary to deviate from the experimental design to test the hypotheses for febrile patients. Because the fever was due to differences in disease or physiological condition the febrile patients were used as their own controls. The differences in the degree of oral temperature for all hypotheses were significant at the .05 levels. It cannot be definitely stated whether the changes in temperature were due to the fever or to the experimental design, especially since previous investigators found differences in oral temperatures in "normal" subjects.

Prior to data collection three dozen thermometers were calibrated for use. Initial testing showed a wide disparity in individual thermometer readings. Differences of two degrees were evident in some instances. The medium used for testing was water.

Because of the wide disparity in registration of thermometers obtained in previous tests by the investigator the controlled laboratory study was completed. The findings of the laboratory investigation show that differences in readings are minimal. However, because of differences in thermometers it is recommended that individual thermometers be used for each patient throughout his hospitalization.

The results of the laboratory test repeated in an electrically controlled water bath using five brands of thermometers are shown in Table III.

Table III. Variance in Thermometer Register at Selected Controlled Temperature

Brand of Thermometer	Span of Variance at Controlled Temperature Reading*					
	95.6°F	98.6°F	100°F	102°F	102.6°F	105°F
A	0.6°	0.2°	0.6°	0.6°	0.4°	0.8°
B	0.4°	0.2°	0.2°	0.6°	0.2°	0.6°
C	0.4°	0.4°	0.4°	0.4°	0.2°	0.6°
D	0.6°	0.4°	0.6°	0.4°	0.4°	0.6°
E	0.6°	0.6°	0.6°	0.6°	0.6°	0.8°

*An electrically controlled water bath with an electric stirring blade was used for the laboratory test. Thermometers were spaced in a wire rack and left in solution at the designated temperature for a five minute interval.

A series of unstructured interviews with 32 selected nursing service personnel at Walter Reed General Hospital were conducted concurrently with the testing of each hypothesis. The purpose of the interview was to gain information concerning the nurses' consideration of each of the variables prior to taking oral temperatures. The majority of interviewees stated they did not take the patients' temperatures for at least twenty to thirty minutes after the ingestion of hot or cold liquids. In general they felt that smoking and gum chewing did not influence the degree of oral temperature but about twenty percent would wait for a period of at least fifteen minutes after smoking.

The following principles of nursing which underly taking oral temperatures of afebrile patients are formulated on the basis of this experimental study:

1. It is not necessary to wait for a period of time to take the oral temperature of a patient after he has smoked a cigarette. Readings of oral temperature immediately after to twenty minutes following smoking a popular-brand unfiltered cigarette were not significantly different from pre-smoking temperature readings in afebrile patients. The average change in thermometer registration ranged from $\pm 0.2^{\circ}$ - $\pm 0.4^{\circ}\text{F}$.

2. The patients' oral temperatures may be taken immediately following chewing gum. Readings of oral temperatures immediately after to twenty minutes following chewing gum were not significantly different from pre-gum chewing temperature readings in afebrile patients. Change in thermometer registration ranged from $\pm 0.2^{\circ}$ - $\pm 0.6^{\circ}\text{F}$.

3. Before taking a patient's temperature after he has drunk cold liquids it is necessary to wait for at least a twenty-minute period. Immediately after and until a lapse of twenty minutes following the ingestion of cold liquids (4° - 10°C [40° - 50°F]) there was an average decrease in temperature ranging from 1.0° - 1.4°F in afebrile patients. This difference in the degree of oral temperature was significant at the .05 level.

4. It is necessary to wait at least twenty minutes after the patient has ingested hot liquids before taking his temperature orally. Immediately after and until a lapse of twenty minutes following the ingestion of hot liquids ($T = 70^{\circ}$ - 76°C [158° - 168.8°F]), there was an average elevation in temperature ranging from 0.8° - 1.4°F in afebrile patients. This difference in the degree of oral temperature was significant at the .05 level.

The definition of a principle as used in this research is "a truth that has been proven which serves as a guide to action." Principles within the limits of this definition could not be formulated concerning changes in oral temperatures of febrile patients because of the necessarily changed experimental design. With no central group with which to make comparison it was impossible to tell whether the temperatures were due to the introduction of variables or to variations occurring among individual patients.

Summary and Conclusions:

Analysis of variance was used to test the hypotheses that cigarette smoking, gum chewing and ingestion of hot and cold liquids do not make a significant difference in the degree of oral temperature in afebrile and febrile patients. Experimental and control groups of patients were used making this design uniquely different from previous nursing investigations.

Results of the experiments show that, in afebrile patients, cigarette smoking and gum chewing do not make a significant difference in the degree of oral temperature, but ingestion of hot and cold liquids makes a difference in oral temperature readings at the .05 level of significance. The temperatures of febrile patients were different following the introduction of the four variables of cigarette smoking, gum chewing and hot and cold liquids. Because control groups of febrile patients were not used it cannot be concluded that differences in temperature readings were due entirely to the variables tested.

Calibration of thermometers prior to testing each hypothesis showed variations ranging from 0.4° to 0.8° F. Because some variation does exist it is recommended that patients be given individual thermometers for use throughout their hospitalization.

Selected nursing personnel were interviewed to survey their opinions relating to whether the variables tested were taken into consideration prior to taking patients' temperatures. The majority of the nurses felt the variables did make a difference and waited from 20-30 minutes following the ingestion of hot or cold liquids. Less consideration was given to smoking and gum chewing.

On the basis of the experimental findings four principles have been evolved to serve as guides for nurse practitioners in taking oral temperatures in afebrile patients.

List of Publications: To be submitted to Nursing Research for publication (based on interest expressed by editor).

ANNUAL PROGRESS REPORT

Project 6X60-01-001 Internal Medicine

Task 31. Gastrointestinal physiology

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Department of Gastroenterology
Division of Medicine

Department of Molecular Biology
Division of Communicable Disease
and Immunology

Period Covered by Report: 1 July 1960 through 30 June 1961

Principal Investigator: Major Milton E. Rubini, MC

Assistants: Roberta S. Hartman, PhD
Richard E. Hartman, PhD
Captain Marcel E. Conrad, Jr., MC
Captain LeeRoy Jones, MC

Reports Control Symbol: MEDDH-288

Security Classification: Unclassified

ABSTRACT

Project No. 6X6C-01-001

Title: Internal Medicine

Task No. 31

Title: Gastrointestinal Physiology

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Period Covered by Report: 1 July 1960 through 30 June 1961

Authors: Major Milton E. Rubini, MC
Roberta S. Hartman, PhD
Richard E. Hartman, PhD
Captain Marcel E. Conrad, Jr., MC
Captain Lee Roy Jones, MC

Reports Control Symbol: MEDDH-288

Security Classification: Unclassified

Work continues in the study of iron absorption. Method is under investigation to use Fe⁵⁹ as a tag to permit study of the turnover time of intestinal epithelium. Study of calcium absorption has been initiated.

BODY OF REPORT

Project No. 6X60-01-001 Title Internal Medicine

Task No. 31 Title Gastrointestinal physiology

Description: A study of the absorptive process in normal and abnormal conditions of the small bowel.

Progress:

The Department of Gastroenterology was established in 1961. It consists of two officers and one full-time and two shared technicians. Currently, most professional activity is awaiting delivery of equipment, but several projects in studies of calcium metabolism have been initiated:

1. Absorption and skeletal kinetics of Ca^{47} as studied with the whole body counter.
2. Calcium absorption in rats.
3. Myeloma bone disease.

Work has also begun in radiographic techniques for the localization of Fe^{59} as a means of determining the half life of intestinal mucosa. Preliminary experience indicates the practicability of this method.

The electron microscopic investigation of iron from the human intestine was curtailed by the remodeling of the electron microscopic laboratory during the current reporting period. As a result, the work has been limited to further characterization of the ferritin-containing bodies, which are the only ferritin depots thus far noticed in the absorptive cells. These bodies are ovoid in profile, somewhat wider than the mitochondria among which they lie, and are not membrane-bound although their boundary with the cytoplasmic background substance is very sharp. The ground substance of the bodies is granular and portions are sometimes in a regular array suggesting a crystal of apoferritin with iron in only a few of the molecules. The origin of the granule is unknown; no association with endoplasmic reticulum is apparent, and these bodies have never been observed in the Golgi region. Obviously a knowledge of the nature of these bodies will be necessary for a complete understanding of the physiology of iron absorption, but in the interim a number of questions can be attacked more efficiently because the area to be observed has been limited.

Summary and Conclusions:

Work continues in the study of iron absorption. Method is under investigation to use Fe⁵⁹ as a tag to permit study of the turnover time of intestinal epithelium. Study of calcium absorption has been initiated.

List of Publications:

1. Hartman, R. S., Hartman, R. E. and Conrad, M. E.:
Ferritin in the human intestinal epithelium. J. Appl. Phys. 31: 1841, 1961.
2. Crosby, W. H.: A concept of the pathogenesis of anemia applied to disorders of the intestinal mucosa. Amer. J. Dig. Dis. (In press)

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001, Internal Medicine

Task No. 32, The Influence of Bed Bath Procedures on Skin Conditions

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Department of Nursing

Period Covered by Report: 15 February 1961 through 30 June 1961

Principal Investigator: Major Phyllis J. Verhonick, ANC

Assistants: Captain Mary E. Doyle, ANC
1/Lt. Ann E. Yoder, ANC

Consultant: Captain D. Joseph Demis, MC.

Reports Control Symbol: MEDDH-288

Security Classification: Unclassified

ABSTRACT

Project No. 6X60-01-001

Title: Internal Medicine

Task No. 32

Title: Influence of Bed Bath Procedures on Skin Conditions

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Period Covered by Report: 15 February 1961 through 30 June 1961

Authors: Major Phyllis J. Verhonick, ANC, Captain Mary E. Doyle, ANC and 1/Lt. Ann E. Yoder, ANC

Reports Control Symbol: MEDDH-288

Security Classification: Unclassified

A systematic investigation of traditional bed bathing procedures as a contributing factor to drying of the skin during hospitalization is being conducted. This research is designed to test the hypothesis that the use of a specific nonionic emulsifier in giving complete or partial bed baths does not make a significant difference in the skin condition of patients. Selected patients are bathed for 21 consecutive days by nurse investigators. One-half of the body (control) is bathed with soap and water. The other half of the body (experimental) is bathed with a specific nonionic emulsifier and water. A double-blind approach is being used in that the control and experimental portions of the body are not disclosed to observers. Approximately eight independent observers examine the skin condition for dryness and scaldiness each week. Keratin is stripped from the skin by placing cellulose tape on the lateral surfaces of the ankle on the control and experimental sides three times throughout the 21-day period. The strippings are exposed to a densitometer for an objective registration of the amount of epithelium. Medical judgments are made each week by a dermatologist who serves as consultant to the nursing research team.

BODY OF REPORT

Project No. 6X60-01-001

Title: Internal Medicine

Task No. 32

Title: The Influence of Bed Bath
Procedures on Skin Condition

Description:

An experimental investigation is being conducted to study the influence of bed bathing on the skin condition of selected patients. The research is designed to evaluate the effects of the following nursing measures on the skin: (1) conventional soap and water bathing as compared to a specific nonionic emulsifier and water bathing; (2) back massage with alcohol and lotions following bathing; and (3) the use of wash cloths as compared to cellulose sponges for bathing. The hypothesis being tested is that the use of a specific nonionic emulsifier in giving complete or partial bed baths does not make a significant difference in the skin condition of selected patients.

Patient comfort is a primary nursing concern in the administration of care. Hospitalized patients may complain of dry, itchy skin, particularly on the elbows, knees and back where friction with bed linens occurs. It is the premise of the investigators that conventional bed bathing procedures with soap and water contribute toward an increase in dryness of the skin.

Selected patients on orthopedic, neurosurgical and general medical and surgical wards at Walter Reed General Hospital are bathed for approximately twenty-one consecutive days by the investigators. One-half of the body (control) is bathed with soap and water. The other half of the body (experimental) is bathed with a specific nonionic emulsifier and water. The portions of the body which are control and experimental are randomly designated by the investigator bathing the patient and this knowledge is not disclosed to other investigators or observers. A double-blind approach is used in making observations in that the observer is not aware of the experimental and control designations. Patients bathed are placed into one of six groups. One-half of the sample or three groups are bathed with wash cloths and the other half are bathed with cellulose sponges. The sample is again sub-divided into thirds or one-third receive an alcohol back rub following bathing; one-third receive a back rub with massage lotion; and the remaining patients receive no back rub following bathing.

A rating scale has been devised for observers to record an estimated degree of roughness and scaliness of the skin. A

translucent plastic slide is used to scrape lightly the lateral surfaces of the patients' ankles. The amount of epithelium appearing on the slide is judged and recorded by observers.

On the first, tenth and last day of the study a one-time keratin stripping is done by placing a strip of cellulose tape on the anterior surface of the legs of the patients. The tape is removed and mounted on a microscopic slide for examination and measurement of the thickness of the epithelium with a densitometer.

Progress:

To date twenty-six patients between the ages of 35 and 79 have been included in the study. Approximately one-half of the sample are male patients and the other are female. Daily observations are made by each investigator, and in addition, approximately eight independent observers examine the skin for dryness and scaling each week. Keratin strippings are done on the first, tenth, and final day of the study. The dermatologist examines the patients once each week for the three-week period.

The nurse and physician ratings concerning the amount of dryness will be analyzed for differences occurring on the experimental and control sides of the body. The densitometer registrations will be statistically analyzed to ascertain if differences exist. Data collected thus far are currently being analyzed and suggest that differences in experimental and control sides of the patients may exist but the data are still insufficient to justify any firm conclusions. Plans are being made to continue the bathing procedure and increase the sample size.

Summary and Conclusions:

An experimental investigation is being conducted to test the hypothesis that the use of a specific nonionic emulsifier in giving complete or partial bed baths does not make a significant difference in the skin condition of selected patients. One-half of the body is bathed with soap and water (control) and the other half is bathed with a specific nonionic emulsifier and water (experimental). The double-blind approach is used to make independent observations of roughness and scaliness on the experimental and control portions of the body.

Epithelium is stripped from the skin by placing cellulose tape on the lateral surfaces of the ankles on the control and experimental sides three times during the study. The strippings are exposed to a densitometer for an objective reading of the

amount of epithelium.

Data collected to date are insufficient to establish any firm conclusions; therefore the study will be continued until a larger number of patients are included.

Publications: None

ANNUAL PROGRESS REPORT

Project: 6X60-09-001, Metabolism

Task: 1, Pressor Substances

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Department of Biochemistry

Period Covered by Report: 1 July 1960 through 30 June 1961

Principal Investigator: Mr. Benjamin Mehlman

Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

ABSTRACT

Project No. 6X60-09-001

Title: Metabolism

Task No. 1

Title: Pressor Substances

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Period Covered by Report: 1 July 1960 through 30 June 1961

Author: Mr. Benjamin Mehlman

Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

Approximately 200 specimens have been received by reference to this laboratory requesting epinephrine and norepinephrine assays on blood plasma in support of differential diagnosis of hypertension.

Collaborative research has been accomplished in the areas of mode of action of mercaptoethanolamine (MEA) in production of hypertension and hypotension in the experimental animal. In addition assistance has been given in a program involving the interaction of glucagon, insulin, and epinephrine.

A satisfactory method for fluorometric assay of histamine has been standardized and research is now continuing regarding the effect of histamine release in the radiation protected animal. Additional problems are encountered when there are excessive amounts of sulfhydryl compounds in the specimen for assay since these compounds interfere with histamine assay.

BODY OF REPORT

Project No. 6X60-09-001

Title: Metabolism

Task No. 1

Title: Pressor Substances

Description: The study of pressor substances has been undertaken to provide improved diagnostic assistance to those hospitals where the facilities are not adequate for the performance of these complicated techniques. In addition, the physiological response of experimental animals in the research program requires more adequate definition of the role of the pressor substances in radiation protection, in traumatic injury, and in the interplay of these substances with biochemical processes.

Progress: Emphasis has been put on collaboration with other groups and setting up analytical procedures as needed in addition to the screening of samples to assist in the diagnosis of pheochromocytoma.

Weil Malherbe's method was used for plasma, epinephrine and norepinephrine. For adrenal gland catecholamines the method of Montagu was used. The method of Roe was used for Vitamin C analyses of the adrenal gland. In addition, a fluoremetric method for the analysis of histamine in blood and tissues was evaluated and put into use. The procedure found to be most satisfactory in our laboratory is that of Shore et al. (V. Pharm & Exp Therap. 127:182-186 (1959))

Approximately 200 samples were analyzed for possible cases of pheochromocytoma. This is a slight drop from previous years and possibly reflects increased clinical experience with this form of tumor. This laboratory still has no access to the final diagnosis, consequently correlation of high catechol values with confirmed tumors is not available. Most reference samples are submitted by the VA hospitals (71%), followed by Army hospitals (22%) and the Air Force and Navy about (7%). Many of the samples from the VA represent part of a long term collaborative project, now in its fifth year, in studying plasma catecholamine levels in patients with various types of kidney diseases. This should yield some valuable medical information when completed.

A major portion of the effort is directed toward assisting the Pharmacology Section of the Radiobiology Department of the Division of Nuclear Medicine with their problems involving chemistry. Work on dogs led to the belief that histamine was released following the injection of MEA, a potent radioprotective agent. The

fluorometric procedure of Shore et al was adapted to the Farrand Model A fluorometer and found to be normally satisfactory. However, MEA, in any amount, interferes with this method, and since the ratio of MEA over histamine is about 5000 to 1, separation has been very difficult. Amberlite IRC-50, while giving excellent recovery for histamine alone, does not give complete separation. It is hoped that work in progress with paper chromatography will allow the determination of histamine in the presence of MEA.

In addition collaborative studies have been carried out with the Department of Cardiorespiratory Diseases regarding the catechol levels in blood from different vessels of the heart. An additional collaborative research effort, in progress with personnel of Roswell Park Memorial Institute, Buffalo, New York in a project involving the interaction of glucagon, insulin and epinephrine.

Summary and Conclusions: The determination of blood levels of epinephrine and norepinephrine has continued with approximately 200 reference specimens being received during the year. Correlation of elevated catechol levels with adrenal tumors is not possible at this time due to difficulty of knowing final diagnosis on the patient.

Collaborative research has been done in the areas of catechol responses to radiation protection drugs and a proposed mechanism of production of the hypertensive response of the experimental animal has been published. Additional research has involved the interaction of glucagon, insulin, and epinephrine. These studies do not as yet allow full evaluation.

A satisfactory method has been established for assay of histamine in the blood and tissues.

Publications:

1. Mundy, R. L., Heiffer, M. H. and Mehlman, B., The Pharmacology of Radio-protectant Chemicals, Biochemical Changes in the Dog Following the Administration of Beta-mercaptoethylamine (MEA). Arch. Int. Pharmac. et de Therap. 130: 354-367 (1961).
2. Heiffer, M. H., Mundy, R. L., Mehlman, B., Plasma Catecholamine Levels and Adrenal Ascorbic Acid Content Following Beta-mercaptoethylamine (MEA) Administration. Endocrinology - Accepted for Publication. •

Talks presented at Radiation Research Society, Washington 12, D. C.
May 15-17, 1961

1. Mundy, R. L. , Heiffer, M. H. , Mehlman, B. , Mechanism of Beta-mercaptoethylamine Induced Hypotension in the Dog.
2. Heiffer, M. H. , Mundy, R. L. , Mehlman, B. , On Some Effects of Beta-mercaptoethylamine and Cystamine in the Rat.

ANNUAL PROGRESS REPORT

Project 6X60-09-001 Metabolism

Task 2 Bio-assay of biologically active substances by tissue culture

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Department of Surgical Metabolism and Physiology,
Division of Surgery

Period Covered by Report: 1 Jul 60 - 30 Jun 61

Principal Investigators: A. D. Glinos, M. D., and W. T. Brown, Jr., M. S.

Assistant: Pvt. R. J. Werrlein

Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

ABSTRACT

Project No. 6X60-09-001 Metabolism

Task No. 2 Bio-assay of biologically active substances by tissue culture

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Period Covered by Report: 1 Jul 60 - 30 Jun 61

Authors: A. D. Glinos, M. D., W. T. Brown, Jr., M. S., and Pvt. R. J. Werrlein

Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

We have previously reported a marked increase of the serum α_2 globulin within 24 hours after total body irradiation, and a similar α_2 response was noted by other investigators in inflammations, burns, extensive surgery and during the growth of tumors. Our earlier efforts to clarify the physiological significance of this response have led to the demonstration of a selective decrease of the α_2 serum globulins in tissue culture media during periods of active cellular proliferation.

During the current period we sought to ascertain whether this decrease was due to conditions peculiar to tissue culture such as attachment of cells to glass surfaces or whether it was indeed related to cell metabolism and growth. It was found that the relative concentration of the α_2 globulin in tissue culture media is an inverse linear function of the number of cells present at any given time and that the rate of the α_2 globulin decrease depends on the exponential growth rate of the cells.

These results indicate that the α_2 serum globulin is actively involved in cellular growth.

Project No. 6X60-09-001 Metabolism

Task No. 2 Bio-assay of biologically active substances by tissue culture

Description: We have previously reported a marked increase of the serum α_2 globulin within 24 hours after total body irradiation (Glinos, A. D. and Berger, C. M., Fed. Proc., 18: 53, 1959), and a similar α_2 response was noted by other investigators in inflammations, burns, extensive surgery, and during the growth of tumors. The physiological significance of this response is uncertain, as it could be associated either with tissue destruction or with tissue proliferation which also occurs in different degrees and different times in all these conditions. Our earlier efforts to obtain evidence helpful in deciding between these two alternatives have led to the demonstration of a selective decrease of the α_2 serum globulins in tissue culture media during periods of active cellular proliferation.

Progress: During the current period we sought to ascertain whether this decrease was due to conditions peculiar to tissue culture or whether it was indeed related to cell metabolism and growth. The main condition peculiar to tissue culture which could perhaps account for this decrease of the α_2 globulin of the medium is the attachment of the cells to the glass, in the stationary type L cell cultures used in this work. Other investigators reported that fetuin, an alpha globulin found at relatively high concentrations in fetal sera, caused adherence of cells to a glass surface; only in its presence did cells assume a flattened epithelial like appearance and began to grow. The physical and chemical characteristics of fetuin indicated a close relationship to the glucoproteins of the α_2 globulin fraction of adult sera. The question therefore arose whether our observations on the decrease of the α_2 globulin fraction on tissue culture media could be interpreted on the basis of a function similar to the function of fetuin.

In such a case it would be expected that the decrease of the α_2 fraction would manifest itself in the first hours after inoculation of a cell suspension into a culture flask and that its course would be related to the events pertaining to the attachment of the cells to the glass rather than to cell growth. This possibility was investigated as follows: Stock flasks of the L strain were trypsinized and the cells washed and resuspended in a medium consisting of 38% rat serum in Earle's balanced salt solution. After determination of the cell density of these suspensions through electronic cell counts, a number of T-15 tissue culture flasks were inoculated with a volume of the suspension adjusted to yield 600,000 cells/flask. In addition an equal number of control flasks containing the identical medium but without cells were set up. Experimental and control flasks were incubated for periods of time ranging from 2 to 12 hours. At the end of these incubation periods the experimental flasks were examined microscopically and the extent of cell adherence and flattening noted; the media were then removed, the cells were fixed with citric acid, resuspended, and counted. Total protein and its electrophoretically identifiable components were determined in the media of both experimental and control flasks.

These determinations revealed that the relative concentration of the α_2 globulin fraction remained constant in both experimental and control flasks throughout the 12 hour observation period. During this time the free floating cells of the inoculated suspension were observed to adhere to the glass surface of the experimental flasks and to undergo a series of progressive morphological changes leading from the original contracted spherical form through the partially flattened spindle shaped cell to the fully flattened polygonal form characteristic of stationary cultures of this strain. There was no change of the cell number in any of the experimental flasks throughout the 12 hour observation period.

These results clearly indicate that the attachment and flattening of the cells on the glass does not account for the decrease of the relative concentration of the α_2 globulin observed earlier. It would, therefore, seem reasonable to propose at this point that the α_2 globulin is utilized by the cells. This utilization could be related to the mere maintenance of the cells or it could be related to cell proliferation. Obviously, the answer to this question would provide a most significant clue for the understanding of the physiological role of the α_2 globulin in the clinical situations mentioned earlier. Accordingly, a series of investigations was undertaken based on the premise that in a tissue culture system, utilization of the α_2 globulin for cellular maintenance will be manifested with a progressive decrease of the α_2 globulin related to the increasing number of cells in the culture but independent of the rate of cell division. On the other hand, if this protein participates in cell growth it would be expected to disappear from the medium at a rate related to the rate of cell division.

In these experiments replicate cultures of the L strain and control blank flasks containing medium only were set up as described previously. Cell numbers and α_2 globulin levels were determined after 3 and 6 days of incubation. Table No. I shows the results obtained with two different pools of sera. Growth was exponential in both cases, with a rate of 0.44 divisions (doublings) of the population per day for the first serum pool and 0.29 for the second. In both cases the α_2 globulin as measured by the difference between the control blank flasks and the cell containing culture flasks decreased as the cell number increased. When the data pertaining to this relationship are plotted as in Fig. 1 it can readily be seen that the α_2 globulin decrease is a linear function of the number of cells and that the rate of this decrease is determined by the rate of cell division.

These results then lead to the conclusion that the α_2 serum globulin participates actively in the process of cell division.

Summary and Conclusions: We have previously reported a marked increase of the serum α_2 globulin within 24 hours after total body irradiation, and a similar α_2 response was noted by other investigators in inflammations, burns, extensive surgery and during the growth of tumors. Our earlier efforts to clarify the physiological significance of this response have led to the demonstration of a selective decrease of the α_2 serum globulins in tissue culture media during periods of active cellular proliferation.

During the current period we sought to ascertain whether this decrease was due to conditions peculiar to tissue culture such as attachment of cells to glass surfaces or whether it was indeed related to cell metabolism and growth. It was found that the relative concentration of the α_2 globulin in tissue culture media is an inverse linear function of the number of cells present at any given time and that the rate of the α_2 globulin decrease depends on the exponential growth rate of the cells.

These results indicate that the α_2 serum globulin is actively involved in cellular growth.

List of Publications: Glinos, A. D. and Brown, W. T. Cell Division and α_2 serum globulin utilization in replicate cultures of L strain cells. Submitted for publication.

Table I
Cell division and α_2 globulin decrease in L strain cultures in sera with different growth promoting activities

		Days of Incubation			Cell Division Rate $K = \log_2 Y_2 - \log_2 Y_1$ $T_2 - T_1$
		1	3	6	
Serum Pool #1	no. of cells/flask $\times 10^4$	28.7	82.0	173.4	0.44
	relative cell no.	1	2.8	6.2	
	α_2 globulin of blank	8.5	8.6	8.5	
	α_2 globulin of culture	8.5	5.4	2.3	
	difference blank-culture	-	3.2	6.2	
Serum #2	no. of cells/flask $\times 10^4$	28.7	54.6	94.8	0.29
	relative cell no.	1	1.9	3.3	
	α_2 globulin of blank	9.1	9.0	9.3	
	α_2 globulin of culture	9.1	8.0	6.9	
	difference blank-culture	-	1	2.4	

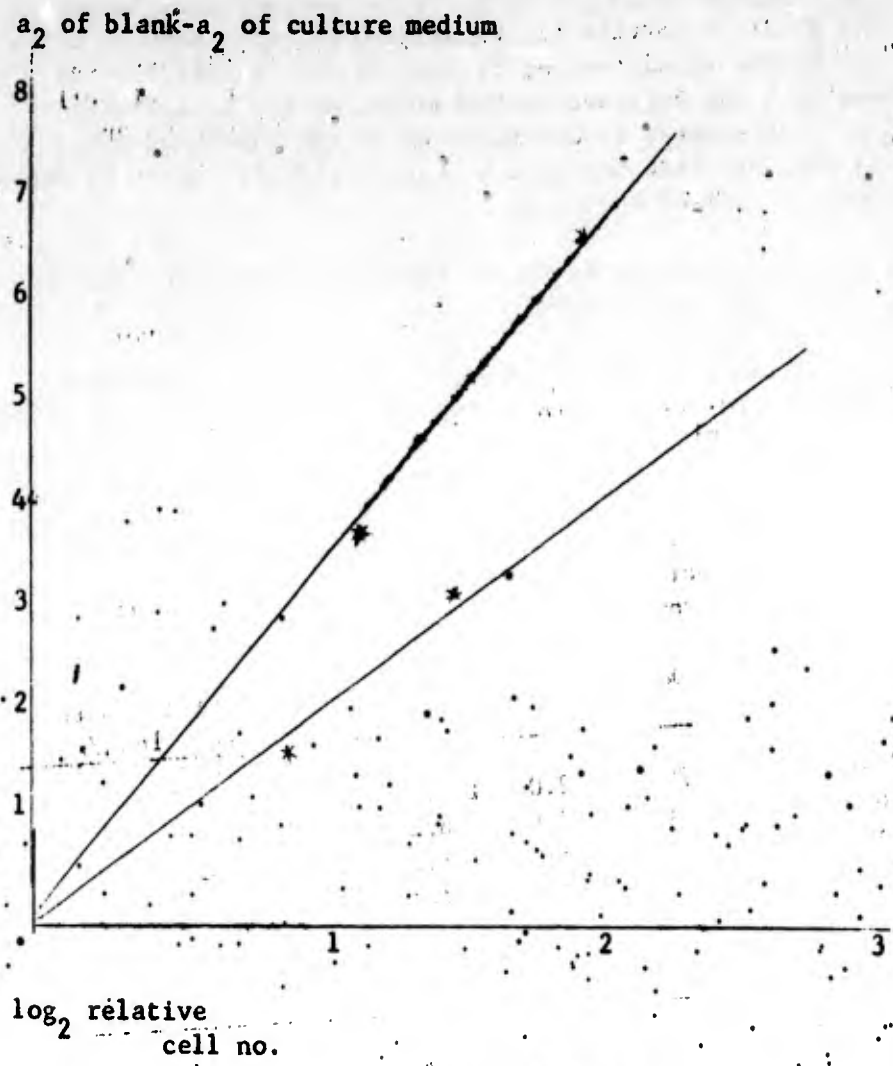


Figure 1

Relationship of the decrease of the a_2 globulin of the culture medium to cell division. The upper line corresponds to the cultures of Experiment No. 1 with an exponential cell division rate $K = 0.44$. The lower line corresponds to the cultures of Experiment No. 2 with an exponential cell division rate $K = 0.29$.

ANNUAL PROGRESS REPORT

Project ~~6Y60~~-09-001, Metabolism

Task 6, Metabolic and Nutritional Problems Associated with Injury

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Department of Metabolism
Division of Medicine

Period Covered by Report: 1 July 1960 through 30 June 1961

Principal Investigator: Lt. Colonel Jacques L. Sherman, ~~LT~~, MC

Assistants: Major Kevin G. Barry, MC
Captain Robert H. Herman, MC
Captain Thomas E. Davis, MC
Captain James P. Knochel, MC*

Reports Control Symbol: MEDDH-288

Security Classification: Unclassified

*Resident in Internal Medicine, WRGH

ABSTRACT

Project 6X60-09-001, Metabolism

Task 6, Metabolic and Nutritional Problems Associated with Injury

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Period Covered by Report: 1 July 1960 through 30 June 1961

Authors: Lt. Colonel Jacques L. Sherman, Jr., MC
Major Kevin G. Barry, MC
Captain Robert H. Herman, MC
Captain Thomas E. Davis, MC
Captain James P. Knochel, MC

Reports Control Symbol: MEDDH-288

Security Classification: Unclassified

Body potassium measurement with the human body counter has added a tool of assistance in metabolic studies. The continued refinement of techniques in the Steroid Laboratory has enabled us to begin the fractionation of certain crude groups of steroids. The suppression-stimulation test for adrenocortical function is standardized for routine clinical use. Additional capabilities in lipid research have been added and it is generally hoped that the direction of our investigations will be toward studies of intermediary metabolism of both lipids and carbohydrates.

Further studies in metabolic edema have been productive and will be extended to try to isolate the defect. Two patients with hyperkalemic paralysis were carefully studied and additional information on the defect has been obtained which is being used in therapy. A method for simultaneous determination of total body water, extracellular space and plasma volume is being developed and may become clinically useful.

BODY OF REPORT

Project 6X60-09-001, Metabolism

Task 6, Metabolic and Nutritional Problems Associated with Injury

Description: For better understanding of numerous disease and injury states, an attempt is being made to study intermediary metabolism of carbohydrates and lipids rather than gross metabolic balances. Similarly, in the field of fluid and electrolyte balance, studies are being directed toward mechanisms of shifts of these constituents rather than measurement of net changes. This change in direction requires development of new techniques which is gradually being accomplished.

Progress:

1. Glucose Intermediates in Metabolic Diseases. Studies on glucose intermediates have nearly been suspended during this past year but are expected to take up a major portion of studies in metabolism with new analytic methods when the gas chromatography will be available. These studies will extend the previous observations and should be extremely important.

2. Studies of Potassium Metabolism. The importance of disturbances of body potassium is increasingly evident in clinical medicine. Because of the intracellular position of most of the body potassium pool and the cation dynamism, metabolic balance data are required for comprehensive study. These are being obtained in hyper- and hypokalemic states, along with correlations of the relationship of potassium to sodium and other blood and tissue constituents. The human body counter has proven to be a tool of great usefulness in studies of total body potassium and further studies are in progress.

3. Studies of Endocrine Function.

a. Adrenal Cortical Suppression and Stimulation

A standardized test of adrenal cortical function has been developed consisting of the assay of urinary adrenal cortical steroids for

17-hydroxycorticoids, 17-ketosteroids, and pregnanetriol during a 2-day control period, a 2-day period of administration of decadron, a second 2-day control period, and a 1-day period of administration of A.C.T.H. This has been further modified by measuring urinary adrenal cortical steroids during a 2-day control period followed by 1 day of A.C.T.H. stimulation.

b. Pregnanetriol Determinations

A technique is now available to determine pregnanetriol. The urinary pregnanetriol levels of 20 normal subjects have been studied so that we have been able to establish a normal urinary pregnanetriol range of 0.5 to 2.6 mg/24 hours. In addition, we have found that pregnanetriol and related compounds can be detected simply by the use of 20% antimony pentachloride. This has enabled us to study the "pregnanetriol fraction" of a patient with metastatic-functioning adrenal carcinoma and the "pregnanetriol fraction" of a patient with hirsutism and elevated 17-ketosteroids and elevated "pregnanetriol". In this latter patient, two unusual steroids have been found which are being investigated further.

c. Total Urinary Corticoid Spectrum

(1) It is now possible to assay urinary adrenal cortical steroids using a simultaneous procedure which isolates a crude "steroid gum". This steroid gum can be fractionated further so that total 17-hydroxycorticosteroids, total 17-ketosteroids, and pregnanetriol can be measured. In addition, by applying the methods of column and paper chromatography the individual steroid members of each fraction can be isolated.

(2) With the acquisition of a fluorospectro-photometer we are setting up the technique for measuring plasma steroids.

(3) With the acquisition of an infra-red analyzer, it is now possible to identify individual steroids in a precise fashion.

(4) A paper scanner has been obtained which is being used to set up a double isotope method for the determination of aldosterone in urine.

d. Studies in Adrenal Carcinoma

A patient with a metastatic functioning adrenal carcinoma and Cushing's Syndrome was found to have very greatly elevated urinary adrenocorticosteroids. With the use of o, p'-DDD the "pregnanetriol fraction" was quickly returned to normal while the 17-hydroxycorticosteroids and 17-ketosteroids declined at a slower rate. Despite chemical improvement, the patient died. Analysis of the "pregnanetriol fraction" before therapy indicated the presence of multiple pregnanetriol-like compounds with not much pregnane-3 α , 17 α , 20 α -triol present. After therapy, only an unknown pregnanetriol-like substance was left.

e. The Use of SU-4885 in Evaluation of Hypopituitarism

A number of patients with hypopituitarism have been studied. It has been found that their urinary 17-hydroxycorticosteroid levels are quite low as are their urinary 17-ketosteroids. Similar findings have been noted in patients with primary myxedema. The compound, SU-4885, distinguishes the presence of A.C.T.H.-producing tissue by releasing the inhibition of A.C.T.H. by compound F (hydrocortisone) and causing a rise in urinary 17-hydroxycorticosteroids. In hypopituitarism, the absence of A.C.T.H.-producing tissue leads to no such rise in 17-hydroxycorticosteroids. However, in primary myxedema the response to SU-4885 has been reported to be decreased. Therefore, it is difficult to distinguish clearly between primary myxedema and the myxedema secondary to hypopituitarism. A study has been devised to investigate the adrenal cortical response of primary hypothyroid patients to SU-4885 before and after thyroid therapy with a view to applying this data to the evaluation of hypopituitary patients with hypothyroidism.

4. Lipid Metabolism. This department has developed several methods for studies of various lipid fractions including a technique to measure the rate of cholesterol synthesis by the use of labeled mevalonic acid. These studies will be continued and applied to patients with occlusive vascular diseases. Silicic acid column chromatography for separation of labeled components of the serum has been developed and will be used in further studies.

5. Refractometer. A total-solid, temperature-compensated refractometer developed by the American Optical Company in collaboration with Dr. Arnold Wolf, formerly of the Department of

Cardiorespiratory Diseases, has been extensively tested in this unit. Its valuable clinical use in determining hydration and urinary specific gravity has been demonstrated. A study in which a valid estimation of total protein content of serum was made by the TS Meter reading has been published. Work has been completed comparing the biuret method, copper sulfate method, and refractometric method of determining the total serum proteins in the fasting and hyperlipemic state. The data show that the TS Meter is a more reliable method of determining total serum proteins than the biuret and that the TS Meter gives readings for serum specific gravity within 0.001 units of the CuSO_4 method determination.

6. Hepatic Coma. Studies of sulfur have shown patients in hepatic coma have decreased urinary SO_4^{--} and total sulfur excretion despite an elevated blood SO_4^{--} level. Other biochemical abnormalities continue to be correlated.

7. Laboratory Methods and Techniques. During this past year the capabilities for automation of several studies have begun, using Technicon analyzers, and studies using these methods will be performed much more efficiently. These include P-A-H, inulin, creatinine, sodium, and potassium, and possibly mannitol.

The method for urinary oxalate has been proven to be accurate and a new method has been developed for plasma oxalate which is expected to be a significant advance in our ability to diagnose patients with hyperoxalate states.

Methods for sulfides, methionine, total sulfur and urinary peptides were all evaluated and can be used clinically.

8. Fructose Metabolism with Particular Reference to Diabetes Mellitus. Patients with diabetes are being screened for excretion of fructose in the urine as part of a routine study of diabetes. This project is being continued.

9. Mannitol for Refractory Fluid Retention. Studies now include over 20 patients. Mannitol is the most effective single diuretic agent evaluated. Mechanisms of fluid retention and its therapy are being studied by these methods.

10. Intravenous Mannitol in Acute Left Ventricular Failure. The administration of mannitol in acute cardiac failure has caused a prompt diuresis with the loss of as much as six pounds in a few hours. We hope to study, in the future, patients with pulmonary edema with the expectation that mannitol diuresis will be as rapid and effective as phlebotomy.

11. Metabolic Edema. Studies of water and solute metabolism have been carried out in six patients who have recurrent generalized edema in the absence of cardiovascular, renal or hepatic disease. In addition to a distinct exaggerated postural influence on edema, these patients have uniformly shown low to low normal basal 17-hydroxycorticoid excretion and a sluggish response to exogenous ACTH stimulation. Further studies which include a comprehensive evaluation of renal and adrenal function are in progress.

12. Diabetes Insipidus. Studies performed on patients with diabetes insipidus and psychogenic water drinking suggest new methods are necessary to differentiate the two entities. These are being sought.

13. The Oxidation of 1-C¹⁴-Glucose by Red Blood Cells of Normal and Hyperlipemic Patients. It has been found possible to determine the state of function of the pentose phosphate pathway of red blood cells by measuring their oxidation of 1-C¹⁴-glucose and measuring the amount of C¹⁴O₂ produced. We have determined the influence of pyridine nucleotides on this system in the red blood cells of normal and hyperlipemic subjects. A number of differences have been noted which are currently under investigation.

14. The Study of Hyperkalemic Paralysis. Two patients with hyperkalemic paralysis have been diagnosed and successfully treated. Multiple metabolic studies have been done to prove the diagnosis and to investigate the mechanism of this metabolic derangement. A number of findings have been uncovered and a number of additional studies are planned to evaluate these findings.

15. External Biliary Fistula. Two patients with complete external biliary fistula have been studied. The fistula in one case was traumatic in origin, the other postoperative. Both developed a characteristic clinical picture of weakness, anorexia, apathy, confusion, circulatory and functional renal failure with hyperkalemia and finally vascular

collapse with severe cardiac arrhythmias. This complication was shown to be secondary to severe Na^+ and HCO_3^- depletion. The Na^+ concentration of bile was greater than normal serum values and remained high despite severe hyponatremia. In one case, Na^+ loss through the fistula averaged 262 mEq/day.

16. Body Fluid Determinations by the Multiple Isotopic-dilution Technique. The status of fluid compartments is being studied in normal dogs and in normal and abnormal humans by means of the isotopic dilution technique. Plasma volume, extracellular fluid, and total body water are being measured simultaneously by a single injection technique using Cr^{51} , H^3 , and S^{35} . The Auto Gamma counter and the Packard Tri-Carb Liquid scintillation machine are being utilized for counting. This procedure seems feasible and accurate.

Summary and Conclusions:

Body potassium measurement with the human body counter has added a tool of assistance in metabolic studies. The continued refinement of techniques in the Steroid Laboratory has enabled us to begin the fractionation of certain crude groups of steroids. The suppression-stimulation test for adrenocortical function is standardized for routine clinical use. Additional capabilities in lipid research have been added and it is generally hoped that the direction of our investigations will be toward studies of intermediary metabolism of both lipids and carbohydrates.

Further studies in metabolic edema have been productive and will be extended to try to isolate the defect. Two patients with hyperkalemic paralysis were carefully studied and additional information on the defect has been obtained which is being used in therapy. A method for simultaneous determination of total body water, extracellular space and plasma volume is being developed and may become clinically useful.

Publications:

1. Goldsmith, R. S., Bartter, F., and Meroney, W. H.: Prominent Peripheral Edema Associated with Primary Aldosteronism Due to an Adrenocortical Adenoma. *J. Clin. Endocrin. & Metab.* 20: 1168, August, 1960.

2. Joy, R. J. T. and Beisel, W. R.: An Exploration of the Antihypertensive Action of Chlorothiazide. *Amer. J. Med. Sciences* 239: 501, May, 1960.
3. Lewis, L. C., Rubini, M. E., and Beisel, W. R.: A Method for Rapid Dehydration of Rats. *Jour. of Applied Physiol.* 15: 525, May, 1960.
4. Barry, K. G., McLaurin, A. W., and Parnell, B.: A Practical Temperature Compensated Hand Refractometer (The TS Meter): Its Clinical Use and Application in Estimation of Total Serum Proteins. *J. Lab. Clin. Med.* 55: 803, May, 1960.
5. Beisel, W. R., Austen, K. F., and Rubini, M. E.: A Comparison of Certain Metabolic Effects of Triiodothyronine, Salicylate, and Dinitrophenol. *Metabolism* 9: 905, October, 1960.
6. Herman, R. H.: The Reaction of Digitalis Compounds with Antimony Pentachloride. *Nature* 190: 268; April 15, 1961.
7. Herman, R. H. and Bruton, J.: The Reaction of Antimony Pentachloride with Pregnane-3 α , 17 β , 20 α -triol, Pregnane-3 α , 17 α , 20 α -triol-11-One, and Other Adrenal Cortical Steroids. Accepted for publication by Nature.

ANNUAL PROGRESS REPORT

Project 6X60-09-001 Metabolism

Task 6, Metabolic and nutritional problems associated with injury

**Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.**

**Department of Surgical Metabolism and Physiology,
Division of Surgery**

Period Covered by Report: 1 Jul 60 - 30 Jun 61

**Principal Investigators: Stanley M. Levenson, M. D., Hyman Rosen, M. A. and
Erving F. Geever, M. D.**

**Assistants: Leo V. Crowley, Maj, MSC, Costan W. Berard, Capt, MC, Clarence E.
Emery, Jr., B. S., PFC Francis S. Poulson, and PFC Ray E. Meacher**

Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

ABSTRACT

Project No. 6X60-09-001 Metabolism

Task 6 Metabolic and nutritional problems associated with injury

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Period Covered by Report: 1 Jul 60 - 30 Jun 61

Authors: S. M. Levenson, M. D., H. Rosen, M. A., E. F. Geever, M. D.,
L. V. Crowley, Maj, MSC, C. W. Berard, Capt, MC, C. E. Emery, Jr.,
B. S., PFC F. S. Poulson, and PFC R. E. Meacher

Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

Studies are continuing with the view of establishing the physiologic and clinical significance of the biochemical and metabolic changes associated with injury (traumatic and irradiation) and thereby provide the basis for improved prophylaxis and care of seriously injured patients. The healing of experimental wounds, collagen biosynthesis, tissue growth, and protein turnover are being followed to gain objective information for the evaluation of certain critical aspects of the response to injury. Concurrent investigations are conducted in vitro, in animals (conventional and germfree) and in man (See Progress Reports for Project 6X60-09-001 Metabolism, Task 14 Wound Healing and Project No. 6X61-08-001 Germfree Animal Studies.)

Investigations of ascorbic acid metabolism following burns are continuing with particular reference to the increased need for this vitamin by the injured patient. Alterations in the metabolism of the aromatic amino acids are among the parameters being investigated.

BODY OF REPORT

Project No. 6X60-09-001 Metabolism

Task 6 Metabolic and nutritional problems associated with injury

Description: Studies are continuing with the view of establishing the physiologic and clinical significance of the biochemical and metabolic changes associated with injury (traumatic and irradiation) and thereby provide the basis for improved prophylaxis and care of seriously injured patients. The healing of experimental wounds, collagen biosynthesis, tissue growth, and protein turnover are being followed to gain objective information for the evaluation of certain critical aspects of the response to injury. Concurrent investigations are conducted in vitro, in animals (conventional and germfree) and in man. (See Progress Reports for Project 6X60-09-001 Metabolism, Task 14 Wound Healing and Project 6X61-08-001 Germfree Animal Studies.

Investigations of ascorbic acid metabolism following burns are continuing with particular reference to the increased need for this vitamin by the injured patient. Alterations in the metabolism of the aromatic amino acids are among the parameters being investigated.

Progress: Neutral Salt Solubility of Rat Tail Tendons in Vitro. Rat tail tendons have long been assumed to be essentially insoluble in neutral salt even though various other collagens may be readily solubilized in such solutions. Previous work in this laboratory revealed the chromatographic alteration of rat tail collagen exposed to increasing molar concentrations of acetic acid and prompted similar exploration of the effect of increasing molar strengths of neutral salt on such preparations. In a series of four experiments we have been able to show that rat tail collagen is, indeed, essentially insoluble in neutral salt solutions of 0.15 molar or less but that both hydroxyproline and total nitrogen content of the extracting salt solution increase as salt concentration is increased up to a peak at 0.75-1.0 molar; as concentration is further increased from 1.0 to 5.0 molar, there is a progressive diminution in hydroxyproline and nitrogen contents of the extract. Although the amounts solubilized represent only a few tenths of a per cent of total exposed rat tail collagen, these results suggest that such collagen may in a limited way be susceptible to the "salting in-and-out" seen with other proteins. Moreover, hydroxyproline nitrogen, expressed as per cent of total nitrogen, was, at peak extraction, between 3.6 and 4.3%. Although this may be the result of solubilization of non-collagenous, "contaminating" protein constituents of the rat tail tendon, it may also be interpreted as additional evidence of the non-homogeneity of such tendon previously demonstrated in this laboratory by chromatography of acetic acid solubilized tendon. It will be recalled that the forepeak of the chromatographed tendon comprised of several low molecular weight peptides with rapid metabolic turnover, also demonstrated a hydroxyproline nitrogen of 3.9% of total nitrogen. This forepeak material was solubilized in distilled water when tail tendons were heated to 60° C and it is possible that neutral salt solutions of appropriate molarity may also solubilize a portion of this loosely found, atypical, precursor material even at 0° C. Additionally suggestive that this neutral salt soluble fraction is precursor material is the finding that

extracts from tendons of 175-250 gram rats contain 6-7 times as much material as those from 400 gram rats. One might expect the amount of precursor material to be appreciably greater in younger animals, whose relatively more rapid growth rate would be accompanied by greater turnover of precursor materials.

Work to elucidate the physico-chemical nature and biologic significance of this neutral salt soluble fraction is contemplated for the future.

Amino Acid Analysis: This department has been working on new techniques for the automatic analysis of amino acids. An experimental gradient elution chromatographic analysis has been set up, according to the method of Piez and Morris (Anal. Biochem., 1, 3, 1960). This apparatus has been found to be of very limited usefulness since, in our hands, at least, the resin consistently packs after only a few runs to yield very high and unworkable back pressures, and the lines through which the reagents run became invariably clogged. In addition, the unstable nature of their reagents complicates the analyses, since these reagents must be protected from air and light by gas and oil.

We have now worked out methods which make possible routine analyses hitherto impossible. These include a new analytical ninhydrin reagent which is stable at room temperature and need not be protected in any way; a buffer system included within the reagent which does not clog at any time nor under any circumstances; a method of backwashing the resin in order to prevent it from packing to give very high back pressures; a new gradient elution device which can be adjusted to yield gradients of any type; and a system of automatic print-out integration of curves. A full report of this system is being prepared for publication.

Growth-Promoting Agents in Wound Fluid: For the past year, work concerning the growth accelerator described by us in previous progress reports has been centered on biological preparation of this material in large amounts. It will be recalled that this substance was isolated from the serous fluid which accumulates within artificially induced wound pockets in dogs. The material promotes the growth of L. casei and rat fibroblast cells, and is non-protein. The preparation of the animals is being carried out by Dr. John Schilling and his associates at the University of Oklahoma Medical School. Stainless steel mesh cylinders are implanted subcutaneously in dogs and remain for varying periods of time before the fluid is withdrawn aseptically. This phase of the work has been planned to determine how the concentration of the growth accelerator varies with the age of the wound, and to find out if it is related to the observed cyclical growth pattern of the collagen within the cylinder. A large quantity of the wound fluid has been obtained for the isolation and chemical identification of the active agent.

Tensile Strength and Histologic Appearance of Healing Skin Wounds: In our studies of the healing of experimental wounds reported last year, we found that skin wounds of rats do not reach maximal strength in 14-21 days as stated in most textbooks of surgery and pathology. In fact, it appeared the wounds continue to gain strength at a maximal rate up to 40-50 days, and then at a lower rate for as long as a year. During the past year, further study has been made

of healing of skin wounds of rats as judged by gain in tensile strength and histologic appearance of the wounds.

Five cm. incisions were made through the dorsal skin of young adult Walter Reed rats (275-300 grams, pathogen free). These wounds were made aseptically under light ether anesthesia, and closed with interrupted fine stainless steel wire sutures. At various intervals postoperatively, the wounds were excised, examined with a variety of histological staining techniques, and their tensile strengths measured on an apparatus designed by this Department.

We have confirmed our impression that the healing of skin wounds continues for many weeks after operation. The increase in tensile strength continues at a very rapid rate for about three months, then appears to have reached a maximum with little further increase in strength. It may be noted that about the same amount of variance in tensile strength (standard deviation expressed as a percentage of the mean) was found for each postoperative interval except for the two-month interval in Experiment A, where a higher variance was found. We have attempted to measure the tensile strength of normal rat skin, but we have been unsuccessful so far in this determination under the conditions of our experiments. Attempts to modify the technics so far used are underway.

We have begun measuring the thickness of the skin at the wound as well as its width. This is because the tensile strength of the wound is properly related to the surface area of the wound, not just its width. Despite this obvious fact, no reported studies, except the early ones of Harvey and Howes 30 years ago, have taken this into consideration. The pitfalls one runs into without measuring the surface area of the wound are readily apparent. For example, in almost every reported study, a difference in tensile strength among wound strips of constant width is interpreted as reflecting fundamental differences in healing rates. But actually the difference in strength may reflect only differences in thicknesses of the wounds. We demonstrated this in an experiment in which 5 groups of young rats of the same age were placed on different amounts of a standard diet which resulted in 5 different rates of weight gain. After several weeks, when the weights of the various groups of rats varied almost 5-fold, dorsal skin incisions were made under ether anesthesia by our usual technique. The tensile strengths of skin strips of fixed width varied five-fold from the smallest to the largest group. But this did not mean that the wounds in the heavier group had healed faster. Utilizing the Dice electronic micrometer, we have been able to measure skin thickness to thousandths of an inch and have demonstrated a linear relation of tensile strength to skin thickness (and, hence, area since the width of the strips is constant) of the wound surfaces. Histologic studies of the wounds confirmed this. It is clear, then, that unless the surface area of the wound is measured, interpretation of tensile strength measurement is almost impossible.

Effect of Age on Healing of Wounds of Guinea Pigs: Studies of wound healing of guinea pigs in this laboratory have centered almost exclusively on 175-225 gram guinea pigs, and the influence of age and growth rate on wound healing and collagen biosynthesis in particular represents an area of vast importance as yet largely unexplored. Does a growth inhibitor exist in the sera of older animals as suggested by Carrel over 40 years ago? A study is

presently being conducted utilizing guinea pigs in groups weighing from 175 to 700 grams. The collagen biosynthesis in implanted ivalon sponges in 14 days, as measured histologically and by hydroxyproline content, shows no significant difference over this range. Tensile strengths of skin incisions are higher and, in general, more variable in the heavier, older animals but, when the tensile strengths are corrected for skin thickness and expressed on the basis of grams/unit area of wound surface, the tensile strengths of all groups are comparable.

Influence of Peracetic Acid on Wound Healing: The following study was prompted by the usefulness of peracetic acid as a sterilizing agent with the plastic isolators for patients under development by this Department in collaboration with the Dept. of Germfree Research. What effects do residual trace amounts of the acid remaining on the cleaned items and thereby introduced into the surgical field have upon the course of wound healing, especially collagen biosynthesis? This point was investigated with 60 guinea pigs, in half of which dorsal skin incisions and implanted ivalon sponges were bathed in 0.005% peracetic acid, saline being used for the control group. Study of the wounds and sponges at 7 and 14 days postoperatively revealed no gross or histologic difference in the appearance of the wounds or sponges and no differences in hydroxyproline content of sponges, but the wounds of the peracetic acid group were slightly, but statistically significantly greater in tensile strength at both 7 and 14 days. This rather surprising finding is to be corroborated and further investigated in studies currently underway.

Effect of Thermal Injury on Ascorbic Acid and Tyrosine Metabolism: It is well established that stress alters many metabolic patterns of the animal organism extensively. The metabolism of Vit. C has been studied in this context, and it appears that a severe injury imposes a need for Vit. C far above normal. This conclusion is based on studies of Vit. C tissue saturation and blood levels, and wound healing. The present investigation was aimed at determining whether the increased need for Vit. C after stress could be demonstrated for an enzymatic function in which this vitamin is known to be involved, namely, metabolism of tyrosine.

Alcaptonuria is marked by appearance in the urine of homogentisic acid (2,5-dihydroxyphenylacetic acid) and is caused by a lack or absence of the enzyme necessary to carry the conversion of tyrosine further. It was subsequently discovered almost simultaneously by Sealock and Levine that Vit. C is required for formation of homogentisic acid from its precursors. Sealock also showed conclusively by differential colorimetry and by preparation of derivatives, that the urinary acids resulting from feeding l-tyrosine or l-phenylalanine to scorbutic guinea pigs are primarily p-hydroxy phenylpyruvic acid and smaller amounts of homogentisic acid; this established that the metabolic defect of scurvy was fundamentally different from inherited alcaptonuria.

We found that intragastric administration of 0.75 mg of l-tyrosine per g body weight to guinea pigs maintained on 2 mg of Vit. C per day did not yield urinary tyrosine intermediate metabolites. Burned guinea pigs to which this test was applied at various times after the burn showed an abrupt abnormal response on the third post-burn day despite a daily maintenance Vit. C dose of 2 mg per day. This response was manifested by appearance of large

amounts of urinary p-hydroxy phenylpyruvic acid, the same compound which appears in the urine of uninjured guinea pigs fed tyrosine when ascorbic acid is removed from their diet. Metabolic abnormality reached its peak on the sixth post-burn day, then declined to almost normal levels by the 20th post-burn day. One hundred mg per day of Vit. C abolished the abnormality after the burn.

This pattern of metabolic reaction to injury as it involves Vit. C agrees with our previous observation (Levenson, S. M., Upjohn, H. L., Preston, J. A., Steer, A., Ann. Surg., 1957, v146, 357) that the healing of wounds of burned guinea pigs was strikingly abnormal during the first postoperative week. These wounds were histologically indistinguishable from those of guinea pigs depleted of Vit. C by nutritional deprivation. The abnormalities were still present 10 days postoperatively, though to a lesser extent, and by the fourteenth day, the wounds were almost normal. These animals received 2 mg Vit. C per day. Addition of 100 mg/day Vit. C to the basal diet allowed the wounds to heal normally, despite the superimposed burn.

Summary and Conclusions: Studies are continuing with the view of establishing the physiologic and clinical significance of the biochemical and metabolic changes associated with injury (traumatic and irradiation) and thereby provide the basis for improved prophylaxis and care of seriously injured patients. The healing of experimental wounds, collagen biosynthesis, tissue growth, and protein turnover are being followed to gain objective information for the evaluation of certain critical aspects of the response to injury. Concurrent investigations are conducted in vitro, in animals (conventional and germfree) and in man. (See Progress Reports for Project 6X60-09-001 Metabolism, Task 14, Wound Healing, and Project 6X61-08-001 Germfree Animal Studies.)

Investigations of ascorbic acid metabolism following burns are continuing with particular reference to the increased need for this vitamin by the injured patient. Alterations in the metabolism of the aromatic amino acids are among the parameters being investigated.

List of Publications: 1. Levenson, S. M., Einheber, A. and Malm, O. J. Nutritional and Metabolic Aspects of Shock, Fed. Proc. (in press):

2. Emery, C. E.; Rosen, H. and Levenson, S. M., Effect of Thermal Injury on Ascorbic Acid and Tyrosine Metabolism, Proc. Soc. Exp. Biol. Med., 106, 267-270, 1961.

ANNUAL PROGRESS REPORT

Project 6X60-09-001, Metabolism

Task 8, Interrelationships of Parathyroid Gland Activity and Calcium
and Phosphorus Metabolism

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Department of Metabolism
Division of Medicine

Period Covered by Report: 1 July 1960 through 30 June 1961

Principal Investigator: Lt. Colonel Jacques L. Sherman, Jr., MC

Assistants: Captain Robert H. Herman, MC
Captain Thomas E. Davis, MC

Reports Control Symbol: MEDDH-288

Security Classification: Unclassified

ABSTRACT

Project 6X60-09-001, Metabolism.

Task 8; Interrelationships of Parathyroid Gland Activity and Calcium and Phosphorus Metabolism

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Period Covered by Report: 1 July 1960 through 30 June 1961 -

Authors: Lt. Colonel Jacques L. Sherman, Jr., MC
Captain Robert H. Herman, MC
Captain Thomas E. Davis, MC

Reports Control Symbol: MEDDH-288

Security Classification: Unclassified

Review of the studies in hyperparathyroidism demonstrates that diagnostic accuracy has approached an ideal level and the methods are being developed for routine use. Work on the endocrine regulation of calcium transport has been extended. The use of Ca^{47} has been added to previous studies on bone metabolism and may give information on the speed of calcium turnover in metabolic bone disease not previously available.

BODY OF REPORT

Project 6X60-09-001, Metabolism

Task 8, Interrelationships of Parathyroid Gland Activity and Calcium and Phosphorus Metabolism

Description: Studies carried out under this task are designed to improve diagnostic methods for abnormalities of parathyroid gland function and to clarify some of the mechanisms of uptake, distribution and excretion of calcium.

Progress:

1. Hyperparathyroidism. With the improved diagnostic technique now available within this department, additional successful surgery on parathyroid adenomas has been carried out in cooperation with the Department of Surgery, WRGH. Studies on the influence of calcium infusion on phosphate clearance have been standardized and appear to show a high degree of correlation with hyperparathyroidism. In addition, phosphorus deprivation studies are being used in cases where the diagnosis is not clear by other methods.
2. Idiopathic Osteoporosis. Detailed balance studies are being carried out in this disease to clarify further the relationship of calcium, Vitamin D, and bone matrix metabolism. Further studies with Sr-85 are being carried out in the human body counter.
3. Intestinal Transport of Calcium in Normal and Hypophysectomized Rats. Using an in vitro tissue sliced technique, the uptake of Ca^{45} by rat duodenal tissue has been studied. The Ca^{45} uptake is enhanced by oxygen and inhibited by a nitrogen atmosphere in both normal and hypophysectomized rats. The normal rat duodenum has a statistically significantly greater uptake of Ca^{45} than the hypophysectomized rat duodenum. This difference is repaired by simultaneous administration of both ACTH and triiodothyronine. ACTH and triiodothyronine alone in both normal and hypophysectomized

rats is not helpful. Growth hormone (bovine) is not effective. ACTH only in pharmacologic doses inhibits Ca^{45} uptake in the hypophysectomized rat only.

4. Hyperoxaluria. In the patients who were previously discovered to have primary hyperoxaluria, a continuation of follow up is being accomplished. New methods for determinations of serum and urine oxalate are being used and new patients are being sought. The patients are being treated with pyridoxine and this seems to have decreased their oxalic acid excretion.

5. Study of Calcium Metabolism Utilizing Ca^{47} . Much of our knowledge of calcium metabolism in the past has been dependent on metabolic balance techniques. Utilizing intravenously administered Ca^{47} , the equilibration dynamics of skeletal accretion and turnover and endogenous secretion into the bowel are being studied. Since Ca^{47} is a gamma-emitting isotope, its energy may be easily detected by external counting over certain bone sites or by using the human whole body counter. Plasma, urine and stool may also be counted by the gamma detection apparatus making this technique feasible. Studies in patients with metabolic bone disease are being carried out in conjunction with the Department of Gastroenterology, Division of Medicine.

Summary and Conclusions:

Review of the studies in hyperparathyroidism demonstrates that diagnostic accuracy has approached an ideal level and the methods are being developed for routine use. Work on the endocrine regulation of calcium transport has been extended. The use of Ca^{47} has been added to previous studies on bone metabolism and may give information on the speed of calcium turnover in metabolic bone disease not previously available.

Publications:

1. Beisel, W. R., Austen, K. F., Rosen, H., and Herndon, E. G. Jr.: Metabolic Observations in Adult Hypophosphatasia. Amer. J. Med. 29: 369, August, 1960.

2. McLaurin, L. W., Beisel, W. R., McCormick, G. J., Scalettar, R., and Herman, R. H.: Primary Hyperoxaluria. Accepted for publication in *Ann. Int. Med.*
3. Beisel, W. R., Gerard, E. S., Barry, K. G., Herndon, E. G. Jr., Meroney, W. H., and Kyle, L. H.: Phosphate Abnormalities in Hyperparathyroidism. Submitted for publication.

ANNUAL PROGRESS REPORT

Project 6X60-09-001 Metabolism

Task 14 Wound Healing

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Department of Surgical Metabolism and Physiology,
Division of Surgery

Period Covered by Report: 1 Jul 60 - 30 Jun 61

Principal Investigators: Hyman Rosen, M.A., Erving F. Geever, M. D. and
Stanley M. Levenson, M. D.

Assistants: Leo V. Crowley, Maj, MSC, Costan Berard, Capt, MC, Clarence E.
Emery, Jr., B. S., John W. Diggs, B. S., PFC Francis S. Poulson,
and PFC Ray E. Meacher.

Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

ABSTRACT

Project No. 6X60-09-001 Metabolism

Task No. 14 Wound Healing

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Period Covered by Report: 1 Jul 60 - 30 Jun 61

Authors: Hyman Rosen, M. A., Erving Geever, M. D., Stanley M. Levenson, M. D.,
Leo V. Crowley, Major, MSC, Costan Berard, Capt, MC, Clarence E.
Emery, Jr., B.S., John W. Diggs, B. S., PFC Francis S. Poulson, PFC
Ray E. Meacher

Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

A comprehensive study of wound healing is underway, the aim of which is to elucidate the basic biochemistry, physical chemistry, and physiology of wound healing with particular reference to the nature of collagen and its relation to other wound components, the mechanism of collagen biosynthesis, and the effects of severe injury (traumatic and radiation) on wound healing in animals and man. Our aim is to provide a sound theoretical basis for improved prophylaxis and treatment of severely injured patients.

ABSTRACT

Project No. 6X60-09-001 Metabolism

Task No. 14 Wound Healing

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Period Covered by Report: 1 Jul 60 - 30 Jun 61

Authors: Hyman Rosen, M. A., Erving Geever, M. D., Stanley M. Levenson, M. D.,
Leo V. Crowley, Major, MSC, Costan Berard, Capt, MC, Clarence E.
Emery, Jr., B.S., John W. Diggs, B. S., PFC Francis S. Poulson, PFC
Ray E. Meacher

Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

A comprehensive study of wound healing is underway, the aim of which is to elucidate the basic biochemistry, physical chemistry, and physiology of wound healing with particular reference to the nature of collagen and its relation to other wound components, the mechanism of collagen biosynthesis, and the effects of severe injury (traumatic and radiation) on wound healing in animals and man. Our aim is to provide a sound theoretical basis for improved prophylaxis and treatment of severely injured patients.

BODY OF REPORT

Project No. 6X60-09-001 Metabolism.

Task No. 14 Wound Healing.

Description: A comprehensive study of wound healing is underway, the aim of which is to elucidate the basic biochemistry, physical chemistry, and physiology of wound healing with particular reference to the nature of collagen biosynthesis, and the effects of severe injury (traumatic and radiation) on wound healing in animals and man. Our aim is to provide a sound theoretical basis for improved prophylaxis and treatment of severely injured patients.

Progress: In the last two progress reports, some details of a chromatographic method have been presented, by which we have been able to show that acetic acid solubilized rat tail tendon collagen is not homogeneous, but actually consists of at least four major chromatographable components. Three of these are high molecular fractions, with amino acid compositions similar to that of whole collagen, while the other is almost entirely dialyzable, with an amino acid composition atypical of the entire collagen macromolecule (Nature, 189, 1640, 1959; Jour. Biol. Chem., 235, 989, 1960). The most striking feature of this latter fraction was its relatively low percentage of hydroxyproline; this feature together with its rapid biological turnover rate, as measured by the incorporation and loss of C^{14} after the injection of C^{14} -glycine (also reported in the last progress report) suggested to us the possibility that this component might be the precursor of the collagen macromolecule.

Previous work by us also suggested that the dialyzable fraction might itself consist of a number of components. We have recently succeeded in fractionating it into at least six peptides, which have been isolated by chromatography on Dowex-50, 12% cross linked. We are now in the process of hydrolyzing them and subjecting them to amino acid analysis by ion exchange chromatography. The first peptide completely analyzed has an amino acid composition containing a predominance of the acidic amino acids.

The above results have significant implications to the over-all structure of collagen; the present concept of the structure of this macromolecule is that it is triple stranded, with a repeating unit called "tropocollagen." Our work indicates that this concept may be incomplete, since it does not account for our findings of chromatographic and biological inhomogeneity, nor does it predict our finding of biologically active peptides. We hope that discovery of the structure of these peptides will enable us to learn their function, and thus more about the "fine" structure of the entire collagen macromolecule.

Other collagens have now also been subjected to our chromatographic procedure, including rat skin, guinea pig skin, and guinea pig Achilles tendon collagen. All show the four component pattern; it is likely that this pattern is characteristic of most, if not all, mammalian collagens. We have, thus far, been unable to apply the method to human skin, since it is extremely insoluble in acetic acid. The problem is being further investigated.

This department is engaged in a long-term study of the molecular structure of collagen in situ. In pursuit of this goal, experiments have been started to determine the effect, if any, of incorporation of deuterium into developing collagen of the rat. Deuterium has been shown to have marked effects on hydrogen bonding of proteins; collagen is very highly hydrogen bonded, and so forms an ideal system for investigation with deuterium. We think that feeding D_2O and deuterated amino acids will lead to incorporation of deuterium into developing collagen as a stable component, and that the physical properties of this collagen will be abnormal; such parameters as extent of incorporation of deuterium, viscosity, solubility, chromatographic properties, etc., will be measured.

These measurements are, in general, indications of the heterogeneity of the collagen molecule, and of its structural features, such as its stability, tendency to random coiling, etc. The importance of these features of the molecule to the actual way in which it is bound into the ground substance is unknown, and these D_2O experiments will, it is hoped, cast light on this subject.

The first preliminary experiment has been a metabolic balance study in which six rats were maintained on water which was 15% D_2O , and six rats were allowed only H_2O . They were subjected to our standard operation, consisting of implantation of four 25 mg polyvinyl sponges between the superficial and deep muscles of the abdomen, and a dorsal skin incision. There were no differences in the amounts of collagen which grew into the sponges in 14 days, nor were there any differences in the tensile strengths of the excised wounds. Table I shows, in summary, the results of the metabolic experiment with respect to nitrogen metabolism. The " D_2O " animals responded to the trauma with an apparently longer period of nitrogen loss, and thus ended the post-operation phase of the experiment with an accumulated negative balance. We feel it is important to establish these points, in order to be able to distinguish any acute effects of such experiments from chronic metabolic effects. A new metabolic experiment is now underway, in which 25% D_2O is being administered to guinea pigs. This is close to the known limit of D_2O compatible with life; we hope to be able to induce, in this way, the maximum incorporation of deuterium in collagen for the studies mentioned below.

The polyvinyl sponge implant technique is being pursued by this department as a model system for the study of wound healing, collagen, biosynthesis, and the factors which affect these processes. We have posed the following questions as fundamental to these studies:

- 1) How accurate is the classical method for the estimation of hydroxyproline, when applied to a total hydrolysate of newly formed tissue in a sponge?
- 2) Is there any "neutral salt soluble" collagen (as measured by hydroxyproline) in these sponges, and if so, how does its concentration change with time?
- 3) If a guinea pig is deprived of vitamin C after sponge implantation and subsequently repleted with vitamin C, what will be the course of collagen synthesis?
- 4) Are there species differences in the way collagen grows into an implanted sponge?

- 5) Does histological estimation of collagen yield results comparable with those obtained by chemical means?
- 6) What are the effects of animal age on the rate of collagen synthesis?
- 7) Are there factors which inhibit collagen formation in the hemorrhagic exudate which collects in the wounds and sponges of scorbutic guinea pigs?

In order to answer the first question, a series of determinations was done in which portions of pooled hydrolysates were analyzed by the Neuman Logan method, and portions were analyzed only after prior isolation of the hydroxyproline on 150 cm. ion exchange columns (Dowex-50). Table II summarizes the results of these analyses as applied to normal guinea pigs, those deprived of vitamin C, and those deprived of vitamin C for 14 days and thereafter given 2 mg Vit C/day.

It can be seen that the application of the Neuman Logan method to these total hydrolysates yields results which are more or less incorrect. The results may be either falsely high or low; after 14 days, however, the results of both the normal and "repleted" sponges were invariably high when compared with the chromatographic method. We conclude from this that chromogens in the hydrolysate mixture render the analysis of hydroxyproline unreliable unless it is first isolated from the hydrolysate. Recovery experiments of the chromatographic procedure gave essentially 100% recovery.

Some sponges from each animal were extracted with 0.2 M sodium chloride at 4° C. for 24 hours. These extracts were then analyzed for hydroxyproline by both the direct and chromatographic methods. It was hoped, by this procedure, to determine whether the extractable hydroxyproline would vary with the age of the developing collagen. Table III shows the results of these analyses in micrograms, and Table IV presents the "chromatographic" figures as per cent of the total hydroxyproline in the sponges. In all cases, the greatest amounts of soluble collagen were found in the earliest samples. This is consistent with the theory that early "precursor" collagen contains the greatest amounts of low molecular weight component.

Results of the "repletion" experiments are shown in Table II; sponges were implanted into guinea pigs deprived of vit C. After 14 days (when little or no collagen growth had occurred), 2 mg/day were given orally. Seven days after this regimen, the hydroxyproline content of the sponges was about the same as the normal "seven day" sponges. After this, however, the rate of incorporation leveled off and took a more gradual course than the normal. At 21 days post-implantation (or repletion), when the "normal" sponge had already started to lose collagen, the "repleted" sponges were still incorporating it. The reasons for these phenomena are as yet unknown. Previous work reported by us on shorter periods of collagen growth, and by Gould, are somewhat at variance with these results. Our histological work, particularly, has suggested that the "repleted" sponges have more collagen than the "normal" after equal times on vitamin C. Again, the reasons for these discrepancies are unknown: The discrepancies between the methods extends to species differences. For example, chemical analysis shows about a twofold difference between rat and guinea pig sponge hydroxyproline for equal times of implantation; histological examination reveals no differences whatever.

Studies are now being planned which we hope will lead to an understanding of the true nature of these phenomena.

Although clinical experience and extensive experimental data have established beyond doubt that wound healing is impaired in scurvy, the pathogenesis remains obscure. Studies have been conducted in this laboratory for several years in young guinea pigs subjected to plastic (ivalon) sponge implants within the anterior abdominal wall. The reparative collagen, deposited therein, is evaluated biochemically and microscopically. The implications of two interesting observations during the past year are being explored: (1) The sponges of the scorbutic guinea pigs often seem to be floating in a pool of fluid blood with little or no clotting, suggesting some local anticoagulant influence. Furthermore, the marginal granulation tissue shows well defined metachromasia, microscopically, with the toluidine blue and other stains. Extraction of scorbutic sponges for heparinoid activity is being undertaken at present using the Monkhouse and Jaques techniques.

Studies of wound healing in germfree animals and investigations of the effect of age, peracetic acid, and growth promoting agents on wound healing are discussed in other Progress Reports

Summary and Conclusions: A comprehensive study of wound healing is underway, the aim of which is to elucidate the basic biochemistry, physical chemistry, and physiology of wound healing with particular reference to the nature of collagen and its relation to other wound components, the mechanism of collagen biosynthesis, and the effects of severe injury, (traumatic and radiation) on wound healing in animals and man. Our aim is to provide a sound theoretical basis for improved prophylaxis and treatment of severely injured patients.

List of Publications: 1. Sabatine, P. L., Rosen, H., Geever, E. F. and S. M. Levenson, Scurvy, Ascorbic Acid Concentration, and Collagen Formation in the Guinea Pig Eye, Arch. Ophthalmol., 65: 32-37, 1961.

2. Rosen, H., Kessler, A. and S. M. Levenson, Metabolic Inhomogeneity of Rat Tail Tendon Collagen: The Distribution of C^{14} in the Chromatographic Components, Arch. Biochem. Biophys., 90: 167-169, 1960.

Table I: Nitrogen Balance of Rats given H₂O and 15% D₂O

Day of Study	Nitrogen Balance (mg /day)	
	Control	D ₂ O
12-14	+28	+32
14-16	+36	+42
16-18	+28	+32
18-20	+29	+32
20-22	+19	+27
	Mean +28	+33
Operation		
22-24	-10	-8
24-26	-30	-20
26-28	-52	-42
28-30	-45	-48
30-32	-19	-48
32-34	+7	-40
34-36	+15	-43

Table II: Hydroxyproline of Whole Sponge (ug/100 mg Dry Implanted Sponge)

Day After Implant.*	Fed 2 mg Vit C/day		Fed No Vit C		Fed 2 mg Vit C/day After 14 days of no Vit C	
	D	C	D	C	D	C
	7	411	547	98	106	1238
14	1340	1375	331	118	1245	694
21	1440	1122			1249	858
28	1160	778				

Table III: Extractable Hydroxyproline (ug/100 mg dry implanted sponge)

7	48	49	57	14	62	37
14	58	51	26	6	48	17
21	29	11				
28	29	27				

D = Direct method. C = Chromatographic method

*In the case of the "repleted" animals, this is in addition to the first 14 days with no vit C.

Table IV: Extractable Hydroxyproline as per cent of total hydroxyproline (chromatographically analyzed)

Day After Implant.*	Fed 2 mg Vit C/day	Fed No Vit C	Fed 2 mg Vit C/day
			After 14 days of no Vit C
7	9.5	11.5	6.4
14	3.8	5.1	2.5
21	1.0		2.6
28	3.5		

ANNUAL PROGRESS REPORT

Project 6X60-09-001 Metabolism

Task 15 Metabolic effects of radiation and combined radiation and traumatic injury

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Department of Surgical Metabolism and Physiology,
Division of Surgery

Period Covered by Report: 1 Jul 60 - 30 Jun 61

Principal Investigators: Stanley M. Levenson, M. D., Maria L. LaConte, Maj,
ANC, Albert Einheber, Ph. D., Major Leo V. Crowley, MSC

Assistant: SFC Thomas J. Winkler

Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

ABSTRACT

Project No. 6X60-09-001 Metabolism

Task No. 15 Metabolic effects of radiation and combined radiation and traumatic injury

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Period Covered by Report: 1 Jul 60 - 30 Jun 61

Authors: S. M. Levenson, M. D., Maria L. LaConte, Maj, ANC, A. Einheber, Ph. D., L. V. Crowley, Maj, MSC, and SFC T. J. Winkler

Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

People purposefully or accidentally exposed to whole body irradiation are particularly susceptible to infection. This assumes special significance when such a patient has suffered other trauma also, operative or accidental. We are working on the design and development of plastic isolators for the effective isolation of such patients from the contaminated environment. These isolators are based on the principles established in the germfree laboratory. Other plastic isolators are being developed for operating in a sterile environment and for the isolation of patients with serious infections.

Studies of the mechanisms of the potentiation of traumatic and hemorrhagic shock by prior whole body x-irradiation are getting underway.

BODY OF REPORT

Project No. 6X60-09-001, Metabolism

Task No. 15 Metabolic effects of radiation and combined radiation and traumatic injury

Description: People purposefully or accidentally exposed to whole body irradiation are particularly susceptible to infection. This assumes special significance when such a patient has suffered other trauma also, operative or accidental. We are working on the design and development of plastic isolators for the effective isolation of such patients from the contaminated environment. These isolators are based on the principles established in the germfree laboratory. Other plastic isolators are being developed for operating in a sterile environment and for the isolation of patients with serious infections.

Studies of the mechanisms of the potentiation of traumatic and hemorrhagic shock by prior whole body x-irradiation are getting underway.

Progress: Plastic Isolators for Patient Care. Hospital-acquired infections have created urgent problems for patients, physicians and hospitals. These problems are ubiquitous on this planet and affect all hospital services. One of the areas of major concern is the problem of wound infection following clean elective surgery. Many of these infections apparently originate in the operating rooms despite the aseptic techniques practiced in contemporary hospitals. The causative agents can be found circulating in the air introduced from the respiratory tracts of the surgical team and patient, brought in unwittingly with the litter, on clothing or linen, or blown in through faulty or dirty air conditioning units. It is clear that complete surgical asepsis is not now attained in even our best operating rooms.

Experiments conducted in germfree animal laboratories show that it is possible to obtain and maintain total asepsis in surgery. Our studies to translate the techniques of the germfree laboratory to problems of patient care continue. The studies involve two isolation systems, the surgical isolator for operating room use and the patient isolator for ward use.

Surgical Isolator: The intent of the surgical isolator is to provide a controlled environment wherein surgery can be performed under truly aseptic conditions. Only the site of operation and necessary surgical equipment are in contact with the sterile interior. The surgeon, assistants and scrub nurse are in jacket-helmet combinations attached to the wall of the isolator. These jacket-helmet combinations are ventilated by an air supply independent of the air supply to the isolator. The isolator is inflated by air which is sterilized by passage through a fiberglas filter similar to the type used in germfree animal tanks. The undersurface of the isolator is adhered to the surgically prepared skin and the incision is made directly through the plastic material.

Considerable time was spent on developing a method of attaching gloves to the gown jacket sleeves so that a more efficient system for changing gloves without contamination during surgery could be accomplished. A fairly satisfactory technique has been devised but investigations will continue towards devising an even better method.

The prototypes of plastic isolators presently being tested are fabricated from heat-sensitive plastic materials requiring sterilization by chemical means. The peracetic acid spray method used successfully in the germfree laboratory and in the initial isolator experiments was considered impractical for hospital use because of the irritating fumes and the space and time factors involved. Sterilization with ethylene oxide is being investigated. Some of the facets are a standard procedure for folding and packing the isolator in order to: (1) obtain sterility, (2) prevent damage to the helmet's rigid face pieces, (3) attain a package small enough to fit the autoclave, (4) unfold and assemble the isolator for use without contamination.

The eight mil vinyl film which we had been using was considered too heavy to meet our requirements and an isolator of four mil vinyl film was made. The results were gratifying, leading us to believe that even thinner materials could be tried with the ultimate goal of constructing disposable units.

Various methods of attaching the plastic adhesive drapes (used to adhere the isolator to the patient) to the isolator prior to sterilization have been tried. Further study is needed not only to improve the method of attaching the drape to the surgical isolator initially but also to obtain a complete seal between the drape and patient's skin. Cultures taken of the wound inside during trials were positive only when the seal between the isolator and the skin was not intact causing the drape to roll back and exposing the skin edges.

Circulation of air to the areas of the patient not glued to the isolator but essentially under the plastic material is necessary to prevent overheating of the patient and unnecessary body fluid loss. The air circulation system for the patient is in the preliminary development stage. A simple and satisfactory air ventilation system for the surgeon and other operating team personnel who will be working in the jacket isolator has been developed.

A study to determine the effects of peracetic acid on wound healing was initiated. The results of the experiments thus far show no detrimental effect on the tissues or on the rate of the wound healing (See Progress Report 6X60-09-001 Metabolism, Task 6 Metabolic and nutritional problems associated with injury).

Elimination of electro-static hazards of the plastic materials, development of an adequate communication system and construction of an electronic glove leak detector to be used in conjunction with the surgical isolator are being studied in collaboration with the Medical Equipment Research Laboratory at Fort Totten, Flushing, New York.

Development of the surgical isolator has progressed to the stage where trial with patients will begin in June 1961 or shortly thereafter.

Patient Isolator for use on the Wards: With the advent of newer surgical techniques such as homografts and organ transplants, infection plays a most important role in the outcome of the procedure since some of the ancillary procedures, such as whole body irradiation, lower the resistance to infection. Sterile rooms in which the patients are protected from exogenous bacteria and elaborate procedures for the reduction of normal bacterial flora of the patient's body are coming into existence. These sterile rooms are elaborate and require an excessive number of personnel for staffing. We believe a plastic isolator will be more practical.

A plastic jacket isolator designed to fit around a regular hospital bed has just been delivered by Professor Trexler of the University of Notre Dame. Studies for establishment of procedures such as sterilization of equipment, decontamination of the patient, feeding, removal of waste materials, administration of medications, and normal patient care are underway. With the arrival of the patient isolator, an intensive program using a healthy volunteer will be initiated.

Through these studies we hope to establish not only a practical solution to the problem of protecting those patients highly susceptible to infections, but also a means of preventing cross-contamination where infection already exists, by effectively isolating patients with serious infections.

Effect of Whole Body Irradiation on Resistance to Shock: We have previously demonstrated that the "shock-resistance" of animals scalded (rats) or tourniqueted (mice and guinea pigs) 7 to 10 days after they have received acute low level whole-body x-irradiation is significantly reduced as judged by their greater 24 and 48 hour mortalities relative to concurrently studied non-irradiated scalded or tourniqueted animals. Scald, tourniquet, or hemorrhagic hypotension (swine) imposed before or after the 7 to 10 day period post-irradiation did not reveal an altered "shock-resistance" of the irradiated animals. McKenna and Zweifach reported similar findings regarding drum shock of rats. We have extended and corroborated the above findings in x-irradiated-scalded rats and mice.

It is now clear that low lethal whole-body x-irradiation sensitizes rodents to early death from various forms of trauma inflicted between the first and second week post-irradiation. The mechanisms behind this reduction in "shock-resistance" are presently unknown and warrant further investigation. The important role of carbohydrate metabolism in bioenergetics and in shock, and the reported (Sestan, N., N. Allegretti, M. Matosic and M. Devcic: Effects of sublethal whole-body x-irradiation on glucose tolerance in the rat and the guinea pig. Radiation Res. 13:25, 1960) impairment of glucose utilization (glucose tolerance) on the eighth post-irradiation (500 r) day (rat), leads us to suggest that work in this area may provide information about the mechanisms underlying the phenomenon we have observed.

Summary and Conclusions: People purposefully or accidentally exposed to whole body irradiation are particularly susceptible to infection. This assumes special significance when such a patient has suffered other trauma also, operative or accidental. We are working on the design and development of plastic isolators for the effective isolation of such patients from the contaminated environment. These isolators are based on the principles established in the germfree laboratory. Other plastic isolators are being developed for operating in a sterile environment and for the isolation of patients with serious infections.

Studies of the mechanisms of the potentiation of traumatic and hemorrhagic shock by prior whole body x-irradiation are getting underway.

Publications:

1. Levenson, S. M., P. C. Trexler, O. J. Malm, R. E. Horowitz and W. H. Moncrief, Jr.: A disposable plastic isolator for operating in a sterile environment. Surgical Forum 11:306, 1960.
2. Levenson, S. M., A. Einheber, L. V. Crowley: Some effects of whole body x-irradiation and thermal injury. Proc. First International Congress on Research in Burns. (In press)

ANNUAL PROGRESS REPORT

Project 6X60-09-001, Metabolism

Task 19, Physiology of Cell Growth and Regeneration

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Department of Surgical Metabolism and Physiology
Department of Experimental Surgery
Division of Surgery

Period Covered by Report: 1 July 1960 through 30 June 1961

Principal Investigators: Andre D. Glinos, M.D.
Capt David Arbiter, MC
Capt George J. M. Slawikowski, MC

Assistant: Pvt Robert J. Werrlein, B.S.

Reports Control Symbol: MEDDH-288

Security Classification: Unclassified

ABSTRACT

Project No. 6X60-09-001 Title: Metabolism
Task No. 19 Title: Physiology of Cell Growth and Regeneration
Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Period Covered by Report: 1 July 1960 through 30 June 1961

Authors: Andre D. Glinos, M.D.; Capt David Arbiter, MC; Capt George J. M. Slawikowski, MC; Pvt Robert J. Werrlein, B.S.

Reports Control Symbol: MEDDH-288

Security Classification: Unclassified

Metabolic Role of Plasma Proteins in Post-injury Tissue Regeneration.
The objective of this task is the investigation of the mechanisms controlling the basic processes of cell growth and regeneration by means of which is effected the repair and healing of lesions produced by trauma, burn, irradiation and other combat-generated conditions. Using liver as the specific experimental subject, a series of important discoveries were made over the last several years representing a major breakthrough in the field of growth control physiology (Glinos, A. D.: The Mechanism of Liver Growth and Regeneration. In: The Chemical Basis of Development, Johns Hopkins Press 1958).

During the period covered by this report this work was extended to the growth responses of another key mammalian organ, the kidney. Following the same experimental approach as before with the liver, the development of methods for the isolation, survival and multiplication in vitro, of rat kidney tubular epithelium was undertaken. Problems related to cell isolation and survival have been resolved and quantitative methods for obtaining stationary replicate cultures of kidney epithelium with a high degree of reproducibility have been developed. Current work deals with the problems of the initiation of active cell division in such cultures.

Tumor Development after Surgical Stress. Development of Walker tumor was studied after surgical stress in rats previously subjected to various endocrine ablations. Surgical stress in normal rats consistently caused an increase in tumor takes, not prevented by adrenalectomy but prevented when cortisone was given 6 days before and 7 days after operation. Hypophysectomy decreased takes in non-operated rats, but aggravated stress: after surgery takes were the same in hypophysectomized and normal rats. Hypophysectomy and adrenalectomy decreased tumor growth. Without surgical stress takes and tumor growth were nearly identical in oophorectomized, orchidectomized, and normal male rats; they were equally increased after stress. In non-operated normal males takes were higher than in females, but tumor growth was the same. Normal females showed increased takes but no change in tumor growth after surgical stress. No effect on tumors was noted in unstressed thyroidectomized rats. After stress the increase in takes was greater and tumor growth was less in thyroidectomized than in normal animals. These data indicate that endocrine ablations do not prevent the increase in tumor takes seen after surgical stress.

BODY OF REPORT

Project No. 6X60-09-001 Title: Metabolism

Task No. 19 Title: Physiology of Cell Growth and Regeneration.

Description:

Metabolic Role of Plasma Proteins in Post-injury Tissue Regeneration.

It is common knowledge that regardless of the various factors causing tissue injury, the repair of all such injury in the mammalian organism is effected by cell growth and regeneration. Yet the basic mechanisms controlling cell growth remain largely unknown despite the urgent clinical need to interfere at will with these mechanisms in order to stimulate post-injury regeneration or to inhibit the growth of tumors.

Our work in this area over the last several years has resulted in the description of a system of humoral control for the growth and regeneration of the liver. (Glinos, A. D., *The Mechanism of Liver Growth and Regeneration*. In: *The Chemical Basis of Development*, Johns Hopkins Press, 1958). During the current period we sought to extend this work to the growth responses of another key organ, the kidney. Contrary to earlier beliefs, our previous work (Glinos, A. D., and Campbell, A. M., *Anat. Rec.*, 24: 11, 1956) as well as the work of others (Rollanson, H. D., *Anat. Rec.*, 104: 263, 1949; Ogawa, K. and Sinclair, J. G., *Texas Reports on Biology and Medicine*, 16: 215, 1958) has shown that the response of the kidney to unilateral nephrectomy or to the loss of tissue from pathological processes, is not limited to cell hypertrophy but involves cell division as well, especially insofar as the tubular epithelium is concerned.

As with the previous work with the liver, the detection of humoral factors controlling these growth processes involves a comparative study of the effects of sera from normal animals and animals undergoing compensatory kidney growth, on kidney cultures *in vitro*. Using our early liver culture work (Glinos, A. D., and Gey, G. O., *Canc. Res.*, 12: 265, 1952) as a springboard, Ogawa and Nowinski (Ogawa, K. and Nowinski, W. W., *Proc. Soc. Exp. Biol. Med.*, 99: 350, 1958) made such a study. Using the mitotic index of primary kidney explants as an indicator of cell division they reported increased cell growth with the sera from unilaterally nephrectomized rats. Increased numbers of mitoses can be a useful indicator of growth only when the duration of mitosis or the kinetics of the entire cell population are known. Information concerning these two other parameters is of primary importance since arrest or slowing down of the mitotic process will also result in increased numbers of mitoses as for example, in the case of colchicine, a powerful cell poison. In the case of sera from rats with nephrectomy or with kidney injury, the presence of toxic metabolic end products is a very real possibility and renders the value of simple mitotic counts for estimating cell growth very questionable, indeed.

Primary kidney explants on plasma clot, as used by Ogawa and Nowinski do not allow cell population studies and consequently our main effort during

the period covered by this report was directed toward the development of quantitative methods for the isolation, survival, and multiplication in vitro; of monodisperse populations of rat kidney tubular epithelium cells.

Tumor Development after Surgical Stress. This is the final report on this phase, covering the period 1 August 1959 through 30 June 1961. The objective of this phase was to complete the series of studies begun by the author at the University of Illinois, aimed at elucidating the role of various endocrine organs in the enhanced tumor development shown to occur in experimental animals after surgical stress.

The major portion of the research performed under this phase was described in the progress report of 30 June 1960 and is covered in the publications cited at the end of this report.

Since the report of 30 June 1960 three immunological experiments were carried out. In one, tumor antigen in the form of 25,000 non-viable tumor cells was injected six weeks before operation and challenge with 25,000 viable cells; the small immunizing dose used had no apparent effect. In the second, 500,000 dead cells served as antigen; here a trend towards higher incidence of takes was observed in the "immunized" rats after operation. In the third, a standard immunizing procedure was followed: four consecutive daily injections of 500,000 dead cells, a booster dose after three weeks, and, five days later, operation plus the challenge dose of 25,000 viable tumor cells. In this experiment, the trend towards increased takes in the "immunized" animals was more definite, and the latent period shorter. Similar tumor enhancement had previously been reported with other tumors. Thus, operation and pre-inoculation with tumor antigen seemed to act synergistically.

TUMOR DEVELOPMENT AFTER SURGICAL STRESS IN RATS PRE-INOCULATED WITH TUMOR ANTIGEN

	DORSAL CELIOTOMY		NO OPERATION (ANESTHESIA ONLY)	
	Number of Rats	Tumor Takes	Number of Rats	Tumor Takes
Inoculation with 25,000 Dead Tumor Cells	38	55.3%	42	30.9%
No Inoculation	39	56.4%	40	32.5%
Inoculation with 500,000 Dead Tumor Cells	30	43.3%	34	29.4%
No Inoculation	32	25.0%	41	17.1%
Multiple Inoculations with Dead Tumor Cells				
Male	27	25.9%	36	2.8%
Female	54	14.8%	50	4.0%
Male and Female	81	18.5%	86	3.0%
No Inoculations				
Male	33	12.1%	36	5.6%
Female	35	8.3%	36	5.6%
Male and Female	68	10.0%	72	5.6%

Some cytopathologic studies on the Walker tumor were carried out in an attempt to correlate the effect of various endocrine ablations (seen in terms of takes or growth of tumors) with changes in the cytology and/or pathology of the tumors. Much variation was observed from tumor to tumor, but this variation was also seen within each tumor so that no clear-cut qualitative or quantitative relationships could positively be established.

Progress:

Metabolic Role of Plasma Proteins in Post-injury Tissue Regeneration.

I. Isolation of renal cells from the rat. In recent years mono-layer epithelial cell cultures from the mammalian kidney have been used extensively in virology. The most common donors have been the monkey and the rabbit. Since an extensive literature survey failed to reveal any data on rat kidney a series of experiments was undertaken aiming at defining the optimal experimental conditions for the isolation of monodisperse cell suspensions from the rat kidney. Using a trypsin concentration of 0.25-0.50% in a Ca and Mg free solution, it was found that the outcome of the isolation procedure is determined by the age of the donor animals and the duration of the trypsinization procedures.

With respect to the age of the donor animals, it was found that trypsinization of kidneys from 3-6 weeks old male rats resulted in liberation of morphologically intact epithelial cells with an efficiency coefficient of approximately 13×10^6 cells/single kidney, whereas identical trypsinization of kidneys from male rats 3-6 months of age resulted in complete cell destruction. These results indicate a remarkable increase of cellular fragility with age, in the renal tissues of the rat, and led to the adoption of 3-6 weeks old male rats as the standard donors for all subsequent experiments.

Effects of the duration of the trypsinization procedures were investigated in a series of experiments where the progress of the dispersion of the renal tissues was monitored by continuous microscopic observation. A number of schemes different with respect to the volumes and concentrations of the trypsin solutions used, the frequency of changes of the trypsin solutions and the length of trypsinizations at room temperature with continuous stirring, and at 37° without stirring, singly and in combination, were thus assayed.

It was found that at room temperature, and with continuous stirring, 2 five-minute trypsinization cycles were necessary in order to loosen up the stroma and release trapped blood cells not removed by the previous washings. The events following this preliminary trypsinization fall into two categories, first the progressive release of formed elements from the renal stroma and second the gradual disintegration of these elements into single cells. Formed elements such as tubules and glomeruli are being released from the stroma for a period of about 80 minutes with a peak yield at the 20'-30' interval. Whereas the total yield is progressively decreasing after this time, the tissue becoming depleted, the degree of dispersion of the released elements increases, with small fragments of tubules, clumps of cells, and single cells replacing the larger structures released at earlier stages.

Maximum efficiency in the release of these formed elements from the renal stroma was obtained when the trypsin solution was changed at intervals of 20 minutes, the whole 80-minute process thus being broken down into four distinct cycles. Dispersion of the formed elements into single cells was progressively more extensive in the four suspensions thus obtained but never complete. Additional incubation for 20' of these suspensions allowed tryptic dispersion to continue until only single cells and a few small cell clumps were obtained. The coefficient of efficiency for isolation of single cells with this procedure varied from 1.1×10^6 cells/kidney for the lighter rats to 5.25×10^6 cells/kidney for the heavier rats in the 3 to 6 weeks age group.

II. Survival of rat kidney cells. Renal cells obtained as previously described, appear microscopically as markedly contracted opaque spherical bodies. Since evaluation of the percentage of cells surviving the trypsin treatment was impossible under these conditions, suspensions of such cells were inoculated into culture flasks, and survival estimated after incubation of the flasks for 3 days at 37° . The qualitative evaluation of survival was based on microscopic visualization of cell attachment on the glass surface of the culture flasks, flattening and clearing of the cell cytoplasm, and formation of cell colonies. The quantitative evaluation of survival was based on Coulter Counter electronic cell counts after removal of the non-attached non-viable cells and citric acid fixation and re-suspension of the cells of the colonies. The results of three survival evaluation experiments are summarized in Table I, where it can be seen that the percentage of surviving cells varies between 15 and 30%. With widely different media and inoculum sizes (Experiments 1 and 2 on one hand, and 3 on the other) the percentage of surviving cells did not vary significantly. This is interpreted to indicate that survival is a function of the trypsin treatment rather than of the ensuing cultural conditions.

III. Cell population kinetics. Qualitatively cell population kinetics of renal cells surviving as described above are as follows: During the first three days the surviving fraction of the highly contracted spherical cells of the inoculated suspension aggregates in clumps of various sizes which become attached to the glass surface of the culture flasks. These attached cell clumps become the centers of an orderly peripheral outgrowth of large flat clear polygonal or round cells with smooth borders thus constituting typical epithelial type cell colonies. During the next three days of culture there is a progressive increase of the size of these epithelial type colonies.

Up to this point the sequence of events described has been seen with all types of culture media used. In contradistinction, events beyond this point are strongly influenced by different media. Thus in balanced saline media supplemented with lactalbumin hydrolysate and fetal bovine serum the typical morphology of these epithelial type colonies is maintained up to the eleventh day, the longest time interval that these cultures have been maintained. In the same balanced saline, or in synthetic media, supplemented with horse or rat serum from the 6th day of culture onward, a progressive transformation of the morphology of cells toward the contracted spindle or

TABLE I
Survival of Isolated Rat Renal Cells

Exp. No.	Incubation Medium*	Inoculum Cell No. x 10 ³	Cell Colony Formation	Final Cell No. x 10 ³	% Cells Surviving
1	Horse serum 30% Synthetic Med-NCTC 109 70%	465	++	78	17%
2	Same as above	378	++	109	29%
3	Calf serum 2% Lactalbumin Hydrolysate 0.5% Hank's BSS 97.5%	150	++	35	23%

*All media contained 100 u. Penicillin + 100 Streptomycin/ml.

TABLE II
Population Kinetics of Rat Renal Cells in Culture

Exp. No.	Culture Medium*	Initial No. of Cells x 10 ³	Cell No. x 10 ³ after days		
			2	4	8
1	a. Horse S. 30%-NCTC(109)70%	107		96	
	b. Rat S. 20%-Hank's 80%	107		118	
	c. Rat S. 40%-Hank's 60%	107		105	
2	a. Horse S. 30%-NCTC 109 70%	78	100		
	b. Rat S. 20% - " 80%	78	69		
	c. Rat S. 20-Hank's 80%	78	92		
3	(Calf S. 2% - Fetal Bovine S. 18% Lactalbumin Hydrolysate 5% (Hank's BSS 79.5%	35	53	54	52
4	a. (Unilat. Nephrectomized (Rat S. 20% - 80% Hank's BSS	109	118	72	
	b. (Normal Rat S. 20% (Hank's BSS 80%	109	121	128	
	c. (Normal Rat S. 20% (Chick Embryo Extr. 10% (Hank's BSS 70%	109	124	99	

*All media contained 100 u. Penicillin + 100 Streptomycin/ml.

stellate form characteristic of fibroblast type cells takes place. In such media at about the 9th day the fibroblastic cell type dominates in cultures almost completely. This sequence of events indicates either a morphological modulation of tubular epithelial cells or a selective survival of the fibroblastic elements of the original cell suspension. Further work is necessary in order to decide between these two alternatives.

Quantitatively, cell population kinetics of these renal cell cultures are summarized in experiments 1, 2 and 3 of Table II. In these experiments the initial number of cells refers to the number of surviving cells, determined as described previously, except that in Experiment No. 1 the determination was performed on the 7th instead of the 3rd day of incubation. A variety of media was used but in no case was there any significant increase in the cell population thus indicating that these renal cell cultures were stationary.

At this point it was considered of interest to investigate the possible effects of sera obtained from rats 48 hours after unilateral nephrectomy on such cultures. This time interval was chosen because it was shown by previous investigations to correspond to the maximum growth response of the remaining kidney, both *in vivo* (Rollanson, H. D., *Anat. Rec.*, 104: 263, 1949) and *in vitro* (Glinos, A. D., and Campbell, A. M., *Anat. Rec.*, 24: 11, 1956). Experiment No. 4 of Table II shows that such sera failed to initiate cell division in the stationary renal cell cultures described.

Current work deals with the problems of initiating cell division in such cultures by other means thereby providing a far more adequate system for the investigation of humoral factors controlling kidney growth.

Summary and Conclusions:

Metabolic Role of Plasma Proteins in Post-injury Tissue Regeneration.
The objective of this task is the investigation of the mechanisms controlling the basic processes of cell growth and regeneration by means of which is effected the repair and healing of lesions produced by trauma, burn, irradiation and other combat-generated conditions. Using liver as the specific experimental subject, a series of important discoveries were made over the last several years representing a major breakthrough in the field of growth control physiology (Glinos, A. D.: *The Mechanism of Liver Growth and Regeneration*. In: *The Chemical Basis of Development*, Johns Hopkins Press, 1958).

During the period covered by this report this work was extended to the growth responses of another key mammalian organ, the kidney. Following the same experimental approach as before with the liver, the development of methods for the isolation, survival, and multiplication *in vitro*, of rat kidney tubular epithelium was undertaken. Problems related to cell isolation and survival have been resolved and quantitative methods for obtaining stationary replicate cultures of kidney epithelium with a high degree of reproducibility have been developed. Current work deals with the problems of the initiation of active cell division in such cultures.

Tumor Development after Surgical Stress. Development of Walker tumor was studied after surgical stress (dorsal celiotomy, i.e., sham-adrenalectomy) in rats previously subjected to various endocrine ablations. Surgical stress consistently increased the takes of the Walker tumor. The overall incidence of tumors was 50.8 percent in 626 celiotomized rats, and only 32.3 percent in 751 rats anesthetized but not operated upon. Adrenalectomy failed to prevent this increase in takes. Other endocrine ablations (hypophysectomy, hypothalamic lesions, orchidectomy, oophorectomy, thyroidectomy) likewise failed to prevent the increase of tumor takes after celiotomy; rather, such endocrine derangements tended to aggravate the deleterious effect of celiotomy on host resistance to implanted cancer cells.

When the gonads were removed 4 weeks before celiotomy (both operations being made through the same incisions) the following observations were made: there was a remarkably close correspondence of data for both takes and growth between oophorectomized, orchidectomized and normal male rats -- as contrasted with normal females. Celiotomy increased tumor takes throughout; but previous oophorectomy caused an increase of both tumor takes and tumor growth after celiotomy as compared to takes and growth in normal celiotomized females. This suggests that the presence of the ovaries may exert an inhibitory effect on the Walker tumor. It should be emphasized that in all the other experiments female rats have been used.

When the thyroid gland was removed 4 weeks before celiotomy, thyroidectomy had no effect on tumor takes in rats not subsequently subjected to celiotomy, but it aggravated the effect of celiotomy. On the other hand, tumor growth was reduced by thyroidectomy, significantly so in the animals having celiotomy. Thyroid function was found to be lowered during the first 48 hours after sham-adrenalectomy (I-131 uptake PBI studies). Cortisone seemed to prevent this increase in takes, but clear interpretation of this finding must await further experimentation since the direct and indirect effects of this hormone on tumors is very complex. Chlorpromazine given three hours before operation increased tumor takes in non-operated rats; in operated rats there was no effect. Electrolytic hypothalamic lesions made ten weeks before surgical stress and interfering with hypothalamic-hypophyseal-adrenal response to stress increased the effect of stress on takes.

There was some indication from immunological studies that surgical stress might enhance tumor takes partly through immunological mechanisms.

Some cytopathological studies on the Walker tumor were also performed but were inconclusive.

Since the Walker tumor is a transplanted tumor, the usual caution must be exercised in translating any data here presented into generalizations applicable to spontaneous human tumors.

List of Publications:

1. Glinos, A. D.: Environmental Feedback Control of Cell Division. Ann. N. Y. Acad. Sci., 90: 592-602, Oct, 7, 1960.
2. Slawikowski, G. J. M.: Tumor Development in Adrenalectomized Rats Given Inoculations of Aged Tumor Cells after Surgical Stress. Cancer Research, 20: 316-320, 1960.
(Abstracted in: Cancer Chemotherapy Abstracts, Vol. 1, No. 4, P. 243, 1960.
Excerpta Medica, Section XVI, Cancer, Abstract No. 4039, P. 1077, July 1960.
Biological Abstracts, Vol. 35, No. 22, Abstract No. 63134, 15 Nov 1960.)
3. Slawikowski, G. J. M.: Enhanced Tumor Development after Surgical Stress: Effect of Ablation or Inhibition of Hypophysis and Hypothalamus. (Promotion del Disvelloppamento Tumoric Post Stresses Chirurgic: Effecto de Ablation o Inhibition de Hypophyse e Hypothalamo). Presented at the First International Congress of Endocrinology, Copenhagen, Denmark, 20 July 1960. Published in Proceedings of the Congress (Suppl. of Acta Endocrinologica), pp. 1323-1324, 1960.
4. Slawikowski, G. J. M.: Endocrinologic Phases of Tumor Take and Tumor Growth as Related to Surgical Stress. Ph.D. Thesis, Univ. of Illinois, 20 Dec 1960. Thesis microfilmed by Univ. Microfilms, Ann Arbor, Mich. Abstract to be published in Dissertation Abstracts 1961.
5. Slawikowski, G. J. M.: Stress and Cancer. Presented by invitation to the Ontario Cancer Foundation, Univ. of Western Ontario, London, Canada, 17 March 1961.
6. Slawikowski, G. J. M.: Effect of Endocrine Ablations on Tumor Take and Tumor Growth after Subsequent Surgical Stress.
(a) Presented to the Amer. Assoc. Cancer Res., Atlantic City, N.J., 9 April 1961.
(b) Abstract published in Proc. Amer. Assoc. Cancer Res., 3: 269, 1961.
(c) Full paper submitted for publication in Cancer Research.
7. Slawikowski, G. J. M.: Reduced Thyroid Function after Celiotomy in Rats: I^{131} Uptake and Protein-bound Iodine Studies. To be submitted for publication in Endocrinology.
8. Slawikowski, G. J. M. and Cole, W. H.: Stress and Hormonal Factors in Cancer — A Review. Submitted for publication in Cancer Research.
9. Data also cited in: Warren H. Cole, et al., "Dissemination of Cancer: Preventive Measures". In Press. Appleton Century Croft, 1961).

ANNUAL PROGRESS REPORT

Project 6X60-09-001, Metabolism

Task 23, Methods for the Chemical and Radiochemical Analysis of Foods

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Department of Veterinary Chemistry
Division of Veterinary Medicine

Period Covered by Report: 1 July 1960 through 30 June 1961

Principal Investigators: E. S. Windham, M. S., and Flo H. Ward, B.S.

Reports Control Symbol: MEDDH-288

Security Classification: Unclassified

ABSTRACT

Project No. 6X60-09-001

Title: Metabolism

Task No. 23

Title: Methods for the Chemical and Radiochemical Analysis of Foods

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.
Department of Veterinary Chemistry
Division of Veterinary Medicine

Period Covered by Report: 1 July 1960 through 30 June 1961

Authors: E. S. Windham, M. S., and Flo H. Ward, B. S.

Reports Control Symbol: MEDDH-288

Security Classification: Unclassified

1. A method for the extraction of benzoates from margarine was developed. Continued work on methods for tocopherol led to slight improvements in sensitivity of the procedure. A method was developed for separation and identification of added antioxidants in dairy products. A method for removing peroxide from ether was developed.

2. Investigations have been continued in the development and evaluation of suitable methods for the detection of radioactive contaminants in foods. Efforts to standardize the application of methods for the identification of P^{32} and I^{131} in aqueous solutions within the Army Medical Laboratories are underway. A specific anion exchange resin to concentrate zinc ions in foodstuffs shows promise. Studies are in progress to perfect a method for the detection and identification of alpha emitters in environmental media.

BODY OF REPORT

Project No. 6X60-09-001 Title: Metabolism

Task No. 23 Title: Methods for the Chemical and Radiochemical Analysis of Foods

Description

1. Research is conducted on chemical analysis of foods to assure that reliable and safe methods for the testing of subsistence are available to the various Army Medical Laboratories. Existing methods are evaluated. New methods are developed and old methods are modified to meet new needs or correct deficiencies in existing methods. Methods developed are distributed to the Army Laboratories and other interested Armed Forces activities.

2. The need for simple, reliable, accurate, and reproducible methods for determining the identity and concentrations of radioactive contaminants in foodstuffs continues to gain in importance with the expansion in number of power and experimental reactors, the increase in concentration of induced radionuclides as by-products of large reactor operations, the dispersion of nuclear devices throughout the defense system, and the continuous build-up of fallout by-products such as Sr^{90} and Cs^{137} . This need is particularly emphasized in the appraisal of the results of reactor and weapons accidents. The radionuclides of prime importance of long-term residual effects (fallout) continue to be Sr^{90} and Cs^{137} . Other nuclides such as Zn^{65} , P^{32} , I^{131} , or certain alpha emitters may assume significance under special circumstances.

Progress

1. a. A method for the quantitative extraction of benzoates from margarine was developed. The method referenced in the specification was unsatisfactory for general use. The method approved by the Association of Official Agricultural Chemists (AOAC) is good for aqueous products, but is not suitable for high fat products such as butter and margarine. By modification of the alkaline aqueous extraction, a procedure that was fairly complete and reproducible in recovering added benzoates from butter or margarine was developed. In this procedure, the sample is placed in a separatory funnel, made alkaline to phenolphthalein and extracted with 12 successive 15-ml. portions of 0.01 N NaOH. The extract is deproteinized by the addition of 15 ml. of 10% zinc sulfate, made alkaline, diluted to 250 ml., and filtered. Using the AOAC method, a 200-ml. aliquot of this filtrate is used for benzoic acid determination.

b. Current investigations of methods for determining tocopherol have not led to any significant improvement in the accuracy or reproducibility of the current method. Minor improvements resulted when the order of addition of the bipyridine and ferric chloride reagents was changed and the color development time was increased from 6 to 15 minutes. The use of Devlin and Mattil's reagent (bipyridine and ferric chloride combined in glacial acetic acid) produced a much lower reagent blank. However, no definite improvement in tocopherol recovery could be made. Some trials favored the new procedure and some favored the previously reported method. Attempts to substitute the stable tocopherol acetate for the unstable tocopherol as the primary standard have not been successful. Although tocopherol determination recoveries vary, this does not prevent the determination of tocopherol as a means to detect significant adulteration of milk fat with vegetable fats.

c. Methods were also developed for detecting foreign animal fats in milk fat by the determination of antioxidants, which are a standard ingredient of animal fats. Usual methods using ammonia, alcohol, ether, and petroleum ether for the extraction of fat either destroy or remove antioxidants. To overcome this deficiency, a detergent separation was developed. To extract the fat in ice cream, 150 g. of melted sample is treated with 100 ml. of a reagent made of 7% sodium tetraphosphate, 3% Triton X 100, and 2% sodium bicarbonate. This sample-reagent mixture is placed in boiling water bath until the fat separates. The separated fat is thoroughly washed with hot water, dissolved in petroleum ether, dehydrated with sodium sulfate, filtered and dried. The AOAC tests for butylated hydroxyanisole and butylated hydroxytoluene are applied to the fat. Since antioxidants are not permitted in dairy products, their presence indicates that the products tested probably contain foreign animal fat.

orig.
12 (1)
10 30
10
1000
no further
sic of sin.
but only
4000 2
1015 11
100

d. The ether used in fat extractions often contains peroxides. These peroxides, when concentrated in ether extraction procedures, may be explosive. Peroxides also react with the fat and produce erroneous high fat analyses. Preliminary studies were undertaken to develop a simple method of removing peroxides from ether. A chromatographic method involving the passage of ether through a column of potassium permanganate overlaying a column of Merck chromatographic alumina not only removed the peroxide, but also a colored nonvolatile material present in the ether. Though no heating occurred on the columns and no incident to indicate danger has shown up in the trials, the reaction products may be explosive. Since the chemistry involved has not yet been elucidated, the method cannot be recommended until further study is completed.

2. a. Further efforts were directed to the standardization of analytical methods for the assay of I^{131} and P^{32} by the various Army Medical Laboratories. To this end, an Army Medical Laboratory

evaluation program with samples of I^{131} and P^{32} was initiated. To keep this program as simple as possible, the first samples were made up in aqueous solutions. After proficiency is demonstrated with the aqueous solutions, samples of contaminated food-stuffs will be utilized.

b. The first samples were sent with a step-by-step procedure instruction sheet. The procedure did not provide guidelines for calculations, for data collection, or systematic reporting of each step. The analyst was requested to report the identity of the radionuclide and its activity at a specified time.

c. The results were so varied that it was obvious that more detailed instructions, standardization of equipment, and systematic reporting of intermediate steps were necessary. A form, outlining the performance of each step in detail, was devised to provide a means for the control laboratory to better assist the Army Medical Laboratories. This form allows the reviewer to make a more thorough evaluation of the analytical procedures and determine where errors had occurred if the results were not in order.

d. Throughout this year, four evaluation samples have been submitted (to the Army Medical Laboratories). The results of these studies indicate the proficiency in analyzing for the presence of I^{131} and P^{32} in aqueous solutions is not sufficient to justify progressing to the more complex problem of analyzing food-stuffs for possible radioactive contamination at this time.

e. Studies of the concentration of zinc ions on two exchange resins (Dowex 1, (200 mesh) and Amberlite IRA 400) were continued. Columns 20 cm. long and approximately 0.03 square cm. in cross section, made of Pyrex tubing, plugged with glass wool, were filled with the pretreated chloride form of the respective resins. Solutions prepared from food ashes containing 5×10^{-3} microcuries, 2.5×10^{-3} microcuries, 1.25×10^{-3} microcuries, and 6.2×10^{-4} microcuries, respectively, were passed through the columns. Once the test solutions seeped the columns, an appropriate hydrochloric acid eluent was added, the eluate was collected, concentrated, and its radioactivity determined in a scintillation well counter having an efficiency of 41% for Zn^{65} . To confirm that the observed radioactivity was attributable to the gamma ray energy of Zn^{65} , a series of pulse height analyses was performed on a combined aliquot of the respective activities from each resin. The experimental results of the concentration of zinc ions on the exchange resin indicate that more consistent and efficient extraction can be accomplished with Dowex 1 than with IRA 400. Further, if the concentration can be adjusted to approximately 2.5×10^{-3} microcuries per sample, more efficient operation can be achieved. Since only two resins were examined in detail, it is obvious that additional studies are necessary before final resin selection is made and methods published.

f. A critical comparison and experimental evaluation of chemical and physical procedures for the detection and identification of alpha contamination in environmental media revealed the

following two methods that may be useful: (1) Method of Schubert of Argonne National Laboratory, and (2) Method of Kennedy, KAPL - A-H-P-3 UC-41 TID 4500, 13th edition. These are presently under investigation.

Summary and Conclusions

1. A method for the extraction of benzoates from margarine was developed. Continued work on methods for tocopherol led to slight improvements in sensitivity of the procedure. A method was developed for separation and identification of added anti-oxidants in dairy products. A method for removing peroxide from ether was developed.

2. a. Investigations have been pursued in the development and evaluation of suitable improved methods for the detection of radioactive contaminants in foods. Special attention was given to methods for the detection, identification and quantification of radioactive phosphorus and iodine in aqueous solutions and of radioactive zinc in foods.

b. Studies are being continued to perfect a method for the detection and identification of alpha emitters in environmental media. Greater proficiency within the AMEDS laboratory system in analyzing for P^{32} and I^{131} in aqueous solutions is necessary before proceeding to the more complex problem of their analysis in food-stuffs. Additional studies are necessary on other resins for the concentration and removal of zinc ions before a method for the analysis of Zn^{65} in foods can be formalized and distributed to the Army Medical Laboratories.

ANNUAL PROGRESS REPORT

Project: 6X60-09-001, Metabolism

Task: 24, Investigation and Analytical Determination of Drugs and Compounds of Toxicological Importance

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Department of Biochemistry

Period Covered by Report: 1 July 1960 through 30 June 1961

Principal Investigators: Lt Col Edward C. Knoblock
Mr. Leo Kazyak

Assistant: Pfc Joe Colony

Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

ABSTRACT

Project No: 6X60-09-001

Title: Metabolism

Task No: 24

Title: Investigation and Analytical Determination of Drugs and Compounds of Toxicological Importance

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Period Covered by Report: 1 July 1960 through 30 June 1961

Authors: Lt Col Edward C. Knoblock and Mr. Leo Kazyak

Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

The techniques of infrared and ultraviolet spectroscopy, were used in investigation of adequate methodology for detection of Librium, Artane, Danilone and Octin in biological specimens. These drugs were brought to the attention of this laboratory through incidents of suspected overdose. Pertinent details of the Librium determination are discussed.

The spectrofluorometric characteristics of digitoxin, digitoxigenin, and digoxin were studied in relation to analysis of blood specimens for digitalis. The technique was found to lack the necessary sensitivity for clinical studies of blood levels of these substances.

Investigations are being conducted to include the analysis of alkaloids and other similar organic compounds in the program of vapor chromatography which has proven very useful in analysis of volatile compounds. Polarography has been utilized with good success in cation analysis.

BODY OF REPORT

Project No: 6X60-09-001 .

Title: Metabolism

Task No: 24

Title: Investigation and Analytical Determination of Drugs and Compounds of Toxicological Importance

Description: A trend toward increased complexity of toxicology cases due to the many new drugs marketed each year, has required maximum use of the newer techniques for analytical procedures in order to provide adequate and timely consultation services in toxicology to the various agencies submitting specimens for analysis. In keeping with the expressed purpose of this function, all new drugs and compounds of toxicological interest which come to the attention of the toxicological laboratory are screened for ultraviolet, infrared, and fluorescent spectral characteristics. Periodically, this information is compiled and distributed to the US Army Area Medical Laboratories and those government facilities engaged in toxicological analyses, e. g. AFIP, FBI, and Naval Medical School. Whenever quantitative identification procedures for certain of these compounds are unavailable, research and development efforts are directed toward this end.

The technique of polarography has found definite applications to cation analysis and has been utilized in a number of instances for blood lead determinations and in selenium and thallium intoxication. Specificity of analysis is improved by this technique

Progress: 1. New Drugs

Suspected drug overdose prompted the detailed study of Librium (7-Chloro-2-methylamino-5-phenyl-3H-1,4 benzodiazepine 4-oxide). This compound exhibited an ultraviolet spectra with maximum absorbency in dilute acid at 245 mu ($E_{1\%}^{1\text{cm.}} = 1000$). It was also found that the compound could be excited with ultraviolet light at 375 mu with the resultant fluorescence measurable at 480 mu. Librium was recovered unchanged from a blood specimen in concentration of 1.5 mg-%. The procedure is simple since it involves only an extraction of the blood with chloroform and re-extraction of the chloroform with 0.5 N sulfuric acid. With a single extraction, recovery of the drug from the blood was 81%.

Although phenandione (Danilone) may be identified by a characteristic spectrum no intact phenandione was recovered from biological specimens submitted to this laboratory. Attempts to identify a metabolite excreted in urine suggested separation of a compound

having a similar ultraviolet absorption spectra to that of the parent compound; however, this work is not fully confirmed.

Octin (methylisooctenylamine) and Artane (α -Cyclohexyl- α -phenyl-1-piperidinepropanol hydrochloride) have come to the attention of this laboratory within the past year. Analytical procedures in the literature were lacking; therefore, infrared spectroscopy was used as a means of identification of these drugs. This method leaves something to be desired in sensitivity and the film-from-solution technique is being investigated to resolve this.

The problem of following blood levels of cardiac glycosides has commanded the interest of clinicians for patients under therapy. Reports of fluorescence by digitalis compounds supported a brief investigation of digitoxin, digitoxigenin, and digoxin by means of spectrofluorophotometry. Although these compounds did fluoresce, the sensitivity was insufficient to extend this study to serum specimens of patients receiving normal therapeutic doses.

2. New Techniques

Although infrared spectroscopy cannot be considered new, its application to analytical toxicology has been limited until recently when new methods for sample preparation have extended this valuable tool to both qualitative and quantitative evaluation of drugs. The two most useful developments are the potassium bromide pellet method and the film-from-solvent techniques. The KBr pellet requires 1.0 mg or more of crystalline material. With the film-from-solvent procedure samples are dissolved in a volatile solvent and quantitatively applied by micro-syringe or pipet to planchets of sodium chloride which have a niche in the center to concentrate the solution upon evaporation. Crystalline silver chloride planchets have previously been employed to minimize the effect of clouding if small amounts of moisture are present in solvent extracts. The gradual darkening of the silver chloride on exposure to sunlight and the expense of replacement prompted the change to sodium chloride which can be easily polished as absorbed moisture diminishes transparency. The most successful application of this technique has been for meprobamate (Miltown, Equanil) determinations. Since as little as a 0.1 mg of meprobamate can be detected in this manner, this sensitivity is adequate for cases of drug intoxication.

The recent development of highly sensitive detectors for gas chromatography, has increased usefulness of this technique for the identification and quantitation of compounds. Gas chromatography offers a definite advantage in sensitivity, and in simplicity of sample preparation.

The major limitation has been in finding suitable column materials to effect desired separation of classes of compounds. Ethanol, methanol, acetaldehyde, fusel oil, trichloroethylene, methyl cellosolve, and carbon tetrachloride have been satisfactorily determined on specimens submitted for toxicological analyses to this laboratory using tetraethylene glycol dimethyl ether as the stationary phase at column temperatures ranging from 50°-70°C. Diisodecyl phthalate has been used for the higher boiling alcohols and hydrocarbons. Other materials still to be investigated include nujol, for amines and halides, silicone oil 200, for aromatic amines and pyridines, and carbowax 20M, for ketones and whiskey congeners. Most important in the area of proposed research in gas chromatography is its application to those compounds which are solid at normal temperatures but which can be converted in appropriate solvents at elevated temperatures to exhibit sufficient vapor pressure for chromatographic development and subsequent identification. In this respect some success has already been reported by other investigators in the field of alkaloid analysis.

Compilation and distribution of ultraviolet spectra to the US Army Medical Laboratories engaged in toxicological analyses has continued. The spectra of twenty-five compounds were sent to these installations during the past year and another set is nearing completion. The usefulness of this program has been confirmed by canvassing recipients of these spectra. Most of the laboratories expressed a definite need of this information, and stated that the compilation activity was their principal source of ultraviolet spectral data.

Techniques for determination of reversibly oxidizable cations have been applied to polarographic determination of lead, thallium, selenium, copper, zinc and iron. Determinations for lead and zinc have proven to be improved in specificity and sensitivity since the half wave potential of these ions in KCl is very reproducible and interfering substances, formerly a problem in dithizone methods, are eliminated. The technique developed in this laboratory for selenium determination has found application to a human case of reaction to a proprietary shampoo containing selenium compounds. Determination of copper in blood is reproducible although not sufficiently sensitive to allow analysis of small amounts of blood. The problem of iron analysis has not been met since no suitable buffer has been found for the iron analysis.

The sensitivity of the thallium analysis approaches spectrographic emission; however, specificity is a problem. Work is continuing to improve on this method.

Summary and Conclusions: The techniques of infrared and ultraviolet spectroscopy have been used in investigation for adequate methods of assay for Librium, Artane, Danilone, and Octin in biological specimens.

The spectrofluorometric characteristics of digitoxin, digitoxigenin, and digoxin were studied in relation to analysis of blood specimens for digitalis. The technique was found to lack the necessary sensitivity for clinical studies of blood levels of these substances.

Investigations also included use of vapor-phase chromatography and polarography. Vapor-phase chromatography has proven to be very useful for volatile solvent assays, and recent work suggests the possibility of doing alkaloid assays by this technique. Polarographic analysis has been used effectively for determination of lead and zinc in blood and urine and additional techniques have been applied to selenium and thallium poisonings. Polarography has not been a satisfactory answer to copper and iron determinations from blood. The compilation of ultraviolet spectra has continued since it has been found that this service provides other laboratories with their principal reference data for compounds of toxicological interest.

List of Publications:

1. Kazyak, Leo: Determination of Dilantin (Sodium Diphenylhydantoin) - submitted for publication.
2. Ransone, James W., Captain, MC; Scott, Norman M., Lt Col, MC, and Knoblock, Edward C., Lt Col, MSC: Selenium Sulfide Intoxication, New England J. Med, 264:384-385. (Feb 23) 1961.

ANNUAL PROGRESS REPORT

Project 6X60-10-001: Neuropsychiatry & Stress

Task 1: Analysis of Therapeutic Methods

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Departments of Psychiatry and Experimental
Psychology, Division of Neuropsychiatry

Period Covered by Report: 1 July 1960 - 30 June 1961

Principal Investigators: Lt Col Kenneth L. Artiss, MC
Maj Joseph V. Brady, MSC
Murray Sidman, Ph.D.

Assistants: Lt Col Ralph W. Morgan, MSC
Lt Col Marguerite Lines, ANC*
Maj Harold S. Kolmer, MC
Capt Leslie J. Shellhase, MSC
Capt Lawrence T. Stoddard, MSC
1st Lt Robert Clark, MSC
Donald L. Slye, M.A.
Gerald A. Tolliver, B.A.
James A. Jackson, B.A.
Sp-7 Herman A. Becker
Pfc Douglas E. Franc

Reports Control Symbol: MEDDH-288

Security Classification: Unclassified

*Dept Neuropsychiatry, WRGH

ABSTRACT

Project 6X60-10-001: Neuropsychiatry & Stress

Task 1: Analysis of Therapeutic Methods

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Departments of Psychiatry and Experimental
Psychology, Division of Neuropsychiatry

Period Covered by Report: 1 July 1960 - 30 June 1961

Authors: J. Aronson, M.D.*
K. L. Artiss, Lt Col, MC
J. V. Brady, Maj, MC
R. Clark, 1st Lt, MSC
R. W. Morgan, Lt Col, MSC
C. R. Rodeman, Lt Col, ANC**
L. J. Shellhase, Capt, MSC
M. Sidman, Ph.D.
L. T. Stoddard, Capt, MSC

Reports Control Symbol: MEDDH-288

Security Classification: Unclassified

Use of a therapeutic milieu as a treatment regime for young persons suffering from schizophrenic psychosis has furnished the central method and laboratory for a series of related studies, including the family and related social units, patient-government and group psychotherapy behavior, specific uses of symptoms to achieve social ends and their disappearance when no immediate interaction with the fate-determining staff is possible. Application of social psychiatry principles has spread to the follow-up study of these patients with remarkable results.

Operant conditioning techniques are being used in the milieu therapy setting to investigate quantitatively some of the differential processes involved in normal and schizophrenic behavior. The relations between performance on or preference for various reward schedules has been studied. The degree to which the patients' ward behavior is reflected in their operant performance is being examined. Techniques are being refined for more rapid and economical behavioral analysis.

*Center for Community Studies, Harvard Univ.

**97th G.H., Frankfurt, Germany

The experimental analysis of drug effects upon animal behavior has been focused on both alterations in psychological processes (timing and motor performance) and alterations in fluid self-injection levels under conditions of behavioral variations. Changes in the time course of action for central nervous system stimulants have been investigated and a method for automatically programming and recording a continuous positioning response with advanced primate subjects has been evaluated in relation to tranquilizing drug effects.

REPORT

Project 6X60-10-001: Neuropsychiatry & Stress

Task 1: Analysis of Therapeutic Methods

Description:

a. Human Clinical Experimental Studies of Schizophrenic Soldiers and Milieu Therapy; to explore the feasibility of combat-psychiatric, ward-environmental and group-oriented therapeutic techniques in a sample of the most serious type of mental illness, in order to severely test the methods and derive reasonable inferences concerning certain aspects of "normal" behavior which are usually kept secret.

b. Operant Studies of Schizophrenic Behavior; to explore the use of operant conditioning techniques for the analysis of human behavior in a milieu therapy setting. Volunteer staff members and schizophrenic patients enter a booth, located on the ward, where they can pull a lever and obtain token rewards, which they can then exchange for items of value. Participation by both patients and normal subjects has been maintained at a high level.

c. Animal Experimental Studies upon the Behavioral Effects of Pharmacologic Agents; to develop animal behavioral assessment techniques for the evaluation of potentially useful pharmacological agents, and to investigate the nature and extent of the behavioral changes which follow both acute and chronic administration of selected neuropharmacologically active compounds.

Progress:

a. A modified treatment closed-type psychiatric ward, operational as a laboratory since July 1956 has continued studying the total milieu of two contrasting groups of first-break schizophrenic patients: (1) the basic training soldier (new recruit) and (2) the soldier with 1 to 8 years of military service. Individual as well as group psychotherapy is conducted and analyzed in concert with those aspects of group process which aid in the formation of therapeutically beneficial sub-groups.

Field-type social-anthropological studies have been extended to the point that the investigator has at times been allowed to "live-in" for short periods with family groups related to patients present on the treatment ward. Details of these visits, together with new findings which cast substantial doubt upon the prima-facie value of conventional "psychiatric histories" are being reported in the literature.

Final compilation of data concerning systematic observation of the behavior of the Milieu Therapy treated schizophrenic

soldiers, as group members, has been completed. This work, annotating and analyzing via an IBM coded Bales Interaction Process Analysis system, has contrasted group behavior in the group therapy situation with that occurring in a patient-government group. The important finding that symptoms are not used transactionally unless the treatment staff is present is one aspect of the 40,000 word doctoral dissertation which reports upon the study.

The year has also seen the organization and refinement of techniques for obtaining a ten-year follow-up on the results of treatment in the experimental ward. With the simple addition of "human factors" such as supplying needed or helpful information to families, their cooperation in providing follow-up data has improved to a previously unheard-of degree, in that none of the treated patients have been "lost."

Training in those principles already found effective continued throughout the year, aiding a new ward officer and nurses in becoming familiar with milieu therapy techniques in order that they might then readily apply them to a new population of patients, the "chronic alcoholic sergeant" in an experiment to begin 1 August 1961.

In addition to arranging for the study of alcoholism in Army personnel, arrangements have been partially completed for instituting a study of the natural history of the youthful offender (AWOL) at Ft. Meade, Ft. Belvoir and MDW.

b. Most of the effort during the past year has been devoted to the refinement of techniques, with the end in view of securing information more rapidly and efficiently. Schedule preferences have been studied by examining the subjects' performances on four reward schedules, each under stimulus control: the schedules were variable interval, tandem variable interval and spaced responding; fixed interval with limited hold, and fixed ratio. The first of these requires neither counting nor timing on the part of the subject; the next two require precise timing, and the last one generates counting behavior. A preference hierarchy was determined by permitting the subjects to choose among these schedules, with each schedule being paired off against each of the others. The relations between the subjects' performance on each schedule and the location of the schedule on the preference hierarchy are now being examined.

Previous results had indicated that many of the patients were not sensitive to the frequency of reward factor in the same way as were the normals. It was not clear, however, whether rewards per unit time or rewards per response -- time or work -- was the critical factor. A progressive ratio schedule is being programmed in which the subjects can reset the ratio to its

minimum value by pulling a second lever and producing a period of time-out during which they cannot get any tokens. In this way, the temporal and work factors can be balanced and the contribution of each assessed.

The impression remains, and is now being checked by examination of ward protocols, that the ward behavior of the patients is reflected in the character of their fine-grain performance in the operant booth. An analysis is also being made of the effects of ECT and drugs upon the operant performance.

c. Activities on this task have focused upon the development and refinement of motor assessment and self-injection techniques with advanced primates and upon further experimental analysis of the timing behavior process in lower animals and its relationship to centrally active compounds. A method has been developed for automatically programming and recording continuous positioning behavior with monkeys and stable performance baselines have been refined using this procedure. The effects of several centrally acting compounds including Nembutal, Benzedrine, Serpasil, LSP, and Chlorpromazine have been investigated using this technique and behavioral changes are being evaluated in relation to environmental factors (lever weight, etc.) which have also been shown to affect this response pattern.

In an experiment directed at a refinement of self-injection procedures for drug administration with advanced primates, two Rhesus monkeys, restrained in chairs, served as subjects. Following surgical implantation of permanently indwelling catheters in the internal jugular vein of each monkey, operant levels of responding on a lever switch (a modified telegraph key mounted on the chair within easy reach of the monkey's paw) were determined for both animals. Following stabilization of the operant levels under both satiated and water-deprived conditions, each lever response was programmed to activate an infusion pump and produce an injection of saline through the indwelling catheter. Under this condition of continuous reinforcement, each lever response delivered 1.95 cc of saline at the rate of 1 cc per minute. With both animals, introduction of the saline infusion reinforcement contingency produced a significant increase in the lever pressing response rate which remained consistently above operant levels and which exhibited the typical characteristics of behavior under continuous reinforcement conditions. Extinction of the instrumental lever pressing response was observed in both monkeys following discontinuation of the saline infusion. With only the tube from the pump to the catheter disconnected and all other aspects of the circuit in operation, a typical lever pressing extinction curve was generated by both monkeys with a concomitant return to operant level response rates. Following reconditioning of the self-infusion behavior by replacing the pump connection both monkeys were provided with an ad libitum supply

of water provided through an easily accessible bottle and mouth spout. Under these conditions of free access to water via the normal oral ingestion route, a marked decline in the self-infusion response rate was again observed for both monkeys. This finding indicated that the reinforcement properties of normal saline infused in this manner are at least partially sensitive to variations in "drive" level. In the final phase of this experiment an attempt was made to bring the instrumental self-infusion behavior under exteroceptive stimulus control. Lever responses were reinforced with saline infusion only in the presence of a dim light stimulus presented on a panel in front of the monkey. Responses in the absence of the light were not reinforced. Following several days of exposure to these conditions, response rates resembled the typical continuous reinforcement performance in the presence of the light and approximated operant levels in its absence. Preliminary observations have also indicated that much self-infusion responding can be brought under reinforcement schedule control by requiring a fixed ratio of lever responses to produce each saline injection. Introduction of such a ratio requirement has been observed to produce a marked increase in the response rate over both operant and continuous reinforcement levels.

A series of experiments have been continued for analyzing the changes which occur in timing behavior under conditions of chronic administration of dl-Amphetamine. Previous reports have shown that animals treated chronically with dl-Amphetamine show initially a marked disruption in their timing behavior performance. However, with continued administration an improvement in timing behavior occurs. In a pharmacologic sense the animals develop tolerance to the drug. Subsequent studies have demonstrated that general activity does not reflect the development of tolerance to chronically administered dl-Amphetamine. In experiments conducted during the last year, rats were trained on a 2-ply multiple schedule. The first component of this schedule required the animal to space his responses 30 seconds apart in order to receive a food reinforcement (DRL-30"). Premature responses recycled the timing sequence so that the animal had essentially postponed the food reinforcement. In the second component of the multiple the animal was reinforced for the first response occurring after 30 seconds (FI-30"). In this component premature responses had no delaying effect upon the time when the food reinforcement could occur. dl-Amphetamine was chronically administered to animals trained in the foregoing procedure. The response rate in both components showed initially a marked increase. In the case of the DRL-30" component this resulted in a reduction in the number of food reinforcements received. In the case of the FI-30" component the increased rate of responding had no significant effect on the number of food reinforcements received. After approximately 5 sessions timing behavior in the DRL-30" component began to improve and at the end of 15 days was

approaching the pre-drug efficiency of performance. In the FI-30" component the animals' performance under drug condition did not change over the course of the chronic drug regimen. These studies indicate that the development of tolerance to dl-Amphetamine seen in timing performance is essentially a reflection of the animals' readjustment to the scheduled reinforcement requirements. Since in the FI-30" component the drug-induced performance changes did not affect reinforcement frequency, the behavior in this component did not reflect the development of tolerance to dl-Amphetamine.

Summary:

A Milieu Therapy ward serves as an excellent laboratory for the study of deviant human behavior, in addition to its value as a treatment method. Findings highly relevant to "normal" behavior emerge from such a study. Alcoholism will be studied by these established methods.

Operant conditioning techniques are being used in a milieu therapy setting to investigate quantitatively some of the differential processes involved in normal and schizophrenic behavior. The relations between performance on or preference for various reward schedules has been studied. The degree to which the patients' ward behavior is reflected in their operant performance is being examined. Techniques are being refined for more rapid and economical behavioral analysis.

The experimental analysis of drug effects upon animal behavior has been focused on both alterations in psychological processes -- (timing and motor performance) and alterations in fluid self-injection levels under conditions of behavioral variations. Changes in the time course of action for central nervous system stimulants have been investigated and a method for automatically programming and recording a continuous positioning response with advanced primate subjects has been evaluated in relation to tranquilizing drug effects.

List of Publications:

Aronson, J., Polgar, S. Pathogenic Relationships in Schizophrenia. Read at 116th Annual Meeting of American Psychiatric Assn, 13 May 1960; to be published in Journal of A.P.A.

Artiss, K. L. Milieu Therapy in Schizophrenia. Grune & Stratton, Inc., New York, (In press) 1961.

Brady, J. V. Emotional Behavior. Handbook of Physiology, Sec. 1: Neurophysiology, Vol. III, American Physiological Society, Washington, D. C., 1960.

Brady, J. V. and Ross, S. Testing Drug Effects on Controlled Animal and Human Behavior. In L. Uhr and J. G. Miller, (Eds.) Drugs and Behavior, Wiley and Company, 1960.

Clark, R., Schuster, C., and Brady, J. V. Instrumental Conditioning of Jugular Self-infusion in the Rhesus Monkey. Science (in press).

Clark, R., Jackson, J., and Brady, J. V. Assessment of Drug Effects on Continuous Positioning Behavior in the Monkey. Psychonomic Society Convention, New York, 1961.

Glass, A. J., Artiss, K. L., Gibbs, J. J. Current Status of Army Psychiatry. Presented at APA, May 1960. (Published in APA Journal, February 1961).

Morgan, R. W. The Social Work Home Visit as a Data Gathering Device in Psychiatric Research. American Journal of Orthopsychiatry (In press).

Rodeman, C. R. The Nursing Service in Milieu Therapy. Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington 12, D. C. Govt. Printing Office 1960.

Shellhase, L. J. Acceptance of Role and Resultant Interaction in Group Psychotherapy of Schizophrenia. Group Psychotherapy, Vol XIII, No. 3-4 (Sep-Dec 1960) pp 208-229.

Shellhase, L. J. A Study of the Self-Governing Activities of a Schizophrenic Group. To be published in The International Journal of Social Psychiatry.

Shellhase, L. J. Abstract of The Group Life of the Schizophrenic Patient: A Social Work Investigation. Washington: Catholic University of America Press, 1961, 64 pp.

Sidman, M. Operant Techniques, In A. J. Bachrach (Ed.), Experimental Foundations of Clinical Psychology, Basic Books, New York, 1961.

Sidman, M. Some New Research Opportunities. Proceedings of Woods Schools Conference on Mental Retardation, May 1961.

Stoddard, L. T., Sidman, M., and Brady, J. V. Conditioning and Maintenance of Human Behavior by Multiple Schedules of Intermittent Reinforcement. Eastern Psychological Association Convention, April 1960.

Stoddard, L. T., Sidman, M., and Brady, J. V. Reinforcement Frequency as a Factor in the Control of Normal and Psychotic Behavior. Psychonomic Society Convention, September 1960.

ANNUAL PROGRESS REPORT

Project 6X60-10-001, Neuropsychiatry and Stress

Task 3, Psychological, Physiological, Metabolic, and Endocrinological Homeostatic Mechanisms in Health and Disease

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Departments of Neuroendocrinology,
Experimental Psychology and Psychiatry
Division of Neuropsychiatry

Period Covered by Report: 1 July 1960 through 30 June 1961

Principal Investigators: John W. Mason, MD
Maj Joseph V. Brady, MSC

Assistants:

Lt Col Kenneth L. Artiss, MC	Walle J. H. Nauta, MD
Bernard Beer, BA	Capt Edwin Polish, MC
Charles E. Cain, MS	*Israel Posner, PhD
1st Lt William Hodos, MSC	James A. Robinson, BS
*Mary Ann Hurley, MS	Robert E. Robinson, BS
*J. A. Jones, MA	Capt Edward J. Sachar, MC
PFC Gabor A. Kalman, MSC	Murray Sidman, PhD
Maj Harold S. Kolmer, Jr, MC	Donald L. Slye, MS
Esther P. Lawrence, BA	*Elizabeth D. Taylor, MS
PVT Robert T. Lawrence, MSC	Gerald A. Tolliver, BA
Lt Col Sidney L. Marvin, MC	Wiley W. Tolson, PhD
1st Lt Bernard M. Migler, MSC	Elliot S. Volenstein, PhD
E. H. Mougey, MS	Mary H. Wikie, MS

Reports Control Symbol: MEDDH-288

Security Classification: Unclassified

* National Institute of Mental Health, NIH.

ABSTRACT

Project 6X60-10-001, Neuropsychiatry and Stress

Task 3, Psychological, Physiological, Metabolic, and Endocrinological Homeostatic Mechanisms in Health and Disease

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Period Covered by Report: 1 July 1960 through 30 June 1961

Authors: John W. Mason, MD; Maj Joseph V. Brady, MSC; Lt Col Kenneth L. Artiss, MC; Bernard Beer, BA; Charles E. Cain, MS; 1st Lt William Hodos, MSC; Mary Ann Hurley, MS; J. A. Jones, MA; PFC Gabor A. Kalman, MSC; Maj Harold S. Kolmer, Jr, MC; Esther P. Lawrence, BA; PVT Robert T. Lawrence, MSC; Lt Col Sidney L. Marvin, MC; 1st Lt Bernard M. Migler, MSC; E. H. Mougey, MS; Walle J. H. Nauta, MD; Capt Edwin Polish, MC; Israel Posner, PhD; James A. Robinson, BS; Capt Edward J. Schar, MC; Murray Sidman, PhD; Donald L. Slye, MS; Elizabeth D. Taylor, MS; Gerald A. Tolliver, BA; Wiley W. Tolson, PhD; Elliot S. Valenstein, PhD; Mary H. Wilkie, MS

Reports Control Symbol: MEDDH-288

Security Classification: Unclassified

Establishment of several new biochemical hormone assay methods this year has permitted us to gain considerable new information about the organization of psychological and neural influences upon the endocrine systems, which in turn affect virtually every biochemical process in the body. It has been learned that not just the adrenal hormones, but every hormone we have been able so far to measure undergoes marked changes during and following psychological stress. The responses of each endocrine system are arranged in characteristic patterns with respect to their direction and temporal features. The extensive medical implications of these findings are outlined.

The experimental behavioral analysis of stress has been directed at temporal and environmental factors involved in behavioral stress situations, both appetitive and aversive. Future work will involve attempts to isolate the critical factors involved in these situations, and to study the interactions when such factors are experimentally combined.

A number of studies are reported in which further information has been gathered on the variables determining the rate of electrical self-stimulation of the brain. These studies generally indicate that certain of the motivationally induced increases in self-stimulation rate may reflect general activity changes rather than changes in reinforcement properties. Furthermore, the experiments on timing behavior (DRL) suggest that a side effect of brain stimulation in certain neural areas may interfere with certain complex responses which animals emit as a means of estimating the passage of time.

Studies on the cardiovascular responses to psychological stimuli of schizophrenic patients have shown physiological changes which appear related to interpersonal involvement in the experimental situation. Progress has also been made in the development of improved methods for reliable measurement of cardiovascular functions in collaboration with physical scientists.

BODY OF REPORT

Project 6X60-10-001, Neuropsychiatry and Stress

Task 3, Psychological, Physiological, Metabolic, and Endocrinological Homeostatic Mechanisms in Health and Disease

Description:

Investigation of the relationships between the brain and the endocrine systems is being pursued in interdisciplinary studies involving blood and urinary hormone measurements in monkeys under acute and chronic experimental psychological stress and in human subjects in a variety of stressful life situations. These studies continue to yield abundant and unique fundamental information about the organization of emotional and psychosomatic processes. The psychoendocrine approach permits a direct view of the major linkages between the brain and the biochemical processes throughout the body, an objective long sought in the field of psychosomatic medicine, but which has become possible only with the rapid and dramatic advances in biochemical hormone assay methods during the past few years. Within the past year we have found that many hormones in addition to those of the adrenal are undergoing marked changes during stress and that the study of patterns of hormonal change promise to give us far greater insight into the organization of integrative processes than the conventional studies of single endocrine or visceral systems.

The experimental analysis of behavioral stress phenomena in laboratory animals continues to focus upon behavioral adaptation to current and anticipated aversive events and upon behavioral mechanisms of temporal orientation. The following investigations have been carried out or are in the process of completion.

A number of studies are presently in progress in the area of intracranial self-stimulation. The program has two purposes: (1) to gather further information on the variables which control this behavior, and (2) to utilize this technique as a means of further study of inter-relationships between structures within the central nervous system.

Studies of cardiovascular responses in schizophrenic patients using pulse rate, blood pressure, finger pulse and ballistocardiograms are yielding insight into dynamic aspects of the illness which have been extremely difficult to evaluate by psychiatric observation alone.

Progress: ..

I. Neuroendocrine Studies of Stress

A. Acute Stress

In order to determine if the levels of hormones other than those of the adrenal gland, hydrocortisone, epinephrine, and norepinephrine, undergo significant changes during psychological stress, four reliable stress situations were chosen for study:

1. Prolonged conditioned avoidance sessions (monkey)
2. Adaptation to a restraining chair (monkey)
3. Final college examinations (normal humans)
4. Hospital admission (normal humans)

In addition to the adrenal hormones listed above, the androgens, the estrogens, and the thyroid hormones (BEI) were measured in these four stress situations. The results uniformly indicated that every hormone studied underwent marked and characteristic changes during and after stress. These changes were perhaps most strikingly demonstrated in their full temporal sequence in the rigidly controlled 72 hour conditioned avoidance experiments in the monkey, as follows:

17-OH-CS. Levels showed a three-fold elevation during the three-day stress period and returned to baseline within three to six days following stress.

Epinephrine. Levels showed a two-fold increase of only two or three days' duration, the briefest change of any hormone. In some instances epinephrine levels remained below baseline for several days following stress.

Norepinephrine. Levels were slightly elevated during stress, but then showed marked, prolonged, two-fold elevation following stress, in some cases lasting two weeks or longer.

Androsterone. Levels dropped 50% during stress, but then rose to about twice baseline from three to six days after stress.

Estrone. Levels dropped during stress and rose afterwards in a pattern very similar to androsterone.

Thyroid. BEI levels rose very slowly, reaching a peak of twice baseline, usually after the stress was over, then remained elevated for as long as three weeks in association with a three-day stress.

Aldosterone. Preliminary experience indicates that aldosterone levels often drop initially during stress but then show marked secondary elevations after stress.

The above patterns of endocrine change have not previously been described, particularly the prominent changes in the stress aftermath involving norepinephrine, androgens, estrogens, aldosterone and thyroid hormones. The sequence of changes suggests that hormones promoting catabolic mobilization of energy resources predominate initially during stress, while those promoting anabolic restorative or reparative processes predominate during the recovery period. Very similar patterns were observed in human subjects during and following final college examinations.

B. Chronic Stress

Previous studies have established the considerable value of conditioning procedures for producing chronic psychological stress in an experimental psychosomatic approach to the study of disorders such as infections, edema, gastrointestinal lesions, and other diseases in the monkey. A new series of chronic avoidance experiments has been initiated in which detailed hormone pattern measurements are being made and correlations sought between the clinical disorders which develop and the preceding and associated hormonal changes. One of the first two monkeys so studied developed severe, dependent edema during the third week of stress, which was associated with a strikingly different hormonal balance from that observed in the other monkey which showed no signs of clinical illness during six weeks of stress. The pattern of change in the monkey with edema involved every hormone measured, but perhaps most marked were progressive increases in aldosterone and norepinephrine to levels far in excess of those so far observed in acute stress. We hope to study many additional monkeys in this fashion during the coming year.

An unusual opportunity to observe healthy human subjects during prolonged stress has been afforded through studies of parents of children with leukemia, in collaboration with the Adult Psychiatry Branch of the NIMH. A substantial percentage of these subjects have shown, surprisingly, 17-OH-CS levels below normal and have a tendency to suppress levels even lower during superimposed acute stress. Preliminary indications are that these endocrine changes may correlate with the type of coping behavior or psychological defenses employed by these subjects rather characteristically during stress. Efforts are being made to develop psychological and psychiatric methods for detecting, classifying and rating the behavioral characteristics which may be associated with the altered pattern of endocrine responsiveness

to stress. Preliminary evidence also indicates that similar changes, along with marked imbalances in endocrine profile, may be found in patients with certain medical illnesses:

The study of severely depressed psychiatric patients and acute schizophrenics have shown that endocrine changes of extremely marked intensity and duration may accompany psychiatric disorders. Levels of epinephrine and aldosterone may rise 20 to 25 times normal during acute periods of disturbance and 17-OH-CS levels may remain three to five times normal for several weeks. Such changes appear to be especially marked during turmoil states, such as those associated with the formation of a psychosis, and may be relieved, with a return of levels closer to a normal balance, when the psychosis becomes well established or set. It appears that the further study of both psychiatric and medical patients from the psychoendocrine approach offers us an exceptional opportunity to evaluate the possible role of disorders of integrative mechanisms in the development of some, or perhaps many, human diseases.

C. Other Studies

Studies of neuroanatomical mechanisms concerned with endocrine regulation have been limited this year to the study of additional amygdalotomized animals in a further evaluation of the preliminary finding that marked increases in urinary androgen levels and in androgen response to LH occur following amygdalotomy. Findings are still only partially complete, but suggest that the effect on androgen levels may be a rather selective or specific one.

Major emphasis continues to be devoted to the crucial matter of biochemical hormone assay methodology. A greatly simplified and rapid BEI method for thyroid hormone measurement was developed which takes only six hours instead of the two or three days of existing methods. A new double isotope derivative method for blood testosterone measurement is in the final stages of development and validation. Many new advances have been made in the development of a radio-immunochemical method for insulin measurement, but this work is being restricted by radiation safety problems. Work has just been initiated on gas chromatographic methods which promise to revolutionize the steroid hormone assay field, with sensitivity, specificity, and rapidity greater than any existing methods.

II. Analysis of Behavioral Stress Methods

With rats, a "limited-hold" avoidance procedure has been investigated. Shocks are scheduled at regular time intervals unless the animal presses a lever during a brief period within the inter-shock interval.

If the critical period is located elsewhere than at the end of the inter-shock interval, the animals fail to maintain their avoidance behavior. As the behavior disappears, however, a nonadaptive temporal discrimination becomes evident, which prevents the animals from avoiding shock successfully.

A concurrent two-response avoidance schedule has provided evidence that the reduction of shock frequency is a critical determinant of the acquisition and maintenance of avoidance behavior.

A methodological analysis of behavioral research has been completed and published in book form.

An investigation is underway, with rats, to determine the role of intervening behavior in temporally-spaced responding. Before they can produce reinforcement by pressing one lever, the animal must press another lever a fixed number of times, and must also allow a fixed period of time to elapse.

Rats have been shown to be able to maintain avoidance responding with very long shock-shock intervals if a short response-shock interval is programmed in tandem with the shock-shock interval. The timing mechanism in this case is adaptive, and permits a successful adjustment.

Animals required to press a lever to terminate shocks have been observed to spend most of their time holding the lever down. This holding behavior cannot be eliminated by punishing it with additional shocks. Spurious escape from the additional shocks apparently serves to override the effects of the punishment.

Schedule preference has been investigated in rats with a two-lever multiple chain procedure. Preference is being assessed by measuring response rates and schedule choices to determine whether these two aspects of behavior covary.

In order to determine whether the previously demonstrated aversive properties of the spaced responding (DRL) procedure result from the timing process itself or merely from the spacing of responses, the preference by rats for internally and externally cued spaced-responding schedules is being investigated.

An experiment on timing is being carried out in which the intervening behavior is limited by requiring the animals to hold the lever down during the timing period.

A progressive ratio procedure has been used to determine how various reinforcement and deprivation conditions will determine the

size of fixed ratio at which rats will cease responding. With decreasing nutrient concentration and decreasing volume per reinforcement; the animals will maintain behavior at lower ratios. With increasing food deprivation, higher ratios will maintain the behavior. As the size of the step by which the ratio increases is made larger, the animals respond at higher ratios, but do not receive as many reinforcements.

III. Analysis of Neurobehavioral Relationships

During the past two years, the progressive ratio technique has been developed in these laboratories as an index of relative reward strength. This technique requires that the animal emit a progressively increasing number of responses to obtain each reward. Our experiments have shown that the "breaking point" of the animals' behavior on this schedule correlates well with variations in reward and deprivation parameters. The technique has now been applied to the measurement of the reward strength of brain stimulation.

Animals have been compared in their progressive ratio performance for food and for brain stimulation. While the analysis is still incomplete, the preliminary findings do not seem to indicate any striking differences in reward strength between food and brain stimulation, despite the strikingly higher response rates in the case of the latter. Moreover, while food deprivation clearly elevates the self-stimulation rate, it does not appear to affect strength of brain stimulation reward as measured by the progressive ratio method. This seems to suggest that the increases in rate may be due to general activity changes.

Another series of experiments is studying temporally spaced responding (DRL) using intracranial stimulation as the reward. At the present time animals with both septal and posterior hypothalamic electrode placements are being trained in a 20 sec. DRL procedure. Septal animals are observed to perform more efficiently in DRL than posterior hypothalamic placements. An increase in stimulus intensity, however, in either placement results in improved DRL performance.

Closely related to these experiments are those in which EEG responses are being studied during DRL with food as the reward. Preliminary data suggest that the animals perform a complex pattern of muscle responses which mediates the passage of time.

Additional studies in progress are investigating the effects on self-stimulation behavior of different electrode types and stimulation parameters, lesions under the electrode and radiation of the central nervous system. In addition, we are studying the resistance to extinction of behavior maintained by brain stimulation as compared with

food. At this time, however, sufficient data has not been gathered to enable us to report any preliminary findings.

IV. Autonomic Functions and Psychological Stress

Studies have been continued on the cardiovascular responses to psychological stimuli of schizophrenic patients under treatment. During periods of tension and withdrawal from social relations, the pulse rate, blood pressure, finger pulse and ballistocardiographic records remain rigidly flat. Slight changes which may occur are not related to the formal stimuli. With improved socialization cardiovascular responses remain minimal, but appear in relation to interpersonal involvement.

A new method for determining diastolic blood pressure has been developed. The rate of pulse propagation (measured between the ECG and finger plethysmographic records) is correlated with blood pressure over short periods of time. The rate increases as pressure is reduced in the cuff. Systolic pressure is measured by the first finger pulse wave to appear and diastolic pressure is the pressure at which the pulse propagation rate reaches its maximal constant value. Careful comparison of these pressures with pressures recorded through intra-cardiac and intra-arterial catheters demonstrates that the method has high reliability and for diastolic pressures is superior to methods dependent upon auscultation.

Future plans include continued studies of schizophrenic and other psychotic patients, with particular attention to correlating autonomic with endocrinological patterns of response. A number of new developments in physics lead to the expectation that improved pressure transducers and improved photo-electric devices will permit the construction of a number of more adequate transducers for measuring changes in various somatic functions. Collaborative work with physicists and engineers of the Diamond Ordnance Fuse Laboratories is being extended. Attention is also being given to the use of computers for determining significant relationships between different cardiovascular functions. Further studies in this area are indicated before it can be decided which aspects of the raw data are most relevant for describing the functional state of somatic systems.

Summary and Conclusions:

Establishment of several additional biochemical hormone assay methods has permitted us to gain considerable new information about the organization of psychological and neural influences upon the endocrine systems, which in turn affect virtually every biochemical process in the body. It has been learned that every hormone we have been able so far to measure undergoes marked changes during and

following psychological stress. The responses of each endocrine system are arranged in characteristic patterns with respect to the direction and temporal features. The extensive medical implications of these findings are outlined.

The experimental analysis of stress has been directed at temporal and environmental factors involved in behavioral stress situations, both appetitive and aversive. Future work will involve attempts to isolate the critical factors involved in these situations, and to study the interactions when such factors are experimentally combined.

A number of studies are reported in which further information has been gathered on the variables determining the rate of electrical self-stimulation of the brain. These studies generally indicate that certain of the motivationally induced increases in self-stimulation rate may reflect general activity changes rather than changes in reinforcement properties. Furthermore, the experiments on timing behavior (DRL) suggest that a side effect of brain stimulation in certain neural areas may interfere with certain complex responses which animals emit as a means of estimating the passage of time.

Studies on the cardiovascular responses to psychological stimuli of schizophrenic patients have shown physiological changes which appear related to interpersonal involvement in the experimental situation. Progress has also been made in the development of improved methods for reliable measurement of cardiovascular functions in collaboration with physical scientists.

List of Publications:

1. Mason, J. W., Mangan, G., Jr., Brady, J. V., Conrad, D., and D. McK. Rioch. Concurrent Plasma Epinephrine, Norepinephrine and 17-Hydroxycorticosteroid Levels During Conditioned Emotional Disturbances in Monkeys. Psychosom. Med., (in press).
2. Posner, I. A Simplified Procedure for the Determination of Butanol Extractable Iodide in Serum or Plasma. J. Lab. and Clin. Med., 57: 314-321, 1961.
3. Mason, J. W., Brady, J. V., Polish, E., Bauer, J. A., Robinson, J. A., Rose, R. M., and Taylor, E. D. Patterns of Corticosteroid and Pepsinogen Change Related to Emotional Stress in the Monkey. Science, 133: 1596-98, 1961.
4. Rose, R. M., Jones, J. A., and Mason, J. W. The Measurement of Urinary and Plasma Pepsinogen in the Rhesus Monkey. (submitted for publication).

5. Rose, R. M., Mason, J. W., Jones, J. A., Lawrence, E. P., and Poe, R. O. Correlation of Gastric Pepsin, Blood and Urinary Pepsinogen During Vagal Stimulation in the Monkey. (submitted for publication).
6. Rosenthal, N. R., and Mason, J. W. Alterations in Urinary 17-Hydroxycorticosteroid Excretion Associated with Administration of Reserpine in the Rhesus Monkey. Psychopharmacologia, 2: 55-62, 1961.
7. Mason, J. W., Nauta, W. J. H., Brady, J. V., Robinson, J. A., and Sachar, E. J. The Role of Limbic System Structures in the Regulation of ACTH Secretion. Acta Neurovegetativa, (in press).
8. Polish, E., Brady, J. V., and Thach, J. S., Jr. Gastric Contents and Avoidance Behavior in the Rhesus Monkey. I. Relationships Under Acute Experimental Conditions. (submitted for publication).
9. Polish, E., Brady, J. V., Mason, J. W., Thach, J. S., Jr., and Niemeck, W. Gastric Contents and Avoidance Behavior in the Rhesus Monkey. II. Relationships Under Chronic Experimental Conditions. (submitted for publication).
10. Tolson, W. W., Mason, J. W., Sachar, E. J., Hamburg, D. A., Handlon, J. H., and Fishman, J. R. Urinary Catecholamine Responses. Associated with Hospital Admission in Normal Human Subjects. (submitted for publication).
11. Mougey, E. H., and Mason, J. W. The Measurement of Butanol Extractable Iodide in the Rhesus Monkey. (submitted for publication).
12. Handlon, J. H., Fishman, J. R., Sachar, E. J., Hamburg, D. A., and Mason, J. W. Mild Stress and Adrenocortical Function: Psychological Factors in Lowering Plasma Hydrocortisone Concentrations. (submitted for publication).
13. Mason, J. W., Sachar, E. J., Bauer, J. A., Hurley, M. A., Hamburg, D. A., Fishman, J. R., and Handlon, J. H. Effect of Hospital Admission on Urinary and Plasma 17-Hydroxycorticosteroid Levels in Normal Human Subjects. (in preparation).
14. Mason, J. W., Brady, J. V., Tolson, W. W., Robinson, J. A., Taylor, E. D., and Mougey, E. H. Patterns of Thyroid, Gonadal, and Adrenal Hormone Secretion Related to Psychological Stress in the Monkey. (Abstract) Psychosom. Med.

15. Sidman, M. Stimulus Generalization in an Avoidance Situation. J. exper. anal. Behav., 4: 157-169, 1961.
16. Hodos, W. Progressive Ratio as a Measure of Reward Strength. Science. (in press).
17. Sidman, M. Tactics of Scientific Research: Evaluating Experimental Data in Psychology. Basic Books, New York, 1960.
18. Sidman, M. Classical Avoidance Without a Warning Stimulus. J. exper. anal. Behav. (in press).
19. Sidman, M. Reduction of Shock Frequency as Reinforcement for Avoidance Behavior. J. exper. anal. Behav. (in press).
20. Hearst, E. and Sidman, M. Some Behavioral Effects of a Concurrently Positive and Negative Stimulus. J. exper. anal. Behav. (in press).
21. Brady, J. V. Emotional Behavior. Handbook of Physiology. Section 1: Neurophysiology. Volume III, American Physiological Society, Washington, D. C., 1960.
22. Brady, J. V. and Polish, E. Performance Changes During Prolonged Avoidance. Psychological Reports, 7: 554, 1960.
23. Beer, B. and Valenstein, E. S. Discrimination of Tones During Reinforcing Brain Stimulation. Science. 132: 297-298, 1960.
24. Hodos, W. and Valenstein, E. S. Motivational Variables Affecting the Rate of Behavior Maintained by Intracranial Stimulation. J. comp. physiol. Psychol. 53: 502-508, 1960.
25. Valenstein, E. S. and Beer, B. Stereotaxic Measurements of the Calvarium of Macaca Mulatta. Amer. J. Physiol. 199: 975-977, 1960.
26. Valenstein, E. S., Hodos, W. and Stein, L. A Simplified Electrode Assembly for Implanting Chronic Electrodes in the Brains of Small Animals. Amer. J. Psychol. 74: 125-138, 1961.
27. Hodos, W. and Valenstein, E. S. An Evaluation of Rate as a Measure of Rewarding Intracranial Stimulation. J. comp. physiol. Psychol. (in press).

28. Valenstein, E. S. and Beer, B. A Comparison of Bipolar and Unipolar Electrodes in Self-Stimulation Experiments. Amer. J. Physiol. (in press).
29. Beer, B., Hodos, W. and Valenstein, E. S. Effects of Some Parameters of Electrical Stimulation on the Size of Lesions in the Brain. (in preparation).
30. Valenstein, E. S. and Beer, B. A Comparison of the Relative Reinforcement Strength of Intracranial Stimulation and Several Other Reinforcements. (in preparation).
31. Hodos, W., Ross, G. S. and Brady, J. V. Complex Response Patterns During Temporally Spaced Responding. (in preparation).
32. Ross, G. S., Hodos, W. and Brady, J. V. EEG Changes During Two Types of Temporally Defined Reward Schedules. (in preparation).
33. Brady, J. V. Behavior and the Nervous System. In R. Waters, D. Rethlingshafer, W. Caldwell (Eds.) Principles of Comparative Psychology, New York, McGraw-Hill, 1960.
34. Brady, J. V. Temporal and Emotional Effects Related to Intracranial Electrical Self-Stimulation. In Ramey, E. R. & O'Doherty, D. S. (Eds.), Electrical Studies on the Unanesthetized Brain. Hoeber, Inc., New York, N. Y., 1960.
35. Brady, J. V. and Conrad, D. Some Effects of Limbic System Self-Stimulation Upon Conditioned Emotional Behavior. J. comp. physiol. Psychology. 53: 128-137, 1960.
36. Brady, J. V. and Conrad, D. Some Effects of Brain Stimulation on Timing Behavior. J. exper. anal. Behav. 3: 93-106, 1960.
37. Brady, J. V. Motivational-Emotional Factors and Intra-Cranial Self-Stimulation. In D. Scheer (Ed.). Houston Symposium on Brain Stimulation. Subcortical Integrative Systems. University of Texas Press, Austin, Texas, 1961.
38. Geller, I. and Brady, J. V. Effect of Electroconvulsive Shock on an Extinguished "Fear" Response. Science. 133: 1080-1081, 1961.
39. Marvin, Sidney L. Section in volume The Manipulation of Human Behavior: New York, Wiley, (editors) Biderman and Zimmer, 1960.
40. Robinson, R. E., Marvin, S. L., Thompson, W. M., Jr., and Dammann, J. F. An Accurate Method for Indirect Determination of Systolic and Diastolic Blood Pressures. (in press)

ANNUAL PROGRESS REPORT

Project 6X60-10-001 Neuropsychiatry and Stress

Task 4, Anatomical and Physiological Substrata of Behavior

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D.C.

Department of Neurophysiology
Division of Neuropsychiatry

Period Covered by Report: 1 July 1960 through 30 June 1961

Principal Investigator: Walle J. H. Nauta, M.D.

Assistants: Mildred Albrecht, B.S.
Jay B. Best, Ph.D.
H. G. J. M. Kuypers, M.D.*
William R. Mehler, Ph.D.
Capt. Ronald E. Myers, MC
Enrique Ramon-Moliner, M.D. *
Elliot S. Walenstein, Ph.D.

Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

* University of Maryland,
Baltimore, Md.

ABSTRACT .

Project No. 6X60-10-001 Neuropsychiatry and Stress

Task No. 4, Anatomical and Physiological Substrata of Behavior:

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D.C.

Period Covered by Report: 1 July 1960 through 30 June 1961

Authors: Mildred Albrecht, B.S.; Jay B. Best, Ph.D.; H. G. J. M. Kuypers,
M.D., William R. Mehler, Ph.D; Capt. Ronald E. Myers, MC.;
Walle J. H. Nauta, M.D.; Enrique Ramon-Moliner, M.D.; Elliot S.
Valenstein, Ph.D.

Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

Anatomical studies of the central nervous system of various mammalian forms have been continued. Areas of main interest--because of their immediate relation to contemporary research in animal and human behavior--are (a) the Limbic System; (b) the Brain Stem Reticular Formation; (c) the Basal Ganglia; (d) the Corpus Callosum. The over-all purpose of these investigations is: to provide a more detailed and more realistic concept of the anatomical organization of the brain as a substratum for behavior.

Project No. 6X60-10-001, Neuropsychiatry and Stress.

Task 4, Anatomical and Physiological Substrata of Behavior.

1. The Limbic system

Neural mechanisms related to the amygdaloid complex. The previous studies on this subject were extended during the past year. The results have demonstrated a common plan in the neural associations of the amygdaloid complex and the hippocampal formation, respectively. Both structures project to a thalamic nucleus (n. anterior for the hippocampus, n. dorsomedialis for amygdala) which in turn projects to a cortical region (gyrus cinguli and caudal orbitofrontal cortex, respectively), which in turn projects back to the structure of origin. A comparable analogy is encountered in the subcortical projections arising from the respective neural organizations. For both, the general system of the medial forebrain bundle appears to represent the major discharge channel. A publication of these findings is currently in preparation.

2. Reticulo-cortical connections

New material for this study is being prepared. At this time nothing can be added to last year's report.

3. Efferent pathways of the basal ganglia

This study of the efferent projections from the lentiform nucleus in the monkey has been continued. Several of the connections reported by earlier workers could be confirmed. Thus, the putamen was found to project to the globus pallidus and substantia nigra; the globus pallidus to the subthalamic nucleus and the nucleus ventralis lateralis thalami. Projections from either source to the red nucleus appear not to exist; earlier reports to the contrary may have resulted from concomitant lesion of the internal capsule, a surgical complication obviated in the present experiments. Two new observations were made: the globus pallidus projects massively to the centre median of the thalamus, and also to a circumscribed cell group in the caudal midbrain tegmentum (Olzewski's nucleus tegmentosus pedunculo-pontinus, pars caudalis). The projection to the centre median is of special interest as it provides an experimental approach to the controversial problem whether a centre median is present in lower mammalian forms. The results of the present study have been reported to the 74th annual convention of the American Association of Anatomists (Chicago, 1961).

4. Commissural connections of the occipital lobe in the monkey.

Dr. Myers left WRAIR in September 1960, and is currently continuing this project at Johns Hopkins University. The findings reported in the preceding annual report have been written up and were submitted to the Journal of Comparative Neurology for publication.

5. Phylogenetic studies of the mammalian "pain tract"

This study has been extended by an analysis of several human cases of anterolateral cordotomy. The findings in man proved to be quite comparable to those made in the monkey and chimpanzee (see annual reports 1958-1960). A detailed report on the human "pain tract" was contributed to the Symposium on Paraplegia (Los Angeles, February 1961) and will shortly appear in print (Mehler, W.R. 1961. IN; Recent Contributions of Basic Research to Paraplegia, C. C. Thomas and Co.).

6. Histopathological Staining Techniques

The development of an organic staining method for degenerating axons has made good progress. Since the time of the last annual report, silico-tungstic acid has been found to increase considerably the affinity of degenerating axoplasm for acid fuchsin. Certain further intermediate steps in the procedure are currently being tested for consistency of effectiveness. At this time, the results of the technique appear virtually equivalent to those obtained by the much more cumbersome and unpredictable silver techniques. It is expected that publication of the method will be possible within the next year.

7. Golgi studies on cat, rat, and mouse brains

During the past year it has proved necessary to establish more reliable criteria for the identification of "specific" and "non-specific" cell groups in the brain stem (the terms in quotes refer to the relationship of the cell groups to homogeneously organized afferent fiber systems). Such a criterion has now been found in the characteristics of the dendritic expansions emanating from the nerve cells. A classification of dendritic ramification patterns is currently being developed. There are strong indications that such a classification will prove to be a valuable aide in the delineation of specific sensory cell groups from the reticular formation. A publication of the proposed classification method is in preparation.

8. Behavioral experiments with planaria

The study of learning mechanisms in flatworms has been continued until the time of Dr. Best's departure to the University of Illinois. The results obtained have been submitted to the Journal of Comparative and Physiological Psychology. There is now irrefutable evidence that Planaria are capable of instrumental learning of such order as to permit a choice between two alternatives. Several characteristics in the learning process of these invertebrates call for further study. There is, for example, a mechanism which leads to a remarkable post-learning rejection of the adopted choice-behavior, followed by a lethargic state not due to simple fatigue or injury.

SUMMARY AND CONCLUSIONS:

Anatomical studies of the central nervous system of various mammalian forms have been continued. Areas of main interest--because of their immediate relation to contemporary research in animal and human behavior--are (a) the Limbic System; (b) the Brain Stem Reticular Formation; (c) the Basal Ganglia; (d) the Corpus Callosum. The over-all purpose of these investigations is: to provide a more detailed and more realistic concept of the anatomical organization of the brain as a substratum for behavior.

PUBLICATIONS:

1. MEHLER, W. R., M. E. FEFERMAN and W. J. H. NAUTA. Ascending axon degeneration following anterolateral cordotomy. An experimental study in the monkey. Brain, 1960, 83: 718-750.
2. MYERS, RONALD E. and CALVIN O. HENSON. Role of corpus callosum in transfer of tactuo-kinesthetic learning in chimpanzee. Arch. Neurol. (in press).
3. BEST, JAY B. Diurnal cycles and cannibalism in planaria. Science, 1960, 131: 1884-1885.
4. NAUTA, WALLE J. H. Limbic system and hypothalamus: Anatomical aspects. Physiol. Revs., 1960, 40 (Suppl. 4), 102-104.
5. NAUTA, WALLE J. H. Some neural pathways related to the limbic system. Chap. I, pp. 1-16, in: Electrical Studies on the Unanesthetized Brain, 1960, Hoeber.
6. BEST, JAY BOYD. Enzymic Processes in cells. Internat. Rev. Cytology, 1960, IX: 129-186.
7. BEST, JAY BOYD. Thermodynamics of cells. In: Encyclopedia of Biology, Peter Gray, editor (in press).
8. NAUTA, WALLE J. H. Fiber degeneration following lesions of the amygdaloid complex in the monkey. J. Anat. (in press).
9. NAUTA, W. J. H. and W. R. MEHLER. Some efferent connections of the lentiform nucleus in monkey and cat. Anat. Rec. 1961, 139: 260.
10. MEHLER, WILLIAM R. The anatomy of the so-called "Pain Tract" in man. Presented at Symposium on Paraplegia, Los Angeles, Feb. 1961; in: Recent Contributions of Basic Research to Paraplegia, C.C. Thomas and Co., (in press).
11. BEST, JAY BOYD and IRVIN RUBINSTEIN. Maze learning and associated behavior in planaria. J. Comp. Physiol. Psychol. (in press).

ANNUAL PROGRESS REPORT

Project 6x60-10-001, Neuropsychiatry and Stress.

Task 5, Electrophysiological Studies of the Nervous System.

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D.C.

Departments of Neurophysiology and Experimental
Psychology,
Division of Neuropsychiatry

Period Covered by Report: 1 July 1960 through 30 June 1961

Principal Investigators: John C. Armington, Ph.D., Robert Galambos, M.D.,
and Felix Strumwasser, Ph.D.*

Assistants: William R. Biersdorf, Ph.D. Lt. Col. Sidney Marvin, MC
Donald F. Bogdanski, Ph.D.*** George Moushegian, Ph.D. **
Robert M. Chapman, M.S. Capt. Jorge Perez-Cruet, MC
Peter Gouras ***** Robert E. Robinson
Allen M. Granda, Ph.D. Capt. Gilbert S. Ross, MC
Capt. Robert G. Grossman, MC Allen Rupert
Fred F. Hiltz, E.E. **** Guy C. Sheatz, Ph.D.
1/LT William Hodos Donald I. Tepas
Walter J. Kropfl Lawrence J. Viernstein, Ph.D. ****
Lewis W. Lee _____ Capt. Eliot D. Weitzman, MC

Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

* Washington School of Psychiatry
** NIH Fellow
*** University of Maryland
**** Johns Hopkins Applied Physics Lab
***** National Institutes of Health

ABSTRACT

Project No. 6x60-10-001, Neuropsychiatry and Stress

Task No. 5: Electrophysiological Studies of the Nervous System

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D.C.

Period Covered by Report: 1 July 1960 through 30 June 1961

Authors: John C. Armington, Ph.D., William R. Biersdorf, Ph.D., Donald F. Bogdanski, Ph.D., Robert M. Chapman, M.S., Robert Galambos, M.D., Peter Gouras, Allen M. Granda, Ph.D., Capt. Robert G. Grossman, MC, Fred F. Hiltz, E.E., 1/LT William Hodos, Walter J. Kropfl, Lewis W. Lee, Lt.Col. Sidney Marvin, MC., George Moushegian, Ph.D., Capt. Jorge Perez-Cruet, MC., Robert E. Robinson, Capt. Gilbert S. Ross, MC., Allen Rupert, Guy C. Sheatz, Ph.D., Donald I. Tepás, Lawrence J. Viernstein, Ph.D., Capt. Elliot D. Weitzman, MC.

Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

Studies on animals and man designed to relate biological electrical activity with behavioral functions are reported. Microelectrode analyses of single nerve cells have yielded increments of knowledge regarding fundamental properties of synapses and the neural processing of visual, auditory and vestibular information. Gross electrode responses from the eye and brain substance, especially as analyzed by computer methods, have been correlated with stimulus parameters, sleep, and disease states. Brain stimulation through chronic implanted electrodes has been employed to reveal new data about pain and the regulation of cardiovascular responses.

BODY OF REPORT

Project No. 6x60-10-001, Neuropsychiatry and Stress

Task No. 5, Electrophysiological Studies of the Nervous System

A. Single Unit Analysis: A major effort of the laboratory since its inception has been the study, with microelectrode methods, of single brain cells in situ. Progress along several lines during the past year can be summarized as follows:

1. The simple invertebrate "visceral brain"

The parieto-visceral ganglion of the sea-hare Aplysia californica is presently under investigation with the use of multiple intracellularly placed glass micropipettes. The ganglion ~~is of interest~~ because it consists of only a few hundred cells and yet constitutes essentially the entire 'visceral brain' of this organism.

The several general areas presently under investigation can be indicated as follows:

a. Membrane properties of neural and non-neural elements penetrated by the microelectrode;

b. Synaptic organization, and modes of synaptic operation of the neurons;

c. Classification of the endogenous patterns of activity within the ganglion and their long-term changes (as a function of time of day and state of the total organism);

d. The effect of temperature on the activities referred to in a-c above;

e. In addition to the conventional neurophysiological approaches just outlined, an attempt is being made to simulate pattern formation in the ganglion with clusters of interconnected artificial neurons. In connection with this effort a catalogue of the real cells based on their connections, membrane properties, synaptic mode and endogenous pattern, has been achieved in the form of a considerable amount of stored, edited and digitized information of this type recorded on magnetic tape. Computations on this material will, it is hoped, be performed on an IBM 7090 through the cooperation of the Johns Hopkins Applied Physics Lab.

f. A histological and electron microscope study of the ganglion is being pursued by members of this laboratory in collaboration with Dr. Rosenbluth (at the National Institute of Neurological Diseases and Blindness, National Institutes of Health).

A summary of the results and conclusions of experiments completed, or very near completion, can be made under 4 headings:

Neuron activity patterns. There are complex but reproducible, endogenous (i.e., 'spontaneous') and evoked patterns of activity which occur in identifiable nerve cells from preparation to preparation, indicating that at least in part the precise connections and modes of synaptic operation giving rise to these patterns are predetermined. The spontaneous patterns most probably represent at a unitary level, the components of some instinctive reactions (in the visceral sphere) built into the organism through its genetics. There is a powerful tool here for an electrophysiological approach to the mechanisms of specific connections between cells during development by proceeding to a study of different embryological stages of the ganglion.

Facilitation. The growth in amplitude of excitatory post-synaptic potentials with repetition of afferent stimulation has clearly shown that this process can be produced by a single input fiber. Furthermore by recording from at least one other cell receiving simultaneous inhibitory input from the same single fiber, it has been possible to show that recruitment of additional neurons in the pathway is not the mechanism responsible for the facilitation. The facilitation, finally, is not interval sensitive (over a wide range of several seconds) but is linearly related to the total number of prior inputs. This suggests a useful function for the facilitatory process; it allows storage register properties, the register being capable of counting (in the case of these cells) to over 25 bits delivered over a total period as long as several minutes.

The mechanism for this facilitatory process was investigated by searching for parameters of the action potential that systematically changed input stimulus with repetition. Prolongation of action-potential duration is a conspicuous normally-occurring change with such repetition in cell bodies of the ganglion. With two microelectrodes, one in the cell body and the other in the axon of the largest neuron in the ganglion, it has been possible to show that the prolongation appears uniformly in both cell and axon. If this process is extrapolated to the axon terminals, the increase in duration of the spike can provide a simple explanation of the facilitation through increasing the transmitter output.

Neuropile structure. Experiments performed with recordings of up to four close-lying cells show that the neuropile is a highly differentiated structure, since the relative synaptic drive, sign of action and convergence of peripheral inputs differ for the various cells.

Effects of temperature. The ganglion can be electrically silenced by bringing its temperature down to 1°C. After one hour at this temperature and rearming to 17°C, the complex patterns of endogenous activity reappear and do not show any alterations. Persistent circulating neuronal activity does not appear to be necessary for maintenance of these patterns, even those with periodicities of the order of minutes.

2. The frog retina

In these studies nerve impulses from retinal units have been elicited by calibrated light flashes of various intensities, wavelengths, and duration delivered to the eye of the intact bullfrog under scotopic and photopic conditions. In the analysis of them several response measures were used, the latency to the first impulse being particularly useful. A simple linear function relates the reciprocal of latency to log light intensity in the dark-adapted state, while a more complex latency-intensity function is associated with the light-adapted state.

When spectral sensitivity curves are derived from these latency-intensity functions (by determining the relative energy at the various wavelengths required to produce a criterion latency), these vary widely, with some of them being related to retinal photopigment absorption curves. The average shift of spectral sensitivity between scotopic and photopic conditions (Purkinje shift) was for these in approximate quantitative agreement with a shift from the rhodopsin to the iodopsin absorption spectrum. About half of the dark-adapted units yielded curves of the rhodopsin type and about half of the light-adapted units yielded curves of the iodopsin type.

Of special interest to color theory are the units not included in either of these groups. None of Granit's modulators was described in its entirety by any of the spectral sensitivity curves obtained. However, some of the sensitive peaks were at wavelengths that correspond to Granit's green and red modulators (frog). Relatively narrow spectral sensitivity curves with maxima below 450 m μ were obtained from several of the dark-adapted units; these were reliably different from Granit's blue modulator (frog) and were approximated by Dartnall's blue-sensitive pigment (frog).

Intra-retinal slow-wave activity could be compared with the ganglion cell impulse activity because it was simultaneously recorded over the same microelectrode. Under both dark- and light-adapted conditions there was a curvilinear relation between slow-wave amplitude and the light intensity, with the maximum slow-wave at an intermediate light intensity. In some cases the slow-wave spectral sensitivity curve agreed with the curve obtained from the spike data, especially when these approximated the rhodopsin and iodopsin absorption curves. However, there was a consistent lack of agreement between them under light-adapted conditions; here the slow-wave curves tended to have a higher sensitivity to blue light. In those cases where spectral sensitivity of the slow-wave activity did not precisely agree with that of the spike activity, the spike activity presumably comes from a single ganglion cell while the slow-wave activity presumably comes from a somewhat larger population.

It can be concluded that the slow-wave activity recorded in the vicinity of retinal ganglion cells has functional characteristics (spectral sensitivity curves) which are partially dependent on the particular retinal location, since the spectral sensitivity curves of the slow-waves tend to be like those of the ganglion cell in that region.

3. The cat medulla

Analysis of data obtained in the region of the cochlear nucleus of nearly 50 cats is nearing conclusion. Most of these animals were entirely normal and unanesthetized when the observations were made. The results can be summarized under two headings:

Auditory units. The stimulus-response characteristics of single auditory neurons in the cochlear nucleus in anesthetized preparations have by now been reported by several investigators. These include the fact that tones inhibit neuron activity as well as enhance it, and that the cochlea projects in an orderly way into the several subdivisions of the nuclear-mass.

The unanesthetized animal displays these same response features. Units isolated in both dorsal and ventral cochlear nucleus show spontaneous activity, response areas, inhibitory areas, adaptation, and tonotopic localization, and no characteristics clearly distinguishing them from those isolated in anesthetized animals have emerged in the analysis thus far.

Vestibular units. Previous study by others on primary and secondary afferent neurons from the vestibular system of anesthetized cats have defined several classes of activity associated with movements of the head in the three planes. These include inhibition as well as excitation correlated with movement of the head in a particular direction. These results have been fully confirmed in the awake cat. Once again, therefore, barbiturate anesthesia seems to have little or no influence on the afferent input to the brain provided by receptors discharging through the eighth cranial nerve.

During the course of these studies special attention was devoted to a group of neurons apparently not previously studied in cats. Each of these elements discharges for hours at a remarkably stable rate (varying between approximately 10 and 100 discharges per sec. depending on the unit). Each is sensitive to head movement, briefly altering its rate by perhaps 1%, but after the stimulus it promptly returns to its original stable value. The end-organ giving origin to these units has not been identified, but they may originate in the macula which in the dogfish activates its neurons similarly.

About 100 of these stable units have been encountered. In several instances a group of them were isolated one after another as the microelectrode penetrated more deeply into the medulla. Each unit in such a series exhibited a different rate, and, furthermore, the rates varied in an orderly way from beginning to end of the series. This suggests that the end-organ from which they originate projects in some orderly way into the medulla (as does the cochlea), though what significance this could have for vestibular function remains unclear.

4. Primary auditory neurons

Several fundamental questions about the hearing process can be answered only with an adequate knowledge of the discharge characteristics of

primary auditory neurons. Available reports on cat and guinea pig--under anesthesia--leave much to be desired. The purpose of the study to be summarized here was to obtain from unanesthetized cats a body of data from which generalizations could legitimately be made.

The technical problems attending such a study have been discussed in previous reports, and their solution has made the present research possible. The hydraulic microelectrode advancer screwed to a device implanted in the cat's skull over the cerebellum carried a tungsten-wire electrode sharpened at the tip that provided the input to conventional amplifying and recording devices. The electrode was advanced through the medulla toward the internal meatus on the opposite side of the head and, in successful punctures, it penetrated into the eighth nerve. Reconstruction of the electrode track from brain sections postmortem established in every case whether contact had actually been made with primary eighth nerve fibers.

It has been claimed by other investigators that primary auditory neurons show only excitation, never inhibition, to acoustic stimuli. Our evidence does not support this statement, for extensive study of each of at least 5 such units with a wide range of stimuli reveals both excitation and inhibition can occur. The spontaneous activity and response areas of these units cannot be readily distinguished from those of cochlear nucleus neurons, and the same can be said of the tones that inhibit their activity. When they are already activated by one tonal stimulus, a second sound--tone or noise--can abolish their response, again as is true for neurons at higher levels in the acoustic tracts. If demonstration of both excitation and inhibition in a neuron reflects synaptic events at a more peripheral level, it must be concluded from these facts that the complex neurology at the basilar membrane level provides significant processing of auditory input information. The auditory nerve evidently conveys the mechanical analysis achieved at the basilar membrane level toward the brain in terms of both increase and decrease in its neural activity.

Whether the observed inhibition of the primary auditory neurons would occur in the absence of the efferent bundle of Rasmussen has not been ascertained, for no animals with the olivocochlear bundle cut were included in the series.

Two additional observations are worth mentioning here. It has proved possible by careful analysis of the click-evoked slow waves recorded by the advancing microelectrode to predict with good accuracy the position of the microelectrode tip in the brain substance. When the tip lies among auditory nerve fibers within the internal meatus the recorded slow wave is virtually identical with the response at the round window membrane. When it lies more centrally--in the cochlear nucleus--the latency to the first large deflection recorded is greater than that at the round window, and this discrepancy progressively increases as the tip lies farther away from the internal meatus. These facts can be of considerable assistance in estimating electrode location during an actual experimental penetration.

Secondly, units with novel characteristics not previously described

have been encountered. One such unit, spontaneously active, reacted to a steady pure tone by promptly discharging at a higher rate, then slowly dropping its rate to zero. Such enhancement followed by complete inhibition of activity over the course of a minute or two clearly illustrates the complexity of neural reaction to a steady tonal stimulus. The mechanism of this set of events remains to be worked out.

5. The auditory cortex. No new experiments on units in the auditory cortex were performed during the past year. A summary of the data previously obtained is in preparation for publication.

B. The EEG and Behavior. The method of simultaneously recording EEG and behavioral responses from cats and monkeys continues to provide information on brain activity associated with learned and unlearned reactions. No significant new developments can be reported in either the methods of implanting the electrodes or of analyzing the data recorded from them. The studies under way can be summarized under three main headings.

1. EEG, eye movements and behavioral sleep in monkeys

During the past several years the EEG patterns in sleep have come once again under intensive study in this laboratory (see other sections of this report) and elsewhere. The appearance of an EEG pattern virtually indistinguishable from that seen in the awake state has been reported in man when dreaming, and in cats when presumably dreaming. A consistent finding in both these situations is rapid eyeball movement. The occurrence in rhesus macaque of an exactly similar correlation between eyeball movement, behavioral sleep, and an EEG record characteristic of the waking state has been demonstrated during the past year.

During a total of 8 hours of sleep in 6 monkeys, rapid eye movements were seen 16% of the time. A total of 19 such periods occurred, each lasting from 1.5 to 8 min, the average being 4 min. The EEG pattern consistently assumed the pattern of low voltage, fast frequency activity during these rapid eye movement periods. The rhesus macaque can therefore serve as a useful experimental animal for the study of these sleep, eye movement and EEG patterns.

2. EEG and Behavioral analysis of differential reinforcement of low rates (DRL) and avoidance training in Monkeys

Two monkeys implanted with chronic sub-cortical electrodes (for EEG recording) and trained in both DRL and avoidance behavior have been intensively studied to see whether characteristic EEG changes accompany the various behavioral states displayed. Spontaneous movements were also recorded--rapid head movement in one monkey, and a tongue-licking response in the other.

Analysis of the data obtained thus far strongly suggests that the tongue-licking and head movement responses are specifically related to the animal's DRL behavior. They are rhythmic and precise during the DRL period,

erratic or absent during the avoidance and rest periods. When the DRL behavior is eliminated by amphetamine, the motor responses described also disappear.

EEG analysis to date reveals disappointingly minimal differences between the DRL, avoidance or rest periods. Occasionally during a rest period high voltage slow waves associated with drowsiness appear for brief periods but these are not sustained nor do they appear during DRL or avoidance behavior. Further analysis of the EEG and behavioral data is in progress.

2. EEG studies of conditioning

Most of the past year has been spent in analysis of previously recorded data and the preparation of final reports for publication.

C. Physiological Basis of Pain Perception

The behavioral method for study of pain in monkeys (described in the previous progress report) has been used in several studies during the past year. With it shocks of increasing intensity are applied to the gasserian ganglion of an alert monkey via implanted electrodes. The animal reduces the intensity of the shock by pressing a lever, thereby regulating and maintaining a level of tolerated intensity (presumably his 'threshold'), which can be raised or lowered by changing the duration of the pulses. Drugs can be administered via a cardiac catheter. The fact that morphine (0.5 mg/kg) induces the animal to find and maintain a higher level of tolerated intensity has been confirmed. Chlorpromazine and Pentobarbital have been found to abolish level-maintenance behavior and to produce a marked increase in the variability of the animal's responses. Methamphetamine and Procaine by contrast produced both a significant decrease in variability and a small rise of the maintained level.

That the method will prove useful in the study of the anatomical and pharmacological basis of pain perception in animals seems confirmed beyond doubt by our data. Several industrial and university laboratories have, after consultations and visits, begun to employ it successfully in the search for new analgesic drugs and in the study of the pathways involved in pain perception. The results thus far obtained from about a dozen animals in this laboratory are being written up for publication.

D. Studies of the Electrorétinogram

The effort to develop more information about the retinal processes by study of the ERG can be summarized under two headings as follows:

1. Human electroretinogram: An investigation of the effects of colored pre-exposures upon subsequent dark adaptation has been completed. Electroretinograms were recorded in response to chromatic test flashes presented at

the rate of once every second for a period of two minutes after the cessation of the pre-exposures. The sensitivity of the ERG increased more rapidly during dark adaptation at 500 mu than in other parts of the spectrum. Strong selective effects depending upon the color of pre-exposure were found in various portions of the spectrum. In addition to the scotopic and red photopic components, analysis suggested at least one additional photopic component.

Another experiment, now in progress and partially completed, is concerned with the effects of stimulus duration upon spectral sensitivity. Stimulus duration from 10 to 100 milliseconds have been used. The spectral sensitivity curves have exhibited the two prominent components demonstrated in previous work: the scotopic process and a red process with a maximum in the neighborhood of 600 mu. The influence of increased stimulus duration at the short wavelength end of the spectrum has been to increase response sensitivity. In the long wavelength region, however, response sensitivity has been highest for the shortest stimulus duration. A preliminary report of these findings has been presented.

2. Turtle electroretinogram: Work on the electrical responses of the turtle's visual system has been continued. The results, to date, show that although these animals possess nearly pure cone retinas, the electrical responses of their eyes show characteristics that have been associated with dual structure eyes. In agreement with other investigators the dark adapted electroretinogram shows a well-defined response consisting of an a- and b-wave, the front leg of the b-wave being rippled when stimulated by white- and red-light. Under light adaptation, however, the response becomes smaller and sharper showing shorter implicit times and latencies. It would appear that some of the electroretinogram characteristics that are thought to differentiate between rod and cone structures in duplex eyes may be a result of something other than receptor structure. These differences may be interpreted to occur as a result of the action of different cone pigments or as a result of a complex neural interaction in the light- and dark-adapted eye.

The results are being prepared for publication.

E. Computer Analysis of Brain Responses

Evoked potentials are often difficult to detect under conventional recording conditions because of the presence of high amplitude interference arising from tissue not under investigation. This research has been directed toward developing equipment for recording and studying the small-amplitude electroretinograms and evoked cortical potentials in normal and clinical subjects.

Equipment Development. An analog computer system, in part previously described, has been devised for recording the potentials in question. It divides the time intervals between consecutive stimulations into 19 segments and integrates the wave form lying under each of these segments for an entire

series of responses. Each of these integrals is approximately equal to an ordinate along the average response curve. The average wave form is now based upon 12 ordinates along the response wave form. An ink writer attached to the output of the computer provides an immediate picture of the response wave form. In addition, the ink-writer may be made to produce a figure which enables the experimenter to judge whether a consistent response wave is being recorded.

Electroretinogram Recording. Using the computer it has been found possible to record electroretinograms as well as $\frac{1}{2}$ μ V with normal human subjects. Conventional recording methods do not resolve electroretinograms below 20 μ V even under favorable conditions. Several properties of the low-amplitude electroretinograms have been ascertained. For instance, the sensitivity of the electroretinogram is related in a regular way to the area of a stimulus producing it. In addition, the photopic electroretinogram varies in amplitude depending upon the position of the stimulus on the retina, the largest responses being obtained when the stimulus is centered on the fovea. A useful clinical application has been demonstrated in patients with retinitis pigmentosa in whom responses are not detectable when conventional recording procedures are used; the computer has shown that such patients do have a small response which is buried in various kinds of interference.

Evoked Cortical Responses. The computer readily detects visual potentials evoked from the human brain through external scalp electrodes. The response wave form has been found to depend upon a number of variables. In general it is more complex with intense stimuli than with weak ones. At low luminances the amplitude of the response increases regularly with increase in stimulus luminance. At higher luminance the response-amplitude reaches a plateau and the waveform becomes more complicated. The latency of the response decreases as stimulus luminance is increased. Large field diameters produce responses when small ones do not, under the conditions so far investigated. Responses have been detected using flicker rates from 1 to 20 flashes per sec (higher flicker rates have not been explored). When the stimulus field is less than 15° in diameter, evoked cortical potentials may be detected with stimuli of lower luminance than those which are required for a measurable electroretinogram.

Briefly summarized, the computer analysis of biological potentials upon which the laboratory embarked two years ago has succeeded in revealing several events that hitherto have defied analysis.

F. Brain Stimulation and Cardiovascular Conditioning

Experiments have been begun aimed at defining the neurophysiological correlates of cardiovascular conditional reflexes induced by stimulation of brain areas which produce cardiovascular changes such as slowing or acceleration of heart rate. Electrodes have been implanted stereotactically in the anterior and posterior hypothalamus, in the hippocampus, caudate nucleus, cingulate gyrus, central gray periventricular area, and the parvocellular reticulum in several cats.

Electrical stimulation in the anterior hypothalamus (which produces cardiac slowing) and in the posterior hypothalamus (cardiac acceleration) were paired with conditional stimuli (tones of 200 and 3000 cps respectively). Successful cardiac conditional reflexes with the unconditional stimulus in the posterior hypothalamus has been obtained in two cats, and in the anterior hypothalamus in two other cats. Conditioned tachycardia (posterior hypothalamus) seems to be much the more stable of the two and is established earlier. Stimulation of anterior hypothalamus in one cat produced extrasystoles and bizarre EEG patterns, but these EKG changes could not be successfully conditioned even after 350 repetitions of the unconditional stimulus.

Although they do not involve brain stimulation, it will be convenient to describe here two related studies on cardiovascular conditional reflexes. One of these used classical Pavlovian techniques in both normal subjects and a case of postural hypotension (Bradbury-Eggleston syndrome). BCG, EKG, heart rate, blood pressure, right and left finger pulse volume and respiration were recorded simultaneously and continuously in these people.

Of all the cardiovascular conditional reflexes studied the finger pulse volume was the most sensitive and easiest to obtain. In the patient with postural hypotension cardiovascular conditional reflexes were difficult to establish but they did appear after 20 or more reinforcements. Normals usually showed cardiovascular conditional reflexes after 3 reinforcements.

A second study aimed at conditioning extrasystoles in normal subjects. Twenty-eight normal volunteers were first examined to determine the frequency of extrasystoles during forced respiratory maneuvers. Five developed rare extrasystoles and 2 displayed them frequently. In these 2 individuals a conditional visual signal was paired with the forced respiratory maneuver which produced the extrasystoles. After 14 presentations the conditional signal alone was able to produce extrasystoles in the absence of the respiratory maneuver.

SUMMARY AND CONCLUSIONS:

Continued study of single unit responses has yielded several new facts about brain function. The responses from the simple "visceral brain" of the sea-hare are of particular interest. Consisting of only some 200 cells, it displays spontaneous integrated patterns of activity resembling that of far more complicated structures. Because of the large size of the cells, furthermore, several units can be simultaneously penetrated, thus permitting any reciprocal interactions between them to be reliably established. Fundamental questions regarding synaptic actions (excitation and inhibition), facilitation and neuronal organization have been explored in, and partly illuminated by these studies.

The demonstration in awake cats of inhibition in primary auditory nerve fibers, and of vestibular units relatively insensitive to head movements, are additional useful facts. In response to a continuous pure tone, the cochlea evidently delivers to the medulla via the auditory nerve a complex pattern

showing both enhanced and reduced nerve activity; previously only enhancement had been recognized. Otherwise, the experiments on primary and secondary sensory neurons summarized here demonstrate that observations on single neuron responses in anesthetized animals can safely be extrapolated --in a general way at least--to animals in the alert, awake state.

The demonstration of a correlation in monkey between eye-movements, behavioral sleep and an EEG record like that of the waking state adds another species to those already known (cat, man) to display this phenomenon. In man these events are claimed to be positively correlated with dreaming. If this be true, further experiments on the monkey may reveal some of the mechanisms operating in the dreaming state.

The studies on pain reported here modestly extend the information available a year ago. It is clear that no single "threshold" of pain exists. At one point in time a monkey will tolerate shocks at a very high level compared with his "threshold" a few minutes later. There is reason to believe these shifts in tolerance occur spontaneously as well as under the influence of drugs, and these, it is hoped, will be carefully examined in future studies.

A year of experimentation with the average response computer as a method for detecting very weak biological signals in the presence of much biological noise has confirmed most of the hopes and expectations for it recounted in the previous progress report. Threshold light flashes clearly evoke retinal and cortical responses, facts not uncovered until the computer became available. The demonstration of a minute ERG in patients with retinitis pigmentosa--contrary to what had previously been believed--raises the possibility of useful application of the technique to this and similar clinical problems.

The experiments combining brain shocks with conditional stimuli in an attempt to condition heart rate represent a new series of studies for this laboratory. Conditioned autonomic responses represent a class of behavioral reactions much neglected by investigators in this country. While we have few data as yet, future developments in this area of autonomic conditioning are anticipated.

PUBLICATIONS:

1. SIMMONS, F. BLAIR, ROBERT GALAMBOS, and JAMES F. ALBRITTE. Serial studies of the onset and progression of drug-induced cochlear damage in cats: A new electrophysiologic method. Arch. Otol. 1960, 72: 233-239.
2. GROSSMAN, ROBERT G. and ROBERT J. GUMNIT. A method of recording from the cortex while maintaining physiological conditions. EEG Clin. Neurophysiol. 1960, 12: 920-921.
3. WEITZMAN, E. D., G. S. ROSS, W. HODOS, and R. GALAMBOS. Behavioral method for study of pain in the monkey. Science, 1961; 133: 37-38.
4. HUBEL, DAVID H. Electroencephalograms in cats during natural sleep. Arch. Ital. Biol. 1960, 98: 171-181.

5. GALAMBOS, ROBERT. Some neural correlates of conditioning and learning. Chap. 6, pp. 120-132, In: Electrical studies on the Unanesthetized Brain. Hoeber, 1960.
6. GROSSMAN, ROBERT G. Effect of strychnine on components of evoked potentials in the visual cortex. J. Pharmacol. & Exp. Therap. (in press).
7. MARSH, JAMES T., DUNCAN A. MCCARTHY, GUY SHEATZ and ROBERT GALAMBOS. Amplitude changes in evoked auditory potentials during habituation and conditioning. EEG. Clin. Neurophysiol. (in press).
3. GALAMBOS, ROBERT, RONALD E. MYERS, GUY C. SHEATZ. Extralemniscal activation of auditory cortex in cat. Am. J. Physiol. 1961, 200: 23-28.
9. GALAMBOS, ROBERT. Neurophysiological studies on learning and motivation. Fed. Proc. (in press).
10. MOUSHEGIAN, GEORGE; ALLEN RUPERT, JAMES T. MARSH and ROBERT GALAMBOS. Evoked cortical potentials in the absence of middle ear muscles. Science, 1961, 133: 582-583.
11. SIMMONS, F. BLAIR. Post-tetanic potentiation in the middle-ear-muscle acoustic reflex. J. Acous. Soc. Am. (in press).
12. WEITZMAN, ELLIOT D. and GILBERT S. ROSS. A behavioral method to study pain perception in monkey--effects of some pharmacological agents. For presentation at Amer. Acad. Neurology meetings, Detroit, April 27-29, 1961.
13. GALAMBOS, ROBERT. A glia-neural theory of brain function. Proc. Nat. Acad. Science, 1961, 47: 129-136.
14. STRUMWASSER, FELIX. Modes of synaptic operation and their relevance for pattern formation in an integrative ganglion. Fed. Proc. 1961, 20: 338.
15. GALAMBOS, ROBERT And GUY C. SHEATZ. Electrical responses evoked in the brain of the normal animal. Fed. Proc. 1961, 20: 326.
16. GUMNIT, ROBERT J. and ROBERT G. GROSSMAN. Potentials evoked by sound in the auditory cortex of the cat. EEG Clin. Neurophysiol. (in press).
17. GROSSMAN, R. G. and VIERNSTEIN, L. J. Discharge patterns of neurons in the cochlear nucleus. Science (in press).
18. ROBINSON, R. E., S. L. MARVIN, W. M. THOMPSON jr., and J. F. DAMMAN. An accurate method for indirect determination of systolic and diastolic blood pressures. Circulation Research. (submitted to).
19. WEITZMAN, ELLIOT D. A note on the EEG and eye movements during behavioral sleep in monkeys. EEG Clin. Neurophysiol. (submitted to).

20. GALAMBOS, ROBERT. Changing concepts of the learning mechanism. CIOMS 1959 Symposium on Brain Mechanisms and Learning, Montevideo (in press).
21. GALAMBOS, ROBERT. Studies of the auditory system with implanted electrodes. Proceedings of Conference on Neural mechanisms of auditory and vestibular mechanisms. Arthur Thomas, publisher (in press).
22. CHAPMAN, ROBERT M. Spectral sensitivity of single units recorded in bullfrog retina. J. Opt. Soc. Am., 1960, 50, 513.
23. CHAPMAN, ROBERT M. Spectral sensitivity of single neural units in the bullfrog retina. Submitted to J. Opt. Soc. Am., 1960.
24. CHAPMAN, ROBERT M. Spectral sensitivities of neural impulses and slow-waves in the frog eye. Paper read at Eastern Psych. Assoc. Meeting, 1961.
25. ARMINGTON, J. C., DONALD I. TEPAS, WALTER J. KROPFL and WERNER H. HENGST. Summated Retinal Potential. Vision Research Reports, National Academy of Sciences-National Research Council, Washington, Publication 835, 1960, 112-130.
26. KROPFL, WALTER J. and ROBERT E. ROBINSON. An analogue computer for separating evoked physiological potentials from background noise. Inst. Radio Engineering, 4th Nat. Convention on Military Electronics, Washington, D.C. June 27-29, 1960.
27. ARMINGTON, JOHN C., PETER GOURAS, DONALD I. TEPAS and RALPH GUNKEL. Detection of the ERG in Retinitis Pigmentosa. Experimental Eye Research (in press).
28. TEPAS, DONALD I. and JOHN C. ARMINGTON. Some characteristics of photically evoked responses in the human cortex. Eastern Psychological Association, Philadelphia, Penna., April 7-8, 1961.
29. ARMINGTON, JOHN C., DONALD I. TEPAS, WALTER J. KROPFL and WERNER H. HENGST. Summation of Retinal Potentials. To appear in the J. Opt. Soc. Am., July 1961.
30. GRANDA, A. M. and W. R. BIERSDORF. Effects of stimulus duration upon spectral sensitivity of the human electroretinogram. J. Opt. Soc. Am. 1961, 51: 475 (abstract).
31. BIERSDORF, W. R. and J. C. ARMINGTON. The effects of chromatic pre-exposure upon dark adaptation of the human electroretinogram. (Submitted to J. Exp. Psychol.).

ANNUAL PROGRESS REPORT

Project 6X6J-1J-001: Neuropsychiatry and Stress

Task 6: Measurement and Analysis of Psychological Mechanisms and of their Modification by Drugs, Sleep Deprivation, Brain Injury, and Other Agents

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Department of Clinical and Social Psychology
Division of Neuropsychiatry

Period Covered by Report: 1 July 1960 through 30 June 1961

Principal Investigators: Major Harold L. Williams, MSC
Murray Glanzer, PhD*
Ardie Lubin, PhD
Martin D. S. Braine, PhD
William R. Biersdorf, PhD
Allen M. Granda, PhD

Assistants: Lt. John T. Hammack, MSC
Sonoko Ohwaki
D. J. Kozil

Reports Control Symbol: MEDDH-288

Security Classification: Unclassified

*University of Maryland Medical School, Baltimore, Md.

ABSTRACT

Project No: 6X60-10-001 Neuropsychiatry and Stress

Task No. 6: Measurement and Analysis of Psychological Mechanisms and of their Modification by Drugs, Sleep Deprivation, Brain Injury, and Other Agents

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Period Covered by Report: 1 July 1960 through 30 June 1961

Authors: Major Harold L. Williams, MSC
Murray Glanzer, PhD
Ardie Lubin, PhD
Martin D. S. Braine, PhD
William R. Biersdorf, PhD
Allen M. Granda, PhD

Reports Control Symbol: MEDDH-288

Security Classification: Unclassified

The study of impaired performance with sleep deprivation has led to principles for the measurement of performance decrement in many kinds of stress and with brain injury. By changing test procedures, decrement can be minimized or maximized. It appears that these same principles can be used in a kind of systems analysis for the individual subject to identify the psychological operations which are impaired. A series of studies of human problem solving developed measures of storage load and operational load which can be independently varied. Rather complex performance can be elicited from human subjects during periods of EEG sleep. This seems to require re-evaluation of the EEG as an index of the sleep wakefulness continuum. Studies of human data-processing have been extended to children and several experiments have been done on the development of language and thought in children from 4 to 8 years of age. Research has continued on the theory of measurement and the development of new methods of statistical analysis.

Project No: 6X60-10-001 Neuropsychiatry (M.I. Gross)

Task No. 6: Measurement and Analysis of Psychological Mechanisms and of their Modification by Drugs, Sleep Deprivation, Brain Injury and Other Agents

Description: This task has two purposes: (a) to develop methods for the analysis of behavior, particularly vigilance, data-processing and problem solving; (b) to apply techniques developed in this analysis to studies of the effects of brain injury, sleep loss and other stress. The analysis involves both behavioral and physiological measurement, and studies have been completed of normal subjects, sleep-deprived and brain-injured subjects. A series of inter-related projects have been carried out analyzing the effects of information load, uncertainty and monotony on human data processing. Investigation of methods for optimal scoring of tests has continued.

Progress:

- a. Studies of Decision-Making; problem-solving and data processing; concept formation; storage of verbal material; verbal learning; cognitive processes in language learning; studies of size and depth perception.

Concept Formation: The purpose of the work in this area was to determine the factors that determine efficiency in this type of problem solving. Last year's work showed that two main mechanisms were used by the subject in working concept problems - dimension selection and storage. This year's work developed the details of the working of these mechanisms. The following experiments furnished this information.

1. Perceptual encodability: In order to specify the processing load placed on the subject by the examples that make up a concept problem, we carried out a calibration experiment on these examples. We measured the accuracy with which the subjects could perceive them under short exposure (1/2 sec.). This information was used in the subsequent concept experiments and also is being analyzed to discover what specific factors determine perceptual difficulty. Standard information measures can be used to measure the perceptual difficulty of the examples. More adequate measures are being developed, however, on the basis of complexity of the code required to specify the example. These encodability measures require the consideration of figure-ground relations.

2. Information load on storage and selection: Using the information from Experiment 1, we devised a technique for manipulating the information load on the two functions - storage and selection - carried out by the subject in the course of solving concept problems. It was demonstrated clearly that load on either storage or selection reduced efficiency in a highly regular fashion.

3. Reduction rate and information load: Two experiments were carried out to determine the effect of reduction rate - the rate at which successive examples eliminate irrelevant dimensions - and information load. (The two experiments differed in the type of presentation. One used origin-based examples; the other used predecessor-based examples). It was clearly demonstrated that as the reduction rate increases, the efficiency of the concept work decreases. This effect, however, had a significant interaction with the information load on the storage and selection functions. As the information load on either increases, the effect of reduction rate becomes more marked.

4. Effects of spacing: A series of four experiments were carried out to determine the effect of rest pauses between successive concept examples on the efficiency of the concept work. The first experiment indicated that a rest in the series improved efficiency. The subsequent experiments showed that this effect was present only when the reduction rate is high, i.e., when the overall information-processing requirement for the subject is high.

5. Effects of concept size with new materials: Earlier experiments had indicated a curvilinear relation between concept size and efficiency. Problems that required the subject to discover four relevant dimensions out of eight were significantly more difficult than problems with either two relevant dimensions out of eight, or six relevant dimensions out of eight. The relation was interpreted as following from the differences in information load on the selection function. These results were checked with a new set of materials, using one, two, and three relevant dimensions out of four. The same curvilinearity was found, with 2-dimension problems generally being more difficult than either one or three. This experiment supports the generality of the findings for different materials and for different numbers of dimensions.

Three reports were issued, covering the earlier work on the project. Work has begun relating characteristics of the performance on the concept problems to EEG measures.

Storage of Verbal Material: A general model for free recall was constructed which postulated two storage mechanisms: short-term and long-term storage. These mechanisms were postulated to determine serial position effects in free recall.

1. Free recall : Effect of presentation rate and delay: On the basis of the model, it was predicted that increasing rate of presentation should lower the beginning peak, while delay of recall should lower the end peak of the serial position curve. An experiment, carried out to test these predictions, supported them.

2. Free recall mechanisms: Effect of repetition of items: Using the same model, another prediction - that repetition of list items should raise primarily the beginning peak of the curve - was tested and supported.

Verbal Learning:

1. Re-examination of the serial position effect: A series of experiments were carried out to specify the determinants of the serial position effect in rote learning. Three characteristics were considered: Primacy-recency, space between end and beginning of the list, and association break. The experiments indicated that once the multiplicative effect of total number of errors is held constant, the major factor is spacing. Contrary to earlier views, an increase in spacing results in an increase in the serial position effect. A report was issued earlier this year covering the first three experiments in the series. A fourth experiment, replicating and extending the earlier work, is now in progress.

2. Grammatical categories in verbal learning: An experiment was carried out to determine whether there are differences in the difficulty of learning words from various grammatical categories. Paired associate learning (both English word to nonsense syllable and nonsense syllable to English word) was used. A highly reliable ranking of difficulty of grammatical categories was found. An experiment was also carried out on word association to words from these grammatical categories, measuring latency, variety of associations, etc. Significant correlations between the word association measures and the ranking of categories obtained in the paired associates experiment were found.

Cognitive Processes in Language Learning: Parallel investigations of two aspects of language learning are being carried out. The focus of one line of investigation is on grammatical structure; here the main goal is to try to identify the cognitive processes involved in children's learning of the grammatical structure (especially rules governing the order of words in a sentence) of language. The other line of investigation is concerned with the semantic aspect of language development; it assumes that what a child means or understands by many words will to a great extent depend on his level of conceptual or intellectual development, and the ultimate goal of the work is to describe some of these conceptual correlates of language behavior.

The learning of word-order and grammatical structure is being studied partly through experiments on the learning of miniature artificial languages. The words in these "languages" are nonsense syllables and the grammatical rules are determined in advance. The procedure is to present the child subjects with part of a sentence of the language and ask them to choose a word or phrase from an array to complete the sentence, correct completion earning a reward. Five such experiments have been carried out, and the results suggest that what is learned is an association of particular words and phrases with particular words and phrases with particular temporal positions in a sentence (5). The data also indicate that subjects define

temporal position in a digital, rather than ordinal, manner, i.e., they tend to hear a sentence as divided into a first and a last part, and then each part as divided into a first and a last part.

The work with the artificial languages suggested that it would be instructive to gather detailed data on the way in which English grammatical structure develops in very young children. Using tape recordings and written records kept by the mother, a continuous sampling of the word-combinations of three children between 18 and 30 months of age is being obtained (this work is being undertaken through the Washington School of Psychiatry). Initial data suggest, as expected, that the first English word combinations of children have a quasi-grammatical structure from the beginning.

The investigation of conceptual correlates of language development has evolved from earlier work (3, 4) which examined some of Piaget's views of intellectual development. The main focus so far has been on certain relational concepts, particularly concepts of size. For the older child size concepts enter into a network of logical relationships (e.g., that of two objects, one cannot be both larger and smaller than the other; that for any objects $A, B, C, A > B, B > C, \therefore A > C$; that lengths are additive), upon which measurement is contingent. These relationships are probably not understood by the very young child, despite the fact that he may correctly use words like "bigger" or "baby one" to indicate size relationships he can see clearly. Both previous work (3), and a priori considerations, suggest that an important conceptual correlate of this kind of development may be an ability to distinguish between "real" and "apparent" properties of objects. The child, for instance, who can distinguish "looks bigger" from "is really bigger" localizes, as-it-were, the size-attribute in the object and not just globally in the stimulus-field. In the experimental procedures used so far a child is confronted with situations in which an object is made to appear successively larger and smaller, than another object (by the use of a lens, or of well-known visual illusions); how quickly he can learn to ignore the illusory, and respond to the real, attributes of the objects is explored. Later experiments, with attributes other than size, will explore the generality of this developing conceptual distinction between a "real" and a "phenomenal" world.

Studies of Size and Depth Perception: During the past year, research has been completed which shows that judgments of apparent size are strongly affected by instructions to the subjects as well as by certain physiological variables under a reduction (dark-room) environment. With "visual angle" (analytic) instructions, size judgments are correlated with the functioning of the oculomotor adjustments of accommodation and convergence. Pupil diameter does not appear to be an important variable. A four-meter blacked-out "visual tunnel" is now under construction to further reduce stimulus cues from the size matching targets within the tunnel. With this tunnel, research is planned to further isolate the critical correlate of the size judgments: whether accommodation, convergence, or apparent distance.

b. Studies of Sleep Deprivation and Brain Injury.

Psychological and Physiological Effects of Sleep Deprivation: Experimental studies using sleep deprivation were continued with research on sleep as a reinforcing variable. Subjects who had gone about 36 hours without sleep were paid sleep for work. Results suggest that sleep can be used to control performance. The analysis of a large amount of data gathered on 70 sleep deprived subjects in 1959 is virtually complete and several research reports are in preparation. The preliminary results reported in June 1960 are confirmed; increasing the information load on the task produces greater decrement during sleep loss. This decrement is primarily due to slowing of cognitive operations; the ability to store and retrieve information is impaired and this deficit appears in immediate storage rather than long-range recall. A subject can retrieve information learned just prior to sleep deprivation. He has difficulty learning and recalling new information, but whatever is learned shows no further decrement 24 hours later in the period of wakefulness; during sleep deprivation the EEG can be used to signal poor performance. Missed signals on a vigilance task are generally preceded by slow waves (theta). Correct responses are preceded by alpha or faster rhythms. The correlation between these EEG changes and performance on a vigilance task increases with increasing sleep deprivation. The "lead time" for the EEG also increases. After one night of sleep loss, the second which just precedes the signal accounts for all of the valid variance. After two nights of sleep loss the minus two and minus three seconds contribute significantly to the prediction of performance. This relationship between EEG and performance on a vigilance task continues into the first recovery day but disappears before the end of the recovery period. For some subjects the correlation between the electrophysiological variable and reaction time is as high as .75. There are subjects, however, who show no such correlation. They perform vigilance tasks with high accuracy; even when the EEG record is composed primarily of rhythms in the theta (4 - 7 per second) range.

Performance During Sleep: The state of sleep has traditionally rested on several sets of criteria for its definition. Behavioral criteria of sleep as distinguished from neurophysiological ones have referred to generalized patterns of activity such as lack of control and coordination, minimal movement of body parts, and absence of organized activity. Neurophysiological criteria, on the other hand, have been more definite and objective. EEG records, for example, follow quite regular patterns purporting to reflect a behavioral continuum from excited emotion to deep sleep or coma. These patterns have been classified into stages and good correlation of these electrophysiological changes to behavioral observation have been claimed by some investigators.

Two kinds of experiments indicate that some subjects can learn to perform rather complex behavior during sleep. Operant conditioning techniques were employed utilizing shock avoidance and a schedule of fixed ratio reinforcement whereby the subject was freed from all commitments for a specified time period and allowed to sleep.

The results revealed that all five subjects employed in the experiments showed an EEG sleep record and learned to respond appropriately on both microswitches without returning to the EEG waking state. Moreover, the data indicated that patterned activity could occur when the subject was in any EEG sleep stage. These observations have been interpreted to mean that "higher mental processes" operate at some level of the nervous system even during deep sleep.

These results have been reported to the 1961 Eastern Psychological Association meetings.

In a second experiment the subject was presented with one of two auditory signals every three minutes during a normal night's sleep. One of these signals was defined as "critical". If the subject failed to turn it off with a switch taped to his hand, he received a series of electric shocks and a very loud buzzer which served to bring him sharply awake. The other auditory signal was regarded as neutral. The subject was told that he was to try to make the necessary discrimination and responses during sleep. Preliminary results show that some subjects are able to perform correctly while in any stage of EEG sleep. Subjects who perform best seem to be rather tense individuals who describe themselves as very light sleepers. Subjects who are very difficult to arouse have not learned the required behavior in three nights of practice.

Studies of Brain Injury: The experimental analysis of performance under stress has led to a set of general principles for the measurement of performance decrement and these are being applied to the study of brain injured patients. The principles, most of which have been stated in previous reports, are a framework for a systems analysis of the individual patient. It is possible to ask whether decrement in a general performance test is due to changes in accuracy or to slowing. Further, one can discover whether the change in performance is related to perceptual, storage, motor, or cognitive impairment. Several patients with gross brain damage due to head injury have now been put through a sequence of tests designed to ascertain the source of performance decrement. Data are still being analyzed.

c. Exploration of Optimal Test Scoring Methods and Contributions to the Theory of Tests and Measurement.

1. (1959) Peut-on appeler les tests non-parametriques, test independant de la distribution? (Are non-parametric tests distribution-free?) Bull. du Centre D'Etude et Recherches Psychotechniques, 8, 129-134.

Most non-parametric rank-order tests such as the Kruskal-Wallis H test, Friedman's test for differences in correlated means, the Mann-Whitney tests, etc., make the "homonomous" assumption that samples are drawn from identical population distribution. Therefore, when heterogeneity of variance, differences in skewness, etc., are found, these non-parametric tests may not be appropriate substitutes for the t test or F ratio. Strictly speaking, they should only be used with identical non-normal populations. However, if the population distributions are symmetric, the H test is probably a sensitive test of the difference in sample means. The term "distribution-free" should be reserved for those significance tests which do not assume that all populations are identical.

2. (1960) with H. G. Osburn. The use of configural analysis for the prediction of a qualitative criterion. Ed. Psychol. Measurement, 20, 275-282.

Given a qualitative attribute as criterion and qualitative predictors, how does one predict the qualitative criterion? The solution offered here is a polynomial equation which turns out to be a discontinuous case of Taylor's theorem. If there are t dichotomous predictors, then there will be 2^t terms in the polynomial predictor. The main effect of the t items are represented by t terms, the other terms represent item interactions.

3. (1960) Notes pour l'utilisation des fonctions discriminantes. Bull. du Centre D'Etudes et Recherches Psychotechniques, 9, 63-70.

A practical guide, short of worked examples, for those research workers who need to make use of discriminating functions. Four general situations are examined: The two-group case for one variable and for many variables; the multi-group case for one variable and for many variables.

The Rao "maximum likelihood" solution, which leads to a minimum number of misclassifications, is emphasized. G. W. Brown's similar solution for obtaining the minimum misclassification cost is described. Differential prediction procedures can be used when quota restrictions are imposed. Canonical variates are not the solution to problems of classification, unless there is only one significant canonical variate. Rao's maximum likelihood method of classification is better than or equal to any other actuarial or statistical method than can be devised using the criterion of minimum misclassification.

4. (1960) On the repeated-measurement design in biological experiments. Proceedings of the Fifth Conference on the Design of Experiments in Army Research Development and Testing, Report No. 602, pp. 123-132. Office of Ordnance Research, U. S. Army.

The two basic difficulties of repeated-measurement designs are:

- a. Any repeated measurements on the same organism will in general exhibit statistical dependence; therefore multivariate analysis of variance rather than univariate analysis of variance is appropriate;
- b. All standard cross-over designs assume that the carryover effect of a treatment on a succeeding treatment is constant and does not depend on the nature of the succeeding treatment, i.e., carryover is additive and does not interact with succeeding treatments.

The Geisser-Greenhouse approach brackets the significance level of F with the same amount of computation that is used in the usual univariate two-way analysis of variance. Since the computations for the multivariate A of V include the data for a two-way A of V , it always pays to try the Geisser-Greenhouse approach first.

Kendall's W or Jonckheere's rank-order coefficient can be used when the average rank-order rather than the difference between means is in question. The routine application of the usual two-way A of V is invalid unless the covariances between treatments are equal.

The study of impaired performance with sleep deprivation has led to principles for the measurement of performance decrement in many kinds of stress and with brain injury. By changing test procedures, decrement can be minimized or maximized. It appears that these same principles can be used in a kind of systems analysis for the individual subject to identify the psychological operations which are impaired. A series of studies of human problem solving developed measures of storage load and operational load which can be independently varied. Rather complex performance can be elicited from human subjects during periods of EEG sleep. This seems to require re-evaluation of the EEG as an index of the sleep wakefulness continuum. Studies of human data-processing have been extended to children and several experiments have been done on the development of language and thought in children from 4 to 8 years of age. Research has continued on the theory of measurement and the development of new methods of statistical analysis.

PUBLICATIONS

1. Biersdorf, W. R. The Utility of the Anterior lens Purkinje Image as a Measure of Accommodation. Amer. J. Optom., 1960, 37, 352-362.
2. Biersdorf, W. R., Ohwaki, Sonoko, and Kozil, D. J. The Effects of Instructions and Oculomotor Adjustments on Apparent Size. (Sub. to Amer. J. Psychol.)
3. Braine, M. D. S. The Ontogeny of Certain Logical Operations: Piaget's Formulation Examined by Nonverbal Methods. Psychol. Monogr., 1959, 73, No. 5.
4. Braine, M. D. S. Piaget on Reasoning: A Methodological Critique and Alternative Proposals. To be pub. with other papers presented at the SSRC Conference on the Development of Intellectual Processes, held in Boston, Spring 1960.
5. Braine, M. D. S. On Learning the Grammatical Order of Words in Sentences of Artificial and Natural Languages.. To be presented at the Amer. Psychological Assoc. meetings, September 1961.
6. Glanzer, M. Coding and Use of Information in Problem Solving. Progress Report, 1 Feb. 60 to 31 Jan. 61. Contract Number DA 49-007-MD-1004.
7. Lubin, A. and Williams H. L. Sleep Loss, Tremor, and the Conceptual Reticular Formation. Percept. mot. Skills, 1959, 9, 237-238.
8. Lubin, A., Giesecking, C. F. and Williams, H. L. Direct Measurement of Cognitive Deficit in Schizophrenia. J. consult Psychol. (In press)
9. Rubinstein, I. The Influence of One Stimulus on the Prediction of the Alternate Stimulus in Two-Choice Problems: A Supplementary Report. J. exp. Psychol. (In press)
10. Williams, H. L. Giesecking, C. F. and Lubin, A. Interaction of Brain Injury with Peripheral Vision and Set. J. consult. Psychol. (In press)

ANNUAL PROGRESS REPORT

Project 6X60-10-001 Neuropsychiatry and Stress

Task 7. The blood-brain barrier and the responses of cerebral tissues to injury

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

— Department of Neurophysiology,
Division of Neuropsychiatry

Period Covered by Report: January 1961 to June 1961

Principal Investigator: Capt. Lawrence C. McHenry, Jr.

Assistants: Col. Harvey C. Slocum, MC*
Col. George J. Hayes, MC--*

Reports Control Symbol: MEDDH-288

Security Classification: Unclassified

* Walter Reed General Hospital

ABSTRACT

Project No. 6X60-10-001 Neuropsychiatry and Stress

Task No. 7 The blood-brain barrier and the responses of cerebral tissues to injury

Reporting Installation: Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D. C.

Period Covered by Report: January 1961 to June 1961

Authors: Capt. Lawrence C. McHenry, Jr.
Col. Harvey C. Slocum, MC
Col. George J. Hayes, MC

Reports Control Symbol: MEDDH-288

Security Classification: Unclassified

The laboratory has been established and apparatus procured for measuring cerebral blood flow and metabolism. Initial observations will measure these functions before, during, and interval following intracranial surgery, utilizing closed system anesthesia with moderate hyperventilation. These studies are preliminary to investigations of fluid balance between blood, cerebral tissues and cerebro-spinal fluid spaces.

BODY OF REPORT

Project No. 6X60-10-001 Neuropsychiatry and Stress

Task No. 7 The blood-brain barrier and the responses of cerebral tissues to injury

Description:

The blood brain barrier, the intra-cranial fluid dynamics and the fluid and electrolyte shift between cerebral tissue spaces are recognized as critical problems in neurosurgery and for the management of brain injury. In this task a variety of techniques are applied to different aspects of these problems. The initial project investigates cerebral blood-flow by a method utilizing the Fick principle with Krypton ⁸⁵ as the inert gas as described by Albert, et al ("A Rapid and Simple Method for Measuring the Rate of Cerebral Blood Flow in Humans with Krypton," J. Lab. Cl. Med. 56:473-82, Sept. 1960).

Apparatus has been obtained and the technique is in the course of being standardized. A-V oxygen measurements are also made, providing a measure of cerebral metabolism.

Progress:

In the reporting period, a laboratory for cerebral blood-flow has been established on Ward 15 WRGH and necessary equipment for the measurements has been procured. The immediate objectives are to study the cerebral blood-flow and the brain metabolism of patients undergoing intra-cranial surgery. It has been found by Col. Harvey C. Slocum and Col. George J. Hayes that with moderate over-ventilation during anesthesia, the brain can be caused to shrink, resulting in a remarkably open operative field and little need for retraction. The effect appears to be secondary to reduction in the CO₂ content of the blood. It is important to determine the cerebral blood-flow and metabolism under these conditions before, during and at intervals after intra-cranial surgery.

An Astrup apparatus has been procured and is in process of being put into operation. The objective is to measure the CO₂ tension, the HCO₃ content and the pH of the arterial blood at appropriate intervals. With these data and the data on blood-flow, it will be possible to further plan experiments for studying fluid shift between the brain tissue and the cerebro-spinal fluid spaces.

Summary and Conclusions:

As this project is just starting, no conclusions can yet be made.

UNCLASSIFIED

UNCLASSIFIED