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## MEDICAL DEPARTMENT

# RESEARCH PROGRAM



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1 JANUARY - 30 JUNE 1950

MEDICAL RESEARCH AND DEVELOPMENT BOARD  
OFFICE OF THE SURGEON GENERAL  
U. S. ARMY

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RESTRICTED

TABLE OF CONTENTS

Reports Control  
Symbol DDRDB-3

SURGERY - 59

	<u>Page</u>
<u>59-01 - Investigations, Authorized</u>	
6-59-01-01 - Analysis of Medical Records	1
<u>59-02 - Devices, Prosthetic</u>	
6-59-02-01 - Army Prosthetics Research Program	2
6-59-02-02 - Surgical Braces	7
6-59-02-05 - Atlas Project	8
<u>59-03 - Eye, Ear, Nose &amp; Throat</u>	
6-59-03-01 - Ophthalmology Research Program	9
6-59-03-02 - Contact Lenses	10
<u>59-08 - Radiology, Roentgenology</u>	
6-59-08-01 - Prevention of Growth of Hair in Skin Grafts; Irradiation	12
6-59-08-03 - Medical Aspects of Atomic Bomb	22
6-59-08-04 - Radiation and Thermal Burns	23
6-59-08-05 - Ionization Effects	24
6-59-08-06 - Nutritional Requirements in Radiation Injury	26
6-59-08-08 - X-Ray Therapy, 1000-KV	28
6-59-08-09 - Thermal Effects of an Atomic Explosion, Manuscript	29
6-59-08-10 - Irradiation on Enzyme Systems, Effects of	30
<u>59-09 - Resuscitation, Anesthesiology</u>	
6-59-09-01 - Anesthesia & Resuscitation (title being changed)	31
6-59-09-02 - Investigation of Sedatives	32
6-59-09-03 - Anesthesia Death Rate	34
6-59-09-04 - Formed Blood Elements	35
6-59-09-05 - Neurologic Complications of Spinal Anesthesia	39
6-59-09-06 - Preservation of Blood for Transfusion	40
6-59-09-07 - Physiological Studies of Traumatic & Operative Shock	45
6-59-09-08 - Studies on Blood Volume	46
6-59-09-09 - Blood Coagulation	47
<u>59-10 - Specialties</u>	
6-59-10-01 - Case Records, Battle Wounds, MTO	48
6-59-10-02 - Intramedullary Fixation of Fractures	49
6-59-10-03 - Kuntzcher Nails in the Treatment of Non-Union of Fractures	50
6-59-10-04 - Vein Ligation - Acute Arterial Occlusion	51
6-59-10-05 - Extrapleural Thoracoplasty in Treatment of Tuberculosis	53
6-59-10-06 - Traumatic Surgery	55
6-59-10-07 - Reaction of Peripheral Nervous System to Antibiotics	58
<u>59-11 - Special</u>	
6-59-11-01 - Clinical Effects of Fibrinolytic and Pus-Liquefying Enzymes	59
6-59-11-02 - Renal Dysfunction following Thermal Injury	60

27 June 94

RG 330, Secretary Of Defense  
Entry 346A - RDB, Resources Division,  
Reports & Statistics Branch Progress  
Reports, Dec 1946 - 1954  
Box #

~~RESTRICTED~~

TABLE OF CONTENTS (cont'd.)

SURGERY (cont'd.)

	<u>Page</u>
<u>59-12 - Therapeutics</u>	
6-59-12-02 - Value of Antibiotics	62
- 6-59-12-05 - Nutrition and Anemia in Wound Healing	64
6-59-12-06 - Wound Healing	66
- 6-59-12-07 - Irreversible Shock	67
- 6-59-12-08 - Clinical Effects of Burn Treatment	68
- 6-59-12-09 - Application of New Methods of Treatment to Extensive Thermal Burns	69
6-59-12-10 - Purification of Toxins & Other Antigens of the Gas Gangrene Group of Clostridia	72
- 6-59-12-11 - Debridement of Burned Tissue by Enzymatic Means	74
6-59-12-12 - Experimental & Clinical Study of Cutaneous Burns	80
6-59-12-13 - Tissue Culture Studies in Relation to Thermal Injury and Epithelialization	81
6-59-12-14 - Transplantation of Skin	82
6-59-12-15 - Pyruvic Acid Treatment of Burns	83

INTERNAL MEDICINE - 60

<u>60-02 - Cardiology</u>	
- 6-60-02-01 - Problems Dealing with Heart & Circulation	84
<u>60-03 - Dermatology</u>	
6-60-03-01 - Antibiotic Therapy of Certain Virus & Coccogenous Infections	85
<u>60-04 - Diseases, Communicable</u>	
6-60-04-01 - Acute and Chronic Infectious Hepatitis	86
<u>60-06 - Equipment</u>	
6-60-06-04 - Experimental Equipment, Explorations, & Preliminary Screening	90
<u>60-09 - Metabolic</u>	
6-60-09-02 - Studies on Diseases of the Liver	91
6-60-09-03 - Studies of the Liver in Disease & Injury	95
- 6-60-09-04 - Protein and Amino-Acid Metabolism in Liver Disease	97
6-60-09-05 - Studies on Infectious Hepatitis	98
- 6-60-09-06 - Dietary Factors & Hormones in Liver Disease	100
6-60-09 series - Studies by Dr. Bean (no contract)	102
- 6-60-09-07 - Effects of Pantothenic Acid on Environmental Stress in Man	104
- 6-60-09-08 - Hormonal, Metabolic, & Nutritional Factors in Liver Disease	109
6-60-09-09 - Steroid Excretion in Patients with Liver Disease	110
- 6-60-09-10 - Carbohydrate Enzymatic Systems	111
<u>60-10 - Neurology, Neuropsychiatry</u>	
6-60-10-02 - Neurocirculatory Asthenia, Anxiety Neurosis, & Allied States	112
6-60-10-03 - Nerve Injuries	114
6-60-10-04 - Fundamental Physiologic Factors in Neurotic Patterns of Behavior	116

~~RESTRICTED~~

27 June 94

RG 330, Secretary Of Defense  
Entry 346A - RDB, Resources Division,  
Reports & Statistics Branch Progress  
Reports, Dec 1946- 1954  
Box #

TABLE OF CONTENTS (cont'd.)

INTERNAL MEDICINE - 60 (cont'd.)

Page			
62	6-60-10-06 - Manpower Selection & Preventive Psychiatry		119
64	6-60-10-07 - Objective Aids in Assessment of Personnel		123
66	6-60-10-08 - Visual Perception		124
67	6-60-10-09 - Chemical Factors affecting Physiology & Pathology of Nervous Function		126
68	6-60-10-10 - Analysis of Data on Psychiatric Problems in Army		126
	6-60-10-11 - Vision & its Relation to Accidents & Errors in Judgment		127
mal 69	60-11 - Nutrition		
one 72	6-60-11-01 - Techniques for Nutritional Surveys of Large Populations		128
74	6-60-11-02 - Diet in Relation to Physical Efficiency		130
80	6-60-11-03 - Symptoms of Deficiency Disease		133
	6-60-11-04 - Therapeutic Diets, Protein Reinforcement of		134
81	6-60-11-06 - Effect of Cold on Nutritional Requirements		136
82	6-60-11-07 - Nutritional & Metabolic Problems in Health & Disease		138
83	6-60-11-09 - Acclimatization to the Cold in Relation to Vitamin C Metabolism and Endocrines		142
	6-60-11-12 - Relation of Choline to Nutritional Edema & Anemia		143
	6-60-11-13 - Nutritional Requirements - Report		144
	6-60-11-14 - Fat Emulsions for Intravenous Nutrition		145
84	60-13 - Therapeutics		
	6-60-13-03 - ACTH, Armour Laboratories (purchase)		146
ons 85	6-60-13-04 - Chemotherapy of Pathogenic Agents		147
	6-60-13-05 - Therapy of Chronic Malaria		156
	6-60-13-08 - Clinical Trials of Antibiotics		157
	6-60-13-11 - Clinical Study of Bronchiectasis		166
86	6-60-13-12 - Bacterial and Fungous Infections of the Skin		167
	6-60-13-13 - Blood and Blood Substitutes		168
	6-60-13-14 - Study of Bronchial Asthma		170
ing 90	60-15 - Tuberculosis		
	6-60-15-02 - Streptomycin in Treatment of Tuberculosis, Fitzsimons GH		172
91	6-60-15-03 - Streptomycin & Other Agents in Treatment of Pulmonary Tbc		174
95	6-60-15-04 - Tibione in Treatment of Tuberculosis		176
97	6-60-15-05 - Serodiagnostic Test for Tuberculosis		178
98	60-16 - Venereal		
100	6-60-16-01 - Syphilis, False-Positive Blood Tests for		179
102	6-60-16-02 - Syphilis, Laboratory Diagnosis of		180
1 104	6-60-16-03 - Oral Penicillin in Prevention of Gonorrhea		181
se 109			
110			
111			
	PREVENTIVE MEDICINE - 61		
	61-01 - Investigations, Authorized		
es 112	6-61-01-01 - Hearing Test Methods, Development of		182
114	6-61-01-02 - Hearing Diagnostic Instruments		183

27 June 94

RG 330, Secretary Of Defense  
ENTRY 346A - RDB, Resources Division,  
REPORTS & STATISTICS BRANCH PROGRESS  
REPORTS, Dec 1946 - 1954  
Box #

REPRODUCED

TABLE OF CONTENTS (cont'd.)

PREVENTIVE MEDICINE (cont'd.)

	Page
<u>61-03 - Communicable Disease</u>	
6-61-03-03 - Studies in Influenza	185
6-61-03 series - Studies by Drs. Loosli & Buddingh (no contracts)	191
6-61-03-04 - Influenza Virus Strain Study	193
6-61-03-05 - Influenza Studies	195
6-61-03-06 - Studies on Infectious Hepatitis, Polio, etc.	198
6-61-03-07 - Hepatitis, Measles, and Mumps	204
6-61-03-08 - Dengue and Japanese-B Encephalitis	207
6-61-03-09 - Typhus Fever	215
6-61-03-10 - Infectious Hepatitis	217
6-61-03-11 - Field Studies in Japan	219
6-61-03-12 - Infectious and Respiratory Diseases	222
6-61-03-13 - Streptococcal Diseases	239
6-61-03-15 - Acute Respiratory Diseases	242
6-61-03-16 - Blood Complement in Immunity	247
6-61-03-18 - Coccidioides Immitis Infection	255
6-61-03-19 - Acute and Chronic Diseases of the Liver	260
6-61-03-20 - Epidemic Hepatitis	261
6-61-03-21 - Search for North American Animal Susceptible to Hepatitis	268
6-61-03-22 - Influenza Immunization	271
6-61-03-23 - Virus and Rickettsial Diseases	275
6-61-03-24 - Field Studies outside U.S. on Control of Infectious Diseases of Military Importance	289
6-61-03-27 - Antiviral Substances in Respiratory Tract	294
6-61-03-28 - Influenza Vaccine	297
6-61-03-29 - Viral Hepatitis	299
<u>61-04 - Entomology</u>	
6-61-04-01 - Biology, Taxonomy, & Control of <u>Phlebotomus</u> Sandflies	301
6-61-04-04 - Insect Cuticle	305
<u>61-05 - Epidemiology</u>	
6-61-05-02 - Streptococcal Diseases	306
6-61-05-03 - Diarrheal and Other Enteric Diseases	318
6-61-05-04 - Salmonella Infections	319
6-61-05-05 - Epidemiologic Pattern for Influenza	320
6-61-05-06 - Epidemiology of Salmonella Infections	322
<u>61-06 - Health, Public</u>	
6-61-06-01 - Nutrition Survey, Western Europe	323
<u>61-09 - Immunology</u>	
6-61-09-01 - Typhoid Fever	324
6-61-09-02 - Japanese Encephalitis Vaccine	327
6-61-09-03 - Bacillary Dysentery	331
6-61-09-04 - Antigen-Antibody Reaction	332

REPRODUCED

27 June 94

RG 330, Secretary Of Defense  
Entry 346A - RDB, Resources Division,  
Reports & Statistics Branch Progress  
Reports, Dec 1946 - 1954  
Box #

TABLE OF CONTENTS (cont'd.)

	<u>PREVENTIVE MEDICINE (cont'd.)</u>	<u>Page</u>
	6-61-09-05 - Immunization against Gas Gangrene	333
	6-61-09-06 - Substances to Increase Stability of Immunizing Agents	334
185	6-61-09-07 - Inhibition of Antigen-Antibody Reactions with Haptenes	338
191	6-61-09-08 - Allergy following Vaccination	339
193	6-61-09-09 - Scrub Typhus Vaccine	340
195	6-61-09-10 - Fundamental Immunity	344
198	6-61-09-11 - Immunology of the Major & Minor Blood Groups	347
204	6-61-09-12 - Diphtheria in Adults	349
207	6-61-09-13 - Parenteral Agents	351
215	6-61-09-14 - Immunization against Infection by Mumps	352
217	6-61-09-15 - Immunity in Influenza	359
219	6-61-09-16 - "M" Substance Produced by Streptococci	362
222	6-61-09-17 - Development & Isolation of Certain Immunologic Factors from Streptococcus	363
239		364
242	6-61-09-18 - Atoxic Proteins of Diphtheria Bacillus	364
247	6-61-09-19 - Fibrinolytic Enzymes in Experimental Pneumococcal Meningitis	366
255		
260	<u>61-12 - Parasitology</u>	
261	6-61-12-01 - Serodiagnosis of Parasitic Diseases	367
is 268	6-61-12-02 - Molluscicides	369
271		
275	<u>61-13 - Sanitation, Environmental</u>	
	6-61-13-01 - Ventilation and Heating of Arctic Shelters	370
289	6-61-13-02 - Iodine Tablets in Water Purification	372
294		
297	<u>61-15 - Venereal Disease</u>	
299	6-61-15-01 - Phenylarsenoxide for Prevention of Venereal Disease	373
	<u>VETERINARY - 62</u>	
301		
305	<u>62-01 - Investigations, Authorized</u>	
	6-62-01-01 - Possible Effects on Food & Food-Producing Animals of the Use of Atomic Weapons	374
306		
318	<u>62-02 - Analysis, Laboratory Inspection, &amp; Storage of Dairy &amp; Meat Products</u>	
319	6-62-02-01 - Same title	375
320	6-62-02-02 - Development & Evaluation of Methods & Materials used in Examination of Food Products	376
322		
	<u>62-04 - Bacteriology</u>	
323	6-62-04-01 - Bacterial & Protozoan Diseases of Military Significance Occurring in Animals and Animal Parasites	378
324	<u>62-05 - Diseases of Animals</u>	
327	6-62-05-01 - Equine Encephalomyelitis	379
331	6-62-05-04 - Equine Infectious Anemia	380
332	6-62-05-05 - Leptospirosis in Animals	381
	6-62-05-06 - Research on Surra	382

27 June 94  
RG 330, Secretary Of Defense  
Entry 346A - RDB, Resources Division,  
REPORTS & STATISTICS BRANCH PROGRESS  
REPORTS, Dec 1946- 1954  
Box #

~~RESTRICTED~~

TABLE OF CONTENTS (cont'd.)

VETERINARY - 62 (cont'd.)

	<u>Page</u>
6-62-05-07 - Viral & Rickettsial Diseases of Military Significance harbored in Animals and Animal Parasites	383
6-62-05-08 - Rabies	387

DENTISTRY - 63

<u>63-01 - Investigations, Authorized</u>	
6-63-01-02 - Research on Dental Materials	388
6-63-01-03 - Cephalometric Studies	389
6-63-01-04 - Clinical & Laboratory Tests on Dental Amalgam	390
6-63-01-05 - Improved Techniques & Equipment for Use in Prosthetic, Operative, & Surgical Dentistry	391
<u>63-05 - Pathology</u>	
6-63-05-01 - Dental Caries	392
6-63-05-02 - Role of Proteolytic Microorganisms in Dental Caries	393
6-63-05-03 - Cytologic Studies of Oral Epithelium	394
6-63-05-04 - Histochemical Studies of Dental Tissues	396
<u>63-06 - Periodontia</u>	
6-63-06-01 - Research in Periodontal Disease	397
6-63-06-02 - Periodontal Disease & Other Aspects of Oral Pathology	398
<u>63-07 - Physiology</u>	
6-63-07-01 - Fluorine in Nutrition, Essentiality of	400
<u>63-08 - Preventive</u>	
6-63-08-01 - Fluorine and Dental Caries	402

BASIC MEDICAL SCIENCE - 64

<u>64-01 - Investigations, Authorized</u>	
6-64-01-01 - Advisory Services, NRC	404
6-64-01-02 - Hepatectomized Frogs	405
6-64-01-03 - Research on Thirst	406
6-64-01-04 - Serum Proteins in Liver Disease	409
6-64-01-05 - Medical Indexing	410
<u>64-06 - Biophysics and Physics</u>	
6-64-06-02 - Radiation Chemistry of Organic Molecules in Solution	412
<u>64-09 - Pathology</u>	
6-64-09-02 - Regeneration of the Liver	413
6-64-09-03 - Connective Tissue in Different Nutritional & Metabolic Con- ditions	418
6-64-09-04 - Acute Effect of Radium Chloride upon Hemopoietic Organs of the White Rat	424

~~RESTRICTED~~

27 June 94

RG 330, Secretary Of Defense  
ENTRY 346A - RDB, Resources Division,  
REPORTS & STATISTICS BRANCH PROGRESS  
REPORTS, Dec 1946 - 1954  
Box #

RESTRICTED

TABLE OF CONTENTS (cont'd.)

Page	BASIC MEDICAL SCIENCE - 64 (cont'd.)	Page
383	6-64-09-05 - Liver Damage, Study of by Vital Staining	425
387	64-11 - Psychology	
	6-64-11-02 - Tank Turret Controls	427
	6-64-11-03 - Prediction of Success in Medical Residency Training	428
388	64-12 - Physiology	
389	6-64-12-02 - Cold, Study of Physiologic Effects of	430
390	6-64-12-03 - High Temperatures, Physiologic Effects of	434
	6-64-12-05 - Studies of Body Measurements as they Affect Efficiency	435
391	6-64-12-06 - Body Reactions & Requirements under Varied Environmental and Climatic Conditions	436
392	6-64-12-07 - Fatigue in Relation to Military Tasks	444
393	6-64-12-08 - Physiologic & Psychologic Problems of Military Personnel in Relation to Task & Environment	445
394	6-64-12-10 - Peripheral Nerve Injuries	448
396	6-64-12-11 - Research in Peripheral Vascular Diseases and Injuries	450
	6-64-12-12 - Peripheral Blood Vessels	453
397	6-64-12-13 - Effects of Warming & Cooling on Body Temperature & Circulation	455
398	6-64-12-14 - Protein Metabolism in Disease and Injury	456
	6-64-12-16 - Overhead Expenses, Ft. Churchill	458
400	6-64-12-17 - Infused Red Cells as a Source of Protein in Man	459
	6-64-12-18 - Irreversible Shock	464
	6-64-12-19 - Catabolic Reaction to Injury, Investigation of	465
402	6-64-12-22 - Cerebral Edema	471
	6-64-12-24 - Renal Insufficiency	474
	6-64-12-25 - Status of Adrenal Cortical Function in Tuberculosis	475
	6-64-12-26 - Electrolytic Composition of Thermal Sweat	476

BASIC RESEARCH - 99

404	99-02 - Wound Ballistics	
405	6-99-02-01 - Wound Ballistics	478
406	6-99-02-03 - Acute Arterial Injuries under Battle Conditions	483
409		
410		

412

413

418

424

vii

RESTRICTED

27 June 94

RG 330, Secretary Of Defense  
ENTRY 346A - RDB, Resources Division,  
REPORTS & STATISTICS Branch Progress  
REPORTS, Dec 1946 - 1954  
Box # 28



RESTRICTED

INDEX OF ARTICLES BY NAME OF INVESTIGATOR

Allen, H. (NWU)	68	Grundfest	114
Allen, J. G. (Chicago)	24	Gyorgy	100
Altemeier (gas gangrene)	333		
Altemeier (burns)	80	Halstead	123
Alving	156	Ham	412
Avery	362	Hamburger	366
		Hammon	219
Bean (47)	109	Hanson	126
Bean (396)	260	Hardy	322
Bean (no contract)	102	Harvey	83
Beard	359	Havens (445)	98
Becks	402	Havens (403)	217
Beecher (sedatives)	32	Hay	67
Beecher (anesthesia death rate)	34	Heidelberger	247
Berry	393	Hertig	301
Blades (anesthesia)	31	Hollenback	390
Blades (antibiotics)	62	Holmes	406
Blocker	81	Howes (Wound Healing)	66
Breed	45	Howes (14)	74
Buddingh (no contract)	191		
Burch	453	Ivy	405
Burnett	474		
		Katz	455
Capps	299		
Churchill	48	Langer	29
Conn	476	Larkey	40
Curnen	320	Levy	84
Curry	170	Loosli (no contract)	191
		Lucke	261
Davidson	97	Lyons	456
Day	400		
DeBakey (history)	1	MacLeod	242
DeBakey (renal dysfunction)	60	Magill	193
DeBakey (arterial injuries)	483	Mahoney	47
DeGowin	40	Malmo	116
Dingle	222	Mayerson	46
Dohan	110	McGuinness	352
Dripps	39	McKee	195
		McMullen	369
Edsall	349	Meiklejohn	297
Elkin	450	Miller	124
Engel, Milton (Chicago)	396		
Engel, R. W. (Ala.)	143	Nachmansohn	125
Evans, E. I.	23	Neefe	95
Evans, Joseph	471		
		Oughterson	22
Francis	185		
Fry	119	Pappenheimer	364
		Paul	198
Goldman	398	Pendergrass	12
Goodner	334	Pillemer	72
Greene	82		

viii

RESTRICTED

27 June 94

RG 330, SECRETARY OF DEFENSE  
ENTRY 346A - RDB, RESOURCES DIVISION,  
REPORTS & STATISTICS BRANCH PROGRESS  
REPORTS, DEC 1946 - 1954  
Box # 28

RESTRICTED

INDEX - INVESTIGATORS (cont'd.)

114		
100	Pillsbury	Page 167
123		104
412	Ralli	239
366	Rantz	35
219	Ravdin (formed bld elements)	51
126	Ravdin (vein ligation)	413
322	Ravdin (regen of liver)	111
83	Reinhold	305
98	Richards	448
217	Richter	424
67	Roofe	294
247	Rose	
301	Sabin	207
390	Salk	271
406	Shaffer	319
66	Smelser	10
74	Smith, Charles	255
	Smith, Hugh	49
405	Snyder	215
	Stare (final)	136
455	Stare (fat emulsions)	145
	Stokes	204
29	Strong	428
40	Strumia (two contracts)	40,41
84		
191	Tillett (enzymes)	59
261	Tillett (streptococcus)	363
456	Treffers	338
	Turner	409
242		
193	Walter	69
47	Watson	91
116	Watt	318
46	Weinmann	394
352	White	112
195	Williams	425
369	Woodhall	58
297	Woodward	289
124		
125	Yaglou	370
95		
22		
364		
198		
12		
72		

27 Jun 94

RG 330, Secretary Of Defense

NND 813064  
BF 6-27-94

REPRODUCED AT THE NATIONAL ARCHIVE

RESEARCH AND DEVELOPMENT PROJECT CARD (NEW PROJECTS)		2. SEC. U	3. PROJ. NO. 6-59-08-01
1. PROJECT TITLE Prevention of Growth of Hair in Skin Grafts; Irradiation		4. REPORT DATE 30 Jun '50	
6. BASIC FIELD OR SUBJECT		7. SUB FIELD OR SUBJECT SUB GROUP AW-6	
8. COGNIZANT AGENCY	13. CONTRACTOR AND/OR LABORATORY		CONTRACT/W. O. NO.
9. DIRECTING AGENCY			
10. REQUESTING AGENCY	13. RELATED PROJECTS		17. EST. COMPL. DATES
11. PARTICIPATION AND/OR COORDINATION	14. DATE APPROVED		RES.
	15. PRIORITY		DEV.
	16.		TEST
19.		18. FISCAL EST'S.	
20. REQUIREMENT AND/OR JUSTIFICATION			
21. BRIEF OF PROJECT AND OBJECTIVE			
<p>e. <u>Progress.</u></p> <p><u>Animals and Equipment.</u> The population of the animal colony has been maintained at about 250 rats for the past six months. No additional groups of Holtzmann rats have been maintained for study.</p> <p><u>Depilation.</u> The experiments in depilation using the long wave length, high-intensity radiation produced by the Bracke-Seib apparatus are continuing. The back of the neck of human volunteers is being irradiated with a small field in single doses. The effect with added filter is now being studied.</p> <p><u>Follow-up Study.</u> No additional replies have been received from the persons whose thighs were radiated in 1942-43. So far, the irradiated area has been excised in 24 individuals. A second letter is being sent out in an effort to contact those who have not yet responded.</p> <p>It has been demonstrated that the following agents or procedures have a protective effect against local irradiation: pitressin, epinephrine, other vasoconstrictors, tourniquet ischemia, cyanide, and cysteine. The effect of the first four could be due either to reduced blood flow or tissue anoxia. It has been presumed that the effect is due to tissue anoxia because cyanide and cysteine protect. Reviewing the data reveals that the effect of cysteine is not unequivocal by any means. The effect of cyanide in this study is almost certainly real. It is generally believed that the principal effect of cyanide in the intact animal is inactivation of the cytochrome system by the formation of a cyanide-ion complex. This produces tissue anoxia in the presence of an adequate oxygen tension. This is the critical observation upon which future investigation is planned. It is important to point out that the reasoning on this point is based upon assumption only. A vasoconstrictor action of cyanide has not been ruled out, and it is known that vasoconstriction will produce protection. It</p>			
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JRDB FORM 1A, 1 APR 1947

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12

PAGE 1 OF 10 PAGES

27 June 94

RG 330, SECRETARY OF DEFENSE  
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Prevention of Growth of Hair in Skin Grafts: Irradiation

6-59-08-01

seems to be of critical importance to collect evidence bearing upon this point.

If the effect of CN is due to inactivation of the cytochrome system, it is of primary interest to explore the mechanism whereby protection occurs. At the suggestion of biochemical consultants, the summer will be devoted to a study of the effect of other enzyme inhibitors on the reaction to local irradiation. A number of other enzyme inhibitors may be studied later, but those to be investigated this summer are:

1. Sodium azide. ( $\text{NaN}_3$ ). This compound inhibits a variety of intracellular enzymes, but it is thought that its effect is primarily on the cytochrome-cytochrome oxidase system. Its effect should parallel that of cyanide.

2. Malonate. This compound has the specific ability to block the enzyme succinoxidase by virtue of its structural similarity to succinate, the normal substrate. This should interrupt the Krebs cycle and leave the tissue oxygen tension high, and the hydrogen transport system in the oxidized state largely.

3. Iodoacetate. This compound is a specific binding agent for sulphydryl groups. It may be expected to affect a great many different enzyme systems in the intact animal. Triosephosphate dehydrogenase is considered to be the most sensitive of these enzymes.

4. Fluoride. This ion probably exerts its effect primarily by virtue of its ability to bind and inactivate magnesium. The enzyme thought to be most sensitive to fluoride is Enolase. The action of the former is fairly diffuse in that it may inactivate various phosphatases and other Mg-dependent enzymes.

5. Dinitrophenol. This compound has the ability to bypass the hydrogen transport system, allowing intracellular oxidation to proceed without the participation of these enzymes. It increases the rate of tissue oxidation, producing a picture in the intact animal that is remarkably similar to hyperthyroidism. Its effect, therefore, should be of considerable interest. If dinitrophenol protects, it would point quite specifically to the hydrogen transport system as the one most vulnerable to the effects of irradiation.

6. The effects of a high tissue oxygen tension should be explored. It has been repeatedly shown that low oxygen tension protects lower forms from the effects of irradiation, and there is a controversy over whether this is due to absence of molecular oxygen or to the fact that oxidation-reduction systems within the cell are present in the reduced state. If the effect is due to  $\text{O}_2$  concentration, high oxygen tensions should enhance the effect of irradiation.

Because of the interest of this project in intracellular biochemical mechanism a study of irradiation of another tissue seems advisable. Within the near future there will be considerable interest in conducting some *in-vitro* studies of metabolism of tissues which have been irradiated. An organ which is readily accessible, for which there is an adequate control, and for which a suitable technique for irradiation could be developed without too much difficulty is the testicle. Since only male rats are being used in these studies, techniques could be developed for irradiating one of two testicles on an experimental animal, and a suitable control-series with adequate pathologic studies accumulated. The testicle has the advantages of being readily accessible for local irradiation, of being relatively unimportant to the life of the animal, and of being readily accessible with a paired control for removal for *in-vitro* studies. It lends itself well to the Warburg technique, it contains large quantities of the nucleic acids, and it is possible to estimate the testicular tissue.

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27-June-94

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REPORTS, Dec 1946-1954  
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Prevention of Growth of Hair in Skin Grafts; Irradiation

6-59-08-01

Table I. A vasoconstrictor, pitressin, was used to determine whether radiation-sensitivity could be reduced; a very dramatic decrease in sensitivity to local x-radiation resulted. This effect of pitressin has been the basis for further work attempting to explain its protective action in local irradiation effects.

**Methods. Animals.** Male albino rats, Wistar strain, obtained from the Wistar Institute were kept for at least nine days before being used for experimental work. They were fed Purina Dog Chow, presumably an adequate diet, since all the animals were in excellent condition and followed Zucher's weight-age relation curve. At the time of irradiation the rats' weights ranged from 180-230 gm. They were housed, three animals per cage, in an air-conditioned room at a temperature of 25-27° C. (77-80° F.). Each animal was individually marked for identification.

**Anesthesia.** About 15 minutes prior to irradiation the rats were given pentobarbital sodium intraperitoneally: 4.5 mg. per 100 gm. body-weight, prepared as a freshly-made 0.9-per-cent solution in water.

**Technique of Irradiation.** The leg to be irradiated was immobilized by pinning it to a wooden block (2 cm. thick) with a single thumb tack inserted through the web of the toes. The left hind legs of four animals were attached near the center of the block within an area 4 cm. square. The upper portion of the leg and the rest of the animal's body were shielded by lead-plates 2 mm. thick. During irradiation the animals lay on a rice mattress 10 cm. thick. Air dosages of 1400-3000 r were delivered at about 270 r per minute. Radiation factors were 200 KV, 25 cm. distance, and filters of 0.5 mm. of Cu. and 1.0 mm. of Al.

**Pitressin.** Pitressin, the pressor fraction of the posterior pituitary as prepared by Parke, Davis and Company, was given in a single dose intraperitoneally at varying intervals before and after irradiation. Two forms were used, pitressin in water, 20 units per ml. and pitressin tannate in oil, 5 units per ml. Except where indicated the water-solution was used. Dosages of any drug appearing on charts represent the total amount administered per animal.

**Gross Reaction and Method of Evaluation.** The animals were examined daily in a good light and records were kept for each. The un-irradiated leg served as a control for the irradiated leg. Following irradiation there were no gross changes for seven to eight days except for slight erythema in most cases. Then there followed a series of inflammatory changes characteristic of radiation-reaction. The earliest changes seen at seven to eight days were slight swelling of the foot pad, erythema, and venous congestion on the dorsal surface. These increased during the next 24 to 48 hours. When the swelling became maximal (12 to 13 days) ulceration often developed. Depending on the experimental procedure used, any degree of reaction could be obtained, from none to a severe ulcerating lesion. For evaluation of the reaction the following arbitrary categories were set up:

- A. **No Reaction.** When there was doubt about the presence or absence of a reaction it was recorded in this group.
- B. **Mild Reaction.** Definite, but often slight, reaction.
- C. **Moderate Reaction.** Advanced swelling and erythema without ulceration.
- D. **Severe Reaction.** Severe swelling with ulceration.

This classification of course gives only a rough clinical estimate of the severity of the reaction but does provide a crude yardstick for measuring the degree of reaction produced.

Page 3 of 10

RESTRICTED

27 June 94

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ENTRY 346A - RDB, Resources Division,  
REPORTS & STATISTICS Branch Progress  
REPORTS, Dec 1946-1954  
Box #

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Prevention of Growth of Hair in Skin Grafts; Irradiation

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**Results.** The reaction following local irradiation of the rat's leg can be decreased or suppressed by the administration of pitressin. Such protection is influenced by several factors: (1) the dosage of pitressin, (2) the time pitressin is given in relationship to irradiation, and (3) the amount of irradiation.

**Influence of the Pitressin-Dosage.** Five units of pitressin given 15 minutes before irradiation leads to a definite reduction in the subsequent local reaction (Table I). When the dosage of pitressin is decreased, other factors remaining constant, the degree of protection is greatly decreased. With 0.2 units little protection is obtained.

Table I

INFLUENCE OF PITRESSIN DOSAGE ON LOCAL RADIATION REACTION

Pitressin (units)	Number of Rats	Maximal Reaction			
		None	Mild	Moderate	Severe
0.0 (Control)	74	2	13	27	32
0.2	8	1	3	4	-
1.0	9	2	5	-	2
2.0	37	16	9	5	7
5.0	31	26	1	1	3

The figures in the table indicate numbers of animals. Each animal irradiated has been classified according to the maximal reaction attained. Pitressin was given 15-30 minutes before irradiation of the hind leg with 2,000 r.

**Influence of Other Vasoconstrictors.** Other vasoconstrictors were tested for their ability to alter the rat's radiation-sensitivity. Adrenalin and nor-adrenalin gave definite protection. In fact, adrenalin appears to give better protection than pitressin (Table V). Ice (used as a vasoconstrictor and an agent to lower the temperature of the extremity) and Neosynephrin each give definite protection, but not nearly to the degree of adrenalin.

**Influence of the Time of Administration of Pitressin.** Pitressin gives protection only when given prior to irradiation. While the maximum effect is obtained when pitressin is administered about 10 minutes before irradiation, there is considerable effect up to one hour. By two hours the influence is considerably reduced (Table II).

15  
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27-June-94

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Prevention of Growth of Hair in Skin Grafts;  
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TABLE V

INFLUENCE OF OTHER VASOCONSTRICTORS ON LOCAL RADIATION REACTION

Drug	Dosage	Number of Rats	Maximal Reaction			
			None	Mild	Moderate	Severe
Control	---	16	1	1	3	11
Pitressin	5 u	15	12	-	1	2
Adrenalin	0.2mg	5	5	-	-	-
Adrenalin	0.3mg	7	1	6	-	-
Adrenalin	0.06mg	6	5	1	-	-
Adrenalin 1 min. after irradiation	0.06mg	7	-	1	4	2
Nor-Adrenalin	0.2mg	17	5	8	3	1
Nor-Adrenalin	0.3mg	5	5	-	-	-
Neosynephrin	1.0mg	8	-	7	1	-
Ice	-	8	-	3	4	1

The figures in the table indicate numbers of animals. Each animal irradiated has been classified according to the maximal reaction attained. The various vasoconstrictors were given before irradiation of the hind leg with 2,000 r except where indicated for adrenalin. The ice was applied to the leg five minutes before irradiation and remained in contact during the procedure.

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27 June 94

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ENTRY 346A - RDB, RESOURCES DIVISION,  
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REPORTS, DEC 1946-1954  
Box # 28

RESTRICTED

Prevention of Growth of Hair in Skin Grafts; Irradiation

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TABLE II

INFLUENCE OF THE TIME OF ADMINISTRATION OF PITRESSIN  
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Time before or after irradiation of Rats	Number	Maximal Reaction			
		None	Mild	Moderate	Severe
Controls	7	-	1	3	3
2 hr. before	5	-	2	3	-
1 hr. before	7	2	4	-	1
30 min. before	7	6	-	1	1
10 min. before	8	7	-	-	1
1 min. after	8	-	1	2	5
30 min. after	6	-	-	-	6

The figures in the table indicate numbers of animals. Each animal irradiated has been classified according to the maximal reaction attained. Pitressin (5 units) was given before or after irradiation of the hind leg with 2,000 r.

Influence of Pitressin on the Radiation-Threshold. The rats used in these experiments show but little radiation-reaction until about 1,500 r is given (Table III). When 5 units of pitressin are given this local radiation-threshold is increased to about 2,400 r. Thus the radiation-threshold is raised roughly 900 r by pitressin.

Following the study of the limitations of pitressin in protecting against local radiation-reaction, other substances and procedures were used to evaluate their protective properties.

Influence of Posterior Pituitary Fractions. Pitressin in water is not the only factor of the posterior pituitary that is protective (Table IV). Pitressin tannate in oil and pituitrin in water (the whole extract of the posterior pituitary) are probably just as active as pitressin in water. Pitocin, the oxytocic factor, is probably inactive.

Influence of Miscellaneous Factors. Eschatin (the whole adrenal cortex extract) was found to increase slightly the sensitivity to radiation (Table VI). The same result was obtained for ACTH. Colchicine apparently gives a little protection.

Other factors in the sensitivity to irradiation have been observed. In the preliminary studies it was repeatedly seen that rats weighing more than 250 gms. were more resistant to local irradiation; for this reason animals of standard weights were used. It has also been observed many times that a sick rat will not develop the "normal" radiation-reaction but will show almost no reaction.

17

Page 6 of 10

RESTRICTED

27-June-94

RG 330, Secretary Of Defense  
ENTRY 346A - RDB, Resources Division,  
REPORTS & STATISTICS Branch Progress  
REPORTS, Dec 1946-1954  
Box #



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TABLE III

INFLUENCE OF PITRESSIN ON THE RADIATION THRESHOLD\*

Condition of rat	Roentgens	Number of Rats	Maximal Reaction			
			None	Mild	Moderate	Severe
Control	1400	8	6	2	-	-
Control	1500	7	1	6		
Control	1600	8	-	1	3	4
Control	1800	7	-	1	2	4
Control	2000	8	1	1	2	4
Pitressin 5 u	2000	8	6	-	-	2
Pitressin 5 u	2200	5	4	-	-	1
Pitressin 5 u	2400	6	3	2	-	1
Pitressin 5 u	2600	8	3	2	-	3
Pitressin 5 u	2800	7	-	2	-	5
Pitressin 5 u	3000	6	-	1	3	2

Figures in the table indicate numbers of animals. Each animal irradiated has been classified according to the maximal reaction attained. Pitressin (15 units) was given 15 minutes before irradiation of the hind leg.

\*Radiation-threshold is defined as the number of roentgens it takes to produce a definite local reaction in 50 per cent of the test-animals, with no reaction in the remaining animals.

TABLE IV

INFLUENCE OF POSTERIOR PITUITARY FRACTIONS ON LOCAL RADIATION REACTION

Drug and Dosage	Time Before Radiation	Number of Rats	Maximal Reaction			
			None	Mild	Moderate	Severe
Control	-	8	-	-	1	7
Pitressin in water: 5 u	30 min.	8	6	-	-	2
Pitressin Tannate in oil: 5 u	2 hrs.	11	9	-	-	2
Pituitrin: 5 u	30 min.	11	7	-	2	2
Pitocin: 5 u	30 min.	6	-	3	2	1

(See next page for explanation.)

18

Page 7 of 10

~~RESTRICTED~~

27 June 94

RG 330, Secretary Of Defense  
ENTRY 346A - RDB, Resources Division,  
REPORTS & STATISTICS Branch Progress  
REPORTS, Dec 1946 - 1954  
Box # 28

RESTRICTED

6-59-08-01

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(Explanation, Table IV, preceding page: The figures in the table indicate numbers of animals. Each animal irradiated has been classified according to the maximal reaction attained. The various posterior pituitary fractions were given intraperitoneally before irradiation of the hind leg with 2,000 r.)

TABLE VI

INFLUENCE OF MISCELLANEOUS DRUGS OF LOCAL RADIATION REACTION

Drug	Dosage & Route	Time Before Radiation	No. of Rats	Maximal Reaction			
				None	Mild	Moderate	Severe
Control	-	-	11	-	-	1	10
Eschatin	5ml I.M., S.Q. & I.P.	2 hrs.	9	-	-	-	9
ACTH	5 mg S.Q.	5 hrs.	8	-	-	-	8
Colchicine	0.4 mg I.P.	5.5 hrs.	14	1	6	2	5
Pitressin	5 u I.P.	15 min.	15	12	-	1	2

The figures in the table indicate the numbers of animals. Each animal irradiated has been classified according to the maximal reaction attained. The various drugs were administered as indicated in the table, before irradiation of the hind leg with 2,000 r.

For Table VII, see next page.

TABLE VIII

EFFECT OF SODIUM CYANIDE ON LOCAL RADIATION REACTION

Condition of Rat	Number of Rats	Reaction			
		None	Mild	Moderate	Severe
Control	7	-	-	1	6
Sodium Cyanide	13	8	2	1	2

The figures in the table indicate numbers of animals. Each animal irradiated has been classified according to the maximal reaction attained. Sodium cyanide, 1.0 mg. per 200-gm. rat, was given intraperitoneally five minutes before irradiation of the hind leg with 2,000 r.

Table VIII. From the experiments with tourniquets it appeared that the protection against local irradiation given by pitressin might be due to an induced anoxia. Consequently, sodium cyanide (1.0 mg./rat) was tried. Table VIII shows that this agent gives definite protection against local irradiation.

Page 8 of 10

RESTRICTED

27 June 94

RG 330, Secretary Of Defense  
Entry 346A - RDB, Resources Division,  
Reports & Statistics Branch Progress  
Reports, Dec 1946-1954  
Box #

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Prevention of Growth of Hair in Skin Grafts; Irradiation

6-59-08-01

TABLE VII

Condition of rats	Dosage of roentgens	Number of Rats	EFFECT OF THE TOURNIQUET ON LOCAL RADIATION REACTION Number of maximum reactions			
			None	Mild	Moderate	Severe
Control	1400	8	6	2	-	-
Control	1500	7	1	6	-	-
Control	1600	8	-	1	3	4
Control	1800	7	-	1	2	4
Control	2000	7	-	-	1	6
Tourniquet	2000	8	8	-	-	-
Tourniquet	2200	7	7	-	-	-
Tourniquet	2400	7	5	2	-	-
Tourniquet	2600	7	6	1	-	-
Tourniquet	2700	8	8	-	-	-
Tourniquet	2800	8	5	3	-	-
Tourniquet	2900	7	5	2	-	-
Tourniquet	3000	7	-	2	3	2

Figures indicate numbers of animals. Each animal irradiated has been classified according to the maximal reaction attained. The tourniquet was applied five minutes before irradiation to the hind leg, and removed immediately after irradiation.

Table II. Whole-Body Irradiation: Treatment with Pitressin. Results of Experiment 71. Procedure. This experiment was carried out on 108 white male rats of the Holtzman strain ranging in weight from 154 to 220 gms. The average body-weight for the entire group was 167.7 gms. at the time of irradiation. The rats were divided into two groups of 54 rats each. Group A was treated with 5 units of water-base solution of pitressin intraperitoneally 15 minutes before irradiation; Group B was the irradiated control-group.

Irradiation: 800 r in air, whole-body irradiation at 50-cm. distance; filters were 0.5 mm. cu. and 1.0 mm. Al., rate of dose about 70.4 r/min.

Exp. No.	Date	No. of Rats	Procedure	No. Died Pit.	No. Died Con.
71-A	3/4/50	54	Pitressin	30	
71-B	3/4/50	54	Control	--	49
			Total	30	49
			% Dead	55.5	90.7

Pitressin gave 38.7% protection.

20

Page 9 of 10

~~RESTRICTED~~

27 Jun 94

RG 330, Secretary Of Defense  
Entry 346A - RDB, Resources Division,  
Report of Statistics Branch Progress  
Reports, Dec 1946-1954  
Box # 28

6-59-08-01

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Prevention of Growth of Hair in Skin Grafts; Irradiation 6-59-08-01

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Discussion. These animals were in very good condition. Only two were showing signs of respiratory congestion (pneumonia); they died between the period of 82 to 90 days after irradiation. Both were treated animals. The resistance of these animals probably was impaired by irradiation.

The LD 50-800 r and rate of dose for pitressin were calculated from the results of previous experiments with this particular strain of animals.

Conclusion. Pitressin in the manner used gives a significant amount of protection but the percentage of protection is not satisfactory. It is improbable that the rate of protection can be advanced, when pitressin is used in this manner, since 5 units has been calculated to give maximum protection for animals in this weight-range.

f. Future Plans. Studies will be continued as indicated above.

g. Reports. None listed. Material summarized under heading of Table I is being prepared for publication.

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27 June 94  
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1. PROJECT TITLE Medical Aspects of Atomic Bomb				5. REPORT DATE 30 Jn '50	
6. BASIC FIELD OR SUBJECT			7. SUB FIELD OR SUBJECT SUB GROUP AW-6		
8. COGNIZANT AGENCY		12. CONTRACTOR AND/OR LABORATORY		CONTRACT/W. O. NO.	
9. DIRECTING AGENCY					
10. REQUESTING AGENCY		13. RELATED PROJECTS		17. EST. COMPL. DATES	
11. PARTICIPATION AND/OR COORDINATION		14. DATE APPROVED		RES.	
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<p>e. <u>Progress.</u> It had been hoped that the monograph would be completed by July 1950, but this will not be possible because the editors have been ill and because authors engaged in other activities have not been able to complete their sections of the volume./ It was decided to include in the book a concise account of the physics involved in an atomic bomb. Four drafts of Part I have been prepared during the last six months and reviewed by Dr. Ralph E. Lapp. His criticism has been severe, but good from the standpoint of physics. It was decided that more rapid progress in this highly technical field could be made if Dr. Lapp were responsible for the final draft. He has assumed this responsibility and has promised the manuscript by 15 July./ Dr. Joe Howland of the University of Rochester has been slow in submitting his chapter on the biologic effects of radiation. He expects to complete the material by 15 August./ Part II, "Catastrophic Effects," is in final draft./ Part III, "Incidence of Casualties"—a large part of which is statistical—has been checked by Dr. Cuyler Hammond who is at present in Puerto Rico. This manuscript is expected within a few weeks of his return./ Part IV, "Clinical and Pathologic Observations," has been largely completed, and most of the material has been published in the <u>American Journal of Pathology</u>.</p> <p>f. <u>Future Plans.</u> Completion of the manuscript is now slated for autumn, 1950.</p> <p>g. <u>Detailed Reports.</u> None listed.</p>					
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JRDB FORM 1A, 1 APR 1947

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22

PAGE 1 OF 1 PAGES

27 June 94

RG 330, Secretary Of Defense  
Entry 346A - RDB, Resources Division,  
Reports & Statistics Branch Progress  
Reports, Dec 1946-1954  
Box # 28

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PAGE 1 OF 1 PAGES

JRDB FORM 1A, 1 APR 1947

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23

PAGE 1 OF 1 PAGES

27 June 94

RG 330, SECRETARY OF DEFENSE  
ENTRY 346A - RDB, Resources Division,  
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REPORTS, Dec 1946 - 1954  
Box # 28

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BF 6-27-94

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8. COGNIZANT AGENCY	12. CONTRACTOR AND/OR LABORATORY		CONTRACT/W. O. NO.
9. DIRECTING AGENCY			
10. REQUESTING AGENCY	13. RELATED PROJECTS		17. EST. COMPL. DATES
11. PARTICIPATION AND/OR COORDINATION	14. DATE APPROVED		RES.
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19.		18. FISCAL EST'S.	
20. REQUIREMENT AND/OR JUSTIFICATION			
21. BRIEF OF PROJECT AND OBJECTIVE <p>The following is a summary of a detailed report on work completed during Fiscal Year 1950:</p> <ol style="list-style-type: none"> <li>1. The <u>in-vitro</u> clotting determination of whole blood was investigated and evaluated. Data were presented to show the manner in which the whole-blood clotting-time is affected by variations in the experimental conditions and by changes in the clotting-time technique. Of particular importance in achieving maximal usefulness of the test is careful venipuncture technique and meticulous handling of the unclotted blood.</li> <li>2. <u>Summary of the Irradiation Effect on Coagulation Mechanism.</u> The individual and collective roles and possible significance of the various changes seen in the irradiation syndrome that have been studied in this laboratory are discussed. New data are also presented on recently-completed work in which an attempt was made to isolate an inhibitory substance from the blood of irradiated animals. The possible origin and fate of clotting inhibitors are discussed in the light of current knowledge. The effects of irradiation thrombocytopenia and direct damage to the capillary bed are considered as changes capable of altering the hemostatic mechanism following irradiation.</li> <li>3. <u>Whole-Blood Transfusions in Total-Body X-Irradiation Injury.</u> Transfusions of whole blood in sufficient amounts, about 8-10 ml./kg. body weight daily, overcome the anemia of 450r total-body irradiation but do not affect the leukopenia or thrombocytopenia in dogs. While the total plasma protein level is maintained better in the transfused animals than in a control group, the plasma albumin levels show less elevation. Whole-blood transfusions to the irradiated animal fail to prolong the survival time.</li> <li>4. <u>Treatment of the Irradiation Syndrome with Aureomycin.</u> Aureomycin is more effective in increasing the survival time of dogs following total-body</li> </ol>			
22. JRDB SN.	PC.	IC & P.	I. I. C.

JRDB FORM 1A, 1 APR 1947

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24

PAGE 1 OF 2 PAGES

27 June 94

RG 330, Secretary Of Defense  
Entry 346A - RDB, Resources Division,  
Reports & Statistics Branch Progress  
Reports, Dec 1946-1954  
Box #

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# Ionization Effects

6-59-08-05

x-irradiation than any other agent known to the investigators. While aureomycin apparently is capable of decreasing the bacteremia of a dog given 300r total-body irradiation, it seems unable to influence the bacteremia of dogs given 450r. Aureomycin does not produce any significant alteration in the chemical or hematologic determinations made on these animals or in their appearance at postmortem examination.

5. The possible relationship of the method of preservation of plasma to the incidence of homologous serum jaundice. While 16 cases of probable homologous serum jaundice due to whole-blood transfusions are reported, no recognized cases of homologous serum jaundice or hepatitis have occurred in patients given pooled human plasma from the Blood Bank of the University of Chicago Clinics. This plasma has been stored in liquid state without preservative at 26-35° C. for at least two to three months prior to its administration. A survey of the previous reports of transmissible hepatitis from human blood products discloses that the method of preservation for these products was also highly suitable for the preservation of any virus that may have been present.

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Page 2 of 2

25

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27 Jun 94

RG 330, Secretary Of Defense  
ENTRY 346A - RDB, Resources Division,  
REPORTS & STATISTICS Branch Progress  
REPORTS, Dec 1946-1954  
Box #



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PROGRESS REPORT (NC)

RESEARCH AND DEVELOPMENT PROJECT CARD - (NEW PROJECTS)		2. SEC. U	3. PROJ. NO. 6-59-08-06
1. PROJECT TITLE Nutritional Requirements in Radiation Injury		4. REPORT DATE 30 Jun '50	
6. BASIC FIELD OR SUBJECT		7. SUB FIELD OR SUBJECT SUB GROUP AW-6	
8. COGNIZANT AGENCY	12. CONTRACTOR AND/OR LABORATORY		CONTRACT/W. O. NO.
9. DIRECTING AGENCY			
10. REQUESTING AGENCY	13. RELATED PROJECTS	17. EST. COMPL. DATES	
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20. REQUIREMENT AND/OR JUSTIFICATION			
21. BRIEF OF PROJECT AND OBJECTIVE			
<p>e. Progress. Metabolism after Thermal Burns and Radiation Injury. Four young adult male mongrel dogs have been followed through a lengthy control period prior to radiation and thermal injury. Hematologic observations (RBC, WBC, hemoglobin, hematocrit, plasma volume, platelets, and eosinophils) have been normal, as have liver functions (BSP retention, thymol turbidity, thymol flocculation, cephalin flocculation, prothrombin time, and serum bilirubin), direct and indirect. Oral food intakes were fixed, and urinary nitrogen excretion and body weight were relatively constant during the control period. One dog (R) was irradiated (100 r) whole body; one (B) burned (20 per cent deep second-degree); and one (RB) irradiated and burned. The fourth dog (C) served as a pair-fed control for the irradiated and burned animal. Liver functions remained normal. Anemia developed about two weeks after injury in B and RB, while red cells and hemoglobin remained normal in C and R. A mild leukopenia was present in R (10th day) post-radiation. Although leukopenia did not develop in the dog irradiated and burned (RB), the white blood cell count was considerably lower in this dog than in the one (B) only burned. The latter dog showed a leucocytosis for about three weeks. Platelets were normal in C and B, but low (1-2 weeks post-injury) in R and RB. Eosinopenia was present for the first few days after injury in the burned (B) and irradiated and burned (RB) dogs, but not in the control (C) or irradiated-only (R) dogs. The burned dog (B) and the one irradiated only (R) remained in apparently good condition. Their appetites were good, they were alert, and in general were not seriously ill. Nitrogen balance was unchanged in the dog irradiated only, but became moderately negative for two weeks in the burned dog. The dog irradiated and burned (RB) appeared well for the first two weeks after injury, but then became progressively sicker, lost his appetite, stopped eating and died three weeks after radiation. He had lost about six pounds. He consistently excreted somewhat more nitrogen than his pair-fed control. At autopsy</p>			
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JRDB FORM 1A, 1 APR 1947

SECURITY RESTRICTED INFORMATION

26

PAGE 1 OF 2 PAGES

27 Jun 94

RG 330, Secretary Of Defense  
ENTRY 346A - RDB, Resources Division,  
REPORTS & STATISTICS BRANCH PROGRESS  
REPORTS, Dec 1946- 1954  
Box #

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## Nutritional Requirements in Radiation Injury

6-59-08-06

the only positive gross findings were more severe infection and less healing of the wounds than in the dog only burned, and evidence of recent weight loss. Histologic sections are being prepared. These observations will be extended to additional dogs. In some, constant dietary intake will be insured by continuous intravenous feedings. The influence of antibiotics will be investigated.

Dog Blood Bank. In preparation for metabolic and hematologic studies of radiation and thermal injury in dogs a blood-bank system was set up. There are four established blood groups (A,B,C,D) in dogs. To date, anti-A and anti-B sera have been produced.

Eosinophils in Dog Blood. A method for the direct counting of dog eosinophils has been developed. The following procedure is used for staining:

Staining fluids: (a) 0.2% phloxin in propylene glycol  
(b) 0.05 methylene blue in propylene

Equal parts of each are mixed just prior to staining.

Staining procedure: Blood is taken in a white cell pipette up to the 1 mark and then diluted with the staining mixture to the 11 mark. The staining is allowed to continue for two hours before counting is done.

f. Future Plans. Studies will be continued as indicated.

6. Reports. None.

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1 OF 2 PAGES

Page 2 of 2

27

# DEATH RETRIBUTION

27 June 94

RG 330, Secretary Of Defense  
Entry 346A - RDB, Resources Division,  
Reports & Statistics Branch Progress  
Reports, Dec 1946 - 1954  
Box #1

# PROGRESS REPORT (NC)

JRDB FORM 1A, 1 APR 1947

**SECURITY INFORMATION**

28

PAGE 1 OF 1 PAGES

27 June 94

RG 330, SECRETARY OF DEFENSE  
ENTRY 346A - RDB, RESOURCES DIVISION,  
REPORTS & STATISTICS BRANCH PROGRESS  
REPORTS, DEC 1946 - 1954  
Box #

NND 813064  
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NO. 6-59-08-08	
REPORT DATE 30 Jn '50	
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SECURITY RESTRICTED ACTION		PROGRESS REPORT (NC)	
RESEARCH AND DEVELOPMENT - PROJECT CARD - (NEW PROJECTS)		2. SEC. U	3. PROJ. NO. 6-59-08-09
1. PROJECT TITLE Thermal Effects of an Atomic Explosion, Manuscript		5. REPORT DATE 30 Jn '50	
6. BASIC FIELD OR SUBJECT		7. SUB FIELD OR SUBJECT SUB GROUP AT-6	
8. COGNIZANT AGENCY	12. CONTRACTOR AND/OR LABORATORY		CONTRACT/W. O. NO.
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21. BRIEF OF PROJECT AND OBJECTIVE			P. 18. FISCAL EST'S.
e. <u>Progress</u> . Dr. Langer has submitted the manuscript and it is being prepared for duplication, in the SGO.			
f. <u>Future Plans</u> . As soon as the report is typed and corrected the required number of copies will be produced by the multilith process.			
g. <u>Reports</u> . None; the end-product of this work is the manuscript.			
22. JRDB SN.	PC.	IC & P.	I. I. C.

JRDB FORM 1A, 1 APR 1947

SECURITY RESTRICTED ACTION

29

PAGE 1 OF 1 PAGES

27 Jun 94

RG 330, Secretary Of Defense  
ENTRY 346A - RDB, Resources Division,  
Reports & Statistics Branch Progress  
Reports, Dec 1946 - 1954  
Box #

NND 813064  
BF 6-27-94

RESEARCH AND DEVELOPMENT PROJECT CARD - (NEW-PROJECTS)		2. SEC. U	3. PROJ. NO. 6-59-08-10
1. PROJECT TITLE Irradiation on Enzyme Systems, Effects of		5. REPORT DATE 30 Jun '50	
6. BASIC FIELD OR SUBJECT		7. SUB FIELD OR SUBJECT SUB GROUP AW-6	
8. COGNIZANT AGENCY	12. CONTRACTOR AND/OR LABORATORY		CONTRACT/W. O. NO.
9. DIRECTING AGENCY			
10. REQUESTING AGENCY	13. RELATED PROJECTS	17. EST. COMPL. DATES	
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<p>e. <u>Progress.</u> A study of the protective effect of pitressin and epinephrine against local (leg) reaction produced by x-radiation (2000 r) in rats is being carried out in collaboration with the Department of Radiobiology, University of Pennsylvania Hospital.</p> <p>Mice were subjected to whole-body x-radiation totaling 800 r/air delivered in 15 minutes. Preliminary work indicates that irradiation produced a definite depression of the succinic acid dehydrogenase activity of the liver near the time of death. In the kidney the enzyme activity did not vary significantly from that of the control group.</p> <p>There was no significant alteration of the hexokinase activity in muscle or kidney after irradiation when compared with the control group.</p> <p><u>Phosphate Distribution. Liver.</u> There was no definite change in the inorganic phosphate after irradiation. There was a slight but definite decrease in the readily hydrolyzable phosphate content up to the fifth day following irradiation. <u>Kidney.</u> There was no definite change in the inorganic and slight decrease in the labile phosphate. <u>Muscle.</u> There was a definite increase in the inorganic phosphate. A slight but definite decline in the labile phosphate occurred up to the third day following irradiation, then there was a gradual continuous rise above normal until time of death.</p> <p>f. <u>Future Plans.</u> X-radiation studies will be continued along the lines mentioned above.</p> <p>g. <u>Reports.</u> None.</p>			
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JRDB FORM 1A, 1 APR 1947

RESTRICTED

30

PAGE 1 OF 1 PAGES

27 June 94

RG 330, Secretary Of Defense  
 Entry 346A - RDB, Resources Division,  
 Reports & Statistics Branch Progress  
 Reports, Dec 1946 - 1954  
 Box #

6-64-12-12

Behavior of Peripheral Blood Vessels

11 Apr 50

Basic Medical Science

Physiology

PC-11

The Surgeon General, DA

Tulane University

W-49-007-

Med Res & Dev Bd

New Orleans, La.

MD-389

(Dr. Burch)

10-50

17 March 1947

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To study the behavior of the peripheral blood vessels under tropical and cold environmental conditions, with particular reference to acclimatization and injury.

The objectives of this project are to study:

1. The influence of tropical and cold environments on the cardiovascular and peripheral vascular phenomena of man.
2. The turnover of water and sodium and potassium in urine, sweat, blood, and inter-cellular fluids of patients in normal and diseased states and under surgical stress.
3. The influence of tropical and cold environments upon certain phases of the processes of depolarization and repolarization in normal and diseased hearts.

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27 June 94

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ENTRY 346A - RDB, Resources Division,  
REPORTS & STATISTICS Branch Progress  
REPORTS, Dec 1946- 1954  
Box #

2 PAGES

NND 813064  
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RESEARCH AND DEVELOPMENT PROJECT CARD (NEW PROJECTS)		2. SEC. U	3. PROJ. NO. 6-64-12-12
1. PROJECT TITLE Peripheral Blood Vessels		5. REPORT DATE 30 Jn '50	
6. BASIC FIELD OR SUBJECT		7. SUB FIELD OR SUBJECT SUB GROUP PO-11	
8. COGNIZANT AGENCY	12. CONTRACTOR AND/OR LABORATORY		CONTRACT/W. O. NO.
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<p>e. <u>Progress.</u> For a better understanding of disturbance in electrolyte physiology in normal man and in subjects with congestive heart failure as an abnormal state, the behavior of several elements has been observed, with the use of radioisotopes, in control subjects, subjects with congestive heart failure, and subjects with other edematous states.</p> <p>Radiomercury (<math>\text{Hg}^{203,205}</math>; <math>t_{1/2} \approx 45</math> days) labeling a mercurial diuretic (Merouhydrin) was studied with reference to the duration of its retention in the body, decline in serum concentration rates of turnover, and distribution. Intravenous catheterization was employed to determine the time-course of renal, hepatic, and extremital arteriovenous differences. Urinary excretion and serum levels obtained after oral administration of the diuretic in capsular form were also determined.</p> <p>Radiochloride (<math>\text{Cl}^{36}</math>; <math>t_{1/2} \approx 2 \times 10^6</math> yrs.) was studied in dogs in order to determine the biologic decay periods (urinary excretion rates, decline in serum concentration, and recovery rates) and chloride space. Similar studies are now in progress in humans, both in controls and in patients with congestive heart failure and other edematous states. The effects of intake and drugs on these measurements are being studied.</p> <p>Earlier studies with radiosodium (<math>\text{Na}^{22}</math>; <math>t_{1/2} \approx 3</math> yrs.) have been reported, and further observations are contemplated. Technical problems dealing with methods of assaying radioisotopes and some of the physical problems encountered have been reported.</p> <p>As time permits, studies are being carried out concerning the uptake of various elements by erythrocytes, in an effort to establish a model with which may be tested various antidiuretic substances believed to be concerned with the pathogenesis of congestive heart failure, retention of electrolytes, and edematous states. This model may then be used to assay other substances</p>			

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JRDB FORM 1A, 1 APR 1947

RESTRICTED  
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453

PAGE 1 OF 2 PAGES

27 June 94

RG 330, Secretary Of Defense  
ENTRY 346A - RDB, Resources Division,  
REPORTS & STATISTICS Branch Progress  
REPORTS, Dec 1946- 1954  
Box #

RESTRICTED

Peripheral Blood Vessels

6-64-12-12

derived in the future.

Studies with the vectorcardiogram are progressing satisfactorily. The normal spatial vectorcardiogram is and will continue to be under observation because of its extreme variability. The patterns for left ventricular hypertrophy and right and left ventricular bundle branch block have been studied sufficiently to define them generally. These data indicate that the more or less empiric criteria for their electrocardiographic identification is in need of modification. It is hoped that additional studies will yield a more precise definition.

Mr. J. A. Cronvich has practically completed a movable unit which will allow recordings to be made in the wards of Charity Hospital as well as in the laboratory. Various types of electrical interference, which have offered the greatest difficulty, have delayed completion of the apparatus, but it is hoped that the unit will be in operation by autumn. Certain standardization factors have been defined for use in spatial vectorcardiography. Reports of these and other aspects of the problem are in press or in preparation for publication.

In addition to these main programs, several young persons have been in training in various aspects of cardiology to which the research program has contributed significantly. For example, Dr. F. J. Kelly, who is on an American Heart Association Fellowship, has completed a study of the rates of diffusion of radiosodium, radiomercury, and radiochloride across the blister surface of the normal human skin and across that of patients with congestive heart failure. This study, now in preparation for publication, has contributed to a better understanding of the exchange rates and related phenomena of electrolytes across the walls of blood vessels.

Other studies have been concerned with determinations of venous pressure in the superficial veins and the larger veins of the thorax and abdomen of normal resting man which are accessible by means of the cardiac catheter.

f. Future Plans. Studies as indicated above will be continued.

g. Detailed Reports. "Regression of a Radioactive Mercurial Diuretic from the Plasma of Man," Nature, 163:640, April 23, 1949.

"A Stereoscopic Method for Obtaining Spatial Vectorcardiogram," Cronvich, Abildskov, Jackson, and Burch (in press).

"An Approximate Derivation for Stereoscopic Vectorcardiograms with the Equilateral Tetrahedron," Cronvich, Abildskov, Jackson, and Burch (in press).

"A Derivation for Stereoscopic Vectorcardiograms and Analysis of Vectorcardiograms by High-Speed Motion Pictures," Burch, Cronvich, Abildskov, and Jackson (in press).

"The Transfer of Radioactive Mercury across a Membrane Produced by the Application of Cantharides to the Skin of Man," Kelly, Svedberg, and Harp, J. Clin. Investigation (in press).

RESEARCH AND DEVELOPMENT

1. PROJECT TITLE Effluents and Circulation  
2. BASIC FIELD OR SUBJECT

3. COGNIZANT AGENCY

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e. Progress. subjects given continued of the space which one of the best used. Work on is expected the tained in the changes in int. Various treatment of p pressure-regul.  
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JRDB FORM 1A, 1 AF

Page 2 of 2

454

RESTRICTED

27 Jun 94

RG 330, Secretary Of Defense  
Entry 346A - RDB, Resources Division,  
Reports & Statistics Branch Progress  
Reports, Dec 1946-1954  
Box #



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RESEARCH AND DEVELOPMENT PROJECT CARD (NEW PROJECTS)		1. SEC. U	2. PROJ. NO. 6-64-12-14
1. PROJECT TITLE Protein Metabolism in Disease and Injury		3. REPORT DATE 30 Jun '50	
6. BASIC FIELD OR SUBJECT		7. SUB FIELD OR SUBJECT SUB GROUP AW-6	
8. COGNIZANT AGENCY	12. CONTRACTOR AND/OR LABORATORY		CONTRACT/W. O. NO.
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19. Completed.			
20. REQUIREMENT AND/OR JUSTIFICATION			
21. BRIEF OF PROJECT AND OBJECTIVE Final Report. General Statement. Work began in July 1946, using the facilities of the Departments of Physiology and Surgery, Tulane University, and the Charity Hospital. In early 1947 a metabolic unit was established in the Foundation Hospital and the work was transferred to that location during the summer. On 31 May 1949 the metabolic unit was discontinued and the work transferred to the Laboratory of Physiology. The following is a brief summary of accomplishments: 1. Development of methods for the clinical estimation of blood volume. The dye (T1824) method was standardized for clinical use and its limitations described for the determination of plasma volume./ Radioactive phosphorus (P-32) was introduced for the measurement of red-cell volume and the method adapted for clinical use./ Radioactive iodine (I 131) was used for plasma volume and shown to be a very excellent method for plasma-volume determination which is probably superior to the dye method. Clinical studies with this method are projected. 2. Clinical studies. The term "chronic shock" was introduced to describe the condition seen in chronically-ill and debilitated patients who show a significant anemia due to a decreased red-cell volume. While this term is not an ideal one, it does serve to distinguish this particular condition./ The investigators have shown that quantitative replacement of blood designed to restore the red-cell volume serves to make these patients good surgical risks and hastens recovery. This work, amply confirmed by other investigators, has emphasized the importance of blood-volume determinations in these patients./ Studies on protein metabolism indicate that the negative N balance frequently seen after surgery is not necessarily due to an inability to handle protein but rather to increased excretion or some other mechanism./ The work during the last year of the contract (1949-50) was concerned with an experimental study on the exchange of albumin between plasma and lymph. This work has yielded quanti-			
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## Protein Metabolism in Disease and Injury

6-64-12-14

tative data on the disappearance of albumin from plasma as well as information regarding lymph flow and albumin metabolism. Clinical application of this work is projected. Incomplete studies on the fate of infused albumin (and plasma) are being completed and definitive data on this problem are hoped for in the near future.

Reports. "Chronic Shock: The Problem of Reduced Blood Volume in the Chronically Ill Patient," Ann. Surg., 125:618-646, 1947.

"Studies of Blood Volume in the Tetralogy of Fallot and in Other Types of Congenital Heart Disease," J. Clin. Inv., 26:860-868, 1947.

"Essential Therapeutic Adjuvants in the Surgical Arrest of Wolff-Israel Actinomyces," Ann. Surg., 126:568-578, 1947.

"The Surgical Significance of Hemoglobin Deficiency in Protein Depletion," J.A.M.A., 135:9-10, 1947.

"Chronic Shock: The Problem of Reduced Blood Volume in the Chronically Ill Patient," Postgr. Med., 5:227-232, 1949.

"Determination of Circulating Red Blood Cell Volume with Radioactive Phosphorus," Amer. J. Physiol., 155:226-231, 1948.

"Comparison of Results of Measurements of Red Blood Cell Volume by Direct and Indirect Techniques," Amer. J. Physiol., 155:232-238, 1948.

"Effect of Administration of Adrenalin on the Circulating Red Cell Volume," Amer. J. Physiol., 155:239-241, 1948.

"Metabolic Changes Associated with the Administration of Salt-poor Human Serum Albumin in Two Cases of Infectious Hepatitis," J. Clin. Invest., 29:381-388, 1950.

"The Utilization of Amino Acids by Patients following Surgery," J. Lab. & Clin. Med., 35:385-390, 1950.

"The Exchange of Albumin between Plasma and Lymph" (being prepared).

In addition, various aspects of the work have been presented to clinical and experimental groups at their respective meetings. Exhibits have also been presented at such meetings, and the one on chronic shock at the Centennial Meeting, AMA, in June 1947 was awarded honorable mention.

Besides work that has been published, considerable data have been accumulated on various aspects of the general problem of protein metabolism in disease. Much of this work, such as the utilization of glycine, distribution of fluids, etc., will eventually be continued by various members of the group as opportunity arises.

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