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UNITED STATES ARMY MEDICAL RESEARCH INSTITUTE OF INFECTIOUS DISEASES

Frederick, Maryland 21701

404

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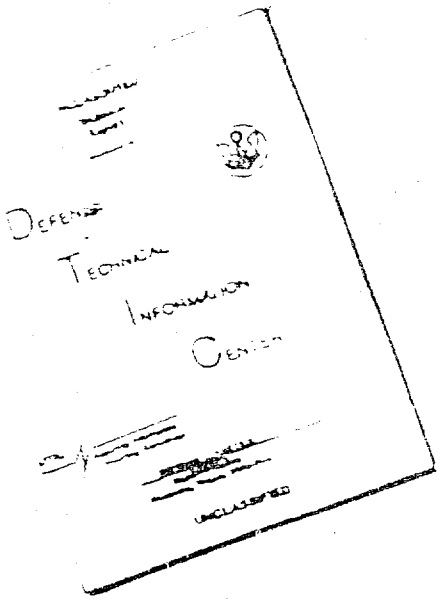

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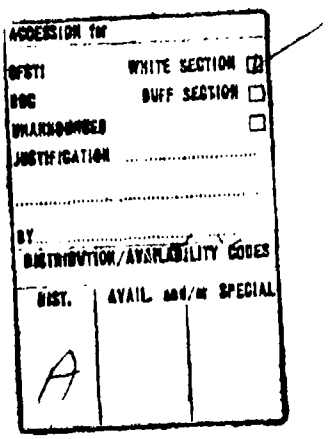

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Colonel Dan Crozier, Medical Corps

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13. ABSTRACT

A report of progress on the research program of the U. S. Army Medical Research Institute of Infectious Diseases on Medical Defense Aspects of Biological Agents (U) for Fiscal Year 1972 is presented. This is the final report under Project No. 1W662711A096. Starting in FY 1973, the number will be 3A062110A834.

KEYWORDS:

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Rickettsial diseases
Host
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U. S. ARMY MEDICAL RESEARCH INSTITUTE OF INFECTIOUS DISEASES

FREDERICK, MARYLAND 21701

ANNUAL PROGRESS REPORT

FISCAL YEAR 1972

RCS-MEDDH-288(R1)

Approved for public release; distribution unlimited.

Project 1W662711A096

New Project 3A062110A834 - FY 1973

30 June 1972

SUMMARY

A report of progress on the research program of the U. S. Army Medical Research Institute of Infectious Diseases on Medical Defense Aspects of Biological Agents (U) for Fiscal Year 1972 is presented. This is the final report under Project No. 1W662711A096. Starting in FY 1973, the number will be 3A062110A834.

FOREWORD

This FY 1972 Annual Progress Report is a general review of research activities of the U. S. Army Medical Research Institute of Infectious Diseases, Frederick, Maryland, conducted under Project 1W662711A096, Medical Defense Aspects of Biological Agents (U) and a small effort under the In-house Laboratory Independent Research Program (ILIR).

1W662711A096 01 - Pathogenesis of Infection of Military Importance.
1W662711A096 02 - Prevention and Treatment of Biological Agent Casualties.
1W662711A096 03 - Laboratory Identification of Biological Agents.

Beginning 1 July 1972, funding will be by The Surgeon General, Army, under Project 3A072110A834.

Seven contracts were in effect with educational institutions or industrial firms. Two of these terminate this date. Reports are available through Defense Documentation Center.

Six appendices are included covering Technology Support Plans (A), Volunteer Studies performed during the year (B), Guest Lecture Series (C), Professional Staff Meetings (D), Formal Presentations and Briefings (E), and Publications of USAMRIID (F). An author index is included.

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council. The facilities are fully accredited by the American Association of Accreditation of Laboratory Animal Care.

30 June 1972

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ANNUAL PROGRESS REPORT -- FY 1972

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PROJECT NO. 1W662711A096: MEDICAL DEFENSE ASPECTS OF BIOLOGICAL AGENTS (U)

ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1W662711A096 01:	Pathogenesis of Infection of Military Importance
Work Unit No. 096 01 001:	Metabolic and Physiological Studies in Experimental Infectious Disease for BW Defense
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Physical Sciences
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Author:	Randall T. Curnow, Major, MC
Reports Control Symbol:	RCS-MEDDH-288(R1)
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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

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11. TITLE (Precede with Security Classification Code): (U) Metabolic and physiological studies in experimental infectious disease for BW defense (11)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical medicine; 007900 Defense; 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
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19. RESPONSIBLE DOD ORGANIZATION
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SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
NAME:
NAME:

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DA

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Glycogen; (U) Glycogen synthetase and phosphorylase; (U) Glucose; (U) Leukocytic endogenous mediator (LEM); (U) Glucose tolerance; (U) Liver

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Study early changes in carbohydrate metabolism and physiology induced by experimental disease. This work unit is an essential element in a comprehensive program for defense against BW agents.

24 (U) A variety of techniques are employed to study metabolic changes associated with infection.

25 (U) 71 07 - 72 06 - Studies have been performed to indicate that early profound changes in carbohydrate homeostasis occur in the host during experimental sepsis. Accelerated liver glycogenolysis is promoted in the rat by pneumococcal and S. typhimurium sepsis and by LEM. The striking changes produced by salmonella sepsis in the adrenalectomized rat strongly implicated that alterations in carbohydrate homeostasis are important determinations of morbidity and lethality during sepsis.

Preliminary studies have shown that glucose intolerance is an early sequela of S. typhimurium sepsis in rat.

Studies utilizing the isolated perfused rat liver have been started.

Publications: Fed. Proc. 31:2:674, 1972
Clin. Res. 20:453, 1972
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Terminated since FY 1973 funding will be under The Surgeon General, Army. New Project No. 3A062110A834; Accession No. DA 0A6410.

* (Available to contractors upon originator's approval)

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE AND [illegible] 1 MAR 68 FOR ARMY USE ARE OBSOLETE

BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 01: Pathogenesis of Infection of Military Importance

Work Unit No. 096 01 001: Metabolic and Physiological Studies in Experimental Infectious Disease for BW Defense

Description:

Study early changes in carbohydrate metabolism and physiology induced by experimental disease.

Progress:

The majority of studies have centered on the effect of infection-related variables on glycogen metabolism in liver, skeletal muscle, and heart of the rat.

Influence of pentabarbital and halothane anesthesia on glycogen metabolism in rat liver.

To determine the most desirable anesthetic agent to use in studies on the influence of infection on liver glycogen metabolism the effects of halothane and pentabarbital anesthesia were compared on liver glycogen synthetase (GS), liver glycogen phosphorylase (GP), and plasma glucose concentration. Animals given halothane were killed within 3 min of induction of anesthesia while those given pentobarbital were killed at 20 min. In fed animals, plasma glucose and liver GS activity were not significantly different regardless of anesthetic agent. However, GP was significantly higher ($p < 0.02$) in halothane-treated animals then those given pentobarbital. In fasted animals plasma glucose concentration was significantly elevated ($p < 0.01$) in animals given pentobarbital. The per cent GS in the active or I form (%GS-I) was significantly lower ($p < 0.01$) in fasted animals given halothane while there was no significant difference in GP activity.

Thus, in using the rat as a model of infectious disease, one must be aware of the effects of the variables of types of anesthesia and nutritional status.

Influence of Diplococcus pneumoniae (DP) and Salmonella typhimurium (ST) sepsis on glycogen metabolism in rat liver.

Studies were performed to demonstrate the sequential changes in liver glycogen metabolism during DP and ST sepsis in the rat. Both infections increased the rate of glycogenolysis over that of controls. In DP sepsis, this was associated with an early decrease in the % GS-I but with a less dramatic effect on GP activity. Thus, an increased rate of glycogenolysis was more closely related to a decreased glycogen synthetic rather than an increased degradative activity. In ST sepsis this was not associated with a significant effect on either GS or GP activities. Thus, the mechanisms responsible for an increased rate of glycogenolysis during ST sepsis are more complex and possibly less applicable to study using the present enzyme assays. ST sepsis promoted a significant diminution in plasma glucose while DP sepsis had no significant effect on this parameter.

Influence of leukocytic endogenous mediator (LEM) on rat liver glycogen metabolism.

Sequential changes in liver GS, GP, glycogen concentrations, and plasma glucose were monitored following intraperitoneal (IP) administration of LEM to fed rats and compared to rats given saline and heat-treated LEM. LEM also promoted an accelerated rate of glycogenolysis compared to controls. This was associated with a decrease in the %GS-I but with no significant effect on GP activity, much like the changes seen during DP sepsis. Thus, it is proposed that LEM may be involved in sepsis-related effects on glycogen metabolism.

Effect of an IP glucose bolus on liver glycogen metabolism in rat during sepsis.

To delineate a possible alteration in dynamic response to various agents of liver glycogen metabolic machinery promoted by sepsis-GS, GP, glycogen concentration, and plasma glucose have been monitored following IP glucose (1 gm/kg) administration. Such a bolus of glucose promotes a very rapid (30 min) increase in the %GS-I and a somewhat later (1-2 hr) increase in total GS (I+D forms) activity. Plasma glucose returns to baseline levels between 1-2 hr in fed and 23 hr in fasted rats.

Glucose given (as above) to rats infected IP 6 hr earlier with 2×10^8 ST organisms promoted a significantly decreased peak plasma glucose concentration at 30 min when compared to noninfected controls. More importantly, plasma glucose remained significantly above baseline at 2 hr, indicating a decreased glucose tolerance in infected animals. Liver GS, GP, and glycogen concentration assays have not been performed on these tissues but when completed should give added insight into alterations in host homeostasis at the molecular level during septic illness.

Effect of ST sepsis on glycogen metabolism in adrenalectomized rats.

To gain information that might reveal mechanisms involved in increased liver glycogenolysis during ST sepsis in the rat, GS, GP, glycogen concentration, and plasma glucose were monitored in adrenalectomized rats infected with 2 x 10^{8} ST organisms. At approximately 3 hr these animals began convulsing, and within 10 min 10 of 18 rats were dead. The remaining rats were immediately anesthetized and killed. At that time mean rectal temperature of the group was 4C below 0-time controls; mean plasma glucose concentration was 34 mg/100 ml compared to 110 for 0-time adrenalectomized animals. At time 0 mean liver glycogen concentration was 15 mg/gm wet-weight-liver, while at 3 hr it had fallen to less than 1 mg/gm. However, there was no demonstrable influence on liver GS activity (GP activity has not yet been measured). At least in rats, the presence of adrenal glands is not essential for infection-promoted accelerated glycogenolysis. Adrenalectomized rats are much more sensitive to the lethal effects of sepsis; this decreased resistance is associated with gross abnormalities in carbohydrate homeostasis as shown by the terminal hypoglycemic and liver glycogen depletion relatively soon after the initiation of sepsis.

Studies utilizing the isolated perfused rat liver.

To determine the influence of sepsis, and factors related to it, on liver metabolic machinery studies utilizing the isolated perfused rat liver have been initiated. The rate of glycogen synthesis and degradation in this organ can be significantly influenced by altering the concentration of perfused glucose concentration with a relative hyperglycemia (300 mg/100 ml), promoting glycogen deposit and euglycemia, and hypoglycemia (< 100 mg/100 ml) promoting glycogenolysis. Glycogen deposition was associated with an increase in %GS-I and a decrease in GP activity, while reciprocal changes were seen during glycogenolysis. It is planned to remove livers from septic animals and study them in above fashion to determine the nature of altered glycogen metabolism at the molecular level in the liver.

Summary:

Studies have been performed to indicate that early profound changes in carbohydrate homeostasis occur in the host during experimental sepsis. Accelerated liver glycogenolysis is promoted in the rat by pneumococcal and S. typhimurium sepsis and by LEM. There is a strong implication that alterations in carbohydrate homeostasis are important determinations of morbidity and lethality during sepsis as revealed by the striking changes produced by Salmonella sepsis in the adrenalectomized rat.

Preliminary studies have shown that glucose intolerance is an early sequela of S. typhimurium sepsis in rat.

Studies utilizing the isolated perfused rat liver have been started.

Presentation:

Curnow, R. T., and R. S. Pekarek. The effect of Diplococcus pneumoniae sepsis and leukocytic endogenous mediator on liver glycogen synthetase and glycogen phosphorylase activity in the fasted rat. Presented at Annual Meeting of the Federation of American Societies for Experimental Biology, Atlantic City, N. J., 9-14 April 1972.

Publications:

1. Curnow, R. T. and R. S. Pekarek. 1972. The effect of Diplococcus pneumoniae sepsis and leukocytic endogenous mediator on liver glycogen synthetase and glycogen phosphorylase activity in the fasted rat. Fed. Proc. 31: 684 (abstract).

2. Curnow, R. T. 1972. Effect of D. pneumoniae and S. typhimurium sepsis on glycogen metabolism in rat liver. Clin. Res. 20: 453 (abstract).

3. Bellanti, J. A., R. I. Krasner, P. J. Bartelloni, M. C. Yang, and W. R. Beisel. 1972. Sandfly fever: Sequential changes in neutraphile biochemical and bactericidal function. J. Immunol. 108: 142-151.

ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	Medical Defense Aspects of Biological AGents (U)
Task No. 1W662711A096 01:	Pathogenesis of Infection of Military Importance
Work Unit No. 096 01 002:	Role of Hormones in Infectious Disease of Military Medical Importance
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Divisions:	Physical Sciences and Bacteriology
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Authors:	Frederick J. DeRubertis, Major, MC (I) Elliot J. Rayfield, Major, MC (II) Richard A. Proctor, Captain, MC (III)
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1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
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71 07 01	H. TERMINATION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

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11. TITLE (Precede with Security Classification Code):
(U) Role of hormones in infectious disease of military medical importance

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003500 Clinical medicine; 004900 Defense; 002300 Biochemistry

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18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
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19. RESPONSIBLE DOD ORGANIZATION
NAME: USA Medical Research Institute of Infectious Diseases
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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
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ASSOCIATE INVESTIGATORS
NAME: Rayfield, E. J. DA
NAME:

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Foreign intelligence considered

22. KEY WORDS (Precede EACH with Security Classification Code):
(U) Thyroid hormone; (U) Kinetics; (U) Pneumococcus; (U) Salmonella; (U) Endotoxin; (U) Radioisotope; (U) E. coli; (U) Growth hormone; (U) Military medicine

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Study the role of hormones in host response to infection. This work unit is an essential element in a comprehensive program for defense against BW agents.

24 (U) Isotope tracer techniques are employed in assessing cellular uptake and subcellular distribution of hormones during acute infection.

25 (U) 71 07 - 72 06 - Acute pneumococcal, Salmonella typhimurium, or Escherichia coli bacteremia result in accelerated host peripheral distribution and metabolism of L-thyroxine with subsequent increase in thyroid gland secretion.

A radioimmunoassay for growth hormone and insulin (IRI) along with a computerized method of computing the data have been discussed. A preliminary study in 4 rhesus monkeys revealed no characteristic hormonal pattern for IRI, HGH, or serum glucose during the course of pneumococcal bacteremia. A model examining these same hormonal parameters after a glucose load during the course of pneumococcal bacteremia is described which may minimize the wide minute-to-minute fluctuations in these polypeptide hormones.

A study was initiated to determine the effect of thyroxine on function of polymorphonuclear leukocytes.

Publications: J. Clin. Invest. 51:788-795, 1972
Endocrinology 90:1384-1387, 1972.
Clin. Res. 20:424, 1972

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BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 01: Pathogenesis of Infection of Military Importance

Work Unit No. 096 01 002: Role of Hormones in Infectious Disease of Military Medical Importance

Description:

Study the role of hormones in the host response to infection.

Progress, Part I:

Accelerated metabolism of thyroid hormone has been observed during acute pneumococcal infections in man and the rhesus monkey.[1,2] In the monkey, cellular uptake of L-thyroxine (T_4) is enhanced early during the course of this illness.[3] Further, concentration of labeled T_4 and triiodothyronine (T_3) has been reported to localize in involved areas of lung in patients with bacterial pulmonary infections.[4] The pathogenesis and significance of these alterations in thyroid hormone distribution and metabolism are unclear. Studies undertaken in the rat suggest that during pneumococcal infection there may be an increased metabolic impact of T_4 at the cellular level.[5] *In vitro* studies with peripheral leukocytes reveal that enhanced thyroid hormone metabolism during infection could be part of a host defense mechanism in which phagocytizing cells utilize T_4 or T_3 as a source of iodine for iodination and killing of ingested microorganisms.[6] However, the extent to which thyroid hormone or iodide participate in this peroxidase-hydrogen peroxide-halide antimicrobial system *in vivo* remains uncertain.[7]

To explore further the effects of bacterial sepsis on host thyroid hormone economy in the rhesus monkey, peripheral metabolism and distribution of ^{131}I-labeled-T_4 (^{131}I-T_4) was examined in monkeys inoculated intravenously (KV) with 10^9 *Salmonella typhimurium*, 10^9 *Escherichia coli*, 10^9 *Diplococcus pneumoniae*, endotoxin, latex or carbon particles. Inoculation of both viable and heat-killed organisms but not endotoxin, latex or carbon particles resulted in a marked acceleration in the fractional disappearance rate of ^{131}I-T_4 (K_{T_4}) within 8 hr. The effect of heat-killed organisms on K_{T_4} was transient and not evident by 16 hr. Monkeys receiving viable organisms demonstrated a more persistent acceleration of K_{T_4} corresponding in duration roughly to that of the septic illness. There was at least a 2-fold increase in the daily absolute metabolic disposal rate of T_4 in the infected monkeys during acute illness.

In additional monkeys, distributive clearance of ^{131}I-T_4 (rapid cellular uptake of hormone) was evaluated at various times after inoculation of viable or heat-killed S. typhimurium. By 4 hr, a marked enhancement of distributive clearance of hormone was noted in both monkeys receiving heat-killed and viable organisms compared to those inoculated with saline. This phenomenon also appeared to be transient in monkeys receiving heat-killed S. typhimurium since no change in distributive clearance of T_4 was noted by 14 and 24 hr in these monkeys, although clearance was still accelerated at these time intervals in monkeys given viable organisms. An increase in hepatic uptake and binding T_4 was also noted at 4 hr in monkeys inoculated with S. typhimurium.

The acute changes in T_4 metabolism and distribution could not be ascribed to decreased extracellular protein-binding of hormone. An increase in the proportion of T_4 in serum was noted only at 48 hr, while the alterations in T_4 peripheral kinetics occurred much earlier. A biphasic change in total serum T_4 concentration was seen during the course of S. typhimurium sepsis consisting of a significant depression by 48 hr and a significant elevation by 192 hr. Further, in monkeys whose intrathyroidal iodine pool had been pre-labeled with ^{125}I a similar pattern of change in serum protein-bound ^{125}I concentration was noted. It would appear likely that the fall in serum T_4 concentration reflects a depletion of the peripheral hormonal pool due to the accelerated T_4 metabolism.

Accelerated host metabolism of T_4 has been reported in other stressful or hypermetabolic states such as exercise, cold exposure, malignancy, salicylate administration and acromegaly. Thus, this response may be a relatively nonspecific one. It is possible, however, that the accelerated metabolism of T_4 noted during bacterial sepsis is related to enhanced phagocytic cell functions.[2,6] This possibility is currently being assessed by examining in vitro T_4 deiodination by peripheral leukocytes harvested during the course of acute S. typhimurium bacteremia. However, preliminary observations in monkeys with radiation induced leukopenia suggest that cellular sites other than circulating leukocytes are involved in T_4 metabolism during S. typhimurium bacteremia. The early disappearance rate of ^{131}I-T_4 from serum in infected, leukopenic monkeys was not significantly different from that of intact monkeys.

Summary, Part I:

Accelerated peripheral distribution and metabolism of T_4 has been observed during several bacteremic illnesses in the rhesus monkey. This is accompanied by a depletion of the peripheral hormonal pool and a subsequent increase in thyroid gland secretion. These changes appear to be mediated by a primary enhancement of cellular uptake and metabolism of hormone since they could not be correlated with appropriate changes in extracellular binding of hormone.

The pathogenesis and significance of these alterations in hormone economy remain unclear and may represent a more general host response to stress. However, a relationship between host phagocytic cell function and T_4 deiodination remains an attractive hypothesis which is currently being more thoroughly evaluated.

Progress, Part II:

A sensitive simultaneous double antibody radioimmunoassay for growth hormone (GH) and insulin (IRI) has been developed.[8] This assay can detect 0.25 ng/ml of GH and 5-7 μU/ml of IRI. In collaboration with CPT Rowberg (Work Unit 096 03 008) a computer program has been devised which draws a standard curve and calculates the final results for this system. We have analyzed about 200 rat serum samples for IRI and GH from Major Curnow's experiments (Work Unit 096 01 001) dealing with the effects of pneumococcal, Salmonella typhimurium, and leukocyte endogenous mediator on carbohydrate metabolism.

A preliminary study of sequential changes in GH, IRI, and serum glucose (glu) during the course of pneumococcal bacteremia in 4 chaired, conscious rhesus monkeys was carried out. After 5 days of baseline equilibration in the chairs, 1 ml of 5×10^8 virulent Diplococcus pneumoniae was injected IV at 0900 hr into 2 monkeys, 1 ml of heat-killed D. pneumoniae was injected IV into one, 1 ml normal saline into the 4th animal. The 2 infected monkeys developed fever and leukocytosis; one died 30 hr postinoculation. The IRI levels pre-inoculation varied from 50-275 μU/ml and fluctuated throughout the course of the infection without any significant change between the experimental and control groups except that the surviving infected animal had a low IRI of 5 μU/ml.

Plasma glucose varied in the pre-inoculation period from 81-131 mg/100 ml, without any recognizable pattern during the infection except for a low level of 66 compared to >100 for the heat-killed and saline controls.

Finally, GH varied from 1.8-21 ng/ml during the baseline period with wide fluctuations in all monkeys during the infected phase and an elevation to 21 ng/ml on day 7 in the surviving infected animal. Additional studies are required to establish a characteristic hormonal profile in macaques during pneumococcal bacteremia.

Investigations are underway in collaboration with AA Division to examine changes in IRI, GH, and serum glucose during IV glucose tolerance tests and pneumococcal bacteremia in monkeys having indwelling bilateral venous catheters. Each animal will receive 3 such tests: Baseline, at 24 hr, and during convalescence.[9] Preliminary baseline data in 3 chaired monkeys who had received a glucose tolerance test (0.5 gm/kg body weight) in the uninfected state showed a mean fasting blood glucose of 44 mg/100 ml

with a peak of 199 at 5 min and a rapid drop to baseline levels of 46 at 30-45 min. The mean fasting serum IRI was 27 μU/ml, with a rapid rise to 90 μU/ml 5 min after glucose infusion; peak levels of 94 μU/ml occurred at 15 min with a sharp fall to baseline levels by 30-45 min.

A radioimmunoassay for ACTH is being developed in collaboration with LTC Collins of Pathology Division. A specific antibody to α_1-39 porcine ACTH in goat has been prepared. The major difficulties we have encountered with iodinating and purifying the ^{125}I-ACTH will be solved by obtaining the labeled ACTH commercially.

Summary, Part II:

A radioimmunoassay for GH and IRI along with a computerized method of computing the data has been discussed. A preliminary study in 4 rhesus monkeys revealed no characteristic hormonal pattern in IRI, HGH, serum glucose during the course of pneumococcal bacteremia. A model studying these same hormonal parameters after a glucose load during the course of pneumococcal bacteremia is described which may minimize the wide minute-to-minute fluctuations in these polypeptide hormones.

Progress and Summary, Part III:

Depression of serum thyroxine (T_4) has been noted during acute infection.[3] In addition, the halide-myeloperoxidase-hydrogen peroxidase system has been implicated as a major bactericidal mechanism of polymorphonuclear neutrophils (PMN).[10]

Consequently, in vitro studies employing the bactericidal assay developed by Quie, et al.[11] have been initiated to determine the effects of T_4 upon PMN function. To prevent nonspecific binding of T_4 by serum proteins introduced as opsonin, Escherichia coli were sensitized with undiluted serum and washed 3 times. No additional opsonin was required in the final reaction mixture which was capable of killing 60-80% of phagocytized bacteria. To assess bactericidal activity of PMN treated with physiological levels of T_4 (100 μg/ml), a 6:1 ratio of bacteria to PMN was employed to provide maximum stress. Preliminary data indicate that actively phagocytizing PMN exhibit increased deiodination but no change in bactericidal activity.

Presentation:

DeRubertis, F. R. and K. A. Woeber. Accelerated host metabolism of L-thyroxine during acute Salmonella typhimurium sepsis. Presented at Annual Meeting, American Federation for Clinical Research, Atlantic City, N. J. 29-30 April 1972.

Publications:

1. DeRubertis, F. R., and K. A. Woeber. 1972. Evidence for enhanced cellular uptake and binding of thyroxine in vivo during acute infection with Diplococcus pneumoniae. J. Clin. Invest. 51:788-795.

2. DeRubertis, F. R., and K. A. Woeber. 1972. Accelerated host metabolism of L-thyroxine (T_4) during acute Salmonella typhimurium (ST) sepsis. Clin. Res. 20:424, 1972 (abstract).

3. DeRubertis, F. R., and K. A. Woeber. 1972. Effect of acute infection with Diplococcus pneumoniae on hepatic mitochondrial alpha-glycero-phosphate dehydrogenase activity. Endocrinology 90:1384-1387.

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2. Woeber, K. A. 1971. Alterations in thyroid hormone economy during acute infection with Diplococcus pneumoniae in the rhesus monkey. J. Clin. Invest. 50:378-387.

3. DeRubertis, F. R. and K. A. Woeber. 1972. Evidence for enhanced cellular uptake and binding of thyroxine in vivo during acute infection with Diplococcus pneumoniae. J. Clin. Invest. 51:788-795, 1972.

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ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1W662711A096 01:	Pathogenesis of Infection of Military Importance
Work Unit No. 096 01 003:	Tissue Enzyme Changes in Infectious Disease of Military Medical Importance
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Physical Sciences
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Authors:	Terry V. Zenser, Captain, MSC
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0L0802	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. GRP'N INSTR'N	8A. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
72 05 15	H. TERMINATION	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	01	003
b. CONTRIBUTING	62711A	1B662711A096		
c. CONTRIBUTING	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)
(U) Tissue enzyme changes in infectious disease of military medical importance

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 Clinical medicine; 004900 Defense; 002300 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
64 10	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER: NA
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 71	2	20
FISCAL YEAR CURRENT 72	0.5	12

19. RESPONSIBLE DOD ORGANIZATION
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21. GENERAL USE
Foreign intelligence considered

ASSOCIATE INVESTIGATORS
NAME:
NAME:

DA

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Prostaglandins; (U) Adenyl cyclase; (U) Enzymes; (U) Military medicine

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) To study serial changes in tissue enzyme systems during the course of experimental infections. This work unit is an essential element in a comprehensive program for medical defense against BW agents.

24 (U) Studies of the adenyl cyclase system are conducted to determine what effect infections have on this endocrine-metabolic parameter.

25 (U) 71 07 - 72 06 - Prostaglandin E-1 significantly stimulates partially purified hepatic adenyl cyclase at high concentrations and significantly inhibits this same enzyme at low concentrations.

Publication: Anal. Biochem. 41:372-396, 1971.

Terminated since FY 1973 funding will be under The Surgeon General, Army. New Project No. 3A062110A834; Accession No. DA 0A6412.

*Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1 1 MAR 66 FOR ARMY USE ARE OBSOLETE

BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 01: Pathogenesis of Infection of Military Importance

Work Unit No. 096 01 003: Tissue Enzyme Changes in Infectious Disease of Military Medical Importance

Description:

Study serial changes in tissue enzyme systems during the course of experimental infection.

Progress:

Recent investigations have led to the development of the second messenger role of cyclic 3'5'-adenosine monophosphate (cAMP) in the mediation of hormone action.[1,2] According to this concept, cAMP has been placed in the cell to serve as a "Director of Foreign Affairs", acting, therein, to regulate cell function in response to changes in the external environment. Since alterations in the adenyl cyclase system can reflect abnormal intracellular metabolism in states of endocrine imbalance, it is hoped that these studies will give more insight into the host response to infection at the molecular level.[3-6]

Certain actions of prostaglandin E_1 (PGE_1) indicate its involvement in infectious disease processes, stimulation of leukocyte chemotaxis[7] and production of a febrile response.[8] Aspirin and indomethacin block these actions by preventing the synthesis of PGE_1. PGE_1 has been shown to increase cAMP production in some tissues, i.e., platelets and thyroid, and to decrease cAMP levels in another tissue, fat. Prostaglandins are released from tissues by hormones which are known to increase cAMP levels, that is, in liver by glucagon and in epididymal fat pad by catecholamines.[9] This has lead to many intriguing postulations about the role of prostaglandins in relation to adenyl cyclase.

Studies are being conducted to determine what effect these prostaglandins may have on the hepatic adenyl cyclase. Preliminary experiments show that PGE_1 is significantly stimulatory at a high concentration and significantly inhibits adenyl cyclase enzyme at a low concentration, Table I.

TABLE I. EFFECT OF PGE_1 ON PARTIALLY PURIFIED HEPATIC ADENYL CYCLASE

Concentration of PGE_1 (M)	% Control ± SEM	Significance P
4.0×10^{-4}	252 ± 21	<0.0005
2.0×10^{-4}	212 ± 5	<0.0005
6.7×10^{-5}	153 ± 18	<0.050
2.0×10^{-5}	152 ± 21	<0.100
6.7×10^{-6}	135 ± 17	<0.100
2.0×10^{-6}	89 ± 13	<0.300
2.0×10^{-7}	59 ± 14	<0.050
2.1×10^{-8}	65 ± 7	<0.0125

Summary:

PGE_1 significantly stimulates partially purified hepatic adenyl cyclase at high concentrations and significantly inhibits this same enzyme at low concentrations.

Publications:

White, A. A., and T. V. Zenser. 1971. Separation of cyclic 3',5'-nucleotide monophosphates from other nucleotides on aluminum oxide columns. Anal. Biochem. 41:372-396.

LITERATURE CITED

1. Sutherland, E. W., G. A. Robison, and R. W. Butcher. 1968. Some aspects of the biological role of adenosine 3',5'-monophosphate (cyclic AMP). Circulation 37:279-306.

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8. Ramwell, P. W., and J. E. Shaw. 1970. Biological significance of the prostaglandins. Recent Progr. Horm. Res. 26:139-187.

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ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1W662711A096 01:	Pathogenesis of Infection of Military Importance
Work Unit No. 096 01 005:	Evaluation of Normal Colony Animals for BW Defensive Research
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Divisions:	Animal Resources and Pathology
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Authors:	Frank E. Chapple, III, Captain, VC (I, II) William P. Czajkowski, Captain, VC (I, II) James L. Stookey, Lt Colonel, VC (III)
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OL080 3	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	01	005
b. CONTRIBUTING	62711A	1B662711A096		
c. CONTRIBUTING	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)
(U) Evaluation of normal colony animals for BW defensive research (11)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 Clinical medicine; 004900 Defense; 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
64 08	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER: NA
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 71	3.0	40
	CURRENT 72	3.0	30

19. RESPONSIBLE DOD ORGANIZATION
NAME: USA Medical Research Institute of Infectious Diseases
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SOCIAL SECURITY ACCOUNT NUMBER:

21. GENERAL USE
Foreign intelligence considered

ASSOCIATE INVESTIGATORS
NAME: Czajkowski, W. P.
NAME: Stookey, J. L. DA

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Disease; (U) Normal values; (U) Tuberculin tests; (U) Hematology; (U) Blood chemistry; (U) Gastroenteritis; (U) Laboratory animals; (U) Military medicine

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Obtain clinical and pathological baseline values. Establish patterns of disease in normal colony animals. This work unit is an essential element in a comprehensive program for defense against BW agents.

24 (U) Conduct studies on colony animals to establish normal values for various biological parameters of interest to investigators using animals as test subjects. Study incidence and patterns of disease in normal colony animals.

25 (U) 71 07 - 72 06 - Animal Resources Division received 292 monkeys, 231 of which were unconditioned animals procured from commercial sources. More than 1000 tuberculin tests were done, all of which were negative. Complete blood counts and selected blood chemistry evaluations were conducted on all unconditioned monkeys upon arrival and periodically during the 90-day quarantine period.

Gastroenteritis was the most common disease problem in the colony, but the principal cause of deaths in the new unconditioned monkeys was viral pneumonia complicated by secondary bacterial infection.

Publication: Lab. Animal Sci. 21:434-437, 1971
J.A.V.M.A. 159:326, 1 Sep 1971
J.A.V.M.A. 160:739, 1 Mar 1972

Terminated since FY 1973 funding will be under The Surgeon General, Army. New Project No. 3A062110A834; Accession No. DA 0A6413.

*Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498 1 MAR 66 FOR ARMY USE ARE OBSOLETE

BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 01: Pathogenesis of Infection of Military Importance

Work Unit No. 096 01 035: Evaluation of Normal Colony Animals for BW Defensive Research

Description:

Obtain baseline clinical values, search for pathological lesions, and establish patterns of disease in normal colony animals.

Progress, Part 1:

Goats, Burros and Sheep - Burros, goats and sheep were periodically screened for internal parasite infestation and treated with anthelminthic drugs as needed.

Due to adverse environmental conditions, the goat herd developed numerous cases of foot rot. The herd was moved inside the barn and the foot rot problem was rectified. Construction of a new pasture area with proper drainage is now in progress which should help prevent recurrence of this problem.

The goat herd was tested and found to be Brucella-free.

Monkeys - A total of 292 rhesus monkeys (Macaca mulatta) was received by the Animal Resources Division (AR). Due to the maximum utilization program now in effect, it has been possible to achieve a greater research utilization from a lower total number of monkeys. Monkeys were received from 2 sources: 61 conditioned monkeys were received from the Animal Farm and Applied Aerobiology Division, Fort Detrick, and 231 nonconditioned monkeys were received from commercial sources. The nonconditioned animals were received in groups of 64, 119 and 48.

[illegible] monkeys was treated for diarrhea, 61 for clinical [illegible], and 40 for miscellaneous problems. Deaths occurred as follows: monkeys - 19, burros - 1 (one adult, one infant), sheep - 1, goats - 5 (3 adults, 2 newborn); euthanized due to maximum utilization: monkeys - 12.

Forty-one (41) monkey deaths occurred in the unconditioned animals during the 90-day quarantine-conditioning period. In the first group of 64 monkeys there were 17 deaths. Six of the deaths occurred during the first

7 days (4 due to measles pneumonia and 2, acute diarrhea); 5 during the 2nd and 3rd week (3 of acute diarrhea and 2, acute pneumonia); and one 30 days later of profuse chronic shigellosis. There were 30 deaths in the 2nd group of 119 monkeys, the majority due to viral pneumonia with secondary bacterial infection. The causative agent of the pneumonia was found to be the measles virus with exposure occurring during transit. In a 3rd group of 48 monkeys received on 1 Mar 72, only 3 deaths have occurred to date (1, each due to viral pneumonia, dysentery, and renal disease). The reduction of deaths in this latter group is due to an intense antibiotic prophylaxis regime initiated upon arrival.

Koch's Old Tuberculin (KOT) was used for 1049 intrapalpebral tests of monkeys; all of which were negative.

AR Division also procures and issues all other laboratory animals utilized by the Institute. To date no significant problems have been encountered in this area.

Summary, Part I:

Gastroenteritis continued to be the most common disease problem in the conditioned monkeys. Viral pneumonia (etiology - measles virus), with secondary bacterial infection, was seen to cause a rapid, often virtually asymptomatic death in newly arrived unconditioned monkeys. This high rate has been reduced in the last shipment by immediate high-level antibiotic prophylaxis regime.

Progress and Summary, Part II:

Complete blood counts and selected chemistry evaluations are being done on all unconditioned monkeys upon their arrival at the Institute and during the quarantine period. The study is being continued with results from the first 2 groups now being evaluated.

Progress and Summary, Part III:

Fifty-two normal colony animals of various species were necropsied by the Pathology Division. These represent animals from the normal colony which either died spontaneously, or were euthanitized because of various illnesses or disorders. In addition to these animals, members of the Pathology Division necropsied and accessioned approximately 950 animals of various species from other divisions in the Institute.

Except for the problems encountered in the unconditioned monkeys, the general health of the colony has been good throughout the year.

Publications:

1. Weil, J. D., M. K. Ward, and R. O. Spertzel. 1971. Incidence of Shigella in conditioned rhesus monkeys (Macaca mulatta). Lab. Anim. Sci. 21:434-437.

2. Stookey, J. L., G. S. Dill, Jr., and G. D. Whitney. 1971. Ceruminal gland neoplasia in the cat. J. Am. Vet. Med. Assoc. 159:326.

3. Stookey, J. L., T. G. Terrell, and G. D. Whitney. 1972. Osteogenic sarcoma in a 9-month-old dog. J. Am. Vet. Med. Assoc. 160:739.

4. Dill, Jr., G. S., U. McElyea, Jr., and J. L. Stookey. 1972. Transitional cell carcinoma of the urinary bladder in a cat. J. Am. Vet. Med. Assoc. 160:743-745.

ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1W662711A096 01:	Pathogenesis of Infection of Military Importance
Work Unit No. 096 01 009:	Host Amino Acid, Proteins and RNA Metabolism during Infectious Disease of Military Medical Importance
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Physical Sciences Division
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Authors:	Robert W. Wannemacher, Jr., Ph.D. (I-V) William R. Beisel, M.D. (I) Peter J. Bartelloni, Lt. Colonel, MC (I) Robert S. Pekarek, Ph.D. (III, IV) Michael C. Powanda, Captain, MSC (II) William L. Thompson, B.S. (V)
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OL0879	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISP'N INSTR'N	8B. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	01	009
b. COMPETITIVE	62711A	1B662711A096		
c. COMPETITIVE	CDOG 1212b (9)			

11. TITLE (Precede with Security Classification Code) (U) Host amino acid, protein and RNA metabolism during infectious disease of military medical importance (11)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 Clinical medicine; 004900 Defense; 002300 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
65 07	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE
EXPIRATION
b. NUMBER
c. TYPE NA
d. AMOUNT
e. KIND OF AWARD
f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 71	2.0	30
	CURRENT 72	2.0	116

19. RESPONSIBLE DOD ORGANIZATION
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TELEPHONE: 301 663-7181
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ASSOCIATE INVESTIGATORS
NAME: Thompson, W. L.
NAME: Beisel, W. R. DA

21. GENERAL USE
Foreign intelligence considered

22. KEYWORDS (Precede each with security classification code)
(U) Amino acids; (U) Sandfly fever; (U) Typhoid fever; (U) Afebrile infections; (U) RNA synthesis; (U) Protein synthesis; (U) Human volunteers; (U) Military medicine

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with security classification code.)

23 (U) Study changes in amino acids of blood and tissues in infectious disease or conditions induced by other variables. Relate these changes with RNA and protein metabolism. This work unit is an essential element in a comprehensive program for defense against BW agents.

24 (U) Free amino acid concentrations are determined by ion-exchange chromatography in plasma and tissue of experimental subjects infected with bacterial or viral organisms. Radioactive nonmetabolizable and metabolizable amino acid traces are utilized to study amino acid flux. Labeled precursors of RNA and protein metabolism are used to study effects of infection on RNA and protein synthesis.

25 (U) 71 07 - 72 06 - A humoral mediator has been detected in serum of subjects with various infections which stimulates the movement of amino acids to liver of recipient animals.

In animal models both infections and a leukocytic endogenous mediator stimulate a flux of 2 nonmetabolizable amino acids, cycloleucine or AIB, from muscle to liver. In animals infected with Salmonella typhimurium there is a greater flux of amino acids than those infected with Diplococcus pneumoniae. Metabolizable amino acids which do not increase in concentration in liver must be rapidly utilized for protein synthesis. Utilization appears to vary with the infectious organisms. This movement of amino acids is correlated with changes in RNA synthesis and polysomal profiles.

Publications: Biochem. J. 124:385-392, 1971; Infec. Immun. 4:556-562, 1971; Proc. Soc. Exp. Biol. Med. 139:128-132, 1972; Metabolism 21:67-76, 1972; J. Infect. Dis. 1972, In press.

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*Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 38 AND 1498-1 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE

BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 01: Pathogenesis of Infection of Military Importance

Work Unit No. 096 01 009: Host Amino Acid, Protein and RNA Metabolism during Infectious Disease of Military Medical Importance

Description:

Investigate the pathogenesis of changes in free amino acid of blood and tissues during infectious disease or in conditions induced by other variables.

Progress, Part I:

Earlier data obtained by paper chromatographic separation of whole-blood amino acids revealed that both bacterial and viral infections in man resulted in a drop in total blood amino acids.[1-5] More recently we have measured serial changes in individual plasma free amino acids which were obtained from volunteers that were exposed to either sandfly fever virus,[6] (Medical Division Protocols FY 70-1 and 70-3), Salmonella typhi[7], adenovirus type 21,[8] (Medical Division Protocol FY 71-2), or 17-D yellow fever vaccine virus (Medical Division Protocol FY 71-3). With both the febrile (sandfly fever and S. typhosa) and afebrile (adenovirus type 21 and 17-D yellow fever virus) infections there was a significant depression in total plasma free amino acids. The magnitude and pattern of individual amino acid response appeared to be characteristic for each infection and can be summarized as follows:

a. Sandfly fever virus: Depression of all amino acids 47 hr after inoculation with maximal responses in leucine, isoleucine and valine;

b. Typhoid fever: Early depression of many individual amino acids with maximal response in alanine, glycine, glutamine, proline, and threonine;

c. Adenovirus type 21: Maximal depressions on day 6-7 with greatest decreases in proline followed by alanine, glutamine, valine, and threonine; and

d. 17-D yellow fever vaccine virus: Maximal depression on day 3 with greatest decreases in threonine followed by lysine, aspartate, histidine, and methionine.

During the febrile stages of the inf[illegible] there was a marked increase in plasma phenylalanine-to-tyrosine ra[illegible] but [illegible] change was absent in the afebrile infections. These data indic[illegible] that [illegible] a mild asymptomatic infection can result in alterations in in[illegible]vidual [illegible]lasma amino acid content. The changes appear to be characteristic o[illegible] the individual infection and do not appear to be related to anorexia or t[illegible] febrile state.

Previous observations have suggested that serum from volunteers infected with S. typhi contained a humoral factor which when injected into recipient rats would stimulate a flux of amino acids into liver.[8] More recently serum has been obtained from subjects with various types of naturally acquired infections (a cooperative study with Dr. Klainer, Ohio State University Medical School, Contract No. DADA 17-68-C-8080). The sera were divided into the following groups: pneumococcal infections, mixed infections, cellulitis, polynephritis, and staphylococcal abscesses. When the millipore-filtered sera were injected into recipient rats which had been equilibrated with ^{14}C cycloleucine, a nonmetabolizable amino acid analog, a significant accumulation of the cycloleucine was observed in the liver 4 hr after the injection of any of the sera from the various types of infections. When heated, 90 C for 30 min, serum from an infected subject was injected into a recipient rat, no effect was noted on the distribution of labeled-cycloleucine. Thus, it was concluded that a humoral factor was released in subjects with various viral and bacterial infections which influenced the distribution of amino acids in host tissues, such as liver. Current experiments are being devised to attempt to develop a quantitative bioassay for the concentrations of this mediator of amino acid transport and to determine its presence during various stages of infectious processes.

A computer program is currently being developed to evaluate the changes in individual amino acids and the presence of the mediator of amino acid flux as a possible means for early detection of infection.

Summary, Part I:

Both viral and bacterial infections in man are characterized by early depression of plasma free amino acids. The magnitude and pattern of change in individual amino acids appear to be characteristic for a particular infection. These changes in plasma amino acids have been observed in mild asymptomatic viral infections but the marked elevation in plasma phenylalanine-to-tyrosine ratio is only observed during the febrile stages of the infectious process. The changes in plasma amino acids appear, in part, to be related to the release of a humoral mediator which stimulates a flux of amino acids into tissues such as liver.[9] The presence and concentration of the mediator as well as changes in individual plasma amino acids may afford the biochemical means of detecting the exposure of the host to a particular infectious organism.

Progress, Part II:

The concentration of 21 individual free amino acids in serum, liver, and skeletal muscle was determined in rats during incubation, acute illness, and terminal stages of experimental infection with Diplococcus pneumoniae.[17] By 4 hr after subcutaneous inoculation the concentration of total and many individual free amino acids in serum, liver and muscle was significantly decreased in comparison to findings in noninfected, pair-fed control rats. By use of a nonmetabolizable amino acid analog (cycloleucine) it was possible to detect a flux of amino acids from muscle to liver in the infected rat. The endogenous amino acids which moved to liver were rapidly utilized in biosynthesis of serum proteins.

In order to elucidate further the mechanisms by which plasma amino acids were depressed in volunteers or experimental animals infected with either viral or bacterial organisms, studies were continued in animal models on alteration and distribution of 2 nonmetabolizable amino acids, ^{14}C-cycloleucine and α-aminoisobutyric-1-^{14}C acid (AIB). Cycloleucine is transported at the "so-called" L site on the cell membrane and is a model for transport of neutral amino acids with branch-chains (e.g., leucine) or aromatic rings (phenylalanine). AIB, on the other hand, is transported by the "alanine-preferring" A system on the cell membrane and is a model for transport of alanine, glycine, and other neutral amino acids with small or polar side chains. These model amino acids are subject to intracellular transport and cellular concentrations gradients but are not incorporated into protein or further metabolized.

Rats were injected subcutaneously with 1 μCi/100 gm body weight of either ^{14}C-cycloleucine or ^{14}C-AIB and 24 hr later, when equilibrium distribution had been established for the model amino acid, were injected subcutaneously with 5 x 10^6 virulent D. pneumoniae or intraperitoneally (IP) with 2 x 10^8 Salmonella typhimurium organisms. Food-restricted controls were inoculated with heat-killed cultures of either organism and all animals were killed 24 hr later. Two hours before killing those rats that had received the cycloleucine were injected IP with 10μCi/100 gm body weight of ^{3}H-leucine and those given AIB with an equivalent amount of ^{3}H-glycine. Liver and muscle intra- and extracellular volumes were calculated from total water determinations and chloride space measurements.

The livers from the rats infected with both organisms contained more intracellular and less extracellular water than pair-fed, noninfected controls. When compared on the basis of intracellular water content, the livers from rats infected with pneumococci, as assessed by bacteremia and elevated body temperatures, had twice as much cycloleucine or AIB as those of control animals, while livers from rats infected with S. typhimurium contained 7 and 5 times as much cycloleucine and AIB respectively, as liver from control animals. Even though there was an increased rate

of amino acid transport, the livers from animals with either infection contained 86% as much free leucine as those of controls, while ^{3}H-glycine content of the livers from rats infected with D. pneumoniae or S. typhimurium were 80 and 40%, respectively, of that found in the control animals. Therefore, in both infections, these amino acids were transported into the liver at an increased rate and were also utilized or further metabolized at an equal or greater rate for protein synthesis. This flux of amino acids into liver and utilization appeared to be greater in the animals infected with the S. typhimurium than those infected with pneumococci.

While the previous studies had shown that infection-related depression in plasma amino acids is related to a flux of amino acids from muscle and tissues such as liver, in certain infections which involve hepatic damage, such as viral hepatitis, a marked increase in plasma amino acids has been observed. One possible explanation for this increase in plasma amino acids could be the fact that the utilization by liver is decreased due to hepatic damage. To test this hypothesis, rats were given various doses of inhibitors of protein synthetic mechanisms and subsequently infected to see what effect this would have on plasma amino acid flux of cycloleucine into liver, and subsequent synthesis of serum proteins. When rats were infected with D. pneumoniae and injected with 12 mg of puromycin over a 24-hr period, they developed typical febrile responses and bacteremia as observed in infected animals without puromycin. Similarly, the rats receiving the protein inhibitor had an increased flux of ^{14}C-cycloleucine into liver as compared to noninfected controls but the concentration of serum unbound ^{3}H-leucine was significantly increased in both control and infected animals. As observed earlier, infection resulted in a 2-3-fold increase in synthesis of serum proteins; however, in the rats receiving puromycin, serum protein synthesis was decreased in both groups of rats. The total serum amino acid concentrations were decreased in rats infected with pneumococci, but, when puromycin was given to these rats there was a significant increase above control values, with a 2-fold increase in serum alanine.

If rats were infected with D. pneumoniae and given 0.6 mg of cyclohexamide IP over 24 hr (another inhibitor of protein synthesis) none of the rats survived for 24 hr. In control rats receiving cyclohexamide there was a very marked accumulation of serum amino acids; serum protein synthesis was only 15% of that in untreated controls. When infected rats received lower doses of cyclohexamide (0.3 or 0.6 mg/24 hr) they survived for 24 hr after infection with D. pneumoniae and had bacteremia very similar to that of untreated rats. In these rats there was an increased flux of amino acids into liver but there was no increased synthesis of serum proteins, as observed in untreated infected rats. Similarly, the concentration of metabolizable leucine was increased in the serum of rats receiving the cyclohexamide. From these data it may be concluded that by blocking the utilization of amino acids for protein synthesis an infection will result in a marked increase in serum free amino acids. Thus, the increase in serum

amino acids in subjects with viral hepatitis may be related to an elevated flux of tissue amino acids into serum and a decreased hepatic utilization as the result of liver damage.

When weanling rats were fed an amino acid deficient diet (6% casein) the liver had a decreased protein biosynthetic capacity.[11] If these rats were then challenged with a subcutaneous dose of D. pneumoniae there was a marked accumulation of cycloleucine in the liver and a 2-3-fold increased incorporation of radioactive leucine into serum proteins as compared to noninfected diet-controls. Thus even though these rats were severely protein-depleted they did utilize their meager body stores to mobilize amino acids for flux to liver and subsequent increased synthesis of serum proteins. This suggests that the movement of amino acid into liver and subsequent synthesis of serum proteins is a basic defense mechanism against infectious organisms and takes place at the expense of other body tissues.

Summary, Part II:

From the evidence obtained from 2 model infections, D. pneumoniae and S. typhimurium in rats, it may be concluded that the infection-related changes in plasma amino acids reflect a flow of amino acids from muscle to liver and that the increased movement of amino acids into intracellular fluids takes place at both the "L" and "A" sites on the hepatocyte membrane. There appears to be a greater movement of amino acids into the liver of rats infected with S. typhimurium as compared to D. pneumoniae and may be related to an increased rate of gluconeogenesis and/or ketogenesis in these rats. When serum and hepatic protein synthesis was blocked by inhibitors, puromycine or cyclohexamide, the plasma amino acid concentrations were markedly elevated in the infected rats even though there is marked accumulation of cycloleucine in their livers. This would suggest that decreased utilization of the amino acids for serum protein synthesis can result in an increase in plasma amino acid concentrations in infections such as viral hepatitis. This flow of amino acids into liver and subsequent increased synthesis of serum proteins takes place even in severely protein-depleted rats, suggesting that this is a vital process in the host defense against infections.

Progress, Part III:

Recently it has been reported that a saline wash from incubated peritoneal leukocytes that have been stimulated with glycogen will mediate a marked increase in the transport of ^{14}C-cycloleucine into liver cells of rats.[9] These studies have been extended to show that this leukocytic endogenous mediator (LEM) also has a significant effect on the hepatic accumulation of ^{14}C-AIB. With one injection a maximal effect is noted in 3 hr; by 9 hr the concentration of the amino acid analogs are back to that observed in saline-injected rats. If the rats receive repeated injections

of LEM at 4-hr intervals, the cycloleucine accumulates logarithmically and by 12 hr attain a liver-to serum concentration gradient similar to that seen in animals infected with S. typhimurium. Thus, it may be concluded that LEM has a relatively short biological half-life as to its effect on serum amino acid flux into liver and, in addition, repeated injections result in continual stimulation of the amino acid transport sites on the membrane of hepatocytes.

Studies have continued in attempts to characterize the LEM prepared from different species of experimental animals. LEM prepared from rat, rabbit, monkey, and dog all stimulate a flux of amino acids into liver when injected into recipient rats, while LEM from goat and burro had no activity. Both pronase and trypsin inactivated the amino acid flux activity of LEM prepared from rabbit; however, it took approximately 20 hr for either pronase or trypsin to completely inactivate the LEM. The LEM is stable for at least 7 days when stored at either 4 or -20 C at a pH range of 4.7-8.0.

When the crude preparations of LEM from rabbits were fractionated on G-200 Sephadex columns, amino acid flux was observed in a fraction of high molecular weight, > 200,000, and a low molecular weight fraction, approximately 10,000. A crude extract of LEM from dogs had similar distribution of activity when fractionated on G-200 Sephadex. When the various fractions were dialyzed against water and back-extracted with either DEAE cellulose and pH 6.5 buffer or CM cellulose and pH 5.0 buffer, the amino acid flux activity of the high molecular weight fraction was in the CM supernatant while for low molecular weight fraction it was in the DEAE supernatant. In another study in which unfractionated rabbit LEM was extracted immediately with either DEAE or CM cellulose, most of the amino acid flux activity was present in the DEAE supernatant fluid. This would suggest that the higher molecular weight fraction merely contains aggregates of the lower molecular weight material. This conclusion was supported by another experiment in which crude LEM was first centrifuged at 105,000 x g for 40 min to remove any particulate matter. This material was then passed through an Amicon ultrafiltration apparatus with a 100,000 m.w. filter. In 5 studies in which the concentration and pH of the crude LEM were varied, an approximately equal distribution of the amino acid flux activity of LEM was found in both fractions, greater than and less than 100,000 m.w. Since both of these subfractions of LEM lose activity when heated to 90 C for 30 min, it may be concluded that the higher molecular weight is not an endotoxin contaminant. The most plausible explanation for the appearance of activity at these different molecular weights is that the LEM exists in an aggregated configuration.

While the purest fractions following fractionation on Sephadex G-200 column and batching with either CM or DEAE cellulose still contain 3 to 4 protein bands when electrophoresed on acrylamide gel, attempts are continuing to purify the mediator of amino acid flux. It is hoped that eventually we

will be able to obtain enough material to produce an antibody against this protein and subsequently develop a radioimmunoassay. Once this has been accomplished it will be possible to analyze sera rapidly for the presence of this endogenous mediator. This may be one of the earliest indications of the presence of an inflammatory state of the host.

Summary, Part III:

A mediator is released from phagocyizing peritoneal leukocytes which will stimulate a flux of cycloleucine or AIB into liver of recipient animals. This mediator can be produced from a variety of animals but has a certain degree of specificity for the homologous species. The LEM is heat-labile, protein in nature and apparently of lower molecular weight (10-20,000 m.w.) but is capable of forming higher molecular weight aggregates. The mediator is in extremely low concentration relative to the other contaminating proteins present in the crude extracts.

Progress, Part IV:

In an attempt to determine the site of action of LEM in regulating the rate of amino acid flux into liver cells, studies have been initiated in endocrine deficient animals. In hypophysectomized rats which have been maintained for at least 3 weeks postsurgery, a crude extract of LEM would markedly stimulate a flux of ^{14}C-cycloleucine into the liver as compared to similar injection of heat-inactivated LEM (90 C for 30 min) or saline. If rats were adrenalectomized and maintained on 1% NaCl for 3 days and subsequently injected IP with 1 ml of crude LEM, there was a marked stimulation in the flux of cycloleucine to liver tissue while heat-inactivated LEM or saline injections had no effect. These data would suggest that neither the anterior pituitary (adenohypophysis), which contains thyrotropic, anenocorticotropic, gonadotrophic, and growth hormones, nor the posterior pituitary (neurohypophysis) are necessary for the effects of LEM on amino acid transport. Similarly, the adrenal corticoids or catecholamines do not appear to be involved in the LEM-mediated effects on amino acid flux into liver. Additional studies are currently being carried out in thyroidectomized and diabetic animals to see what effect these have on LEM-stimulated amino acid transport into liver.

Summary, Part IV:

The action of LEM is apparently not mediated through the pituitary or adrenal glands.

Progress, Part V:

Methods have been developed for the in vitro determination of the protein synthetic ability in the liver of various animals in response to either a viral or bacterial infection. This work is based on the work of previous investigators at USAMRIID[12-15] who showed alterations in overall host protein synthesis in response to an infection. The methods

employed are: amino acid incorporation,[16] percentage of active ribosomes,[17] and ribosomal patterns.[18]

The amino acid incorporation procedure employs the use of a labeled amino acid to determine the rate of incorporation of a ribosomal preparation. Whereas the % active ribosome test indicates the percentage of the total ribosomes present that are active, by the use of labeled puromycin which forms a peptidyl-puromycin bond and removes the polypeptide from the ribosome and at the same time shuts it down from further protein production. The ribosomal patterns are obtained by layering the ribosomal preparation [illegible] sucrose gradient and separating fractions by ultracentrifugation. [illegible] of the heavier to the lighter polysomes gives one an idea of the [illegible] or breakdown or aggregation as compared to the normal ratios.

There is an early increase in amino acid incorporation, % of active ribosomes, and % in the polysome area of the ribosomal patterns in both diplococcal and Salmonella infections. This is followed by a return to normal or below normal values in all 3 tests as the time of infection progresses.

Recently work was begun on in vivo incorporation of labeled orotic acid into the RNA of the following fractions of rat liver; total homogenate, nuclei, nonsedimentable RNA (S-RNA), total ribosomes, and free ribosomes.[19,20] Preliminary work on subfractionation of rat liver after 16-20 hr of pneumococcal infection has revealed a marked increase in incorporation of orotic acid into total ribosomes of infected animals as compared to controls. Smaller increases were noted in the free ribosomes, S-RNA and total homogenate with no change in the nuclear fraction. This indicates that while the production of ribosomes involved in the formation of intracellular proteins (free ribosomes) is significantly stimulated, the formation of ribosomes responsible for the production of extracellular proteins (bound ribosomes which make up a part of the total ribosome preparation) are more greatly stimulated.

Studies will also be performed on infected and control rats raised with a deficient protein diet[11] to determine their ability to incorporate orotic acid into RNA in response to an infection.

Summary, Part V:

The effects of infection on the ribosomes of rat liver are characterized by an early increase in the production of ribosomal RNA, especially that associated with the bound ribosomes, followed by a decrease back to or below control levels.

Presentations:

1. Wannemacher, Jr., R. W. Interrelationship between nutrition and infection with regard to changes in plasma amino acids. Presented at Western Hemisphere Nutrition Congress III, Miami, Fla., 30 August - 2 September 1971.

2. Wannemacher, Jr., R. W. Several factors affecting plasma free amino acid concentrations. Presented at meeting of Federation of American Societies for Experimental Biology, Atlantic City, N. J., 9-14 April 1972.

3. Wannemacher, Jr., R. W., R. S. Pekarek, and W. R. Beisel. An endogenous mediator(s) of plasma amino acid flux and trace metal depression during experimentally induced infection in man. Presented at 12th Annual Meeting American Society for Clinical Nutrition, Atlantic City, N. J., 29 April 1972.

4. Wannemacher, Jr., R. W. Ribosomal ribonucleic acid synthesis and function as influenced by amino acid supply and stress. Presented at 527th Meeting, The Biochemical Society, School of Agriculture, Aberdeen, Scotland, 1-2 June 1972.

Publications:

1. Wannemacher, Jr., R. W. 1971. Interrelationship between nutrition and infection with regard to changes in plasma amino acids. In Program Western Hemisphere Nutrition Congress III, p. 42.

2. Wannemacher, Jun., R. W., C. F. Wannemacher, and M. B. Yatvin. 1971. Amino acid regulation of synthesis of ribonucleic acid and protein in the liver of rats. Biochem. J. 124:385-392.

3. Wannemacher, Jr., R. W., M. C. Powanda, R. S. Pekarek, and W. R. Beisel. 1971. Tissue amino acid flux after exposure of rats to Diplococcus pneumoniae. Infec. Immun. 4:556-562.

4. Wannemacher, Jr., R. W., R. S. Pekarek, P. J. Bartelloni, R. T. Vollmer, and W. R. Beisel. 1972. Changes in individual plasma amino acids following experimentally-induced sandfly fever virus infection. Metabolism 21:67-76.

5. Wannemacher, Jr., R. W., R. S. Pekarek, and W. R. Beisel. 1972. Mediator of hepatic amino acid flux in infected rats. Proc. Soc. Exp. Biol. Med. 139:128-132.

6. Wannemacher, Jr., R. W. 1972. Several factors affecting plasma and free amino acids concentrations. Fed. Proc. 31:270 (abstract).

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9. Wannemacher, Jr., R. W. 1972. Ribosomal ribonucleic acid synthesis and function as influenced by amino acid supply and stress. Proc. Biochemical Soc., p. 5-6.

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ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1W662711A096 01:	Pathogenesis of Infection of Military Importance
Work Unit No. 096 01 010:	Effect of Irradiation on Infection and Immunity for BW and RW Defensive Research
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Divisions:	Animal Assessment and Medical
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Authors:	Richard O. Spertzel, Lt Colonel, VC (I) John C. Holder, Major, MC (II) Douglas W. Mason, Captain, VC (I) Garrett S. Dill, Jr., Captain, VC (I) James W. Brown (I, II)
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OL0883	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	01	010
b. [illegible]	62711A	1B662711A096		
c. [illegible]	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)

(U) Effect of irradiation on infection and immunity for BW and RW defense

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical medicine; 004900 Defense; 014100 Radiobiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
67 07	CONT	DA	C. In-house

17. CONTRACT, GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER: NA

c. TYPE d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
PRECEDING FISCAL YEAR 71	2.0	30
CURRENT FISCAL YEAR 72	2.0	31

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Holder, J. C.

NAME: Mison, D. W. DA

21. GENERAL USE

Foreign intelligence considered.

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Radiation; (U) Mice; (U) Infectious diseases; (U) Vaccine; (U) Military medicine; (U) Encephalomyelitis, equine (VEE)

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Investigate interrelationships between acute or chronic irradiation and disease processes. This work unit is an essential element in a comprehensive program for defense against BW agents.

24 (U) Acute or protracted whole body irradiation is delivered to selected animal species before, simultaneously with, or after infection. Clinical and immune responses are observed and measured serially.

25 (U) 71 07 - 72 06 - Radiation and immunity studies were continued. Viremia is prolonged in irradiated, vaccinated mice, thus contributing to the eventual development of protection with a living vaccine. Histopathological examination of this material revealed that (1) no lesions were produced in mice with the TC-83 strain of VEE virus whether the mice were irradiated or not, (2) the meningoencephalitis, produced in mice by infection with Trinidad strain of VEE virus, was altered to neuronal necrosis by the irradiation, presumably because of depression of the immune reponse; and (3) infection with Trinidad strain prolonged the depletion of the bone marrow and lymphoid elements produced by irradiation. Several mechanical failures of the 1-MEV X-ray unit have hindered the progress of this project throughout the year.

A satisfactory cobalt-60 source has been unavailable. Procurement action has been initiated.

Terminated since FY 1973 funding will be under The Surgeon General, Army.

New Project No. 3A062110A834; Accession No. DA 0A6415.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 68 AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 01: Pathogenesis of Infection of Military Importance

Work Unit No. 096 01 010: Effect of Irradiation on Infection and Immunity for BW and RW Defensive Research

Description:

Investigate interrelationships between acute or chronic irradiation and disease processes.

Progress, Part I:

In previous studies,[1] 600 r irradiation was shown to delay but not to prevent the onset of protection from the attenuated form (TC-83) of Venezuelan equine encephalomyelitis (VEE); but the smaller the time interval between irradiation and TC-83 inoculation, the greater the delay of onset of protection. Since the ultimate result of vaccination, protection, with the attenuated VEE virus was the same in irradiated and nonirradiated mice, the period of viremia in irradiated mice following TC-83 immunization was probably prolonged until the host immune mechanisms had recovered from irradiation damage. Limited studies in 1966[a] showed that 500 r irradiation delivered 24 hr prior to TC-83 inoculation prolonged viremia by 3-6 days. An experiment was designed to determine to what extent viremia was prolonged in irradiated mice and how this prolongation correlated with the previously observed delay in onset of protection relative to challenge.

Thirty-day-old white, Swiss mice, obtained from Microbiological Associates, Inc., Walkersville, Md., were irradiated as previously described.[1] Irradiation was timed so that inoculation with 5000 median guinea pig intraperitoneal immunizing doses ($GPIPID_{50}$) of TC-83 occurred at 0, 2, 7, and 14 days postirradiation. The experiment was designed, however, so that all mice were vaccinated on the same days. Viremia levels were followed for 15 days. Each mouse was bled every 5th day and marked by ear punching. This enabled us to study any cyclic changes in the viremia and determine whether it was prolonged in any one mouse. One hundred microliters of blood were drawn retrobulbarly from each mouse and mixed with 0.4 ml of phosphate buffered saline; 0.3 ml of this mixture was injected IP into a recipient mouse. The recipients were challenged 14 days later with 1000 median mouse intracranial lethal doses ($MICLD_{50}$) of Trinidad strain VEE. If virus was present in the donor blood, then the recipient should resist challenge.

The results, Table I, show that irradiation prolongs the TC-83 VEE viremia in mice; the shorter the time interval between irradiation and immunization the longer the viremia is prolonged. Viremia of the unirradiated

TABLE I. DURATION OF VIREMIA IN IRRADIATED AND NORMAL MICE GIVEN TC-83 VEE AS MEASURED BY SURVIVAL AFTER TRINIDAD CHALLENGE

DAY OF BLEEDING POSTVACCINATION	SURVIVORS/TOTAL BY DAY OF IRRADIATION PREVACCINATION				VACCINE CONTROLS
	-14	-7	-2	(hr -1)	
1	3/7	1/12	2/11	5/12	4/11
2	5/12	5/11	6/12	3/12	4/11
3	4/7	3/11	1/12	3/11	5/11
4	5/10	1/6	2/10	5/11	1/11
5	1/7	4/11	2/9	4/12	0/11
6	0/7	3/7	3/8	4/12	0/12
7	0/11	2/12	2/7	3/11	0/12
8	0/7	1/10	1/7	1/11	0/12
9	0/10	0/5	1/5	1/11	0/12
10	0/8	0/9	0/5	1/10	0/12
11	0/7	0/6	0/7	1/11	0/11
12	0/12	0/7	0/6	0/6	0/12
13	0/6	0/11	1/3[a]	0/6	0/11
14	0/10	0/5	0/5	1/8[a]	0/11
15	0/6	0/9	0/4	0/9	0/12
		% by Day			
1	43	8	18	42	36
2	42	45	50	25	36
3	57	25	8	25	45
4	50	17	20	45	9
5	14	36	22	33	0
6	0	43	38	33	
7		17	28	27	
8		10	14	9	
9		0	20	9	
10			0	10	
11				9	
12				0	

a. Probably aberrant figures.

vaccine controls persisted for only 4 days, which correlated closely (5 days) with those animals irradiated on day -14. However, when the mice were irradiated 7 days prior to immunization, viremia lasted 8 days, 2 days prior, 9 days, and at hour -1, 11 days. A spurious viremic response was observed in one mouse in day -2 and day 0 group on 13 and 14 days postimmunization, respectively. In mice vaccinated 1 hr postirradiation, viremia was present in individual mice on at least 2 consecutive bleedings, e.g., viremia persisted in individual mice $>$ 5 days. However, in all other groups, although viremia of the group was detected up to 9 days postirradiation, in each instance this represented viremia in different mice; thus, in these mice viremia could not have been present more than 5 days.

Although most of the irradiation work has been done in 30-day-old mice, some studies were completed in adult mice (2-9 mon old). As previously reported,[1] the median lethal dose ($LD_{50(30)}$) for the adult mice was 760 r, about 85 r higher than for 30-day-old mice. A series of studies was designed utilizing the adult mice in order to evaluate the effect of age of the mouse. Mice were irradiated with 650 r from 21 days pre- to 9 days postvaccination with 5000 $GPIPID_{50}$ TC-83. From days 1-28 postvaccination, groups of mice were challenged IP with 1000 $MICLD_{50}$ of Trinidad strain VEE, Table II. As observed with the 30-day-old mice,[1] irradiation before vaccination delayed the onset of protection to Trinidad VEE. The delay was not as pronounced as that seen with young mice; however, the same general trend was noted: the shorter the irradiation-to-vaccination time interval, the greater the delay of onset of protection to Trinidad VEE. Also, as in 30-day-old mice, irradiation of adult mice after vaccination inhibited the onset of protection, but a time factor, if involved, was not as obvious as when irradiation preceded vaccination.

The effects of 600 r irradiation on hemagglutination-inhibition (HI) antibody titers produced by TC-83 VEE in 30-day-old mice have been reported.[1] However, because of a few aberrant figures and statistical inadequacies, further studies were planned. Before the project began, the 1-MEV X-ray unit became inoperable. It was found that by placing the mice 90 cm in front of the Autorad 500 Cobalt source, a 2 r/min-dose could be achieved when utilizing both Cobalt sources. The project was redesigned so that the mice received 300 r ($2\frac{1}{2}$ hr in front of the Cobalt source) on days -14, -10, -7, and -1 preimmunization IP with 5000 $GPIPID_{50}$ of TC-83 VEE. The mice were then bled from days 1-60; results are pending.

A joint project with Pathology Division involving the determination of the histopathology of Trinidad and TC-83 strains of VEE in 30-day-old CD_1 mice (obtained from Charles River Breeding Laboratories, Inc., Wilmington, Mass.) with and without radiation was initiated. The mice were placed in one of 6 groups: (1) control, (2) irradiated, (3) infected with TC-83, (4) infected with Trinidad, (5) infected with TC-83 and irradiated, and (6) infected with Trinidad and irradiated. The irradiated animals were exposed to 600 r, infected 4 days later, and then serially sacrificed with 8 mice per group on days 3, 4, 5, and 6.

TABLE II. EFFECT OF TIMING[a] OF 650 r IRRADIATION AND TRINIDAD VEE CHALLENGE ON VEE-IMMUNIZED ADULT MICE

DAY OF VEE CHALLENGE POSTVACCINATION	SURVIVORS/TOTAL BY DAY OF IRRADIATION PREVACCINATION												VACCINE CONTROL
	-21	-14	-8	-4	-2	-1	0	+1	+2	+4	+7	+9	
+ 1	4/8	4/8	0/9	0/10	1/6	1/6	0/18	2/16					11/18
+ 2	7/8	5/8	1/9	2/10	1/6	0/6	1/18	2/16	9/12				14/18
+ 3	6/6	6/8	0/8	0/9	0/6	0/6	4/17	2/13	9/12				17/18
+ 4	6/6	5/8	3/8	2/9	0/6	0/5	5/18	7/16	5/12	8/12			21/22
+ 5	5/5	5/5	3/6	0/10	1/5	2/5	4/6						10/10
+ 7	6/6	7/7	7/7	2/9	1/5	1/4	7/18	7/15	9/12	8/12	7/12		21/22
+10	6/6	6/6	7/7	5/7	3/4	2/5	11/15	12/15	5/12	10/11	7/11		19/22
+14	6/6	6/6	5/6	6/6	3/3	3/3	17/17	8/10	8/8	10/12	4/8	4/12	19/21
+17	6/6	5/6	3/4	6/6	2/2	2/2	15/16	12/12	8/9	8/9	5/10	6/7	19/20
+21	4/5	2/2	5/5	3/3	2/2	2/2	17/17	11/12	12/12	9/9	8/8	2/7	15/17
+28	5/5	4/5	3/3				13/15	11/11	6/7	11/11	4/4	4/4	17/18
Radiation Controls	8/10	9/10	7/10	9/10	6/8	6/8	18/20	9/12	8/12	8/12	9/12	10/12	

a. Negative numbers indicate irradiation before vaccination; positive numbers indicate irradiation following vaccination; day 0 is day of immunization.

The control animals were essentially normal histologically except for moderate infection with chronic murine pneumonia (CMP) in a few. Animals inoculated with TC-83 showed no significant differences from the controls. Irradiated controls had CMP only and expected depletions of bone marrow and spleen. The regeneration of the lymphoid and erythroid elements had almost corrected the depletions by day 6. Irradiated animals inoculated with TC-83 did not differ significantly from irradiated controls.

Animals infected with Trinidad VEE, on the 3rd and 4th days postinfection had a mild-to-severe, acute, lymphoid necrosis in germinal centers of the spleen, lymph nodes and/or lymphoid nodules of the small intestine. This became less apparent on day 5 and had almost disappeared the next day. A minimal encephalitis in the olfactory lobe was observed in 2 of 8 mice on day 3, which by day 4 was manifested by a neuronal necrosis in 7 of 8 mice. On days 5 and 6, all 8 had a minimal-to-severe encephalitis or meningoencephalitis with neuronal necrosis. No other significant deviations from controls were observed except some myeloid depletion on days 3 and 4 postinoculation.

Irradiated animals infected with Trinidad had depletions of lymphoid and bone marrow elements similar to the irradiated controls on days 3 and 4, but the effect persisted longer, so that on days 5 and 6 they were still moderately depleted. Unlike those nonirradiated mice infected with Trinidad VEE, a true encephalitis was present minimally in only one irradiated mouse, but neuronal necrosis was progressive (0 of 8 on day 3, 5 of 8 on day 4, 8 of 8 on day 5, and 7 of 8 on day 6).

Additional studies on the histopathology of irradiated infected mice are planned, but are held in abeyance pending repair of the 1-MEV unit.

Summary, Part I:

Radiation and immunity studies were continued. Viremia is prolonged in irradiated, vaccinated mice, thus contributing to the eventual development of protection with a living vaccine.

Histopathological examination of this material revealed that no lesions were produced in mice with the TC-83 strain of VEE virus whether the mice were irradiated or not; the meningoencephalitis, produced in mice by infection with Trinidad strain of VEE virus, was altered to a neuronal necrosis by irradiation, presumably because of depression of the immune response. Infection with Trinidad strain prolonged the depletion of the bone marrow and lymphoid elements produced by irradiation.

Several mechanical failures of the 1-MEV X-ray unit have hindered the progress of this project throughout the year.

Progress and Summary, Part II:

A satisfactory Cobalt-60 source has been unavailable. Procurement action has been initiated.

Publications:

None.

LITERATURE CITED

1. U. S. Army Medical Research Institute of Infectious Diseases. 1 July 1971. Annual Progress Report, FY 1971. p. 41 to 54. Fort Detrick, Maryland.

2. U. S. Army Medical Unit. 1 July 1966. Annual Progress Report, FY 1966. p. 147 to 152. Fort Detrick, Maryland.

ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1W662711A096 01:	Pathogenesis of Infection of Military Importance
Work Unit No. 096 01 011:	Rapid Electron Microscopic Assay for Virus Particles of Diseases of Military Medical Importance
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Physical Sciences
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Author:	Anne Buzzell, Ph.D.
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0L0810	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	01	011
b. CONTRIBUTING	62711A	1B662711A096		
c. CONTRIBUTING	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code): (U) Rapid electron microscopic assay for virus particles of diseases of military medical importance

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical medicine; 004900 Defense; 003200 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
62 02	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER: NA
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
PRECEDING FISCAL YEAR 71	1.0	15
CURRENT FISCAL YEAR 72	1.0	36

19. RESPONSIBLE DOD ORGANIZATION
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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME:
NAME:

21. GENERAL USE
Foreign intelligence considered

DA

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Virus; (U) Electron microscopy; (U) Negative staining; (U) Military medicine

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) To develop a rapid electron microscopic assay for virus particles. This work unit is an essential element in a comprehensive program for medical defense against BW agents.

24 (U) A method is being developed which involves the transfer of virus from a millipore filter to an electron microscope grid with negative staining.

25 (U) 71 07 - 72 06 - Pending completion of a series of papers on the model for mechanism of membrane transport, experimental work on the electron microscopic assay for virus particles has been temporarily halted. A survey of the virus literature will be undertaken shortly to extend the membrane model to the process of virus penetration and release. It is expected that this survey will be useful also in providing new ways to improve and simplify the virus assay.

Terminated since FY 1973 funding will be under The Surgeon General, Army. New Project No. 3A062110A834; Accession DA 0A6416.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. AF FORMS 1498A AND 1498B, 1 MAR 68, FOR ARMY USE, ARE OBSOLETE

BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 01: Pathogenesis of Infection of Military Importance

Work Unit No. 096 01 011: Rapid Electron Microscopic Assay for Virus Particles of Diseases of Military Medical Importance

Description:

To develop a rapid electron microscopic assay for virus particles.

Progress and Summary:

As reported previously, experimental work on the electron microscopic assay for virus particles has been halted, pending completion of a series of papers on a model of membrane transport, a model which appears potentially capable of accounting also for the ability of virus particles to enter a cell. The papers covering the basic aspects of the membrane model have now been almost completed, so that it is expected that work on the virus assay can be resumed shortly.

The survey of the literature, necessary for extending the membrane model to cover the mechanism of virus penetration and release, should also be useful in connection with the virus particle assay. New methods may now be available to make possible a closer identification of the particles from their image in the electron microscope. Such methods would simplify development of the assay, which may otherwise have to include the use of ferritin labelled specific antisera.

Publications:

None.

ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1W662711A096 01:	Pathogenesis of Infection of Military Importance
Work Unit No. 096 01 012:	Biophysical Studies of Membrane Transport in Infections of Military Importance
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Physical Sciences
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Authors:	Anne Buzzell, Ph.D.
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OL0889	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	8A. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NL	XX YES ☐ NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	01	012
b. CONTRIBUTING	62711A	1B662711A096		
c. CONTRIBUTING	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code): (U) Biophysical studies of membrane transport in infections of military medical importance

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical medicine; 004900 Defense; 010100 Microbiology; 002300 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
61 07	CONT	DA	C. In-house

17. CONTRACT GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER: NA
c. TYPE: d. AMOUNT:
e. KIND OF AWARD f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 71	1.0	30
FISCAL YEAR CURRENT 72	1.0	36

19. RESPONSIBLE DOD ORGANIZATION
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SOCIAL SECURITY ACCOUNT NUMBER:

21. GENERAL USE
Foreign intelligence cosidered

ASSOCIATE INVESTIGATORS
NAME:
NAME: DA

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Latex particles; (U) Membrane; (U) Pore; (U) Phospholipid; (U) Ion; (U) Chelate; (U) Ultracentrifugation; (U) Micelle; (U) Military medicine

23. TECHNICAL OBJECTIVE; 24. APPROACH; 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Apply a membrane model to explain mechanisms of action of infectious agents. This work unit is an essential element in a comprehensive program for medical defense against BW agents.

24 (U) The literature will be surveyed for applications of the membrane model to virus-host cell interactions and other infectious disease processes.

25 (U) 71 07 - 72 06 - After revision of the first two papers on the proposed model for membrane structure and function for resubmission for publication, and completion of a paper on sugar transport now underway, a literature survey will be undertaken to see if the model can be used to account for the penetration and release of virus particles from host cells. The model can account for the behavior of myxoviruses in some detail and appears likely to do so for other viruses as well.

Terminated since FY 1973 funding will be under The Surgeon General, Army. New Project No. 3A062110A834; Accession No. DA 0A6417.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE AND 498A, 1 MAR 68 FOR ARMY USE ARE OBSOLETE

BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 01: Pathogenesis of Infection of Military Importance

Work Unit No. 096 01 012: Biophysical Studies of Membrane Transport in Infections of Military Importance

Description:

Apply a membrane model to explain mechanisms of action of infectious agents.

Progress and Summary:

Work has been completed on the theoretical model for biological membrane structure and function described in the previous report.[1] The key feature of the theory is the lipid micelle, bilayers of limited extent, of which 2 major types are envisioned. Phospholipid micelles, with molecules arranged in a linear fashion, would occupy a substantial fraction of the surface, the linear micelles being arranged for the most part in parallel rows. Soap micelles, of equal importance but occupying a far smaller area, would be scattered throughout the membrane, the bylayered clusters of a few hundred ionized fatty acid molecules being trapped between neighboring pairs of linear phospholipid micelles.

These 2 types of micelle would constitute the actual transport sites in the membrane; the soap micelles with their strong negative charge allowing rapid transport of monovalent cations and some types of cationic molecules. The slits between the phospholipid micelles would allow transport of a wide variety of ions and metabolites. The arrangement of various individual phospholipids would provide specific binding sites along the edges of the slits to arrest passing ions or molecules and orient them properly for passage through the slit into the cell. The principal classes of phospholipids found in biological membranes could account for the broad categories of transport: phosphatidylcholine and phosphatidylethanolamine accounting for transport of anions; phosphatidylserine, for amino acids, mainly small, hydrophilic ones; and phosphatidylinositol, for sugars. A study of molecular models shows that the required specificities could be produced by simple physical adjustments of molecules about the slit.

Enzymes embedded in the membrane would regulate transport activity by altering the compression of the membrane or by shifting phospholipid micelles relative to one another, the conformation of the enzymes changing as they interact with substrates.[2] Sudden motion of an enzyme embedded

near a row of linear phospholipid micelles would loosen molecules already oriented across the slit orifice, the molecules then entering the cell through the widened slits. Transport through a soap micelle would be regulated by compression, the spacing between individual soap molecules determining the size of the cation that could be sequestered. Thus, enzymes would not have to move back and forth across the membrane to regulate transport, as theorized in the carrier protein model.

The concepts, based originally on the properties of the external membranes of cells, have been extended to include mitochondrial membranes. Realizing the peculiarities in the lipid composition of these membranes, the theory can account in detail for the characteristics of carboxylic acid transport as well as other properties of these membranes. Molecular models have been built which illustrate the steric factors underlying the transport specificities.

Two papers were written, covering in detail all features of the model described in the previous report[1] except for sugar transport. A paper on that subject is now well underway. The 2 completed papers were submitted for publication but rejected. The validity of the theory was not questioned. The principal grounds for rejection were (1) that the papers were too long for the first presentation of a new theory; and (2) that the subject of transport specificity was not covered completely. Therefore, it seems advisable to finish the topic of sugar transport before re-submitting the papers for publication, as had initially been planned.

On considering how to shorten the papers it was decided that reorganization of the first 2 papers into 3, one devoted to amino acid transport exclusively, would solve much of the problem, as well as giving greater emphasis to the coverage of amino acid transport specificity. Ideas for reorganization with respect to other topics covered also indicate that the presentation can be shortened considerably without excluding any of the evidence needed for support of the theory. Reorganization of one of the papers, covering phospholipid structure, is now complete and writing tightened so that it is much shorter and easier to read. Furthermore, a new section covering anion transport has been added. It is intended to complete the reorganization of the remaining material, into papers on cation transport and amino acid transport, before proceeding with the sugar transport. It now appears possible that the total length of the presentation, even with inclusion of the paper on sugar transport, can be kept within reasonable bounds, so that length will not be a hindrance to acceptance of the papers for publication.

Publications:

None.

LITERATURE CITED

1. U. S. Army Medical Research Institute of Infectious Diseases. 1 July 1971. Annual Progress Report, FY 1971. p. 59 to 64. Fort Detrick, Md.

2. Koshland, Jr., D. E., J. A. Yankeelov, Jr., and J. A. Thoma. 1962. Specificity and catalytic power in enzyme action. Fed. Proc. 21:1031-1038.

ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1W662711A096 01:	Pathogenesis of Infection of Military Importance
Work Unit No. 096 01 013:	Host Lipids in Infectious and Toxic Illnesses for Defense Against BW
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Physical Sciences
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Authors:	Robert H. Fiser, Jr., Major, MC Michael C. Kastello, Captain, VC Alan T. Rowberg, Captain, MC Joseph Kaplan, Major, MC Virginia G. McGann, Ph.D. William R. Beisel, M.D.
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OL0900	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	01	013
b. [illegible]	62711A	1B662711A096		
c. [illegible]	CDOG 1212b(9)			

11. TITLE (Precede with security classification code)

(U) Host lipids in infectious and toxic illnesses for defense against BW

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical medicine; 004900 Defense; 002300 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
69 07	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER: NA

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 71	2.0	60
FISCAL YEAR CURRENT 72	2.0	85

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: Fiser, R. H.

NAME: Beisel, W. R. DA

21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Precede each with security classification code)

(U) Lipids; (U) Lipoproteins; (U) Free fatty acid; (U) Cholesterol; (U) Triglycerides; (U) Endotoxin; (U) Bacterial illness; (U) Viral illness; (U) Military medicine

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with security classification code.)

23 (U) Study early changes in lipid metabolism during infectious and toxic illnesses. This work unit is an essential element in comprehensive program for medical defense against BW agents.

24 (U) Measure serum lipids and lipoproteins during induced illness and examine the kinetics of fatty acid metabolism.

25 (U) 71 07 - 72 06 - The kinetic data from monkeys infected with Diplococcus pneumoniae or Salmonella typhimurium have been subjected to multicompartmental computer analysis. Results suggest an increased mobilization of fatty acids and utilization for synthesis of triglycerides and for catabolism as a source of energy in the infected animals. Feeding a high fat, high cholesterol diet resulted in marked alterations in cholesterolgenesis and increased humor and cellular immunity.

Publications: Proc. Soc. Exp. Biol. Med. 138:605-609, 1971
Amer. J. Vet. Res. 33: 323-327, 713-719, 1972
J. Infect. Dis. 125:54-60, 1972
Clin. Res. 20:233, 271, 1972
Fed. Proc. 31:727, 1972
Proc. Soc. Exp. Biol. Med. 140:314-318, 1972

Terminated since FY 1973 funding will be under The Surgeon General, Army. New Project No. 3A062110A834; Accession No. DA 0A6418.

* Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498 [illegible]

BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 01: Pathogenesis of Infection of Military Importance

Work Unit No. 096 01 013: Host Lipids in Infectious and Toxic Illnesses for Defense Against BW

Description:

Study early changes in lipid metabolism during infection and toxic illness.

Progress, Part I:

As part of a continuing study on lipid metabolism during illness, the data from rhesus monkeys infected with either Diplococcus pneumoniae or Salmonella typhimurium were subjected to multicompartmental computer analysis. Free fatty acid (FFA) metabolism was studied using ^{3}H-palmitic acid as previously described.[1] The data from about 200 experiments involving injection of labeled FFA into control and infected monkeys are being analyzed. The raw data (counts of radioactivity) from the FFA and triglyceride compartments have been corrected for counter efficiency and for an impurity present in the triglyceride fraction in the early time periods. The model was extended to include the triglyceride compartment and has been fitted to about a quarter of the experiments. When all are satisfactorily fitted, the amount of free fatty acid being transformed into triglycerides can be found as a function of time and compared between control and experimental groups. Reversible and irreversible loss from the FFA compartment can also be calculated so that the transformed lipid may be expressed as a fraction of total irreversible loss from that compartment.

In order to elucidate further the effects of infection on conversion of FFA to triglycerides, control and D. pneumoniae-infected monkeys were given a dose of a nonionic detergent, Triton WR 1339, which blocks the utilization of triglycerides by peripheral tissues. This resulted in marked increases in the serum triglyceride concentrations of the animals treated with the detergent. In the animals infected with the pneumococci, however, serum triglycerides were increased to almost twice that found in the noninfected animals treated with the detergent. These data suggest that in infected animals there is a more rapid synthesis of triglycerides

than in the noninfected group and may be related to increased mobilization of fatty acids as a source of energy.

Summary, Part I:

The monkey model for infection and endotoxemia has been expanded and the data have been subjected to multicompartmental computer analysis. Preliminary results suggest that there is an increased mobilization and utilization of fatty acids in the infected animals.

Progress, Part II:

A number of investigators have suggested that the feeding of high caloric diets can result in depressed immune response.[2] In order to study the effects of diet on the immune responses, rhesus monkeys were fed a high fat, high cholesterol diet for 9 months and compared to a group fed a low fat diet. The animals fed this high cholesterol, high fat diet had serum cholesterol levels of 400-600 mg/100 ml. Cholesterogenesis during infection in these monkeys was markedly depressed when compared with the normal diet group of infected monkeys. Both prior to and after infection with D. pneumoniae a marked effect was noted on both humoral as well as cellular immunity in monkeys fed the high fat, high cholesterol diet. Changes included: TB skin test became positive, quantitatively and qualitatively different precipitin response to ovalbumin, lessened immunoglobulin response, RE system clearance of colloid impaired with morphological changes noted by electron microscopy of the polymorphonuclear leukocytes. This work is preliminary but suggests that the marked effects on immune defense mechanisms can be altered by manipulating the dietary fat intakes.

Summary, Part II:

When monkeys were fed a high fat, high cholesterol diet marked effects were noted on cholesterol metabolism and both humoral as well as cellular immunity in these animals. These studies stress the importance of prior dietary intake on host response to infection and also suggest the important feedback mechanisms on cholesterol synthesis.

Publications:

1. Fiser, R. H., J. C. Denniston, R. B. Rindsig, and W. R. Beisel. 1971. Effects of acute infection on cholesterogenesis in the rhesus monkey. Proc. Soc. Exp. Biol. Med. 138:605-609.

2. Fiser, R. H., J. B. Rollins, and W. R. Beisel. 1972. Decreased resistance against infectious canine hepatitis in dogs fed a high-fat ration. Am. J. Vet. Res. 33:713-719.

3. Fiser, R. H., J. C. Denniston, and W. R. Beisel. 1972. Infection with Diplococcus pneumoniae and Salmonella typhimurium in monkeys: Changes in plasma lipids and lipoproteins. J. Infect. Dis. 125:54-60.

4. Fiser, R. H., J. C. Denniston, J. Kaplan, V. G. McGann, and W. R. Beisel. 1972. Hypercholesterolemia and altered immunity in rhesus monkeys. Clin. Res. 20:271 and Fed. Proc. 31:727 (abstracts).

5. Fiser, R. H., J. C. Denniston, and W. R. Beisel. 1972. Gram negative septicemia versus endotoxicosis: Differential effects on lipid metabolism. Clin. Res. 20:233 (abstract).

6. Fiser, R. H., J. C. Denniston, M. C. Kastello, R. B. Rindsig and W. R. Beisel. 1972. Cholesterogenesis during acute infection in chronically hypercholesterolemic rhesus monkeys. Proc. Soc. Exp. Biol. Med. 140:314-318.

LITERATURE CITED

1. U. S. Army Medical Research Institute of Infectious Diseases, 1 July 1970. Annual Progress Report, FY 1970. p. 55 to 61. Fort Detrick, Maryland.

2. Beisel, W. R., and R. H. Fiser, Jr. 1970. Lipid metabolism during infectious illness. Am. J. Clin. Nutr. 23:1069-1079.

ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1W662711A096 01:	Pathogenesis of Infection of Military Importance
Work Unit No. 096 01 014:	Development, Calibration and Standardization of Aerosol Equipment and Model Systems for Testing Military Vaccines
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Animal Assessment
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Author:	Ralph W. Kuehne, B.S.
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OLO909	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISS'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	01	014
b. [illegible]	62711A	1B662711A096		
c. [illegible]	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)

(U) Development, calibration and standardization of aerosol equipment and model systems for testing military vaccine

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical medicine; 004900 Defense; 008700 Laboratories, test facilities

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 01	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER: NA
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR PRECEDING 71	1.0	8
FISCAL YEAR CURRENT 72	0	0

19. RESPONSIBLE DOD ORGANIZATION

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ADDRESS: Fort Detrick, MD 21701

RESPONSIBLE INDIVIDUAL
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TELEPHONE: 301 663-2833

20. PERFORMING ORGANIZATION

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ADDRESS: Fort Detrick, MD 21701

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Kuehne, R. W.
TELEPHONE: 301 663-7244
SOCIAL SECURITY ACCOUNT NUMBER

21. GENERAL USE

Foreign intelligence considered

ASSOCIATE INVESTIGATORS
NAME:
NAME:

DA

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Aerosols; (U) Aerosol generator; (U) Infectious diseases; (U) Military medicine

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Prepare specialized equipment for use in aerosol exposures of man and laboratory animals to biological materials and their products. This work unit is an essential element in a comprehensive program for medical defense against BW agents.

24 (U) Based upon specific requests and criteria, testing will be performed of all facets of aerosol exposure with the view of obtaining accurate and precise data.

25 (U) 71 07 - 72 06 - Due to higher priorities, no work was conducted during the report period.

Terminated since FY 1973 funding will be under The Surgeon General, Army. New Project No. 3A062110A834; Accession No. DA 0A6419.

* Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498

BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 01: Pathogenesis of Infection of Military Importance

Work Unit No. 096 01 014: Development, Calibration and Standardization of Aerosol Equipment and Model Systems for Testing Military Vaccines

Description:

Prepare specialized equipment for use in aerosol exposures of man and laboratory animals to biological materials and their products.

Progress and Summary:

Due to higher priorities, no work was conducted on this project during the reporting period.

Publications:

None.

ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1W662711A096 01:	Pathogenesis of Infection of Military Importance
Work Unit No. 096 01 015:	Evaluation of Myocardial Contractility during Infectious Disease of Military Medical Importance
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Physical Sciences
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Author:	William H. Zech, Captain, VC
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OL0911	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISB'N INSTR'N	8b. SPECIFIC DATA: CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	01	015
b. [illegible]		1B662711A096		
c. [illegible]	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code): (U) Evaluation of myocardial contractility during infectious diseases of military importance

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical medicine; 004900 Defense; 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 03	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER: NA
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR — PRECEDING 71	1.0	50
FISCAL YEAR — CURRENT 72	1.0	71

19. RESPONSIBLE DOD ORGANIZATION
NAME: USA Medical Research Institute of Infectious Diseases
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SOCIAL SECURITY ACCOUNT NUMBER:

21. GENERAL USE
Foreign intelligence considered

ASSOCIATE INVESTIGATORS
NAME: Boucher, J. H. DA
NAME:

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Myocardium; (U) Physiology; (U) Infectious diseases; (U) Military medicine

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Determine to what extent various infectious diseases cause changes in myocardial function. This work unit is an essential element in a comprehensive program for medical defense against BW agents.

24 (U) Using conventional methods of quantitating myocardial contractility, determine baseline values and then measure changes induced by infections induced in rhesus monkeys.

25 (U) 71 07 - 72 06 - A method for the implantation of long-term ventricular pressure transducers in the rhesus monkey has been devised. Cardiac indices evaluated in their response to increased myocardial inotropism included: Peak dp/dt, Peak dp/dt over P-O, Peak Vce and Vmax. The second index showed the most sensitive and accurate response to increased myocardial contractility achieved through graduated doses of isoproteronol. Infection with Diplococcus pneumoniae caused a slight, transient decrease in all parameters studied in 1 of 2 monkeys given 100 million organisms intravenously.

Terminated since FY 1973 funding will be under The Surgeon General, Army. New Project No. 3A062110A834; Accession No. DA 0A6420.

DD FORM 1498

BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 01: Pathogenesis of Infection of Military Importance

Work Unit No. 096 01 015: Evaluation of Myocardial Contractility during Infectious Disease of Military Medical Importance

Description:

Determine to what extent various infectious diseases cause changes in myocardial function.

Progress:

The established methods of measuring cardiac performance leave much to be desired in describing the innate contractility of the myocardium. Cardiac output, stroke volume, stroke index, etc. measure the heart's ability as a pump, rather than as a muscle, due to the fact that the loading conditions of the heart at the time of measurement affect these parameters.[1] In addition, these parameters frequently do not decrease until late in the course of myocardial disease.[2,3]

Recently, attempts have been made to define myocardial contractility from high fidelity intraventricular pressure measurements and the corresponding first derivative of pressure (dp/dt). These studies have dealt with the isovolumic phase of systole as there is little change in ventricular geometry at this time which would be another variable with which to contend.[2] A single heartbeat may be described during its isovolumic phase by relating the velocity of the contractile elements (Vce) to the corresponding isovolumic pressure (IP).

A controversy exists as to which derived index, Peak dp/dt, $\frac{\text{Peak dp/dt}}{P_o}$, Peak Vce, or Vmax, is the most valid for measuring the contractility of the myocardium. It is the purpose of this study to discuss the use of these indices in conscious chaired rhesus monkeys.

Electromechanical pressure transducers with head diameters of 6.2 mm (Model 1017 Dynasciences, Chatsworth, Cal.) and 3.0 mm (P-15 Konigsberg Instruments, Pasadena, Cal.) were implanted in the apex of the left ventricle of the heart in male rhesus monkeys via a left 4th intercostal thoracotomy. Simple interrupted sutures were used to anchor the cable to the intercostal muscles where it exited the thorax and a pre-placed suture in the skin was anchored to the cable emerging from the skin by taping the suture ends to the cable.

The monkeys were sufficiently recovered from anesthesia in a few hours to be placed in their restraint chairs, which were constructed of plexiglass and allowed limited arm and leg motion but prevented access to the transducer cable. They were fed a ration of commercial monkey feed and fresh fruit and given at least 1 hr free exercise daily after immobilizing the transducer to their body with a protective bandage.

The monkeys were acclimated to the chairs and general environment for 1 week prior to surgery; data collection began 1-2 weeks after operatively. They were kept as basal as possible by limiting access and visual stimuli in their quarters and by duplicating daylight and evening hours with artificial light. All recording devices were kept in an adjoining room and interfaced to the monkeys via wires.

Pressure within the left ventricle was detected using the Dynasciences Model 1017 and Konigsberg Model P-15 implantable transducers. The signal from each transducer was amplified to a level of approximately 1 volt full scale by a Dynasciences Model BE-3 preamplifier. The preamplifiers were powered from a regulated supply to overcome calibration errors from battery aging. The signal was conducted via a multiconductor cable from the animal room to the separate, sound-insulated room where the recording apparatus was located.

In the recording room, the signal went through a Brush Universal amplifier with differential inputs for hum cancellation. The hum induced through the approximately 50 ft of cable between the rooms was unmeasurable when the differential inputs were used.

A Brush Mark 200 pressurized-ink recording system was used (Brush Instruments, Cleveland, Ohio). Tracing were made of left ventricular pressure, and of its first derivative with respect to time, as developed by a Biotronex Laboratory Model 620 analog computer (Biotronex Laboratory, Silver Spring, Md.). System frequency response was maintained at maximum bandwidth throughout the system, to obtain a maximum amount of detail on the tracings. The high-frequency limit of the differentiator was 320 hz; the high-frequency limit of the pen drives of the Brush Mark 200 system was approximately 100 hz for small deflections. Because of the mass of the pens, a simple expression for frequency response cannot be given as the response changes with respect to both frequency and amplitude.

Isoproteronol studies were conducted by taking a baseline recording, injecting a bolus of isoproteronol intravenously (IV) and then recording at 30 sec postinjection. The change in the parameters studied is expressed as % of change (% Δ) from baseline values, by use of the equation:

$$\% \Delta = \frac{\text{Experimental value-baseline value}}{\text{Baseline value}} \times 100$$

When more than one injection was given per day, at least 15 min were allowed for recovery between trials. The dose range was from 0.001-1.600 ug/kg isoproternol.

Diplococcus pneumoniae (1×10^8 organisms) were injected IV into 2 monkeys (A-607 and B-12). Continuous monitoring of core body temperature was achieved by use of implanted thermocouples connected to a continuous print-out recorder (Model 16 Electronic Honeywell, Inc., Philadelphia, Pa.).

Pressure recordings were taken every 2 hr during the first day of infection and then every 4 hr for the next 2-3 days. Blood cultures were taken every 12 hr until at least 3 consecutive samples showed no growth of D. pneumoniae.

Vce was calculated at 5-msec intervals, throughout isovolumic systole, by the equation $Vce = \frac{dp/dt}{TP.32}$. Vmax is obtained by extrapolating back to zero pressure that portion of the Vce curve that is linear. Po was defined as that pressure which occurred simultaneously with peak dp/dt. Peak Vce was defined as the largest Vce which was used in the regression analyses for Vmax and is not usually synonymous with the absolute peak Vce. All presures used are developed pressure (DP) which is total pressure (TP) less end diastolic pressure (EDP).

The monkeys recovered well from the surgical procedure. They began to eat a day or two following surgery and at no time was there swelling of the incision line. They were allowed at least a 1-hr period of exercise each day to maintain muscle tone and prevent decubital sores from the chairs. This is believed to be the major factor that allowed us to keep 2 monkeys for the period of 4 and 5 mon respectively. During this time they gained in body weight and muscle mass and never developed decubital sores or infection along the transducer cable entering the body.

In order to assess the reliability of choosing one hearbeat to analyze, rather than averaging results from a number of heartbeats, we analyzed the results from 10 consecutive heartbeats. These data are presented in Table I. It can be seen there is no significant difference among these 10 curves and therefore we feel analysis of one systolic event is sufficiently accurate to describe the heart's contractile element velocity and pressure relationships at a given time.

A total of 358 observations were made on 5 chaired, conscious monkeys. These data are presented in Table II. The smallest number of observations contributed by a monkey was 12 and the greatest was 162. Data collection was not begun until 2 weeks postoperatively in order to help assure a basal state.

TABLE I. ANALYSES OF 10 CONSECUTIVE HEARTBEATS

HEARTBEAT	PEAK dp/dt mm Hg/sec	$\frac{\text{PEAK dp/dt}}{\text{Po}}$	PEAK Vce sec^{-1}	Vmax sec^{-1}
1	3464	52.4	2.585	2.857
2	3472	50.3	2.359	2.844
3	3522	51.0	2.609	2.930
4	3581	51.9	2.585	3.072
5	3564	51.6	2.942	3.034
6	3531	51.2	2.942	3.073
7	3506	51.5	3.054	3.144
8	3589	52.0	2.871	2.990
9	3506	50.1	2.507	3.033
10	3405	49.3	3.094	3.189

Investigation of the Myocardial Indices:

A good index of myocardial contractility should respond to inotropic intervention and be unresponsive to changes in the loading conditions of the heart. We have investigated the first criterion by measuring the amount of change, after increasing doses of isoproteronol, of the following indices:

1. Peak dp/dt
2. $\frac{\text{Peak dp/dt}}{\text{Po}}$
3. Peak Vce
4. Vmax

The data are tabulated in Table III. There is extremely good correlation between the log dose of isoproteronol and both Peak dp/dt and $\frac{\text{Peak dp/dt}}{\text{Po}}$. There is less correlation between Peak Vce and the dose of isoproteronol and no correlation whatever between Vmax and the levels of isoproteronol challenge.

TABLE II. SUMMARY OF DATA COLLECTED ON NORMAL, CONSCIOUS, CHAIRED RHESUS MONKEYS
(Mean ± 2 SD)

ANIMAL (n)	WEIGHT kg	PEAK dp/dt mm Hg/sec	PEAK dp/dt Po	PEAK Vce sec^{-1}	Vmax sec^{-1}	HR beats/min	EDP mm HG	Po mm Hg
A-804 (29)	4.0	4307 ± 1734	56.6 ± 14.8	2.44 ± 0.40	2.67 ± 0.30	196 ± 43	2.7 ± 2.6	75 ± 12
A-957 (12)	4.3	3602 ± 1802	49.1 ± 8.8	2.50 ± 0.62	2.79 ± 0.50	148 ± 38	3.0 ± 3.0	73 ± 26
A-607 (162)	5.2	2799 ± 1150	49.1 ± 11.6	2.85 ± 0.66	3.18 ± 0.48	138 ± 41	2.7 ± 2.7	57 ± 14
A-840 (80)	4.6	3195 ± 1616	50.6 ± 14.6	3.01 ± 0.68	3.38 ± 0.54	128 ± 44	3.7 ± 3.0	63 ± 15
B-12 (75)	4.7	2358 ± 1060	42.7 ± 16.8	2.67 ± 0.90	2.99 ± 0.90	120 ± 36	2.8 ± 2.0	55 ± 14
ALL MONKEYS (358)		2944 ± 1670	48.7 ± 15.4	2.80 ± 0.78	3.13 ± 0.72	137 ± 56	3.0 ± 2.8	60 ± 19
COEFFICIENT OF VARIATION		23%	10%	8%	9%	20%	14%	14%

TABLE III. RESULTS OF INCREASING DOSES OF ISOPROERONOL ON THE INDICES OF MYOCARDIAL CONTRACTILITY

INDEX	CORRELATION COEFFICIENT r	SLOPE b	F
Peak dp/dt	0.98	71.49	325.60**
$\frac{\text{Peak dp/dt}}{\text{Po}}$	0.93	30.48	89.65**
Peak Vce	0.78	7.99	22.19**
Vmax	0.0	0.13	0.01ns
HR	0.93	20.89	94.00**

** $P < 0.01$

It appears that the most sensitive indices of increased myocardial inotropism are Peak dp/dt and $\frac{\text{Peak dp/dt}}{\text{Po}}$, while the other indices are much less sensitive to changes in the inotropic background of the myocardium.

Pneumococcal Infection:

The IV administration of 1×10^8 D. pneumoniae to 2 monkeys was performed as a pilot study to determine if myocardial depression or enhancement occurred during pneumococcal septicemia dn to determine which index would demonstrate these changes. Both monkeys had an initial febrile response of 105 F within 4 hr which lasted no more than 6 hr. Monkey A-607 never again became febrile or showed any sign of clinical illness although pneumococci were cultured from his blood for 48 hr. Monkey B-12 had a diphasic fever pattern and became clinically ill, exhibiting anorexia and depression, for 2 days during the 2nd febrile period. Positive blood cultures were obtained for 60 hr.

Monkey A-607, which never became clinically ill, at no time exhibited any significant change in the parameters studied. Monkey B-12, however, had a decrease in all the parameters studied (Vmax, Peak Vce and $\frac{\text{Peak dp/dt}}{\text{Po}}$ which corresponded to the time between his 2 febrile states and prior to the time he became clinically ill. This decrease was in relation to the control monkey's values but were well within the sick monkey's normal range as established by the mean ± 2 SD.

The use of Vmax has been critized on both theoretical and practical grounds.[4] Our experience with it has shown it to be a quantity extremely subject to minute errors in analyses. Table IV shows the effect on the parameters of interest due to an increase and decrease of only 2 mm Hg pressure throughout isovolumic systole. This could occur as a result of

TABLE IV. RESULT OF A CHANGE OF 2 mm Hg ON A SINGLE HEARTBEAT[a]

HEARTBEAT	PEAK dp/dt	$\frac{\text{PEAK dp/dt}}{\text{Po}}$	PEAK Vce	Vmax
Original	2505	43.2	2.199	2.508
+ 2 mm Hg	2505	41.7	1.900	2.369
- 2 mm Hg	2505	44.7	2.565	2.898

a. See Table I for units.

error in analyses both due to the difficulty of finding the true zero pressure point on the pressure curve and as a result of errors in visual discrimination, which we feel to be in the range of ± 0.5 mm Hg at the sensitivity levels used. Therefore, extreme care must be taken when hand analyzing these pressure curves if meaningful, comparable results are to be obtained.

The use of total pressure or developed pressure has similar effects on the Vce curve. The use of DP (DP = TP - EDP) and hence lower than TP, results in consistently higher Vce and Vmax values than those obtained using TP. We analyzed the results from the isoproteronol study, using both TP and DP; there was no difference. The results from the normal observations were also calculated using both TP and DP and are reported from the DP data.

Peak dp/dt and $\frac{\text{Peak dp/dt}}{\text{Po}}$ showed, by far, the greatest sensitivity to increased inotropism of all the indices studies. Peak dp/dt, however is said by many to be influenced by the loading conditions at the time of measurement and hence is not ideally suited for use under conditions when preload and aferload change.[6-9] Others claim that preload, as measured by EDP, has little if any effect on Peak dp/dt.[10,11] It has been stated, however, that EDP is not a reliable indicator of end-diastolic volume, which is the better index of preload. We did not investigate the effects of preload on these indices in this study, but preload, as measured by EDP, did not change much, 2.9 ± 2.8 (mean ± 2 SD) in over 300 observations over a period of several months in normal rhesus monkeys, nor did it change significantly after isoproteronol challenge. Therefore, if EDP is a useful indicator of preload, and preload does affect Peak dp/dt, our values for Peak dp/dt were not influenced by preload.

Changes in afterload (aortic diastolic pressure), however, may have affected our values for Peak dp/dt, but it was not measured.

Peak dp/dt_{PO} has been claimed to be sensitive to changes in myocardial inotropism and to be free of variations in the heart's loading conditions.[7,12,13] If this latter statement is true, it would appear that this index would offer the most reliable, sensitive and accurate measurement of myocardial contractility in the rhesus monkey, as it showed extremely good response to increasing doses of isoproteronol. We believe any value for this index out of the limits of 33.3-64.1, i.e. 2 SD below and above the mean, indicates either a depressed myocardium (< 33.3) or increased contractility (> 64.1) in the chaired, conscious rhesus monkey.

D. pneumoniae caused a small, transient depression of all the parameters studied in 1 of 2 monkeys to which it was administered. This decrease began from 10-16 hr postinfection and lasted from 10-14 hr, depending on the parameter studied. This depression occurred during the initial febrile peak which was before the monkey became clinically ill. During the period of clinical illness, the monkey exhibited the second peak of diphasic fever but the cardiac indices we used did not decline as they did during the initial febrile peak, even though blood cultures were positive for pneumococci during this second febrile period. It may be that the initial febrile response and myocardial depression were due to metabolites present in the inoculum of pneumococcus as myocardial depression did not occur during the second febrile peak even though pneumococcus was isolated from the blood.

Summary:

A method for the implantation of long-term ventricular pressure transducers in the rhesus monkey has been devised. Cardiac indices evaluated in their response to increased myocardial inotropism included: Peak dp/dt, Peak dp/dt_{PO}, Peak Vce and Vmax. Peak dp/dt_{PO} showed the most sensitive and accurate response to increased myocardial contractility, achieved through graduated doses of isoproteronol. D. pneumoniae caused a slight, transient decrease in all parameters studied in 1 of 2 monkeys given 1×10^8 organisms IV.

Publications:

None.

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ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1W662711A096 01:	Pathogenesis of Infection of Military Importance
Work Unit No. 096 01 105:	Capillary Ultrastructure in Bacterial Infections of Military Medical Importance
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland 21701
Division:	Pathology
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Author:	Thomas H. Hudson, Captain, MSC
Reports Control Symbol:	RCS-MEDDH-288 (R1)
Security Classification:	UNCLASSIFIED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OL0905	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISB'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	01	105
b.	62711A	1B662711A096		
c.	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)
(U) Capillary ultrastructure in bacterial infections of military medical importance

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
004900 Defense; 010100 Microbiology; 002600 Biology (Pathology)

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
70 12	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER: NA
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 71	1.0	5
	CURRENT 72	1.0	47

19. RESPONSIBLE DOD ORGANIZATION
NAME: USA Medical Research Institute of Infectious Diseases
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RESPONSIBLE INDIVIDUAL
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20. PERFORMING ORGANIZATION
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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME:
NAME: DA

21. GENERAL USE
Foreign intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Bacterial diseases; (U) Pathogenesis; (U) Molecular biology; (U) Circulatory system; (U) Military medicine

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Investigate ultrastructure and permeability changes of capillaries resulting from bacterial infection. This work unit is an essential element in a comprehensive program for medical defense against BW agents.

24 (U) Infect white mice, introducing protein markers at varying times; study the tissues ultrastructurally.

25 (U) 71 07 - 72 06 - Results of protein marker localization have been inconsistent and could not be reproduced with a high degree of accuracy even in controls. Procedures have been undertaken to standardize results obtained from both infected experimental animals and noninfected controls. Progress has been made to the point that differences can be observed and reproduced between tissues from animals sacrificed immediately and at 1, 2, 5, 10 and 20 min after peroxidase injection. The progression of pneumococcal infection has also been standardized so that infected animals can be compared with controls; the differences are repeatable.

Terminated since FY 1973 funding will be under The Surgeon General, Army. New Project No. 3A062110A834; Accession No. DA OA6421.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 01: Pathogenesis of Infection of Military Importance

Work Unit No. 096 01 105: Capillary Ultrastructure in Bacterial Infections of Military Medical Importance

Description:

Investigate ultrastructure and permeability changes of capillaries resulting from bacterial infection.

Progress:

Several procedures were undertaken to insure standardization in the localization of exogenous protein tracers. The specific enzymatic activity of different stocks of horseradish peroxidase (HP) were shown to be different. Therefore, only one enzyme stock was used for any one experiment. HP has a tendency to aggregate when dissolved in saline for long periods of time. Therefore, the solutions were made-up immediately before injection. The timing was determined to be critical. Variations of as little as 30 sec could produce differences in the patterns of localization. All manipulations, therefore, were carefully timed and repeated identically in all experiments. Length of incubation, thickness of tissue blocks, area of liver from which the tissues were taken were all influencing the localization, and were all standardized. One of the most critical areas of the experiment was the method of anesthesia. Various types of anesthetics were tested and, in general, shown to produce erratic localization. The experiment was redesigned so that the animals were sacrificed at the time of tissue harvesting by cervical dislocation with no anesthesia.

The progression of Diplococcus pneumoniae, Type I, infection was observed to differ according to the animal. This variation was overcome in two ways: (1) the virulence of the culture was increased by 8 passages through mice, and (2) monitor the rectal temperature of infected mice and sacrifice at the same time during the infection as observed from temperature charts.

After standardization of procedures, mice were infected and HP was injected as tracer.

Ten seconds after peroxidase was injected intravenously into mice, reaction product was found in the sinusoids, the space Disse and adjacent portions of the intercellular spaces of the liver. As exposure time increased, peroxidase activity progressed up the intercellular spaces until, 5 min after injection of peroxidase, the deepest intercellular

spaces were labeled from the space of Diesse to the pericanalicular tight junctions; labeled pinocytotic vesicals could also be seen in the hepatocytes.

Pinocytotic activity is peaks at 10 min postinjection. Collecting vacuoles are also formed at this time. The sinusoid is often empty at this time. Twenty minutes after injection, the only activity is found in pericanalicular lysosomer. At no time is reaction product positively located within the bile canaliculus.

After 6-hr exposure to D. pneumoniae, Type I, 3 changes in peroxidase uptake could be seen. A definite gradient across the endothelium was observed immediately after HP injection. Pinocytotic activity was observed regularly after 1-min exposure. After 5 min, the bile canaliculi contained reaction product.

Summary:

Procedures were standardized for anesthesia dosages of HP, and pneumococcal infection in a study of capillary ultrastructure.

Publications:

None.

ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	**Medical Defense Aspects of Biological Agents (U)**
Task No. 1W662711A096 01:	Pathogenesis of Infection of Military Importance
Work Unit No. 096 01 401:	Effect of Bacterial and Virus Infections on Host Cell Biosynthetic Mechanisms in BW Defense Research
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Physical Sciences
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Authors:	Michael C. Powanda, Captain, MSC, (I, II) Robert W. Wannemacher, Jr., Ph.D. (I, II) Gary L. Cockerell, Captain, VC (II) H. Shelton Earp, M.D., Captain, MC (III)
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OL0809	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISS'N INSTR'N	8b. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	01	401
b. CONTRIBUTING	62711A	1B662711A096		
c. CONTRIBUTING	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code): (U) Effect of bacterial and viral infections on host cell biosynthetic mechanisms in BW defense research

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical medicine; 004900 Defense; 002300 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
62 10	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER: NA
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 71	2.0	30
FISCAL YEAR CURRENT 72	2.0	116

19. RESPONSIBLE DOD ORGANIZATION
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ASSOCIATE INVESTIGATORS
NAME: Earp, H. S.
NAME: DA

21. GENERAL USE
Foreign intelligence considered.

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Human volunteers; (U) Rats; (U) Sepsis; (U) Nitrogen metabolism; (U) Tryptophan metabolism; (U) Chromatin

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Study alterations in nitrogen and tryptophan metabolism, RNA biosynthesis, and template activity chromatin in host cells during infection. This work unit is an essential element in a comprehensive program for medical defense against BW agents.

24 (U) Urinary excretion of nitrogen and metabolites of tryptophan, as well as the extent and/or pattern of serum protein synthesis are measured during infections of man and laboratory animals. The incorporation of radioactive precursors of RNA into RNA isolated from various subcellular fractions is measured during infection.

25 (U) 71 07 - 72 06 - Increased excretion of metabolites of tryptophan and alterations in the urinary output of the catabolites of the pyridine nucleotides were noted in man during sandfly and typhoid fever without resort to an oral dose of tryptophan and thus allow discrimination between viral and bacterial illnesses.

Sepsis caused by either Diplococcus pneumoniae or Salmonella typhimurium induces a loss of body nitrogen and increased synthesis of specific serum proteins, the latter can be demonstrated to occur even in malnourished rats suggesting the essentiality of the response.

Diurnal variation in the template activity isolated from rat liver has been demonstrated.

Publications: Infec. Immun. 4:556-562, 1971
Biochim. Biophys. Acta 252:239-245, 1971
Fed. Proc. 31:710, 1972 (abstract)

Terminated since FY 1973 funding will be under The Surgeon General, Army. New Project No. 3A062110A834; Accession No. DA 0A6422.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498 1 MAR 68 FOR ARMY USE ARE OBSOLETE

BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 01: Pathogenesis of Infection of Military Importance

Work Unit No. 096 01 401: Effect of Bacterial and Virus Infections on Host Cell Biosynthetic Mechanisms in BW Defense Research

Description:

Study alterations in nitrogen and tryptophan metabolism, RNA biosynthesis, and template activity of chromatin in host cells during infection.

Progress, Part I:

Tryptophan metabolism in human subjects, as measured by the urinary excretion of tryptophan metabolites, can be shown to increase during typhoid fever even in the absence of a 3-gm oral load of tryptophan. Eleven volunteers who contracted typhoid fever during a typhoid vaccine evaluation study conducted by the Division of Infectious Diseases, University of Maryland[1] demonstrated significant increases in kynurenine, acetylkynurenine and o-aminohippurate during manifest clinical illness. No increases in kynurenate, xanthurenate or anthranilate were observed unless a 3-gm oral dose of tryptophan was administered 6 hr before urine collection. N-methylnicotinamide, a catabolite of the pyridine nucleotides, was found in lessened amounts on the first day of febrile illness but was within the control range during the height of illness. This observation suggests that changes in pyridine nucleotide metabolism occur in infectious illness but are not necessarily linked to alterations in tryptophan catabolism.

Preliminary analysis of data from volunteers with experimentally-induced sandfly fever revealed a significant increase in urinary kynurenine excretion and evidence of increases in 3-hydroxykynurenine and 3-hydroxyanthranilate output. The change in kynurenine excretion appeared to parallel fever index and was in some measure apparent before dietary intake lessened. Variations in N-methylnicotinamide excretion appeared not to correlate with those noted in tryptophan catabolites.[2] Further analysis must be undertaken to assess whether the excretion patterns of tryptophan metabolites in the absence of an oral load of tryptophan differ significantly during bacterial and viral illness.

In order to study the mechanisms of the enhanced excretion of tryptophan metabolites, rats were put in metabolic cages and allowed to equilibrate

before being infected with either Diplococcus pneumoniae or Salmonella typhimurium. In both instances pair-fed infected animals excreted significantly more nitrogen but, on the other hand, significantly less diazotizable material (i.e., metabolites of tryptophan) than their controls.[3] The explanation for the decreased excretion of tryptophan catabolites may lie in the essentiality of tryptophan to protein synthesis in rodents[4,5] and the marked increase in serum protein synthesis observed during sepsis.

Summary, Part I:

Increased excretion of metabolites of tryptophan and alterations in urinary output of catabolites of the pyridine nucleotides were noted during sandfly and typhoid fever. These changes were demonstrated in the absence of an oral dose of tryptophan and thus may allow discrimination between viral and bacterial illnesses. Lessened excretion of degradation products of tryptophan were noted in rats exposed to D. pneumoniae and S. typhimurium.

Progress, Part II:

Rats fed a diet adequate for growth eliminated more nitrogen when exposed to D. pneumoniae than their pair-fed controls which had been injected with heat-killed pneumococci. The average increase in nitrogen excreted amounted to 37 mg/24 hr on day 1 and 25 mg/24 hr on day 2. These differences were significant at the 0.005 and 0.05 level, respectively as judged by the paired t-test. Analysis of the urine samples for specific nitrogen metabolites showed urea nitrogen, ammonia, α-amino nitrogen and creatinine to be excreted in greater quantities by the infected animals than the controls. The significantly increased excretion of creatinine on day 2 is consistent with the data from studies in man[6] and with the hypothesis that infection induces a flow of amino acids from skeletal muscle to liver.[7]

In contrast to the enhanced overall catabolic activity on day 1 in the infected animals, there was a significant increase in the incorporation of radioactive leucine into serum proteins at 18 and 24 hr postexposure, corresponding in time to the onset of fever and bacteremia in these animals. Little change was noted in total liver protein incorporation at these times.[3]

Rats were infected with 10^6 D. pneumoniae; 22 hr later they were injected with 100 μCi ^{3}H-leucine/100 gm body weight. Two hours later they were killed. Cellulose acetate strip electrophoresis of the sera revealed significant increases in the α_2 and β protein fractions and marked decreases in the albumin and γ globulin fractions. When the strips were stained for glycoproteins and the data rendered as mg of protein bound carbohydrate,

the α_1, α_2 and β-glycoglobulin fractions displayed significant increases while the albumin and γ fractions remained unchanged.[8] Radiochromatograms of the electrophoretic strips as well as analysis of individual fractions indicated that the α_1, α_2, and β serum protein fractions of the infected animals contained more ^{3}H-leucine than similar fractions in non-infected pair-fed controls, while radioactivity in the albumin fraction was significantly decreased.

A marked increase in serum protein synthesis is also observed in rats fed a protein deficient diet (6% protein) for 28 days after weaning and prior to their exposure to infecting organisms. The increase is of the same magnitude as that found to occur in rats fed a diet optimal for growth (18% protein) and thus suggests that serum protein synthesis by the liver is a fundamental host response to infection.

When S. typhimurium was used as an infectious agent, pair-fed infected rats excreted on the average 135 mg more nitrogen than the saline-injected controls over a 5-day period following exposure. Though there was a significant increase in nitrogen output by the infected rats on day 1, the preponderant outpouring did not occur until day 3. By day 5, the difference between control and infected rats was minimal. Preliminary data indicate that salmonellosis also enhances serum protein synthesis.

Very recently we have been able to induce a pattern of increased serum protein synthesis resembling that observed during pneumococcal sepsis in the rat, by the administration of an extract from peritoneal leukocytes. This same leukocytic extract has been shown to induce a movement of amino acids from muscle to liver tissues.[9] Similar extracts have also been shown to elicit the synthesis of acute phase globulins.[10] We therefore suggest that the concomitant outpouring of body nitrogen and the enhanced synthesis of serum protein observed in these studies have a common origin and are interrelated facets of the liver-mediated response to a proliferating infectious organism.

Summary, Part II:

Sepsis caused by either D. pneumoniae or S. typhimurium induces a greater loss of body nitrogen than occurs in pair-fed controls. Concomitant with this outpouring of nitrogen there is an enhanced synthesis of serum proteins which occurs even in rats fed a protein deficient (6% protein) diet for 28 days after weaning and prior to exposure. Cellulose acetate electrophoresis reveals that the α^2 and β protein and α^1, α^2 and β glycoglobulin fractions are increased in pneumococcal sepsis. This is confirmed by the analysis of ^{3}H-leucine incorporation into serum proteins.

The increased synthesis of serum proteins does not become evident until after the onset of fever and bacteremia. This infection-induced increment in serum protein synthesis can be mimicked by the injection of an extract of peritoneal leukocytes as can the flux of amino acids from muscle to liver. These data suggest that the enhanced excretion of body nitrogen and synthesis of serum proteins in infection have a common origin and are interrelated facets of the liver mediated host response.

Progress, Part III:

During the past year the transition to a new investigator has occurred. Previous work done at this Institute has shown diurnal variation in genetic template expression in the mouse[11] and a change in this normal variation during D. pneumoniae infection of the mouse.[12,13] The system used to gather this data consisted of isolated mouse chromatin and a bacterial DNA-dependent RNA polymerase isolated from Escherichia coli. Progress for this year has centered on the investigation of the basic findings in the rat.

Preliminary data obtained from time intervals more frequent than previously studied has been used to construct a day curve of template activity. The data supports the hypothesis that diurnal variation is present in the rat and that the magnitude of the variation is greater than that reported in the mouse.[11] This work has also utilized the above mentioned bacterial enzyme.

In order to standardize further the assay system and to allow further study of the multifactorial mammalian transcriptional process, work is in progress to isolate the rat liver DNA-dependent RNA polymerases. This species-specific in vitro system will enable further studies into the earlier observations[14] concerning the increased incorporation of precursors into ribonucleic acids during infection.

Summary, Part III:

The study of the template activity of isolated mammalian chromatin has been extended to another species, the rat. Diurnal variation in the template activity of this animal has been established.

Presentation:

Powanda, M. C., R. W. Wannemacher, Jr., and G. L. Cockerell. 1972. Nitrogen metabolism during sepsis in rats. Presented at, Annual Meeting of Federation of American Societies for Experimental Biology, Atlantic City, N. J. 9-14 April 1972.

Publications:

1. Wannemacher, R. W.,Jr., M. C. Powanda, R. S. Pekarek, and W. R. Beisel. 1971. Tissue amino acid flux after exposure of rats to Diplococcus pneumoniae. Infec. Immun. 4:556-562.

2. Powanda, M. C., and R. W. Wannemacher, Jr. 1971. Tryptophan availability as a control of hepatic pyridine nucleotide concentration in mice. Biochim. Biophys. Acta 252:239-245.

3. Powanda, M. C., R. W. Wannemacher, Jr., and G. L. Cockerell. 1972. Nitrogen metabolism during sepsis in rats. Fed. Proc. 31:710 (abstract).

LITERATURE CITED

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2. Powanda, M. C. 1971. (Discussion of Dr. Rapoport's Paper, "Tryptophan Metabolism in Infectious Illness"). Am. J. Clin. Nutr. 24:813-814.

3. Powanda, M. C., R. W. Wannemacher, Jr., and G. L.Cockerell. 1972. Nitrogen metabolism during sepsis in rats. Fed. Proc. 31:710 (abstract).

4. Jefferson, L. S., and A. Korner. 1969. Influence of amino acid supply on ribosomes and protein synthesis of perfused rat liver. Biochem. J. 111:703-712.

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6. Beisel, W. R., W. D. Sawyer, E. D. Ryll, and D. Crozier. 1967. Metabolic effects of intracellular infections in man. Ann. Intern. Med. 67:744-779.

7. Wannemacher, R. W., Jr., M. C. Powanda, R. S. Pekarek, and W. R. Beisel. 1971. Tissue amino acid flux after exposure of rats to Diplococcus pneumoniae. Infec. Immun. 4:556-562.

8. Cockerell, G. L. 1972. Plasma protein and glycoprotein changes in inflammation, infection, and/or starvation. Fed. Proc. 31:710 (abstract).

9. Wannemacher, R. W., Jr., R. S. Pekarek, and W. R. Beisel. 1972. Mediator of hepatic amino acid flux in infected rats. Proc. Soc. Exp. Biol. Med. 139:128-132.

10. Eddington, C. L., H. F. Upchurch, and R. F. Kampschmidt. 1971. Effect of extracts from rabbit leukocytes on levels of acute phase globulins in rat serum. Proc. Soc. Exp. Biol. Med. 136:159-164.

11. Steinhart, W. L. 1971. Diurnal rhythmicity in template activity of mouse liver chromatin. Biochim. Biophys. Acta 228:301-305.

12. Steinhart, W. L. 1971. Role of the chromatin template during protein synthesis in mouse liver. Fed. Proc. 30:517 (abstract).

13. Steinhart, W. L. 1971. Alteration of template activity of chromatin from livers of pneumococcus-infected mice. Proc. Soc. Exp. Biol. Med. 137:619-622.

14. Kehoe, J. M., and G. Lust. 1969. Studies of the host response to infectious disease: Alterations of RNA metabolism in mouse tissues. J. Infect. Dis. 120:411-418.

ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1W662711A096 01:	Pathogenesis of Infection of Military Importance
Work Unit No. 096 01 403:	Host-parasite Relationships in Pathogenesis of Virus Infections of Military Medical Importance
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Animal Assessment
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Authors:	Richard O. Spertzel, Lt Colonel, VC Timothy G. Terrell, Captain, VC Carroll L. Crabbs
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OL0812	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. OMB'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	01	403
b. [illegible]	62711A	1B662711A096		
c. [illegible]	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code): (U) Host-parasite relationships in pathogenesis of virus infections of military medical importance

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical medicine; 004900 Defense; 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
61 07	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER: NA
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 71	1.0	30
	CURRENT 72	2.0	15

19. RESPONSIBLE DOD ORGANIZATION
NAME: USA Medical Research Institute of Infectious Diseases
ADDRESS: Fort Detrick, MD 21701

RESPONSIBLE INDIVIDUAL
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20. PERFORMING ORGANIZATION
NAME: Animal Assessment Division USAMRIID
ADDRESS: Fort Detrick, MD 21701

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Terrell, T. C. DA
NAME:

21. GENERAL USE
Foreign intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Encephalitis (VEE, EEE); (U) Virus diseases; (U) Arboviruses; (U) Yellow fever; (U) Teratology; (U) Military medicine

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Identify and study factors involved in the pathogenesis of virus diseases. This work unit is an essential element in a comprehensive program for medical defense against BW agents.

24 (U) Extend investigations of the effect of arboviruses on the fetus, in order to understand how the observed effect on fetuses occurs, etc.

25 (U) 71 07 - 72 06 - Studies were conducted to elucidate the site of action of TC-83 virus which results in fetal death when mouse dams are inoculated on the days 10-12 of gestation. Developing viremia levels in maternal and fetal tissues following inoculation of the dam indicated virus levels in embryos as high as 2 logs more virus/gram of tissue than observed in maternal blood. However, the highest virus levels were seen in uterine tissues. Histopathologic studies suggest that placental lesions precede fetal death.

Terminated since FY 1973 funding will be under The Surgeon General, Army. New Project No. 3A062110A834; Accession No. DA 0A6423.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 01: Pathogenesis of Infection of Military Importance

Work Unit No. 096 01 403: Host-parasite Relationships in Pathogenesis of Virus Infections of Military Medical Importance

Description:

Identify and study factors involved in the pathogenesis of virus diseases.

Progress:

Studies have shown that pregnant mice inoculated with live, attenuated Venezuelan equine encephalomyelitis (VEE) vaccine virus (TC-83) during a certain stage of gestation had diminished litter size, more stillbirths and increased mortality of young.[1] The same studies suggested that transmission of TC-83 from dams to young occurred in utero. Previous studies had shown that the maximum detrimental effect on the fetuses and newborn occurred when dams were inoculated on days 10-12 of gestation.[2] To determine relative viremia titers of maternal blood and embryonic tissues, dams were inoculated on the 10th or 12th day of gestation with 5000 median mouse intraperitoneal (IP) immunizing doses ($MIPID_{50}$). In Experiment 1, selected dams inoculated on day 10 were killed 12 and 24 hr postinoculation and at 24-hr intervals thereafter. Maternal blood and an embryo suspension from each dam were examined for virus titers. Results are shown in Table I. Peak embryonal viremia was attained by 48 hr postinoculation of the dam and sustained through 96 hr. This period of maximum embryonal viremia correlated with the peak maternal viremia; however, the maternal blood virus titers were approximately 2 logs lower in magnitude.

In Experiment 2, dams were inoculated on the 12th day of gestation and killed daily, as in experiment 1. Maternal blood, uterine tissue and embryonic suspensions were assayed for virus titers. Results are shown in Table II. The virus titers in the uterine tissue were exceptionally high, exceeding values attained in either embryos or maternal tissues. Future studies will attempt to separate placental and uterine tissues and further evaluate these exceptionally high virus titers.

To investigate the mode of action of TC-83 in producing the severe detrimental effects previously observed, the gross and histopathological effects of TC-83 virus on the fetus, placenta and uterus were initiated. Ninety-six pregnant mice were inoculated IP on the 12th day of gestation with 5000 $MIPID_{50}$ TC-83 virus. Twelve infected and 12 noninfected dams were necropsied at 24-hr intervals until 1 day post partum. A gross determination of number of fetuses, viability, sites of resorption and placental sites were made in

TABLE 1. VIREMIA LEVELS IN MATERNAL BLOOD AND EMBRYO SUSPENSIONS AFTER INOCULATION OF DAMS WITH 5000 $MIPID_{50}$ TC-83 VIRUS

HOURS POST-INOC.	DAM-EMBRYO PAIRS												Total $\bar{x}$ 1 S.E.M.	
	1		2		3		4		5		6			
	D[a]	E[b]	D	E	D	E	D	E	D	E	D	E	D	E
17	+[c]	+	<1.0[d]	+	+	<1.0	+	+	+	+	<1.0	+	+	+
24	2.9	3.1	3.4	3.4	2.4	2.0	3.0	+	3.2	2.0	e		3.0 ± 0.1	2.5 ± 0.3
48	2.3	7.0	2.3	6.2	4.1	6.0	3.6	6.8	2.3	5.7	2.8	3.5	2.9 ± 0.3	5.8 ± 0.4
72	5.1	7.0	4.0	4.0	2.7	3.6	2.7	6.0					3.6 ± 0.6	5.2 ± 0.6
96	3.4	6.5	4.5	7.0	4.0	5.8	2.6	5.6	3.3	6.0	3.7	5.5	3.6 ± 0.3	6.1 ± 0.2
120	+	+	2.7	4.0	3.5	3.4	2.9	6.7	2.2	3.3	2.2	7.0	2.6 ± 0.2	4.4 ± 0.8
144	<1.0	3.4	<1.0	4.8	<1.0	3.4	<1.0	2.6	<1.0	2.6			<1.0	3.4 ± 0.4
168	<1.0	<1.0	<1.0	4.5									<1.0	+

a. Maternal blood.
b. Embryo suspension.
c. Viremia present; too low to quantitate.
d. No detectable viremia; 1.0 = limit of detection.
e. No sample.

TABLE II. VIREMIA LEVELS IN MATERNAL AND FETAL TISSUES AFTER INOCULATION OF DAM WITH 5000 $MIPID_{50}$ TC-83 VIRUS

HOURS POST-INOCULATION	TISSUE		
	Maternal Blood	Embryo	Uterus
24	+[a]	+	4.4 ± 0.1
48	+	3.9 ± 0.3	5.5 ± 0.2
72	3.9 ± 0.2	5.4 ± 0.7	8.0 ± 0.1
96	+	4.2 ± 0.7	8.9 ± 0.4
120	-[b]	4.9 ± 0.8	5.5 ± 0.3
144	-	+	4.1 ± 0.2

a. Viremia present; too low to quantitate.

b. No detectable viremia; limit of detection 2.0.

each mouse. Portions of the uterus and its contents were collected from each mouse necropsied, fixed in 10% neutral buffered formalin solution and evaluated histologically. Maternal blood and fetal tissue were collected for viremia assay, but a laboratory accident prevented an accurate virus determination.

Results of macroscopic observations are summarized in Table III. While placental attachment sites were relatively uniform in all groups, significant fetal death with resorption was seen in the experimental group by 5 days post-inoculation. By day 6, the maximum effects were observed, as the mean number of live fetuses did not change significantly thereafter. It would appear that resorption of most of the dead embryos began shortly after fetal death.

The histopathologic observations are summarized as follows: No lesions were observed in either the fetuses or dam which could be attributed specifically to the effects of TC-83 virus. Necrotic foci in the lumen of the uterus which were either resorption or abortion sites were observed in both control and inoculated dams with about the same frequency on days 1 and 2. In these cases, the lesions obviously had been present more than 2 days; they are considered to be the result of early embryonic death, rather than an effect of TC-83 virus. On the other hand, in mice necropsied after day 2, the resorption sites occurred primarily in the infected mice. Marked placentitis and/or placental necrosis also were seen primarily in inoculated mice. Results

TABLE III. EFFECTS OF MATERNAL INFECTION ON FETUSES AND NEONATES WHEN DAMS ARE INOCULATED ON DAY 12 OF GESTATION WITH TC-83 VIRUS

HOURS POST-INOCULATION		PARAMETERS OBSERVED				
		No. Dams	Healthy Fetuses	No. Dead Fetuses/ No. Dams	No. Resorption Sites/ No. Dams	Total Placental Sites
24	C[a]	10	8.9 ± 0.5	0	3/3	9.2 ± 0.5
	E[b]	5	8.6 ± 0.5	0	1/1	8.8 ± 0.5
48	C	9	8.6 ± 0.4	0	5/4	9.1 ± 0.4
	E	8	8.5 ± 1.0	1/1	8/3	9.6 ± 0.4
72	C	8	9.6 ± 0.5	0	0	9.6 ± 0.5
	E	2	10.5	0	0	10.5
96	C	8	8.6 ± 0.7	1/1	1/1	8.9 ± 0.6
	E	7	7.4 ± 0.7	2/2	2/2	8.0 ± 0.7
120	C	5	8.4 ± 0.7	0	0	8.4 ± 0.7
	E	9	7.0 ± 1.1	10/6	9/3	9.1 ± 0.7
144	C	5	8.8 ± 0.4	0	0	8.8 ± 0.4
	E	7	4.6 ± 1.1	19/6	11/2	8.4 ± 0.5

168[c]	C	8	8.9 ± 0.5	2/2	1/1[d]	9.2 ± 0.4
	E	11	3.7 ± 0.7	20/6	37/8[d]	8.2 ± 0.4
192[c]	C	9	8.1 ± 0.6	0	6/4[d]	8.7 ± 0.4
	E	9	4.7 ± 0.8	3/2	34/9[d]	8.7 ± 0.5
216[e]	C	10	8.6 ± 0.8	0	6/4[d]	9.2 ± 0.7
	E	5	4.6 ± 2.2	0	20/4[d]	8.6 ± 0.7

a. Control.

b. Experimental.

c. Values include fetuses and neonates.

d. Data point obtained by subtracting embryos and neonates from the placental attachment sites observed.

e. Values are neonates only.

suggest that the primary lesion resulting in the severe effects observed may be in the placenta; however, these results are limited. The exceptionally high virus titers in the uterine tissues (which may be entirely a result of placental tissue) would further suggest that this rapidly growing tissue may be a primary target organ for this virus. Additional studies will be oriented toward further elucidation of these observations.

Summary:

Studies were conducted to elucidate the site of action of TC-83 virus which results in fetal death when mouse dams are inoculated on the 10th-12th days of gestation. Developing viremia titers in maternal and fetal tissues following inoculation of the dam indicated virus titers in embryos as high as 2 logs more virus per gram of tissue than observed in maternal blood. However, the highest virus titers were seen in uterine tissues. Histopathologic studies suggest, but are too minimal to be conclusive, that placental lesions precede fetal death.

Additional studies are in progress.

Publications:

Spertzel, R. O., C. L. Crabbs, and R. E. Vaughn. 1972. Transplacental transmission of VEE virus in mice. Infec. Immun. (In press).

LITERATURE CITED

1. Spertzel, R. O., C. L. Crabbs, and R. E. Vaughn. 1972. Transplacental transmission of VEE virus in mice. Infec. Immun. (In press).

2. Commission on Epidemiological Survey. Dec 1970. Annual Report, FY 1970, to the Armed Forces Epidemiological Board. p. 275 to 281. Fort Detrick, Maryland.

ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1W662711A096 01:	Pathogenesis of Infection of Military Importance
Work Unit No. 096 01 404:	Mouse Brain Ultrastructure in Viral Infections of Military Medical Significance
Report Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland 21701
Division:	Pathology
Period Covered by Report:	1 April 1972 to 30 June 1972
Professional Author:	Leo Corelkin, Major, MC
Reports Control Symbol:	RCS-MEDDH-288 (R1)
Security Classification:	UNCLASSIFIED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OL0906	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	01	404
b. [illegible]	62711A	1B662711A096		
c. [illegible]	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)
(U) Mouse brain ultrastructure in arthropod-borne viral encephalitis of military medical significance

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 Clinical medicine; 004900 Defense; 010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
70 12	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER: NA
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 71	1.0	5
FISCAL YEAR CURRENT 72	1.0	78

19. RESPONSIBLE DOD ORGANIZATION
NAME: USA Medical Research Institute of Infectious Diseases
ADDRESS: Fort Detrick, MD 21701

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TELEPHONE: 301 663-2833

20. PERFORMING ORGANIZATION
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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
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TELEPHONE: 301 663-7211
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME:
NAME:

21. GENERAL USE
Foreign intelligence considered

DA

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Encephalomyelitis, equine (VEE); (U) Pathogenesis; (U) Molecular biology; (U) Nervous system; (U) Military medicine

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Determine the ultrastructure of arbovirus infection in the mouse central nervous system. This work unit is an essential element in a comprehensive program for medical defense against BW agents.

24 (U) Mice infected with VEE virus are killed at regular time intervals and their brains examined by electron microscopy.

25 (U) 72 04 - 72 06 - The new investigator has studied normal brain and other tissues. Animals will be infected with VEE virus in the near future.

Terminated since FY 1973 funding will be under The Surgeon General, Army. New Project No. 3A062110A834; Accession No. DA 0A6424.

*Available to contractors upon originator's approval

DD FORM 1498 (1 MAR 66) PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. OD FORMS 1498A 1 NOV 66 AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

ANNUAL PROGRESS REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 01: Pathogenesis of Infection of Military Importance

Work Unit No. 096 01 404: Mouse Brain Ultrastructure in Viral Infections of Military Medical Significance

Description:

Determine the ultrastructure of arbovirus infection in the mouse central nervous system.

Progress and Summary:

The outcome of a viremia is dependent on a multitude of factors, among which are virus concentration, organotropism (if any), condition of the host, and virus size.[1]

Large viruses, such as vaccinia (250 mμ) are cleared in minutes by the reticuloendothelial system, while smaller ones, such as Venezuelan equine encephalomyelitis (VEE) (60-75 mμ)[2] have longer half-lives.[1] This longer half-life may be decisive in favoring endothelial invasion by a circulating virus. Indeed numerous investigators have shown viral antigen in significant amounts in the endothelium of the central nervous system.[1,3-6] Neural infection and spread along axons, perineural space and endoneural cells[7] is probably secondary to endothelial invasion and growth.

Adult mice will be inoculated with 1000 median intracerebral lethal doses by the intravenous route. They will be killed serially in time and tissues taken (principally brain) for electron microscopy study, titrations and H & E section.

Normal brain (and other tissues) have been processed and studied by electron microscopy, in anticipation of actual inoculation and harvesting of infected tissue.

Publication:

None.

LITERATURE CITED

1. Mims, C. A. 1964. Aspects of the pathogenesis of virus diseases. Bact. Rev. 28:30-71.

2. Bykovsky, A. F., F. I. Yershov, and V. M. Zhdanov. 1969. Morphogenesis of Venezuelan equine encephalomyelitis virus. J. Virol. 4:496-504.

3. Johnson, R. T. 1964. The pathogenesis of herpes virus encephalitis. I. Virus pathways to the nervous system of suckling mice demonstrated by fluorescent antibody staining. J. Exp. Med. 119:343-356.

4. Johnson, R. T. 1965. Virus invasion of the central nervous system. Amer. J. Path. 46:929-943.

5. Cole, G. A., N. Nathanson, and H. Rivet. 1970. Viral hemorrhagic encephalopathy of rats. II. Pathogenesis of central nervous system lesions. Amer. J. Epidemiol. 91:339-350.

6. Mims, C. A. 1957. The invasion of the brain by yellow fever virus present in the blood of mice. Brit. J. Exp. Path. 38:329-338.

7. Johnson, R. T., and C. A. Mims. 1968. Pathogenesis of viral infections of the nervous system. New Engl. J. Med. 278:23-30, 84-92.

ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1W662711A096 01:	Pathogenesis of Infection of Military Importance
Work Unit No. 096 01 405:	Lymphoid Tissue Ultrastructure in Viral Infections of Military Importance
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland 21701
Division:	Pathology
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Author:	Timothy G. Terrell, Captain, VC
Reports Control Symbol:	RCS-MEDDH-288 (R1)
Security Classification:	UNCLASSIFIED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OL0916	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISP'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	01	405
b. [illegible]	62711A	1B662711A096		
c. [illegible]	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)

(U) Lymphoid tissue ultrastructure in viral infections of military importance

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical medicine; 004900 Defense; 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY			16. PERFORMANCE METHOD
71 07	CONT	DA			C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER: NA
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 71	0	0
	CURRENT 72	1.0	50

19. RESPONSIBLE DOD ORGANIZATION

NAME: USA Medical Research Institute of Infectious Diseases
ADDRESS: Fort Detrick, MD 21701

RESPONSIBLE INDIVIDUAL
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TELEPHONE: 301 663-2833

20. PERFORMING ORGANIZATION

NAME: Pathology Division USAMRIID
ADDRESS: Fort Detrick, MD 21701

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Terrell, T. G.
TELEPHONE: 301 663-7211
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME:
NAME:

21. GENERAL USE

Foreign intelligence considered

DA

22. KEYWORDS (Precede each with Security Classification Code)

(U) Yellow fever; (U) Viral infections; (U) Ultrastructure; (U) Pathogenesis; (U) Encephalomyelitis, Equine (VEE)

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) To evaluate the ultrastructural changes in lymphoid tissue of mice infected with virulent and attenuated strains of VEE virus. This work unit is an essential element in a comprehensive program for medical defense against BW agents.

24 (U) Groups of mice are infected with Trinidad VEE and the TC-83 vaccine strain VEE. At various time intervals following inoculation, mice are sacrificed. Sections of spleen are collected and prepared for electron microscopy to study the lymphoid elements. The remaining tissues are formalin fixed for later light microscopic evaluation. Fluorescein tagged antibody is to be used to localize the virus in the tissues.

25 (U) 71 07 - 72 06 - Light microscopic evaluation of lymphoid changes early in infection with virulent and attenuated strains of VEE have been compared. Tissues have been prepared for electron microscopic study but technical difficulties have delayed this study.

Fluorescein conjugated anti-VEE antibody of rooster origin was prepared and is being tested.

Terminated since FY 1973 funding will be under The Surgeon General, Army. New Project No. 3A062110A834; Accession No. DA 0A6425.

*Available for signature upon originator's approval

DD FORM 1 MAR 66 1498 (PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68, FOR ARMY USE ARE OBSOLETE)

BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 01: Pathogenesis of Infection of Military Importance

Work Unit No. 096 01 405: Lymphoid Tissue Ultrastructure in Viral Infections of Military Importance

Description:

Study ultrastructural changes of lymphoid tissue lesions produced by VEE and yellow fever.

Progress:

Lymphoid necrosis has been observed in mice infected with the virulent strain of Venezuelan equine encephalomyelitis (VEE) virus. Hyperplasia of reticuloendothelial (RE) cells is described in mice infected with the attenuated vaccine strain of VEE (TC-83). These mice develop an enhanced antibody response to antigenic challenge. It has been theorized that this enhanced immunity is related to the effect of the virus on the RE system. It does appear that the lymphoid system is a target organ for VEE virus. An ultrastructural study of the lymphoid changes during infection should help explain the pathogenesis of VEE infection. It may explain the mechanism of enhanced antibody response produced by attenuated VEE infection in mice.

Fifty 35-day-old white mice were divided into 3 groups. One group was inoculated with 5000 median mouse intracerebral lethal doses ($MICLD_{50}$) of TC-83 by the intraperitoneal route (IP) route. The 2nd group received 1000 $MICLD_{50}$ of Trinidad strain VEE, and the 3rd group served as a control group. Mice from each group were killed at 12-hr intervals through 48-hr post-inoculation and then at 24-hr intervals through the 6th day.

Sections of spleen were collected from each mouse and prepared for electron microscopy. The remaining tissues were formalin fixed and prepared for light microscopy. Special interest was directed toward the presence and severity of lymphoid changes. These findings have been summarized in Table I. RE cell hyperplasia was not pronounced in any of the infected mice. Technical difficulties with the electron microscope have delayed the examination of the tissues at the ultrastructural level.

TABLE 1. PRESENCE OF NECROSIS IN LYMPHOID TISSUES

TIME POST-INOCULATION hr	PRESENCE OF LYMPHOID NECROSIS[a]		
	CONTROL	TC-83	TRINIDAD
12	-	-	-
24	-	-	++
36	-	++	+++
48	-	++	+++
72	-	-	++
96	-	-	++
120	-	-	+
144	-	-	-

a. - No pathology
+ Minimal
++ Moderate
+++ Severe

Anti-VEE antibody was produced by inoculating roosters with Trinidad VEE. The serum was collected, fractionated and conjugated with fluorescein isothiocynate. The conjugated antibody will be used for localization of the virus in tissue sections. Initial attempts to test the conjugated antibody on VEE-infected tissue culture cells have yielded poor results, and it has not yet been determined if the conjugated antibody is satisfactory for use.

Summary:

Lymphoid changes in mice infected with virulent and attenuated strains of VEE have been compared by light microscopy. Tissues have been prepared for electron microscopic study, but technical difficulties have delayed this study.

Fluorescein conjugated anti-VEE antibody of rooster origin was prepared and is being tested.

Publications:

None.

ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1W662711A096 01:	Pathogenesis of Infection of Military Importance
Work Unit No. 096 01 800:	Biological Effects of Microbial Toxins of Military Medical Importance
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Divisions:	Animal Assessment and Bacteriology
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Authors:	Michael D. Kastello, Captain, VC (I, II) Thomas W. Davis, Captain, VC (II) Joseph C. Denniston, Captain, VC (I) James W. Stiles, Captain, MSC (I) Virginia G. McGann, Ph.D. (I, II) Richard O. Spertzel, Lt Colonel, VC (1, II)
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OL0871	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NL	☑ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	01	800
b. CONTRIBUTING	62711A	1B662711A096		
c. CONTRIBUTING	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)

(U) Biological effects of microbial toxins of military medical importance

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical medicine; 004900 Defense; 016800 Toxicology; 002300 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
66 10	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER: NA

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 71	3.0	40
FISCAL YEAR CURRENT 72	3.0	60

19. RESPONSIBLE DOD ORGANIZATION

NAME: USA Medical Research Institute of Infectious Diseases

ADDRESS: Fort Detrick, MD 21701

RESPONSIBLE INDIVIDUAL

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20. PERFORMING ORGANIZATION

NAME: Animal Assessment Division USAMRIID

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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Davis, T. W.

NAME: Spertzel, R. O. DA

21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Staphylococcus; (U) Enterotoxin; (U) Toxins; (U) Lung pathology (U) Military medicine; (U) Cannulation; (U) Histiocytes

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Study the biological effects of microbial toxins. This work unit is an essential element in a comprehensive program for medical defense against BW agents.

24 (U) The effects of microbial toxins on animal hosts are determined by measuring various physiologic parameters.

25 (U) 71 07 - 72 06 - Techniques have been perfected for surgical implantation of single or multiple cannulation devices in the rhesus monkey to facilitate introduction of toxins at specific gastrointestinal sites. In addition, preliminary studies utilizing intestinal loops have demonstrated no gross or histopathologic differences between loops containing SEB and control loops containing saline.

Preliminary data obtained in this study suggest that the dosage of purified staphylococcal alpha toxin employed was lethal for the rhesus monkey and that postmortem lesions were compatible with those seen in deaths from endotoxins. IV administration of delta toxin at the dosage indicated appeared to have no adverse effect. Additional studies employing significant numbers of rhesus monkeys are needed to fill the existing void in data collected from subhuman primates and to better elucidate the in vivo mechanism of action of purified samples of staphylococcal alpha toxin.

Publication: Lab. Invest. 25:617-625, 1971.

Terminated since FY 1973 funding will be under The Surgeon General, Army. New Project No. 3A062110A834; Accession No. DA OA6426.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE AND 1498-1 1 MAR 68 FOR ARMY USE ARE OBSOLETE

BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 01: Pathogenesis of Infection of Military Importance

Work Unit No. 096 01 800: Biological Effects of Microbial Toxins of Military Medical Importance

Description:

Study the biological effects of microbial toxins in the animal host.

Progress, Part I:

Permanent cannulation devices developed for use in the dog have been successfully modified for use in the rhesus monkey. Techniques have been perfected for surgical implantation of single- or multiple-cannulation devices to facilitate introduction of toxins at specific gastrointestinal sites. There are indications that clinical illness, as a result of intoxication with staphylococcal enterotoxin B (SEB), depends on the level of the gastrointestinal tract at which the toxin is introduced. Studies are currently in progress to determine if this difference is real.

Preliminary work has been completed utilizing SEB instilled into the lumen of intestinal loops using methods employed in the investigation of cholera toxin. Forty µg of Lot 14-30 SEB in 1 ml of saline were introduced into loops in the duodenum, jejunum or ileum. Saline was used in alternate loops as controls. In addition, corresponding loops in another monkey contained only saline. When observed after 4 hr, no differences in fluid accumulation were noted between loops containing SEB and saline. Histopathologic examination revealed minimal to moderate dilation of lacteals, edema of the submucosa, histiocyte and necrotic cellular debris near the tips of villi in control as well as SEB test loops.

Summary, Part I:

Techniques have been perfected for surgical implantation of single- or multiple-cannulation devices in the rhesus monkey to facilitate introduction of toxins at specific gastrointestinal sites. In addition, preliminary studies utilizing intestinal loops have demonstrated no gross or histopathologic differences between loops containing SEB and control loops containing saline.

Progress, Part II:

A pilot project was initiated to determine the pathophysiologic responses of rhesus monkeys to intravenous (IV) injection of purified samples of

staphylococcal alpha (α) and delta (Δ) hemolytic exotoxin.*[1] Alpha and Δ hemolytic exotoxins are only 2 of a large number of potentially toxic factors and enzymes produced by various strains of Staphylococcus aureus; these are not to be confused with staphylococcal enterotoxins which likewise bear letter designations.

Three rhesus monkeys were inoculated IV with a Δ, α-10 (most purified) or α-11 toxin preparation; a 4th monkey, inoculated with borate buffered saline (BBS), served as a control. After surgical implantation of necessary instrumentation, monkeys were placed in restraint chairs and allowed to stabilize for 24 hr prior to collection of baseline data and toxin injection. Physiologic parameters measured included arterial pressure (BP), mean arterial pressure (MAP), electrocardiogram (EKG), respiratory rate (RR), heart rate (HR), body temperature, and various hematologic values [white blood cell (WBC) and differential white count, hematocrit (HCT), blood urea nitrogen (BUN) and blood chemistry]. Complete gross and microscopic pathological studies were performed on monkeys that died.

Table I is a summary of the data sent by Dr. Rahal, indicating the lethality of the various toxins for rabbits, their respective activities in terms of hemolytic units (HU) per μg protein, and the amounts of the respective toxins that were administered to each of the 4 monkeys.

TABLE I. CHARACTERISTICS OF STAPHYLOCOCCAL ALPHA AND DELTA HEMOLYTIC TOXINS

IDENT.	MONKEY NO.	mg INJECTED (IV)	SPECIFIC ACTIVITY (HU/μg)	μg LETHAL (45 MIN)/ 3-kg RABBIT
α-10	B-137	1.0	10	75
α-11	B-147	3.5	3.6	<140
Δ	B-142	10.0	--	--
Control	B-156	2 ml BBS	--	--

Monkeys receiving BBS or Δ toxin showed no abnormal clinical signs. However, the monkey receiving α-10 toxin demonstrated severe signs consisting of emesis, lethargy, dyspnea and respiratory arrest terminally; the monkey which received α-11 toxin showed similar clinical signs.

*Supplied by Dr. James J. Rahal, Chief, Division of Infectious Diseases, Veterans Administration Hospital, New York, N. Y.

Physiologic data collected on the monkey receiving Δ toxin were consistent with values recorded for the control monkey throughout the 72-hr observation period. The α-10 and α-11 monkeys showed early elevation in body temperature and blood pressure, believed to be associated with emesis, retching and struggling. From 10 min postinjection to death at 95 min, the α-10 monkey showed a progressive drop in BP, RR and HR. Although the α-11 monkey remained more physiologically stable throughout the observation period, both α-10 and α-11 monkeys showed a precipitous fall in blood pressure terminally. Physiological data are summarized in Table II.

The control and Δ monkeys showed similar hematologic values except for a marked leukopenic change with subsequent rebound demonstrated by the Δ monkey. Both the α-10 and α-11 monkeys showed leukopenic changes in the hemogram, but rebounded prior to death. Similarly, both had terminal elevation in the hematocrit. Hematologic values are summarized in Table III. Additionally, alterations in blood chemistry values included early elevation in blood glucose in both the α-10 and α-11 monkeys. These 2 monkeys demonstrated terminal elevation in lactic dehydrogenase (LDH) values.

Electrocardiographic changes were numerous in the α-10 monkey and included periods of both tachycardia and bradycardia, arrythmia, altered P and T waves, marked AV dissociation with varying degrees of heart block and extra beats. Similar electrocardiographic abnormalities were noted in the monkey administered α-11 toxin, but were not as pronounced.

Monkeys given α-10 and α-11 toxins were necropsied immediately following death. Significant lesions in the α-10 monkey included tracheal fluid, edematous lungs, acute hemorrhage of the myocardium, gastric mucosa, and brain, and moderate congestion of all tissues. The lesions described are compatible with those observed in deaths from endotoxins. The immediate cause of death appeared to be acute pulmonary edema.

In addition to the previously described lesions noted in the α-10 monkey, the α-11 monkey demonstrated gastric dilation, subendocardial hemorrhage, severe peracute infarction of the left kidney, edema of the submucosa of the stomach, small intestine and colon, thrombosis of the major arteries of the lungs and kidney, splenic necrosis, and lymphoid hyperplasia of the spleen. Cystic dilations in the mucosa of the stomach were unique in the pathologist's experience.

TABLE II. PHYSIOLOGIC DATA ON 4 MONKEYS ADMINISTERED DELTA, ALPHA-10, ALPHA-11 TOXINS AND SALINE

TIME	BP mm Hg	RR	HR	TEMPERATURE °F	BP mm Hg	RR	HR	TEMPERATURE °F
	Control - Saline					α-10		
min								
0	122/84	32	180	101.4	116/84	20	180	98.3
5	120/76	30	180	101.4	160/140	75	80	98.0
10					200/140	60	20	
15	132/80	32	180	101.4	180/130	40	120	97.3
25					140/100	40	90	
30	122/80	30	180	101.4	170/125	40	90	97.0
45					136/100	40	200	
60	128/80	30	180	101.5	130/110	60	240	96.4
67								
70					80/56	30	90	
85					84/56	30	90	
90					90/70	6	90	
93					10/8		30	95.8
						Death		
hr								
2	120/80	30	180	101.5				
6	156/80	35	180	101.5				
12	160/94	40	180	101.2				
24	138/76	35	180	101.4				
48	140/80	30	180	101.1				
72	140/92	32	180	101.0				

		Δ				α-11		
min								
0	144/100	30	180	100.7	180/120	25	200	101.9
1					184/124	25	200	
3					196/144	30		
5	140/100	25	180	100.6	180/128	40	200	101.5
10	140/100	35	180		130/100	50	240	
15	136/96	25	180	101.0	140/120	40	240	100.7
30	136/100	27	180	101.2	148/116	40	200	99.6
45	156/120	32	240					
60-66	136/96	30	200	101.2	160/120	34	240	97.5
90	140/100	32	200	100.9	172/120	38	240	98.0
hr								
2	148/104	35	240	99.9	168/120	30	240	98.0
2.5	140/100	30	200	99.8	172/120	30	240	98.0
3	136/110	30	200	99.8	168/120	32	220	98.0
4	136/100	30	240	100.4	170/126	30	200	98.2
6	126/100	32	200	101.0	120/90	50	240	98.4
+10"					108/68	60	240	
+15"					24/12		120	
+25"					12/8		60	
+30"							60	
12	140/104	32	180	101.6		Death		
24	160/92	32	180	101.0				
36	168/108	20	180					
48	158/108	20	180	100.9				
72	164/100	23	180	101.1				

TABLE III. HEMATOLOGIC VALUES, STAPHYLOCOCCAL ALPHA AND DELTA HEMOLYTIC TOXIN STUDIES

TIME	WBC no./cu. cm.				HCT %			
	Control	Δ	α-10	α-11	Control	Δ	α-10	α-11
min								
0	11,990	16,900	19,800	9,350	32	38	42	39
5	11,440	8,580	9,850	3,000	32	38	40	38
15	12,320	9,000	8,250	2,500	32	38	25	38
30	14,740	7,150	12,100	4,180	31	38	42	38
60	14,410	2,750	8,250	4,290	32	38	47	38
90		6,820	12,000	5,000		36	70	37
hr								
2	15,180	11,700	Dead	5,500	31	35	Dead	38
2.5		9,600		9,680		34		38
3	9,790	9,460		10,350	25	32		37
4		12,100		12,430		32		36
6	14,630	10,500		13,860	25	31		40
+15"				11,000				52
12	10,450	12,800		Dead	21	30		Dead
24	8,690	11,100			27	28		
36		6,710				26		
48	11,440	9,240			27	24		
72	11,000	13,640			27	24		

Summary, Part II:

Preliminary data obtained in this study suggest that the dosage of purified staphylococcal α toxin employed was lethal for the rhesus monkey and that postmortem lesions were compatible with those seen in deaths from endotoxins. IV administration of Δ toxin at the dosage indicated appeared to have no adverse effect.

Additional studies employing significant numbers of rhesus monkeys are needed to fill the existing void in data collected from subhuman primates and to better elucidate the in vivo mechanism of action of purified samples of staphylococcal α toxin.

Publication:

Stiles, J. W., and J. C. Denniston. 1971. Response of the rhesus monkey, Macaca mulatta, to continuously infused staphylococcal enterotoxin B. Lab. Invest. 25:617-625.

LITERATURE CITED

1. Rahal, J. J., Jr., M. E. Plaut, and L. Weinstein. 1968. Effect of purified staphylococcal alpha toxin on active sodium transport and aerobic respiration in the isolated toad bladder. J. Clin. Invest. 47:1603-1614.

ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1W662711A096 01:	Pathogenesis of Infection of Military Importance
Work Unit No. 096 01 801:	Mediators of Microbial Toxin Activity in BW Defense Research
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Physical Sciences
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Author:	Anthony C. Jung, Captain, MSC
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OL0872	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	8A. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	01	801
b. CONTRIBUTING	62711A	1B662711A096		
c. CONTRIBUTING	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)

(U) Mediators of microbial toxin activity in BW defense research

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical medicine; 004900 Defense; 016800 Toxicology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
66 10	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER: NA

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 71	2.0	50
	CURRENT 72	1.0	70

19. RESPONSIBLE DOD ORGANIZATION

NAME: USA Medical Research Institute of Infectious Diseases

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME:

NAME:

21. GENERAL USE

Foreign intelligence considered

DA

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Bradykinin; (U) Kinin system; (U) Hypotension; (U) Radioimmunoassay; (U) Military medicine

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Evaluate the role of host mediator in the action of microbial toxins. This work unit is an essential element in a comprehensive program for medical defense against BW agents.

24 (U) A radioimmunoassay technique is employed to determine bradykinin plasma levels in animals which have been infected or intoxicated with agents known to have hemodynamic manifestations.

25 (U) 71 07 - 72 06 - Progress is being made in producing bradykinin antiserum of greater binding capacity. Valid and reproducible standard binding inhibition curves have been derived, but in actual practice, the precision of the assay is still inadequate.

Terminated since FY 1973 funding will be under The Surgeon General, Army. New Project No. eA062110A834; Accession No. DA 0A6427.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 01: Pathogenesis of Infection of Military Importance

Work Unit No. 096 01 801: Mediators of Microbial Toxin Activity in BW Defense Research

Description:

Evaluate the role of host mediators in the action of microbial toxins.

Progress:

Research into the role played by bradykinin in the action of microbial toxins has been slowed by problems encountered in the radioimmunoassay technique. The major problem has been in producing a suitable bradykinin antiserum.

Since the last report,[1] work on this problem has taken the following direction: Two additional rabbits were immunized with antigen previously prepared, and the resulting antisera gave no improvement in binding activity. Fresh immunogen was prepared and injected into 2 rabbits, with similarly poor results. Gamma globulin fractions were isolated from previously harvested antisera in the hope of concentrating the antibodies, but the resultant binding activity was still inadequate. A close examination of all antisera data up to that point showed that those rabbits immunized with the first immunogen prepared in this laboratory gave the best binding activity. Unfortunately, these were rabbits lost because of a thermostat malfunction in the animal chamber. Batches of immunogen prepared after that first one may have contained a partially (or completely) deteriorated bradykinin molecule, resulting in antibodies which would not bind well to labeled tyrosyl-8-bradykinin.

Therefore, fresh bradykinin was obtained from a different supplier and the bradykinin-ovalbumin complex again prepared in the previous manner. Upon the recommendation of Dr. Talamo, the originator of this assay,[2] a dose of approximately 15 mg of immunogen, as compared to 6 mg previously used, was administered to each of 3 rabbits. The method of immunization was also altered. Previously the entire dose was injected into the toepads of rabbits. This time, however, the rabbit's back was shaved, the dose divided in half, and 2 series of injections given subcutaneously 3 weeks apart.

At this stage of the research an ^{125}I-labeled bradykinin product became commerically available and was obtained. Use of this hapten has improved the chances success with this immunoassay, since it is a more standardized product than the one we tried to label ourselves and has a known specific activity.

Antisera were collected from the rabbits immunized by the newer method and larger dose and tested for their binding capacity with the commerically-obtained hapten. Results from the binding studies show that the antibody response from the newer method is somewhat better. However, the binding capcity must still be greater and the antisera diluted further before the assay is suitable.

Therefore, in an attempt to stimulate further antibody response in rabbits previously immunized by the new method, a 3rd series of subcutaneous injections of immunogen was given. Antisera were collected 8 weeks later and analyzed for their binding capacity. One rabbit yielded an antiserum which could be diluted 20 times and still give a binding capacity above 700-800 counts. This antiserum was utilized in the assay procedure, and standard binding inhibition curves were derived using the linear regression method. In the case of each curve, the level of significance and correlation coefficient gave an extremely favorable verdict ($P < 0.001$) as to the line's validity. In addition, the inhibition curves was very reproducible.

Consequently, an attempt was made to utilize the assay procedure in analyzing the bradykinin concentration in processed blood from a rabbit. Four random samples previously collected and processed were chosen and assayed 5 times.

TABLE I. BRADYKININ LEVELS OF 4 RANDOM RABBIT SERA.

SAMPLE	ng/ml PLASMA mean ± SEM	Range
I	10.2 ± 2.7	7.2 - 15.0
II	13.2 ± 10.8	2.6 - 31.2
III	7.8 ± 3.4	2.2 - 12.5
IV	8.8 ± 5.3	4.8 - 18.8

It is plainly evident from Table I that the assay was not consistent in its ability to measure constant concentrations of bradykinin in the same sample. The variation between samples is not unexpected, since plasma bradykinin concentrations is dependent on a variety of processes and/or factors. The reason(s) why the assay was not more consistent is now being explored. It is expected that a more suitable antiserum, with a much greater binding capacity, would contribute much to solving this dilemma. Steps are still being taken to produce such an antiserum.

In cooperation with LtC Collins, bradykinin has been coupled to bromacetyl cellulose and used to immunize a goat. However, 4-week and 7-week antisera have failed to show an antibody response.

Summary:

Progress is being made in producing bradykinin antiserum of greater binding capacity. Valid and reproducible standard binding inhibition curves have been derived, but in actual practice, the precision of the assay is still inadequate.

Publications:

None

LITERATURE CITED

1. U. S. Army Medical Research Institute of Infectious Diseases, 1 July 1971. Annual Progress Report, FY 1971. p. 121 to 125. Fort Detrick, Maryland.

2. Talamo, R. C., E. Haber, and K. F. Austen. 1969. A radioimmunoassay for bradykinin in plasma and synovial fluid. J. Lab. Clin. Med. 74:816-827.

ANNUAL PROGRESS REPORT

Project No. 1B662711A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1B662711A096 01:	Pathogenesis of Infection of Military Importance
Work Unit No. 096 01 802:	In vivo Distribution of Microbial Toxins
Reporting Installations:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland 21701
Division:	Pathology
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Authors:	Joseph F. Metzger, Colonel, MC Anna D. Johnson
Reports Control Symbol:	RCS-MEDDH-288 (R1)
Security Classification:	UNCLASSIFIED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OL0873	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	8A. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	6271IA	1W662711A096	01	802
b. CONTRIBUTING	6271IA	1B662711A096		
c. CONTRIBUTING	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)

(U) In vivo distribution of microbial toxins of military medical importance

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical medicine; 004900 Defense; 016800 Toxicology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
66 10	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER: NA
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 71	1.0	40
FISCAL YEAR CURRENT 72	1.0	50

19. RESPONSIBLE DOD ORGANIZATION

NAME: USA Medical Research Institute of Infectious Diseases
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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Johnson, A. D.
NAME: DA

21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Enterotoxin; (U) Staphylococcus; (U) Immunofluorescence; (U) Immunoenzymatic techniques; (U) Isotopic tracers; (U) Antigen; (U) Antibody; (U) Military medicine

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Study the transport and localization of microbial toxins in animal tissues and the pathogenesis of the corresponding intoxications. This work unit is an essential element in a comprehensive program for medical defense against BW agents.

24 (U) Use an in vivo isotopically labeled enterotoxin and study the pharmacology utilizing this preparation.

25 (U) 71 07 - 72 06 - Isotopically labeled SEB utilizing tritium resulted in a low energy label which was difficult to demonstrate by radioautography with exposure times of less than 3 months. SEB labeled in vivo with C-14 resulted in a marked increase in energy which was reflected in short exposure times necessary for radial diffusion identification. In addition, the labeled SEB could be measured directly by scanning electrophoretic strips.

Terminated since FY 1973 funding will be under The Surgeon General, Army. New Project No. 3A062110A834; Accession No. DA 0A6428.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

BODY OF REPORT

Project No. 1B662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1B662711A096 01: Pathogenesis of Infection of Military Importance

Work Unit No. 096 01 802: *In vivo* Distribution of Microbial Toxins of Military Medical Importance

Description:

Study transport and localization of microbial toxins in animal tissues and the pathogenesis of the corresponding intoxications.

Progress:

Various *in vitro* labeled staphylococcal enterotoxin B (SEB) preparations have been utilized to determine the dynamic pharmacology of enterotoxin B.[1-3] SEB was labeled with ^{131}I, ^{125}I, fluorescein, peroxidase, and tritium. *In vitro* labels separate either spontaneously or through the actions of enzymes when these *in vitro* labeled toxins are tested in animals. Fluorescein and ^{131}I are both separated almost completely when the toxin is administered via the gastrointestinal tract. Therefore, the *in vitro* labeled toxin has in no way elucidated toxin adsorption, distribution, or fate by this route. By the intravenous and intrapulmonary routes a similar displacement of the label occurs and most measurements have been made utilizing TCA precipitation as an indication of that portion of the label which is still attached to SEB. It has been demonstrated by all routes that from ^{125}I or ^{131}I can attach *in vivo* to proteins other than SEB. Therefore a portion of the TCA precipitable activity may well represent free ^{125}I or ^{131}I which has attached to proteins other than the toxin and previous localization studies may represent either the toxin or an extraneous protein.

U. S. Army Biological Laboratories[4] demonstrated an *in vivo* labeled SEB with the isotope ^{14}C. This material was purified by the method of Schantz et al.[5] and the activity was verified by immunological and toxicological methods.

Tritium labeled SEB was prepared by adding tritiated protein hydrolysate and amino acids to both complete and partially synthetic media. The production of toxins utilizing isotopically reagents was not decreased. The levels of incorporation of tritium remained low and exposure times for autoradiographs were at least 3 months. Though specific localization of ^{3}H.SEB was demonstrable in the kidneys, areas with smaller amounts could not be visualized. The highest levels of tritium incorporation occurred utilizing

a complete media (4% N-Z Amine + 1% yeast extract) augmented by isotopically labeled glutamic acid.

Since tritium has a low energy, ^{14}C glutamic incorporation was attempted. Trapping procedures for containment of the radioactive CO_2 generated during incubation resulted in diminution in amounts of SEB produced. The ^{14}C-SEB produced had an activity of 1 mCi/mg which was a higher rate of incorporation than had been achieved with tritium. The electrophoretic strips scanned with a gas flow radioactive counter revealed isotope counts only in the area of SEB. Tritium labeled material scanned previously could not be identified due to low energy. In addition, the radioactivity could be demonstrated in the immune precipitate with radioautography.

Preliminary in vivo studies utilizing isolated small intestine pouches to determine histological differences in control, nonimmune, and immune animals are in progress. Initial studies were accomplished utilizing non-labeled SEB in most animals and one animal in each group with labeled ^{14}C-SEB. Fluorescein labeled SEB was also utilized in one animal as a control for an in vitro labeled SEB type.

Summary:

A labeled SEB has been prepared by utilizing a complete media with added ^{14}C-glutamic acid. This material has a high specific activity. It can be readily demonstrated by radioautography and is to be utilized in in vivo test systems.

Publications:

None.

LITERATURE CITED

1. Morris, E. L., L. F. Hodoval, and W. R. Beisel. 1967. The unusual role of the kidney during intoxication of monkeys by intravenous staphylococcal enterotoxin B. J. Infect. Dis. 117:273-284.

2. Rapoport, M. I., L. F. Hodoval and W. R. Beisel. 1967. Influence of thorotrast blockade and acute renal artery ligation on disappearance of staphylococcal enterotoxin B from blood. J. Bacteriol. 93:779-783.

3. Rapoport, M. I., L. F. Hodoval, E. W. Grogan, V. McGann and W. R. Beisel. 1966. The influence of specific antibody on the disappearance of staphylococcal enterotoxin B from blood. J. Clin. Invest. 45:1365-1372.

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ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1W662711A096 01:	Pathogenesis of Infection of Military Importance
Work Unit No. 096 01 803:	Subcellular Biological Effects of Microbial Toxins and Microbial Diseases of Military Medical Importance
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Bacteriology
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Author:	Peter G. Canonico, Captain, MSC
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OL0896	72 06 30	DD-DR&E(AN)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	8a. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	01	803
b. CONTRIBUTING	62711A	1B662711A096		
c. CONTRIBUTING	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code): (U) Subcellular biological effects of microbial toxins of military medical importance

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical medicine; 004900 Defense; 016800 Toxicology; 002300 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
60 07	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER: NA
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 71	2.0	40
	CURRENT 72	1.0	69

19. RESPONSIBLE DOD ORGANIZATION
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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME:
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21. GENERAL USE
Foreign intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Enterotoxin; (U) Staphylococcus; (U) Lysosomes; (U) Enzymes; (U) Pneumococcus; (U) Pulmonary endothelium; (U) Military medicine; (U) Q fever

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Study the subcellular effects of microbial toxins and other informational molecules and determine the role of lysosomal enzymes in the catabolism of exogenous proteins. This work unit is an essential element in a comprehensive program for medical defense against BW agents.

24 (U) A variety of techniques, e.g., fractionation, electron microscopy, are used to study subcellular action of toxins.

25 (U) 71 07 72 06 - In response to pneumococcal infection rat liver endoplasmic reticulum was found to undergo a change in its biochemical morphology: a progressive loss of cytoplasmic catalase and peroxisomes was also observed. In tularemia infection, only endoplasmic reticulum changes were seen. Techniques of cellular fractionation were applied to the isolation and purification of Coxiella burneti from egg yolk sac slurries. A 100-fold purification was achieved.

Lysosomal enzymes were capable of hydrolyzing staphylococcal enterotoxin B at acid pH. The extent and pH optimum of hydrolysis was shown to be dependent on the extent of toxin denaturation.

Publications: J. Infect. Dis. 124:372-378, 1971.
Appl. Microbiol. 23: 1972, In press.

Terminated since FY 1973 funding will be under The Surgeon General, Army. New Project No. 3A062110A834; Accession No. DA 0A6429.

*Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE

BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 01: Pathogenesis of Infection of Military Importance

Work Unit No. 096 01 803: Subcellular Biological Effects of Microbial Toxins and Microbial Diseases of Military Importance

Description:

Study the subcellular effects of microbial toxins and informational molecules and determine the role of lysosomal enzymes in the catabolism of exogenous proteins.

Progress, Part I:

Enzymatic and physical properties of rat liver subcellular organelles during the infectious process were determined by zonal centrifugation techniques. Livers from rats, inoculated 8-48 hr previously with 10^7 Diplococcus pneumoniae or 10^9 Francisella tularensis, were homogenized and subjected to isopycnic centrifugation in a Spinco B XIV zonal rotor.[1] Thirty-three zonal fractions were obtained and analyzed for protein, RNA and 6 enzyme markers of mitochondria, lysosomes and peroxisomes. A computer program was developed to calculate and represent enzymatic activities in the form of standardized equilibrium density distribution patterns.

The quilibrium density distribution patterns of cytochrome oxidase indicated that progressive degeneration of mitochondrial structural integrity occurred during the course of pneumococcal infection. Activity of peroxisomal markers, catalase and urate oxidase, were reduced by 82 and 76% respectively during more advanced stages of pneumococcal infection, and were accompanied by a substantial increase in mean equilibrium density of peroxisomal particles. The activity and distribution of particle-bound cathepsin D, acid phosphatase, and β-glucuronidase (β-gluc) were not significantly different from controls. However, a moderate increase in soluble activity of lysosomal enzymes was observed in experimental animals, suggesting that in the pneumococcus-infected animals, lysosomes may be larger, thus more susceptible to rupture during homogenization of the tissue.

The distribution pattern of β-gluc demonstrated the presence of a minor sedimentable component with a non-lysosomal localization. The equilibrium density of this component was approximately 1.14 and coincided with the

localization of the endoplasmic reticulum (ER) marker, glucose-6-phosphatase. This observation supported the known dual localization of β-gluc in hepatic cells.[2] A loss in ER-associated β-gluc was observed during advanced stages of pneumococcal infection. This finding suggests that the biochemical morphology of ER in hepatocytes is altered during infection and that these changes may signify a reorganization of liver ER and protein synthesizing machinery specifically for synthesis of serum glycoproteins[3] rather than cellular proteins.

In contrast to pneumococcal infection, tularemia infection was marked by sustained rapid loss of ER β-gluc and transient changes in the physical-chemical properties of mitochondria and peroxisomes.

These data demonstrate that tissue fractionation and zonal ultracentrifugation techniques can be employed to evaluate cellular responses and thereby obtain further information regarding pathogenesis of infection.

Summary, Part I:

Fractionation of pneumococcal and tularemia infected rat liver by zonal centrifugation techniques demonstrated alterations in the biochemical morphology of cellular organelles. The most significant changes observed in pneumococcal infection were delayed loss in β-gluc activity and endoplasmic reticulum and progressive loss of cytoplasmic catalase and peroxisomal particles. In tularemia infected animals there was a sustained rapid loss of endoplasmic reticulum β-gluc and a moderate but transient decrease in peroxisomal enzymes.

Progress, Part II:

A formalin killed, Henzerling, phase II, Q fever liquid vaccine was subjected to density gradient fractionation in a B XIV zonal rotor. Two fractions, with equilibrium density values of 1.24 and 1.28, were found to contain Coxiella burneti organisms. Electron micrographs of these fractions showed that organisms in the lighter density fraction were oval shaped with prominent nucleoids and thick cell walls. The heavier fraction was composed primarily of round organisms without prominent nucleoids or thick cell walls. These findings suggest that the 2 populations of organisms represent different pleomorphic forms of C. burneti. However, the possibility cannot be ruled out that chemical modification during production of the original liquid vaccine altered the morphological characteristics of the denser organisms.

Both fractions that contained organisms were judged free of contaminating egg yolk sac materials by electron microscopy and the complement fixation test against anti-egg yolk sac sera. That quantity of protein in the lower density C. burneti fraction which protected 50% of guinea pigs against a live challenge of C. burneti was found to be 0.16 μg

as compared to 1.601 µg for the original liquid vaccine. Fractionation of 2 L of liquid vaccine yielded approximately 600 ml of purified organisms with a protein concentration of 120 µg/ml, representing a total protein yield of 1.98%.

Summary, Part II:

Purification by isopycnic zonal centrifugation of large quantities of inactivated, phase II, C. burneti for use as diagnostic antigen and as a vaccine is described. Fractionation of egg yolk sac derived C. burneti vaccine resulted in the separation of 2 distinct populations of organisms each devoid of microscopically and serologically recognizable components of egg yolk sac. One population of organisms, characterized by an equilibrium density of 1.24, was rod shaped with a thick, densely-stained wall and prominent central body. The 2nd population, with an equilibrium density of 1.28, had a coccobacillary shape, thin cellular walls and lacked a prominent nucleoid.

Progress, Part III:

Studies concerning hydrolysis of staphylococcal enterotoxin B (SEB) by lysosomal enzymes in vitro were continued. Hydrolysis of SEB in vitro was monitored by determining the release of TCA insoluble ^{125}I tagged peptides and amino acids. Iodination of SEB was accomplished by the recently developed enzymatic method of Morrison and Hultquist,[4] which employs bovine lactoperoxidase isolated from raw milk.

SEB was iodinated by mixing 500 µl of SEB (5 mg/ml), which had been dialyzed against 0.05 M phosphate buffer, pH 7.2, with 5 mCi ^{125}I and 25 µg lactoperoxidase. The iodination reaction was initiated by addition of 50 µl of 2.5 mM H_2O_2 and at the end of 1-2 hr the reaction mixture was dialyzed against cold buffer to remove unreacted ^{125}I. An iodination efficiency of 99% was obtained for SEB. Rabbit YG immunoglobulins were enzymatically iodinated with an efficiency of 93% and a specific activity greater than 3 µCi/µg protein. Acetate strip electrophoresis of enzymatically iodinated SEB or YG failed to demonstrate any evidence of denaturation. Both proteins formed precipitin lines with specific antisera using the Ouchterlony technique.

A substantial release of ^{125}I was observed when iodinated SEB was incubated at pH 2.5 with liver, kidney and peritoneal exudate cell homogenates, as well as a purified liver lysosome extract. The amount of ^{125}I released at pH 3.5 and 4.4 was substantially lower (Table I). The ability of SEB to bind with specific antisera was lost after digestion at pH 2.5 but not 3.5 or 4.4

TABLE I. HYDROLYSIS OF SEB-^{125}I BY LYSOSOMAL CATHEPSINS.

SEB TREATMENT		% ^{125}I[a] BY SOURCE OF LYSOSOMAL ENZYMES			
		Liver Tritosome Extract[b]	Kidney G.F.[c]	Liver G.F.	PMN G.F.
Trace Labelled					
	2.5[d]	55	40	59	49
pH	3.5	28	20	19	16
	4.4	13	14	13	6
	7.5[e]	--	--	--	4
Formic Acid Oxidized					
	2.5	92	86	84	
pH	3.5	80	96	89	
	4.4	87	93	90	
100 C for 60 Min					
	2.5	10	27	32	46
pH	3.5	45	63	35	64
	4.4	44	80	44	76
	7.5	--	--	--	32
Extensively Iodinated					
	2.5	11	10		12
pH	3.5	11	10		14
	4.4	9	14		8
	7.5	--	--		10
Chloramine-T Iodinated					
	2.5	10	11		5
pH	3.5	9	10		4
	4.5	8	7		3
	7.5	--	--		3

a. ^{125}I released into a soluble 5% TCA supernatant after 24 hr incubation.

b. Liver lysosomes isolated after Triton WR-1339 injestion by the method of Trouet.[7] Sonified saline extracted lysosomal protein composed 10% of the total protein in the incubation mixture.

c. Large granule fraction isolated by differential centrifugation of homogenate. G.F. was incubated with toxin at a protein to protein ratio of 2:1.

d. Citrate buffer 0.1 M containing 0.01 M cysteine.

e. Phosphate buffer 0.1 M containing 1.0 M KCL.

The pH optima for SEB hydrolysis was found to be between 2.75 and 3.0. Hydrolysis was more effective in citrate buffer than glycine-HCl or acetate buffer. In the presence of iodoacetamide, the pH optima for hydrolysis of SEB shifted slightly to a lower value. Cysteine (0.001 M) increased the extent of hydrolysis nearly 2-fold.

In contrast to trace labeled SEB, toxin oxidized with formic acid was more extensively digested by purified lysosomes and kidney homogenates. SEB iodinated by the chloramine-T method and heavily iodinated SEB were less susceptible to lysosomal hydrolysis. The latter SEB derivative, formed by additional enzymatic iodination of trace labeled SEB in the presence of 0.001 M KI for 12-24 hr as described by Agner,[5] resulted in a yellow product which was electrophoretically distinct from native or trace labeled SEB, and did not react with specific antisera. SEB heated at 100 C for 60 min, on the other hand, was more extensively digested at pH 4.4 and 3.5 than in pH 2.5.

These results indicate that lysosomal cathepsins are capable of hydrolysing SEB at an acid pH. Cathepsin D and probably cathepsin C are primarily responsible for the hydrolysis of the toxin. Susceptibility of the toxin to hydrolysis seems dependent on the conformational state of the molecule, since native toxin is hydrolyzed almost exclusively at pH 2.5, while heat-denatured or oxidized derivatives are appreciably digested at pH 4.4, a value which more reasonably approaches the intralysosomal pH.

These results support the hypothesis that certain proteins and microbial protein toxins must be "denatured" prior to hydrolysis by lysosomal hydrolases within the vacuolar system of intact cells.[6]

Summary, Part III:

Lysosomal cathepsins were capable of hydrolysing SEB at acid pH. Hydrolysis occurred most readily at pH 2.75 - 3.0, were enhanced by cysteine and unaffected by iodoacetamide. Heavily iodinated SEB was found to be less susceptible to digestion while heat-denatured and oxidized toxin were readily hydrolyzed at pH 4.4.

Presentations:

1. Canonico, P. G. 1971. Cellular biology of SEB. Presented to the National Institute of Child Health and Human Development, Bethesda, Md.

2. Canonico, P. G. 1971. Lysosomes and protein catabolism. Presented to the Graduate Student Association, Bureau of Biological Research, Rutgers University, New Brunswick, N. J.

Publications:

1. Canonico, P. G., and M. J. Van Zwieten. 1971. Swelling of mitochondria from rabbit liver induced by staphylococcal enterotoxin B. J. Infect. Dis. 124:372-378.

2. Canonico, P. G., M. J. Van Zwieten and W. A. Christmas. 1972. Purification of large quantities of Coxiella burneti by density gradient zonal centrifugation. Appl. Microbiol. 23. In press.

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1. Canonico, P. G., and J. W. C. Bird. 1970. Lysosomes in skeletal muscle tissue. Zonal centrifugation evidence for multiple cellular sources. J. Cell Biol. 45:321-333.

2. Fishman, W. H., S. S. Goldman, and R. Delellis. 1967. Dual localization of β-glucuronidase in endoplasmic reticulum and in lysosomes. Nature 213:457-460.

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6. Coffey, J. W., and C. deDuve. 1968. Digestive activity of lysosomes. I. The digestion of proteins by extracts of rat liver lysosomes. J. Biol. Chem. 243:3255-3263.

7. Trouet, A. 1964. Immunisation de lapins par des lysosomes hépatiques de rats traités au Triton WR-1339. Arch. Int. Physiol. 72: 698-700.

ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1W662711A096 02:	Prevention and Treatment of Biological Agent Casualties
Work Unit No. 096 02 002:	Evaluation of Efficacy of Experimental Vaccines in Man
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Divisions:	Medical and Virology
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Authors:	Peter J. Bartelloni, Lt Colonel, MC (I, II) Nemesio M. Francisco, Major, MC (I) Charles S. White, III, Captain, MC (I, II) Helen H. Ramsburg (I)
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OL0829	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DOD'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	02	002
b. ~~CONTRIBUTING~~	62711A	1B662711A096		
c. ~~CONTRIBUTING~~	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)

(U) Evaluation of experimental vaccines in man in BW defense research

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical medicine; 004900 Defense; 002300 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
61 10	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION

b. NUMBER: NA

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 71	2.0	190
FISCAL YEAR CURRENT 72	2.0	160

19. RESPONSIBLE DOD ORGANIZATION

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21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Vaccines; (U) Immunization; (U) Encephalitis, equine (EEE); (U) Military medicine; (U) Human volunteers

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Evaluate experimental vaccines developed by various contractors, organizations or other governmental agencies. This work unit is an essential element in a comprehensive program for medical defense against BW agents.

24 (U) Test vaccines are given to experimental animals, and when considered safe, to volunteers.

25 (U) 71 07 - 72 06 - An inactivated EEE vaccine produced by large scale production methods was administered to a limited number of volunteers in 2 different doses 28 days apart. No significant clinical reactions occurred. Significant neutralizing antibody developed in the majority of subjects 14 days after the second dose of vaccine. A booster dose on day 180 was required because of poor persistence of antibody; it was shown to be immunogenic.

Clinical and laboratory studies are being performed on a group of 77 individuals who have undergone intensive immunization to a variety of antigens. These individuals have received an average of over 100 ml of antigen and over 40 skin tests since 1946. These subjects had been previously studied in 1954 and 1964 by other investigators. The studies are in progress and complete data are not available.

Terminated since FY 1973 funding will be under The Surgeon General, Army. New Project No. 3A062110A834; Accession No. DA OB6410.

*Available to contractors upon originator's approval

DD FORM 1 JAN 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DA FORMS 1498A 1 NOV 65 AND 1498 1 JAN 68 FOR ARMY USE ARE OBSOLETE

BODY OF REPORT

Project No. 3W662771A096: Medical Defense Aspects of Biological Agents (U)

Task No. 3W662771A096 02: Prevention and Treatment of Biological Agent Casualties

Work Unit No. 096 02 002: Evaluation of Efficacy of Experimental Vaccines in Man

Description:

Evaluation of experimental vaccines developed by various organizations, contractors, or other governmental agencies.

Progress, Part 1:

Evaluation of Eastern Equine Encephalitis (EEE) Vaccine, Formalin Inactivated, Tissue Culture Origin, NIBR 104 (Medical Division Protocol No. FY '1 5): The clinical responses to the administration of 2 different doses of EEE vaccine, produced by the Merrell National Laboratories, Swiftwater, Pa., under contract to the United States Army Medical Research and Development Command (Contract No. DA 49 193 MD 2179) was described in a previous report.[1]

Blood was obtained prior to vaccination and on days 7, 14, 28, 42, 56, 90, and 180 for neutralizing antibody. A booster dose of 0.5 ml of vaccine was administered to both groups of volunteers on day 180. Additional blood specimens for neutralizing antibody were obtained on days 28, 42, 56, and 90 following administration of the booster dose of vaccine. Neutralization tests have been completed on serum specimens obtained on days 28 and 42 only.

The accompanying table shows the serological responses by day after the administration of the first dose of vaccine. Results are expressed as $\log_{10}$ serum neutralization index (SNI). As shown, the majority of subjects in both groups failed to develop significant neutralizing antibody on days 7, 14, and 28 after the first dose of vaccine. The mean SNI on day 28 was 0.9 in both groups. However, by day 42 (14 days after the 2nd dose of vaccine) all but one subject in Group I and two individuals in Group II developed significant neutralizing antibody. The mean SNI in Group I was 2.4 and in Group II, 1.9. Mean neutralizing antibody titers gradually declined in both groups to low titers by day 180, with a mean SNI of 0.5 in Group I and 1.0 in Group II. In addition, on day 180 only 2 of 16 subjects had SNI 1.7. Because of this a 0.5 ml booster dose of vaccine was administered to each subject.

TABLE I: NEUTRALIZING ANTIBODY RESPONSES IN 16 VOLUNTEERS WITHOUT PRIOR EASTERN EXPERIENCE TO THE ADMINISTRATION OF 2 DOSES OF EEE VACCINE 28 DAYS APART, AND A BOOSTER DOSE ON DAY 180.

VOLUNTEERS	RESPONSE BY DAY AFTER FIRST DOSE								
	7	14	28	42	56	90	180	208	222
*GROUP I (0.5+0.5)									
BCB	0.7	1.7	1.8	3.6	2.8	2.3	2.1	2.9	2.9
JGB	0.4	0.9	0.8	2.0	2.2	1.4	0.6	3.0	2.5
EMC	0	0.8	0.7	2.3	2.7	1.9	1.4	2.7	2.7
RLF	0	0.4	0.9	2.5	1.5	1.4	1.3	2.0	1.5
HGH	0	0.6	0.2	2.1	2.2	2.0	2.2	3.1	2.9
JAL	0	0.4	0.7	1.4	1.4	1.4	0.7	2.9	2.3
WYL	0.8	0.8	1.5	3.0	2.5	2.1	1.4	2.7	-
RWM	0	0.5	0.5	1.9	2.0	1.3	0.5	1.8	1.5
Range	(0.4-0.8)	(0.4-1.7)	(0.2-1.8)	(1.4-3.6)	(1.4-2.8)	(1.3-2.3)	(0.5-2.2)	(1.8-3.1)	(1.5-2.9)
Mean	0.2	0.8	0.9	2.4	2.2	1.7	1.3	2.6	2.3

*GROUP II
(0.25+0.25)

CFM	0.3	1.4	1.4	2.2	2.2	1.7	1.1	2.9	3.2
GFK	0.5	0.5	0.8	1.3	0.9	0.9	0.4	2.2	2.4
LAQ	0	0.8	0.9	1.8	2.0	1.2	1.2	2.2	2.2
WDR	0.4	1.3	1.1	2.1	1.8	2.0	1.5	3.0	2.6
JTR	0.3	0.2	0.2	1.2	0.8	0.4	0.4	1.0	1.3
GAS	0	0.9	0.9	3.1	2.6	2.7	1.3	2.8	2.9
LFT	0.4	0.7	0.9	1.9	1.6	1.7	1.3	3.2	3.1
DLW	0.7	1.1	1.1	1.8	1.3	1.0	1.1	1.3	1.0
Range	(0.3-0.7)	(0.2-1.4)	(0.2-1.4)	(1.2-3.1)	(0.9-2.6)	(0.4-2.7)	(0.4-1.5)	(1.0-3.2)	(1.0-3.2)
Mean	0.3	0.9	0.9	1.9	1.7	1.5	1.0	2.3	2.3

* Groups I & II received vaccine on days 0, 28, and 180. Results expressed as Log_{10} serum neutralization index (LNI). An LNI of $\geq$ 1.7 is considered as representing significant neutralizing antibody.

Subjects in both groups exhibited a secondary antibody response with mean LNI of 2.6 in Group I and 2.3 in Group II. All individuals in Group I had LNI ≥1.8. Two subjects failed to achieve significant neutralizing antibody in Group II. The mean LNI 42 days after the booster dose of vaccine was 2.3 in both groups.

Summary, Part I:

An EEE vaccine produced by the Merrell-National Laboratories, Swiftwater, Pa. utilizing large scale production methods has been administered to a limited number of subjects. It has been found to be of low reactogenicity when administered subcutaneously in 2 different doses 28 days apart. Significant neutralizing antibody developed in the majority of subjects 14 days after the second dose of vaccine. A booster dose on day 180 elicited a secondary response in the majority of subjects.

Progress, Part II:

Clinical and Laboratory Evaluation of Repeated Immunizations in Man: Immunization has become an accepted method to prevent infection in man. With the exception of local and systemic reactions following the administration of a vaccine, few other adverse effects to vaccination have been clearly defined in man. Amyloidosis has been reported as a complication in experimental animals receiving frequent and large amounts of antigen.[2] To determine whether frequent and repeated immunizations are associated with adverse effects in man, a study was initiated in 1958 in 99 individuals at Fort Detrick who were receiving a variety of antigens for an extended period of time.[3] Seventy-seven of the original 99 subjects were re-studied in a similar manner in 1964.[4] No evidence of clinical illness was found in subjects in either study which might be attributed to immunization. However several unexplained laboratory deviations were noted as follows:

1. High incidence of lymphocytosis.

2. An abnormal serum protein electrophoretic pattern in some of the subjects characterized by poor separation of alpha-2 and beta-globulin fractions.

3. Elevated serum hexosamines.

4. Unexplained abnormalities of liver and renal function.

5. A high incidence of serum anti-gamma globulin activity.

No conclusive evidence was presented that these abnormalities represented adverse effects of intensive immunization.

The study to be described represents a 25-year follow-up evaluation of the 99 individuals. Eleven had died; 11 have not yet been seen; the 77 other subjects had received an average of 100 ml of antigen and over 40 skin tests each since 1946.

Each individual was studied with a complete history, physical examination, chest x-ray and electrocardiogram. Blood was obtained from each subject for the following laboratory studies: white blood cell, differential, platelet count, hematocrit, partial thromboplastin and prothrombin time, leucocyte alkaline phosphatase stain of peripheral blood smear, lupus erythematosis cell preparation, sodium, potassium, chloride, copper, zinc, iron, calcium, phosphate, creatinine, uric acid, SGOT, alkaline phosphatase, bilirubin direct and indirect, cholesterol, triglycerides, free fatty acids, blood urea nitrogen, phosphates, venereal disease research laboratory test (VDRL), serum hexosamine, antinuclear antibody, rheumatoid latex factor, serum protein and lipoprotein electrophoresis, IgG, IgM, IgA, IgD, Kappa and Lamba light chains, C'3 and lymphocyte stimulation studies. In addition, 24 urine specimens were obtained from each subject for creatinine, protein and immunoglobulin determinations. The results were compared with 25 control subjects from Fort Detrick.

Although the studies on these subjects are in progress, preliminary data indicate that no clinical illnesses have been detected that might be attributed to immunization. The previously noted laboratory observations of lymphocytosis, proteinuria and alterations in the serum protein electrophoretic pattern were not detected in the present study. Some individuals continue to manifest abnormal liver function studies and elevations of serum hexosamine. The significance of these laboratory deviations are not known. Serum and urine immunoglobulin values did not differ from control subjects.

Summary, Part II:

A 25-year clinical and laboratory follow-up study was conducted in 77 subjects who had received frequent special immunizations. Although studies are still in progress no clinical illnesses were found that could be attributed to intensive immunization. Liver function studies and serum hexosamines were found to be elevated in some of the vaccinees and remain unexplained. Lymphocytosis, abnormal serum protein electrophoretic patterns and proteinuria noted in previous studies were not detected in the present study.

Publications:

None.

LITERATURE CITED

1. U.S. Army Medical Research Institute of Infectious Diseases. 1 July 1971. Annual progress report, FY 1971. p. 143 to 156. Fort Detrick, Maryland.

2. Bailey, C. H. 1916. The production of amyloid disease and chronic nephritis in rabbits by repeated intravenous injection of living colon bacilli. J. Exp. Med. 23:773-790.

3. Peeler, R. N., L. E. Cluff, and R. W. Trever. 1958. Hyperimmunization of man. Johns Hopkins Med. J. 103:183-198.

4. Peeler, R. N., P. J. Kadull, and L. E. Cluff. 1965. Intensive immunization of man. Evaluation of possible adverse consequences. Ann. Intern. Med. 63:44-57.

ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1W662711A096 02:	Prevention and Treatment of Biological Agent Casualties
Work Unit No. 096 02 003:	Chemoprophlaxis and Therapy of Infectious Diseases of Potential Biological Warfare Significance
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Medical
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Authors:	Peter J. Bartelloni, Lt Colonel, Mc
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 010830	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISPN INSTR'N	8b. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	02	003
b. CONTRIBUTING	62711A	1B662711A096		
c. CONTRIBUTING	COOG 1212b(9)			

11. TITLE (Include with Security Classification Code): (U) Chemoprophylaxis and therapy of infectious diseases of potential biological warfare significance

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical medicine; 004900 Defense; 003200 BW, CW, RW

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
62 09	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATE EFFECTIVE: — EXPIRATION:
b. NUMBER: NA
c. TYPE: — d. AMOUNT:
e. KIND OF AWARD: — f. CUM. AMT.:

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
PRECEDING FISCAL YEAR 71	0	0
CURRENT FISCAL YEAR 72	1.0	15

19. RESPONSIBLE DOD ORGANIZATION
NAME: USA Medical Research Institute of Infectious Diseases
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20. PERFORMING ORGANIZATION
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ADDRESS: Fort Detrick, MD 21701

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
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TELEPHONE: 301 663-7281
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: White, C. S.
NAME: DA

21. GENERAL USE: Foreign intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Prophylaxis; (U) Therapy; (U) Infectious diseases; (U) Human volunteers; (U) Military medicine

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Assess the effect of microbials and various drug regimens in various infectious diseases. This work unit is an essential element in a comprehensive program for medical defense against BW agents.

24 (U) Various drugs are tested in volunteers under strict protocol conditions.

25 (U) 71 07 - 72 06 - During the year, no tests were performed. This is a work unit needed for future work as required.

Terminated since FY 1973 funding will be under The Surgeon General, Army. New Project No. 3A062110A834; Accession No. DA 0B6411.

*Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498 — PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 68 AND 1498-1 1 MAR 68 FOR ARMY USE ARE OBSOLETE

BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 02: Prevention and Treatment of Biological Agent Casualties

Work Unit No. 096 02 003: Chemoprophylaxis and Therapy of Infectious Diseases of Potential Biological Warfare Significance

Description:

Assess the effect of antimicrobials and various drug regimens in various diseases.

Progress and Summary:

During the year, no tests were performed.

Publications:

None.

ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1W662711A096 02:	Prevention and Treatment of Biological Agent Casualties
Work Unit No. 096 02 004:	Studies in Combined Antigens for Use in Military Medicine
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Bacteriology
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Authors:	William A. Christmas, Major, MC Harry G. Dangerfield, Colonel, MC
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OL0831	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	02	004
b. CONTRIBUTING	62711A	1B662711A096		
c. CONTRIBUTING	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)
(U) Studies in combined antigens for use in military medicine

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 Clinical medicine; 004900 Defense; 010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
63 04	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER: NA
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE

FISCAL YEAR	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
PRECEDING 71	2.0	80
CURRENT 72	2.0	79

19. RESPONSIBLE DOD ORGANIZATION
NAME: USA Medical Research Institute of Infectious Diseases
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ASSOCIATE INVESTIGATORS
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NAME: Spertzel, R. O. DA

21. GENERAL USE
Foreign intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Encephalitis, equine (VEE, EEE, WEE); Rift Valley fever; (U) Chikungunya; (U) Q fever; (U) Yellow fever; (U) Military medicine; (U) Vaccines; (U) Immunization

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Determine the feasibility of combining various immunizing antigens and establish the compatibility, optimal dose, best schedule for administration, and efficacy of combinations. This work unit is an essential element in a comprehensive program for medical defense against BW agents.

24 (U) Various antigens will be mixed in a variety of proportions and given to experimental animals for challenge studies. Promising combinations will be tested further and eventually may be tested in volunteers.

25 (U) 71 07 - 72 06 - Simultaneous immunization of guinea pigs with an inactivated pentavalent vaccine, containing killed EEE, WEE, CHIK, RVF and Q, neither enhanced nor suppressed immunogenic responses to living, attenuated VEE (TC-83) or tularemia vaccines. Serological responses to WEE and EEE components of the pentavalent vaccine appeared to be suppressed by simultaneous immunization with TC-83 vaccine. However, serological response to the Q fever vaccine component of the pentavalent preparation was enhanced by immunization with TC-83 vaccine.

Terminated since FY 1973 funding will be under The Surgeon General, Army. New Project No. 3A062110A834; Accession No. DA 0B6412.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A AND 1498-1 MAY BE USED FOR ARMY USE UNTIL DISCONTINUED.

BODY OF REPORT

Project No. 1W662713A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662713A096 02: Prevention and Treatment of Biological Agent Casualties

Work Unit No. 096 02 004: Studies in Combined Antigens for Use in Military Medicine

(U) OBJECTIVE:

Determine the feasibility of combining various immunizing antigens and establish the compatibility, optimal dose, best schedule for administration, and efficacy of the combinations.

Progress:

It was previously observed that formalin-inactivated vaccines for Eastern and Western equine encephalitis (EEE and WEE), Chikungunya (ChIK), Rift Valley (RVF) and Q Fever (Q) retained their immunogenic properties after combination into a pentavalent product.[1] These studies have been expanded to investigate the feasibility and efficacy of simultaneous immunization of Hartley strain guinea pigs with the pentavalent preparation and the living, attenuated vaccine for tularemia (LVS vaccine) and/or for Venezuelan equine encephalomyelitis (VEE, vaccine strain TC-83). Other workers have shown that TC-83 vaccine administered alone or simultaneously with LVS vaccine produced no adverse reaction in guinea pigs and protects them against challenge with virulent Trinidad strains of VEE, whereas LVS vaccine, or the LVS-VEE vaccine treatment merely indicated an altered state of resistance, as indicated by prolongation of survival time, but did not protect against fatal infection with virulent *Francisella tularensis*, strain SCHU-S4.[2]

On day 0, one group of guinea pigs was immunized with LVS vaccine, a 2nd group with TC-83 vaccine, and a 3rd group with both vaccines administered simultaneously. Immunization of 3 additional groups corresponded to that of the first 3, except that pentavalent vaccine was administered simultaneously with attenuated vaccine(s) on day 0 and a 2nd dose of pentavalent vaccine was given on day 28. LVS vaccine at a dosage of 2.2×10^8 viable cells was injected subcutaneously (SC) in the groin area; TC-83 vaccine, at a dosage equivalent to 1 human dose, was inoculated intraperitoneally (IP) on one side of the abdomen and 1 ml of pentavalent vaccine (isotonic to body fluids, pH 7.2) IP on the opposite side of the abdomen. The injected dose of pentavalent preparation contained 20 hamster median intraperitoneal effective doses (HD_{50}) of EEE and WEE vaccines, 10 hamster $IPFD_{50}$ of RVF vaccine, 20 mouse $IPID_{50}$ of ChIK vaccine

and 20 guinea pig $LPED_{50}$ of Q vaccine. Immunized and nonimmune control guinea pigs were challenged on day 42 with 400 mouse IP lethal doses of Trinidad strain VEE, with 100 viable cells of strain SCHU-S4 of F. tularensis, or with 10^7 viable cells of the attenuated strain 425 of F. tularensis.

Thirty-four of 35 guinea pigs immunized with TC-83 vaccine, alone or simultaneously with other vaccines, survived an IP challenge with highly virulent Trinidad strain of VEE at a dose lethal for all nonimmune control animals (Table I). Immunization with LVS vaccine protected guinea pigs against subcutaneous challenge with strain 425, a tularemia strain of moderate virulence, and effected significant prolongation of survival time [illegible] of animals challenged SC with highly virulent tularemia.

TABLE I. PROTECTIVE EFFICACY OF VENEZUELAN EQUINE ENCEPHALITIS (VEE) TC-83 VACCINE AND TULAREMIA LVS VACCINE ADMINISTERED SIMULTANEOUSLY WITH OR WITHOUT PENTAVALENT VACCINE (PV).

IMMUNIZATION GROUP	CHALLENGE ORGANISM			
	Trinidad Strain	Tularemia Strain		
	VEE	425	SCHU-S4	
	P/T[a]	P/T	P/T	MTTD[b] days
PV + TC-83	7/7	--	--	--
PV + LVS	--	15/15	1/15	23 ± 3.6
PV + TC-83 + LVS	9/9	10/10	1/9	22 ± 2.3
TC-83	10/10	--	--	--
LVS	--	13/14	0/13	23 ± 2.8
TC-83 + LVS	8/9	14/14	4/14	26 ± 3.6
Unimmunized	0/9	3/17	0/12	6 ± 0.2

a. P/T = Protected/Total.

b. MTTD = Mean time to death ± SEM.

Serum samples were collected for serological evaluation from all animals on day -3 and day 38, from survivors of VEE challenge on day 56 and from survivors of tularemia challenge on day 102. Only preliminary data

can be reported at this time. Preimmunization sera had no detectable antibodies for VEE; hemagglutination inhibition (HI) titers were <1:10, and tularemia agglutinin titers <1:10. Except for 6 animals in the group that received the LVS-TC-83 vaccine treatment, a good VEE antibody response developed following TC-83 immunization (Table II). The 6 refractory animals, members of a group in which some guinea pigs inadvertantly received 0.01 human dose (rather than 1 human dose) of TC-83 vaccine, had prechallenge HI titers <1:10, but 5 of the 6 survived challenge. The reciprocal geometric mean titer (GMT) for the other 4 members of the group was 760, a value compatible with that of other TC-83 immunized groups. Antibody response to tularemia immunization was essentially the same for all groups.

TABLE II. RECIPROCAL GEOMETRIC MEAN TITERS OF PRECHALLENGE SERA FROM GROUPS OF GUINEA PIGS IMMUNIZED WITH TC-83 AND/OR LVS VACCINE, ALONE OR SIMULTANEOUSLY WITH PENTAVALENT VACCINE (PV).

IMMUNIZATION GROUP	PRECHALLENGE VALUES FOR CHALLENGE GROUP		
	Trinidad	425	SCHU-S4
	VEE titer HI	Tularemia agglutinin titer	
PV + TC-83	830	-	-
PV + LVS	-	175	211
PV + TC-83 + LVS	1880	197	160
TC-83	933	-	-
LVS	-	201	150
TC-83 + LVS	37	150	175

Antibody response to 4 of 5 components of the pentavalent preparation was also evaluated (Table III). Simultaneous immunization with live LVS vaccine did not affect the responses to any of the 4 components. Simultaneous immunization with living TC-83 vaccine appeared to suppress WEE and EEE responses but enhanced response to Q vaccine.

TABLE III. RECIPROCAL GEOMETRIC MEAN TITERS 7 DAYS AFTER THE 2ND INJECTION OF PENTAVALENT VACCINE (PV) ADMINISTERED ALONE OR IN COMBINATION WITH TC-83 AND/OR LVS VACCINE.

IMMUNIZATION GROUP	MEAN TITER[a]			
	HI		Complement Fixation	
	WEE	EEE	RVF	Q
PV	2540	930	2.5	7.6
PV + TC-83	697	128	2.4	23.0
PV + LVS	1222	1469	1.4	5.2
PV + TC-83 + LVS	1483	1888	1.5	14.0

a. Test antigens: WEE - Formalin-inactivated Western equine encephalitis virus
EEE - Formalin-inactivated Eastern equine encephalitis virus
RVF - Formalin-inactivated Rift Valley Fever virus
Q - Formalin-inactivated Q fever, phase II

It is obvious that serological techniques employed to measure humoral antibody were ineffective for evaluating protection conferred by immunization with live attenuated vaccines. Guinea pigs lacking HI antibody were resistant to challenge with VEE and most animals with tularemia agglutinins succumbed to challenge with virulent tularemia strain SCHU-S4.

It is difficult to assess the significance of resistance to challenge with tularemia strain 425. Mechanisms for establishing infection are probably quite different from attenuated and fully virulent strains, as is suggested by the numbers of bacteria required to produce fatal infections. Attenuated organisms may exert their effect by accumulation of endotoxin-like products; their ability to invade cells of the host may be blocked or delayed; or their somatic or soluble products may provoke an accelerated host defense response. In any event, some modification of their infectious properties is advantageous to the host and permits effective utilization of specific and nonspecific mechanisms of resistance.

Summary:

Simultaneous immunization of guinea pigs with pentavalent vaccine neither enhanced nor suppressed immunogenic responses to living attenuated viral and/or bacterial vaccines. Serological responses to WEE and EEE components of the pentavalent vaccine appeared to be suppressed by simultaneous immunization with TC-83 vaccine. However, serological response to the Q Fever vaccine component of the pentavalent preparation was enhanced by immunization with TC-83 vaccine.

Publication:

1. Canonico, P. G., M. J. Van Zwieten and W. A. Christmas. 1972. Purification of large quantities of Coxiella burneti by density gradient zonal centrifugation. Appl. Microbiol. 23. In press.

LITERATURE CITED

1. U. S. Army Medical Research Institute of Infectious Diseases. 1 July 1971. Annual Progress Report, FY 1971. p. 161 to 172. Fort Detrick, Maryland.

2. U. S. Army Medical Research Institute of Infectious Diseases. 1 July 1969. Annual Progress Report, FY 1969. p. 171 to 176. Fort Detrick, Maryland.

ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1W662711A096 02:	Prevention and Treatment of Biological Agent Casualties
Work Unit No. 096 02 005:	Vaccine Evaluation Employing a Reference Animal Model System for BW Defense
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Bacteriology
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Author:	Mary H. Wilkie
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OLO886	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. DOWN SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	02	005
b. [illegible]	62711A	1B662711A096		
c. [illegible]	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)

(U) Studies on antibody production and their binding properties in BW defense research

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical medicine; 004900 Defense; 002300 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
67 10	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER: NA

c. TYPE d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 71	1.0	50
	CURRENT 72	1.0	72

19. RESPONSIBLE DOD ORGANIZATION

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TELEPHONE: 301 663-2833

20. PERFORMING ORGANIZATION

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TELEPHONE: 301 663-7341

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME:

NAME: DA

21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Encephalitis, equine (EEE); (U) Antigens; (U) Antibody; (U) Binding strength; (U) Serology; (U) Military medicine

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) To develop an animal reference model system for studying the efficacy of experimental vaccines and to define parameters that, by extrapolation, may be employed to predict immune responses in man.

24 (U) To characterize antibody responses of rabbits to experimental vaccines by employing standard serological titration and gel chromatographic analyses of whole serum and to compare such findings with those from man.

25 (U) 71 07 - 72 06 - Sera from rabbits and volunteers immunized with a killed vaccine for Eastern equine encephalitis (EEE) were analyzed for IgG production by whole serum serological titrations and gel filtration chromatography. No IgG was detected up to 6 mon after immunization of volunteers, except in 3 individuals whose responses were compatible with prior experience to EEE. Rabbits immunized with 2.5 times as much vaccine produced significant amounts of IgG. An unexplained delayed increase in specific antibody activity was observed in both man and rabbits after the initial response decreased.

Discrepancies in the standard hemagglutination-inhibition (HI) serological procedure prompted development of a new HI test for arbovirus antibodies.

Terminated since FY 1973 funding will be under The Surgeon General, Army. New Project No. 3A062110A834; Accession No. DA 086413.

*Available to contractors upon originator's approval

DD FORM 1 MAR 65 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE DD FORMS 1498A 1 NOV 65 AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 02: Prevention and Treatment of Biological Agent Casualties

Work Unit No. 096 02 005: Vaccine Evaluation Employing a Reference Animal Model System for Bw Defense

Objective:

Develop an animal reference model system for studying the efficacy of experimental vaccines and to define parameters that, by extrapolation, may be employed to predict immune responses in man.

Progress:

Studies continued to investigate the feasibility of employing an animal model system to establish optimal dosage and schedule for administration of experimental vaccines to man.

Rabbits were immunized with inactivated Eastern equine encephalitis (EEE) vaccine, NDBR 104, Lot 1; antibody responses were studied in conjunction with those of volunteers immunized with the same vaccine, Medical Division Protocols FY 71-4 and 71-5.[1]

To determine if EEE vaccine was capable of producing a total immune response through the IgG phase and if low serological responses in humans are due to low dosage, 4 rabbits were injected with 1.5 and 1.0 ml of the subcutaneous (SC) sites. Rabbit sera obtained at 7-day intervals for 6 weeks and at 3½ mon were studied in detail.

Whole sera were screened for hemagglutination inhibition (HI) antibody by the method of Clarke and Casals,[2] and for complement fixing (CF) antibody by standard procedures.[3] In addition, 2 ml of fresh serum were chromatographed on upward-flow C-200 Sephadex columns (2.5 x 45 cm) in Tris buffered saline, pH 7.3. Protein content of eluates was determined by UV absorption through a continuous flow monitor. In contrast to titrations with whole serum, HI antibody activity was determined without kaolin absorption on an aliquot from each tube. Chromatographs of each serum yielded the usual 4 UV absorbing peaks. The 1st peak (19S) contained all activity of specific IgM and a nonspecific, β-lipoprotein hemagglutination inhibitor. The 2nd peak (7S) consisted primarily of γ globulin, and some specific IgG, some IgA and no inhibitory activity. IgG was not separated in this study. The 3rd peak (4S) contained albumin and other 4S serum proteins which exhibited some inhibitory activity.

The 4th peak, peptide and/or amino acids, demonstrated no activity. Results are shown in Table I.

TABLE I. HEMAGGLUTINATION-INHIBITION (HI) AND COMPLEMENT FIXATION (CF) RESPONSES IN RABBITS IMMUNIZED WITH EEE VIRUS VACCINE.

ANIMAL NUMBER	DAY AFTER 2ND DOSE	WHOLE SERUM RECIPROCAL ANTIBODY TITER		HI ACTIVITY (UNITS/ml)[a] CHROMATOGRAPHY PEAK	
		HI	CF	1st Peak	2nd Peak
44	0	<20	8	135	56
	7	20	32	67	151
	14	80	64	123	646
	28	40	32	304	884
	42	80	32	176	550
	135	160	4	58	688
	365	80	4	ND[b]	ND
45	0	<20	4	ND	ND
	7	20	32	14	73
	14	40	32	17	145
	28	40	32	0	181
	42	80	32	0	234
	135	320	4	68	335
	365	320	4	ND	ND
46	0	<20	2	44	0
	7	320	128	159	414
	14	160	128	53	308
	28	80	16	70	880
	42	40	8	80	333
	135	320	16	53	452
	270	40	8	0	349
47	0	<20	2	61	23
	7	80	64	174	412
	14	80	64	55	612
	28	20	8	99	329
	42	20	4	196	286
	142	160	4	310	471
	365	160	4	ND	ND

a. Sum of HI activity in a UV peak (area under curve).

b. ND = Not Done

This EEE vaccine preparation produced a total immune response in rabbits. By day 14 after the last injection, whole serum HI and CF titers averaged 1:80 and 1:64 respectively. Specific IgM HI activity, expected in the 1st chromatographic peak, was masked by lipoprotein inhibitors that could not be removed. In the 2nd peak, specific IgG activity generally became maximal by day 28 but subsequently decreased. This finding, in combination with responses observed from whole serum, indicated that conversion to IgG occurred and, therefore, that the capability for immunological memory had been established by the vaccine dosages employed. The increase in HI titers seen in both whole sera and IgG fractions by days 135-142 cannot be explained at this time.

Of considerable interest was rabbit 46. This animal developed neck paralysis 9 mon after vaccination and was killed. Neither viral isolation techniques nor histopathological studies provided a diagnosis. During the following 2 mon, 8 of 15 healthy nonvaccinated animals caged in the same area developed CF and/or HI titers against EEE antigen; IgG conversion was confirmed by chromatography in 2 of these rabbits. Unlike findings observed with vaccinated animals, antibody activity disappeared within 45 days.

For volunteer studies, smaller doses of vaccine were employed. Eight men received 2 doses of 0.5 ml each of EEE vaccine 28 days apart and another eight 2 doses of 0.25 ml each 28 days apart. Whole sera obtained at specified intervals were screened for HI antibodies.[2] Sera of 3 individuals with the highest titers and 3 with low or intermediate responses on days 35 and 42 were selected for detailed study, as described for rabbit sera.

Two vaccinees, GFN and JTR (Table II) developed essentially no antibody response following immunization: no CF antibody, sporadic HI titers, and no significant serum neutralizing (SN) activity as indicated by log neutralization index (LNI). HI activity of chromatographic samples never exceeded baseline values which, influenced by the lipid content of serum, varied from sample to sample of each individual. It should be noted that both individuals were from the low vaccine dose group. Whole serum titers for subject RWM were representative of responses of 9 other vaccinees, i.e. no CF antibody, sporadic HI titers and transient LNI activity. As with nonresponders, HI activity in peak 2 did not exceed baseline levels.

Antibody responses of the 3 volunteers who apparently responded maximally to vaccine are shown in Table III. Although not shown by the tabular presentation of data, inspection of chromatographs combined with serological activity suggests that all 3 individuals had some form of prior experience to antigens in the EEE vaccine. All developed significant levels of whole serum titers for HI, CF and SN antibodies. Significant increases in HI activity were observed in the 2nd chromatographic peak of sera from subjects BCB and GAS. Values for chromatographic analysis of sera from subject HGM resembled those described for volunteer RWM, Table II, a poor responder; however, serological activity for volunteer HGM was confined to a sharp peak upon a low baseline rather than distributed among a 20-fraction tube range.

TABLE II. VALUES FOR VOLUNTEERS WITH POOR ANTIBODY RESPONSES FOLLOWING IMMUNIZATION WITH EEE VACCINE.

SUBJECT	DAY	WHOLE SERUM TITERS			HI ACTIVITY (UNITS/ml)[d] CHROMATOGRAPHY PEAK	
		HI	CF	LNI[c]	1	2
[illegible][a]	0	<10	<2	0	56	18
	7	<10	<2	0.50	75	28
	14	20	<2	0.50	67	32
	28	<10	<2	0.75	86	38
	35	<10	<2	1.25	44	18
	42	10	<2	1.25	62	32
	56	10	<2	0.85	84	19
	90	<10	<2	0.65	150	29
	180	<10	<2	0.35	ND[e]	ND
JTR[a]	0	<10	<2	0	110	30
	7	<10	<2	0.25	63	34
	14	20	<2	0.15	82	24
	28	<10	<2	0.15	56	32
	35	<10	<2	0.90	70	22
	42	<10	<2	1.15	126	11
	56	<10	<2	0.80	78	20
	90	<10	<2	0.40	25	18
	180	<10	<2	0.38	ND	ND
RWM[b]	0	<10	<2	0	82	63
	7	<10	<2	0	121	39
	14	20	<2	0.50	275	59
	28	<10	<2	0.50	90	43
	35	<10	<2	1.50	104	69
	42	20	<2	1.87	384	84
	56	<10	<2	2.00	282	61
	90	<10	<2	1.15	580	117
	180	<10	<2	0.50	ND[e]	ND

a. 0.25 ml EEE vaccine per dose on Days 0 and 28.

b. 0.5 ml EEE vaccine per dose on Days 0 and 28.

c. LNI = Log neutralization index, titration in suckling mice, courtesy Virology Division.

d. Sum of HI units in a UV peak (area under curve).

e. ND = Not Done.

TABLE III. VALUES FOR THE THREE VOLUNTEERS WITH MAXIMAL ANTIBODY RESPONSES FOLLOWING IMMUNIZATION WITH EEE VACCINE.

SUBJECT	DAY	WHOLE SERUM RECIPROCAL ANTIBODY TITER			HI ACTIVITY (UNITS/ml)[d] CHROMATOGRAPHY PEAK	
		HI	CF	LNI[c]	1	2
BCB[a]	0	<10	<2	0	211	43
	7	20	<2	0.65	103	24
	14	160	<2	1.65	521	95
	28	40	<2	1.75	111	140
	35	160	<2	2.75	301	210
	42	160	4	3.55	222	462
	56	80	2	2.75	517	291
	90	320	<2	2.25	262	615
	180	80	8	2.10	ND[e]	ND
HGH[a]	0	<10	<2	0	486	97
	7	<10	<2	0	548	62
	14	20	<2	0.60	429	95
	28	<10	<2	0.20	93	59
	35	40	2	2.10	176	58
	42	80	4	2.10	208	75
	56	160	4	2.20	128	51
	90	80	<2	2.00	168	104
	180	20	<2	2.24	ND	ND
GAS[b]	0	<10	<2	0	3430	37
	7	10	<2	0	42	13
	14	20	<2	0.86	130	28
	28	<10	<2	0.86	65	45
	35	40	<2	2.86	135	121
	42	160	32	3.05	245	216
	56	80	4	2.61	108	83
	90	ND	<2	2.61	144	112
	180	20	<2	1.25	ND	ND

a. 0.5 ml EEE vaccine per dose on Days 0 and 28.

b. 0.25 ml EEE vaccine per dose on Days 0 and 28.

c. LNI = Log neutralization index, titration in suckling mice, courtesy Virology Division.

d. Sum of HI units in a UV peak (area under curve).

e. ND = Not Done.

Six months following the 2nd dose of vaccine only subjects BDB and HGH had significant levels of neutralizing antibody. Therefore, a 0.5 ml booster dose of vaccine was administered to all subjects. With few exceptions antibody responses at 28 and 42 days after the booster resembled in both magnitude and duration those following the 2nd dose of vaccine. We were unable to determine the nature of response to booster, i.e. anamnestic vs. new primary, because earlier bleedings were not obtained.

Of particular interest were 2 individuals, JTR and DLW. Both responded weakly to the 1st 0.25-ml injection but failed to respond to subsequent injections; consequently, the possibility of low dose tolerance must be considered. Of additional interest was an apparent increase between days 56 and 90 in specific IgG activity of chromatographic samples from subjects shown in Table III; this finding was similar to a delayed increase that occurred in the more intensively immunized rabbits. Among factors that may contribute to this phenomenon are: (1) changes in quantity and/or serological efficiency of specific immunoglobulin classes, (2) activation of secondary antigenic systems, e.g. latent viruses, (3) presence of a low concentration of live virus, (4) adjuvant effect of viral nucleic acid and (5) breakthrough from a state of partial tolerance.

It is clear from review of these data that it is feasible to employ a reference animal model, the rabbit, for evaluation of responses to vaccines. However, it is equally clear that serological techniques are not sufficiently sensitive to quantitate antibody responses to arboviral vaccines in a meaningful manner. Therefore, studies were initiated in an attempt to develop an improved HI test.

Nonspecific arboviral hemagglutinin inhibitors in normal sera have been reported to be lipoproteins which could be removed by kaolin absorption prior to antibody titration.[2, 4] In the foregoing experiments, normal inhibitors were found in the 1st and 3rd chromatographic peaks. None were detected in the 2nd peak of sera from nonimmunized humans or rabbits; therefore, specific IgG titers in the 2nd peak of immune sera could be determined directly without treatment. However, in order to titrate IgM in the 1st peak, it was necessary to remove inhibitors. We found that absorption of chromatographic eluates by the standard kaolin method removed all HI activity, antibodies as well as inhibitors. Furthermore, chromatography of immune rabbit sera after absorption with kaolin demonstrated that 60% of IgM and 90% of IgG activity were removed. Therefore, the standard procedure employing kaolin absorption will not yield valid arbovirus antibody titers for either whole sera of serum fractions.

Similar findings have been reported for other viruses, e.g. reovirus and rubella, and the use of polyanion complex as an inhibitor absorbant has been proposed.[4-6] The schema for stepwise precipitation of lipoproteins from sera with Ca^{++} and dextran sulfate[7] was explored as a better method for removal of nonspecific arbovirus inhibitors. Since the borate and phosphate

buffers employed in standard HI procedures are incompatible with a system requiring Ca ions, a new buffer system was required. It was found that the single pH (6.2) buffer system employed in the rubella test was unsatisfactory in the EEE system and possibly for all group A arboviruses; EEE antigen was stable for only 4 hr and inhibitory titers often exceeded 10,000. A new dual buffer system was developed. Antigen and serum were incubated in 0.0125 M 2-(N-morpholino) propanesulfonic acid (MOPS) saline, containing 0.0025% gelatin and 1% bovine serum albumin (BSA), adjusted to pH 7.5. After incubation, goose erythrocytes (RBC) were added in a cell adjusting diluent of 0.05 M 2-(N-morpholino) ethanesulfonic acid (MES) saline with gelatin and BSA, adjusted to pH 6.0. These tests can be completed in 4 hr.

With this dual buffer system, the relationships of virus hemagglutinins, antibodies, and inhibitors could be explored. Viral hemagglutinin titers were equal to or greater than those obtained in the standard borate/phosphate system and were stable for >48 hr at 1:400 dilution of EEE antigen. Antibodies and inhibitors against the same antigen cannot be measured independently in serum; effects of inhibitor removal were evaluated in anti-sheep RBC and anti-goose RBC sera by independent titration of inhibitor with EEE antigen and antibodies with sheep and goose RBC. Two groups of lipoprotein inhibitors could be precipitated from human and rabbit sera. Although the sera of both species contained both groups, their distribution varied. Human sera contained more inhibitor precipitated in the low density (VLDL) and low density group (LDL). Rabbit sera contained more inhibitor in the high density fractions (HDL). Inhibitors can be precipitated from aged or fresh sera. Heating or aging, known to denature lipoproteins, does not affect inhibition. Since delipidation by exhaustive ether extraction does not affect inhibitor activity, viral hemagglutinin may bind to protein, rather than lipid. The arbovirus inhibitors in serum have not been identified.

The final details of volumes and conditions are being completed by application of the new test to normal and immune sera from different species.

Summary:

Sera from rabbits and volunteers immunized with EEE vaccine were analyzed for IgG production by whole serum serological titrations and gel filtration chromatography. No IgG was detected 4, 7, or 9 days after immunization of volunteers, except in 3 individuals whose responses were compatible with prior experience with the illness. Rabbits immunized with 2.5 times as much vaccine produced significant amounts of IgG. An unexplained delayed increase in specific antibody activity was observed in both man and rabbits after the initial response decreased.

Discrepancies in the standard hemagglutination-inhibition serological procedure prompted development of a new HI test for arboviruses and results

Publications:

None.

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ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1W662711A096 02:	**Prevention and Treatment of Biological Agent Casualties**
Work Unit No. 096 02 007:	Evaluation of Experimental Vaccines in Laboratory Animals in BW Defense Research
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Animal Assessment
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Authors:	Richard O. Spertzel, Lt Colonel, VC Robert W. McKinney, Ph.D.
Reports Control Symbol:	RCS-MEDDH-288(R1)
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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
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71 07 01	H. TERMINATION	U	U	NA	NI.	☒ YES ☐ NO	A. WORK UNIT

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a. PRIMARY	62711A	1W662711A096	02	007
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19. RESPONSIBLE DOD ORGANIZATION
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(U) Equine encephalomeylitis (VEE); (U) Epizootic; (U) Central America; (U) Vaccines; (U) Attenuated virus vaccine; (U) Immunization; (U) Military medicine

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number preceds test of each with security Classification Code.)

23 (U) Evaluate experimental vaccines or antigens in laboratory and other animals before use in man. Assist in control of an epizootic. This work unit is an essential element in a comprehensive program for medical defense against BW agents.

24 (U) Promising vaccines are given to laboratory animals and safety tested prior to administration to man. Supply vaccine and technical assistance to conduct a vaccination program in order to create a barrier of immune horses and thereby curb the spread of the disease in these animals.

25 (U) 71 07 - 72 06 - Back-passage of TC-83 virus in burros was accomplished. After 7 consecutive serial passages, no evidence of reversion to virulence was obtained. In addition, field observations were made on the safety and efficacy of TC-83 vaccine during the 1971 Texas VEE epizootic. The results attained were consistent with observations made in the laboratory and in 1969 and 1970 in Central America.

Publications: Proc. Annual Meeting, U. S. Animal Health Assoc., p. 268-275, 1971.
J. Am. Vet. Med. Assoc. 159:731-738, 1971.
Appl. Microbiol. 23:654-655, 1972.

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BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 02: Prevention and Treatment of Biological Agent Casualties

[illegible] No. 096 02 007: Evaluation of Experimental Vaccines in Lab Safety Animals in BW Defense Research

[illegible]:

Evaluate experimental vaccines as to safety and efficacy in animals. Investigate the possible reversion to virulence of live, attenuated Venezuelan equine encephalomyelitis (VEE) virus (TC-83) vaccine. Report observations made during field use of the vaccine during VEE epizootics.

Progress:

After the 1969 Central American epizootic and the large demand for live, attenuated VEE vaccine, numerous attempts were made by personnel of the U. S. Army Medical Research and Development Command to induce the U. S. Department of Agriculture (USDA) to license the vaccine for export and for contingency use within the United States. In spite of extensive use of vaccine in Central America, where it appeared to be both safe and effective, USDA officials continued to express serious reservations about the dangers of reversion to virulence, should horse-to-horse transmission occur.

Back-passage of TC-83 vaccine virus from burro to burro was initiated to determine if such transmission would cause the vaccine virus to revert to virulence. Although no evidence of reversion to virulence was observed during serial passage of the virus by subcutaneous or intraperitoneal (IP) routes in small laboratory animals, several laboratories attempted horse-to-horse passage of the virus.

Vaccine administration to Equidae is characterized by a low, irregular viremia with a transient fever in approximately 50% of animals. Unlike man, where 35-40% of vaccinated individuals may show some reaction to the vaccine, only 1% of horses show even a transient reaction consisting of anorexia and depression for 12-24 hr. Thus, Johnson, who used fever as a guide, failed twice to recover virus beyond the 2nd passage; and McConnell, who selected the 72-hr sample for transmission, was unable to infect recipients on the 4th passage.[1] USDA personnel in Mexico City collected 100 ml of serum from each of 5 animals daily for 5 days postinoculation. These samples were pooled and an aliquot was given to each of 5 additional horses. By this method, they attained 5 passages, with no indication of reversion to virulence.

In our laboratory, a slightly different approach was used. One burro was used for each passage level. Serum was collected at 12-hr intervals postinoculation; a portion of each sample was immediately inoculated IP into weanling mice and intracerebrally (IC) into 1-2-day-old mice. Serum from each bleeding was stored at -20 C in 1- and 10-ml volumes. If tests in mice indicated the presence of virus, the 1-ml sample was titrated for viremia. On the basis of these titrations, the 10-ml sample corresponding to the highest titer of virus was selected for inoculation into the next burro. Results are summarized in Table I. It can be seen that there were an irregular viremia pattern, absence of detectable viremia in Burros 6 and 8, and no fever in burros 3, 4, 5 and 8. Consequently, transmission studies based on these responses could readily be unsuccessful. The prolonged viremia and the high viremia titers observed in burros 2, 3 and 7 are consistent with data on primary vaccination with TC-83 reported previously by our laboratory,[a] and indicate an increase in virulence, as can be seen with Burros 4, 5, 6 and 8.

With this method, 7 passages were attained. No evidence of reversion to virulence, as indicated by lethality by neural or extraneural routes for weanling mice or clinical signs in burros, has been observed.

In addition to the back-passage studies, additional safety studies were conducted in the field. Observation of approximately 22,000 Equidae by USDA and/or U. S. Public Health Service personnel in 5 states indicated a reaction rate of less than 1%. These results were consistent with those reported by our laboratory and with empirical observations in Central America.

In 1969 and 1970, numerous field observations attested to the efficacy of the vaccine; deaths of nonvaccinated animals were documented in herds where all vaccinees survived. These same observations have been made in Texas. A not-uncommon herd report, from an area with active encephalitic cases, follows: On 20 July, 38 working horses were vaccinated, but the owner considered it too much trouble to round up the remaining horses. In mid-August, encephalitis and death began to occur in these nonvaccinated animals. A field investigation was made on 31 August. Horses were pastured in 3 noncontiguous areas on the ranch. The 38 vaccinated horses remained healthy, while 3 of 5 unvaccinated geldings on the same pasture died, and one was sick at the time of investigation. In Pasture A, all 16 colts and 11 of 16 mares died; the other 5 mares were noticeably encephalitic. Similar results were seen for Pasture B.

This striking protection with a single dose of vaccine is consistent with the high degree of serologic conversion[3] observed in field use of the vaccine.

During the 1969 and 1970 Central American epizootics, the empirical observation was made that when the live, attenuated vaccine was used in areas where cases of active encephalitis were occurring, no new cases occurred in animals vaccinated more than 10 days previously. Within the first [illegible] days, however, an unusual pattern of illness was noted; cases of acute encephalitis that occurred on the 2nd-4th day after vaccination frequently developed into progressive illness, terminating in death; in other animals [illegible] symptomatic on the 7th-10th day, the illness generally was less severe and recovery

TABLE I. SERIAL BACK-PASSAGE OF TC-83 VIRUS IN BURROS

BURRO	PASSAGE LEVEL P-	INOCULUM SMICLD$_{50}$	RESULTS BY HOUR POSTINOCULATION[a]													
			12	24	36	48	60	72	84	96	108	120	132	144	156	192
1	TC-83	3.8			tr		(1.7)+	tr		tr		tr				
2	1	2.7			tr+			tr	tr	tr	2.0	(2.5)	2.0+	2.0+		
3	2	3.5	tr	1.7	1.7	1.6+	1.5+	(2.3)	2.1	tr+	+	+	+	+		
4	3	3.3		2.2	(2.3)		tr	tr				tr				
5	4	3.3				(2.6)	2.0	tr								
6	5	3.6														
7	6	110 ml[b]					+	tr+	2.0+	(3.2)+	3.6+	2.7+	+		1.8	2.0
8	7	4.2			+											

a. 180- and 192-hr samples were collected; all samples were negative.
Blank space indicates no detectable virus.
tr = virus present too low to quantitate.
( ) indicates sample used to inoculate next burro.
+ = ≥ 1.5 F rise in body temperature.

b. All blood samples collected from 24-144 hr of Burro No. 6.

was more frequent. This biphasic pattern of disease following vaccination was seen also during the 1971 Texas epizootic. Follow-up information was not available in all cases, but data indicate that the majority of the deaths occurred in horses with early onset of illness.

At the time of the original observations in 1969, the early, severe disease was attributed to an acceleration and exacerbation of a preexisting infection due to vaccination with the attenuated strain; whereas the more mild disease was considered to represent infection of the animal within a few days after vaccination, but before development of effective immunity. A study by Eddy et al.[4] in 1970 in Costa Rica, suggested that both patterns of disease following vaccination represented infection with the virulent virus prior to vaccination and severity of the disease was, in fact, determined by the titer of viremia at the time of vaccination. Of 35 animals studied, 2 developed the early, severe form of illness 2 days postvaccination. A 3rd animal developed overt encephalitis on the 5th day, but eventually recovered. One of the 2 horses which developed the early, severe form of the disease had viremia of 1.6×10^5 logs at the time of vaccination. The other horses which became ill on days 2 and 5 contained trace amounts of virus in prevaccination sera. Two other horses which remained asymptomatic also had trace amounts of virus in their blood at time of vaccination. Horses that did not contain virus at time of vaccination did not develop any evidence of disease.

The data of Eddy[4] suggest that both forms of the disease are probably due to preinfection with the virulent virus and the form of the disease may be related to the stage of viremia at time of vaccination. His data further suggest that the vaccine may serve to protect horses already infected with the virulent virus. This protection concept received some support in a documented case study during the 1971 Texas outbreak. The surviving, nonvaccinated animals in the herd previously discussed were vaccinated at the time of the herd investigation. Nine of the nonvaccinated horses had fevers of 103-105.5 F at the time of vaccination. Virus was isolated from 3 of these animals (only 3 sampled) from sera collected at the time of vaccination. Of these 9 animals, only one died. The other 8 animals recovered without apparent permanent sequelae. At the same time, 3 of 3 horses not vaccinated with fevers of 103-105 F died. In addition, 9 nonfebrile animals at the time of vaccination never developed any clinical illness. On the basis of the 100% virus isolation rate, all febrile animals were presumptively VEE cases. If this presumption is correct, since the 89% survival rate of infected animals at time of vaccination far exceeds the expected recovery rate from VEE infection in the equine, the above information supports the concept that attenuated vaccine affords some protection to horses already infected with virulent VEE virus.

Summary:

Back-passage of TC-83 virus in burros was accomplished. After 7 consecutive serial passages, no evidence of reversion to virulence was obtained. In addition, field observations were made on the safety and efficacy of TC-83 vaccine during the 1971 Texas VEE epizootic. The results attained were

consistent with observations made in the laboratory and in 1969 and 1970 in Central America.

Presentations:

1. Spertzel, R. O. Venezuelan equine encephalomyelitis. Presented at Foreign Animal Diseases Training Course, National Animal Disease Laboratory, Ames, Iowa. 10 Sep 71.

2. Spertzel, R. O. VEE--the clinical picture and production of a vaccine for its prevention. Presented at Horse Health Conference on Venezuelan Equine Encephalomyelitis and Equine Infection Anemia, Rutgers University, New Brunswick, N. J. 11 Sep 71.

3. Spertzel, R. O. Natural history of VEE infection in diseased hosts: equines. (Discussant). Presented at Workshop-Symposium on Venezuelan Encephalitis Virus, Pan American Health Organization, Washington, D. C. 15 Sep 71.

4. Spertzel, R. O. Epizootic control--vaccination and quarantine. Presented at Florida State Veterinary Medical Association Convention, Miami Beach, Florida. 27 Sep 71.

5. Spertzel, R. O. Overview of the 1971 Texas Venezuelan equine encephalomyelitis epizootic. Presented at Annual Meeting, U. S. Animal Health Association, Oklahoma City, Okla. 29 Oct 71.

6. Spertzel, R. O. Venezuelan equine encephalomyelitis. Presented at Standardbred Short Course, New York State College of Agriculture, Cornell University, Ithaca, N. Y. 22 Jan 72.

7. Spertzel, R. O. Venezuelan equine encephalomyelitis. Presented at Annual Meeting, Indiana State Veterinary Association, Indianapolis, Ind. 25 Jan 72.

8. Spertzel, R. O. Venezuelan equine encephalomyelitis in the United States. Presented at 72nd Annual Conference of Veterinarians, Philadelphia, Pa. 7 Feb 72.

9. Spertzel, R. O. Venezuelan equine encephalomyelitis. Presented at Symposium on Military Veterinary Medicine, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D. C. 14 Feb 72.

10. Spertzel, R. O. VEE--epidemiology and the recent spread patterns of the disease in South America, Central America and the U.S.A. Presented at Conference on Venezuelan Equine Encephalomyelitis, Ontario Veterinary Association, Toronto, Canada. 25 Apr 72.

11. Spertzel, R. O. 1971 outbreak of VEE in Texas: spread and control. Presented at Conference on Venezuelan Equine Encephalomyelitis, Kansas City, Kansas. Sponsored by Jensen-Salsbury Laboratories and American Association of Equine Practitioners. 7-8 Jun 72.

12. Spertzel, R. O. VEE--epidemiology and the recent spread patterns of the disease in South America, Central America and the U.S.A. Presented at VEE Meeting, Montreal, Canada. Sponsored by Equine Practitioners Association of Quebec and Blue Bonnets Raceway. 19 Jun 72.

13. [illegible], R. O. Control of VEE epizootic-epidemic by vaccine developed at USAMRIID. Eighth Bi-Annual Army Science Conference, West Point, N. Y. 20-23 Jun 72.

Publications:

1. Spertzel, R. O., and R. W. McKinney. 1971. VEE, a disease on the move. In Proceedings, 74th Annual Meeting, U. S. Animal Health Assoc., p. 268 to 275, Philadelphia, Pa.

2. Spertzel, R. O., and D. E. Kahn. 1971. Safety and efficacy of an attenuated Venezuelan equine encephalomyelitis vaccine for use in Equidae. J. Am. Vet. Med. Assoc. 159:731-738.

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4. McManus, A. T., and D. M. Robinson. 1972. Stability of live attenuated Venezuelan equine encephalitis vaccine. Appl. Microbiol. 23:654-655.

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6. Spertzel, R. O. 1972. Venezuelan equine encephalomyelitis. In Proceedings, Standardbred Short Course, New York State College of Agriculture, Cornell University, Ithaca, N. Y. (In press).

7. Spertzel, R. O., and D. Crozier. 1972. Control of VEE epizootic-epidemic by vaccine developed at USAMRIID. In Army Science Conference Proceedings. (In press).

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1. Johnson, K. M. Personal communication, 1971.

2. Spertzel, R. O., and D. E. Kahn. 1971. Safety and efficacy of an attenuated Venezuelan equine encephalomyelitis vaccine for use in Equidae. J. Am. Vet. Med. Assoc. 159:731-738.

3. U. S. Army Medical Research Institute of Infectious Diseases. 1 July 1971. Annual Progress Report, FY 1971. p. 179 to 185. Fort Detrick, Maryland.

4. Eddy, G. A., D. H. Martin, W. C. Reeves, and K. M. Johnson. 1972. Field studies of an attenuated Venezuelan equine encephalomyelitis vaccine (strain TC-83). Infec. Immun. 5:160-163.

ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1W662711A096 02:	Prevention and Treatment of Biological Agent Casualties
Work Unit No. 096 02 008:	Evaluation of Efficacy of Combined Antigens in Man
Report Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Divisions:	Medical, Virology and Animal Assessment
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Authors:	Peter J. Bartelloni, Lt Colonel, MC Charles S. White, III, Captain, MC David M. Robinson, Major, VC Francis E. Cole, Jr., Ph.D. Richard O. Spertzel, Lt Colonel, VC
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 01.0894	72 06 30	DD DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	02	008
b. [illegible]	62711A	1B662711A096		
c. [illegible]	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code): (U) Evaluation of efficacy of combined antigens in man in BW defense research

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical medicine; 004900 Defense; 002300 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
69 02	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER: NA
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 71	2.0	90
	CURRENT 72	2.0	110

19. RESPONSIBLE DOD ORGANIZATION
NAME: USA Medical Research Institute of Infectious Diseases
ADDRESS: Fort Detrick, MD 21701

RESPONSIBLE INDIVIDUAL
NAME: Crozier, D.
TELEPHONE: 301 663-2833

20. PERFORMING ORGANIZATION
NAME: Medical Division USAMRIID
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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Bartelloni, P. J.
TELEPHONE: 301 663-7281
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME:
NAME:

21. GENERAL USE
Foreign intelligence considered

DA

22. KEYWORDS (Precede Each with Security Classification Code)
(U) Vaccines; (U) Immunization; (U) Equine encephalitis (WEE, EEE); (U) Military medicine; (U) Yellow fever; (U) Human volunteers

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number, precede text of each with Security Classification Code.)

23 (U) Test and evaluate combinations of vaccines in man. This work unit is an essential element in a comprehensive program for medical defense against BW agents.

24 (U) After combination antigens have been safety tested and evaluated in laboratory animals, they are given to man.

25 (U) 71 07 - 72 06 - A study on associated administration to volunteers of yellow fever and VEE vaccines was initiated late in the fiscal year.

Terminated since FY 1973 funding will be under The Surgeon General, Army. New Project No. 3A062110A834; Accession No. DA 0B6415.

* Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE AND WILL BE USED FOR ARMY USE UNTIL OBSOLETE

BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 02: Prevention and Treatment of Biological Agent Casualties

Work Unit No. 096 02 008: Evaluation of Efficacy of Combined Antigens in Man

Description:

Test and evaluate combinations of vaccines in man.

Progress:

Associated Administration to Volunteers of Venezuelan Equine Encephalomyelitis Vaccine, Live, Attenuated and Yellow Fever Vaccine, 17D Strain (Medical Division Protocol No. FY 72-4): A study in volunteers has been initiated to compare single, simultaneous and closely spaced administration of yellow fever (YF) vaccine, 17D strain, and Venezuelan Equine Encephalomyelitis (VEE) Vaccine, Live, Attenuated, NDBR 102, on the basis of neutralizing and hemagglutination inhibition antibody responses to both antigens in volunteers. The onset and duration of viremia and circulating interferon levels resulting from infection with VEE and YF vaccine strains will be studied.

Thirty-two healthy male volunteers, not previously immunized with VEE or YF vaccine and having no history of infection with these viruses will be divided into 4 groups, A, B, C, and D. Groups A and B will consist of 6 subjects each and groups C and D will consist of 10 volunteers each. Individuals in each groups will be administered vaccine as follows:

GROUP	NO. SUBJECTS	VACCINE ADMINISTERED Day of Study[a]	
		YF	VEE
A	6		0
B	6	0	
C[b]	10	0	0
D	10	+10	0

a. 0.5 ml of vaccine will be administered subcutaneously.

b. Subjects in Group C will be administered vaccine simultaneously but at different inoculation sites.

The study begun in May will be reported on in detail in FY 1973.

Summary:

A study on associated administration of yellow fever and VEE virus vaccines was initiated.

Publications:

None.

ANNUAL PROGRESS REPORT

Project No. 3A61101A91C: Independent Laboratory In house Research

Task No. 3A61101A91C 00: (Prevention and Treatment of Biological Agent Casualties)
(1W662711A096 02)

Work Unit No. 91C 00 133: Studies with Human Diploid Cell Cultures
(096 02 069)

Reporting Installation: U.S Army Medical Research Institute of Infectious Diseases
Fort Detrick, Maryland

Division: Virology

Period Covered by Report: 1 July 1971 to 30 June 1972

Professional Author: Albert T. McManus, Captain, MSC

Reports Control Symbol: RCS MEDDH 288(R1)

Security Classification: UNCLASSIFIED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OL0901	72 06 30	DD-Dr&E-AR(A)36

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISS'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A06110191C	00	133
b. [illegible]	62711A	1W662711A096	02/009	
c. [illegible]	CDOG 1212b(9)			

11. TITLE (Precede with security classification code)

(U) Studies with human diploid cell cultures for production of military vaccines

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical medicine; 004900 Defense; 010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
69 11	CONT	DA	C. In-house

17. CONTRACT GRANT

a. DATES/EFFECTIVE — EXPIRATION

b. NUMBER: NA

c. TYPE — d. AMOUNT

e. KIND OF AWARD — f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 71	2.0	40
FISCAL YEAR CURRENT 72	2.0	40

19. RESPONSIBLE DOD ORGANIZATION

NAME: USA Medical Research Institute of Infectious Diseases

ADDRESS: Fort Detrick, MD 21701

RESPONSIBLE INDIVIDUAL

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20. PERFORMING ORGANIZATION

NAME: Virology Division USAMRIID

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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

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SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME:

NAME:

21. GENERAL USE

Foreign intelligence considered

DA

22. KEYWORDS (Precede each with security classification code)

(U) Diploid cell culture WI-38; (U) Immunization; (U) Vaccines; (U) Military medicine

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with security classification code.)

23 (U) Evaluate human diploid cell cultures for use as substrate for preparation of viral and rickettsial vaccines. This work unit is an essential element in a comprehensive program for medical defense against BW agents.

24 (U) The human diploid cell, WI-38, is studied to establish the technical requirements to produce and quality control cell strain substrates for human vaccine.

25 (U) 71 07 - 72 06 - Seed stocks of WI-38 have been and are continuing to be prepared. This cell is available in quantities greater than 10,000 square centimeters per day to investigators of USAMRIID. WI-38 has been examined for its ability to: assay human interferon, produce levels of virus acceptable for vaccines to Q fever, Rocky Mountain spotted fever and selected Group A, Group B, and California Group arbovirus strains. A Mayaro virus pilot vaccine has been produced and is presently being evaluated.

Terminated because the accession number is changed starting FY 1973. New accession number will be DA 0B6416.

DD FORM 1498

BODY OF REPORT

Project No. 3A61101A91C: Independent Laboratory In-House Research

Task No. 3A61101A91C 00: (Prevention and Treatment of Biological Agent Casualties)
(1B662711A096 02)

Work Unit No. 91C 00 133: Studies with Human Diploid Cell Cultures for Production of Military Vaccines
(096 02 009)

Description:

Evaluate human diploid cell cultures for use as substrate for preparation of viral and rickettsial vaccines.

Progress:

A separate laboratory area in USAMRIID has been organized for the production and characterization of human diploid cells. This area meets U.S. Public Health Service requirements for the propagation of cells to be used in vaccine production. The human diploid cell area is presently capable of growing and maintaining cells without contact with other cell culture areas.

Arrangements for procurement of low passage starter cultures of WI-38 cells have been made with Dr. Leonard Hayflick of Stanford University and Dr. John Shannon of the American Type Culture Collection. Frozen seed stock have been made and are continuing to be made from the above cultures. Quality control analysis of seed stock material has shown the absence of Mycoplasma and normal values for isoenzyme patterns and chromosome complement.

Approximately 300,000 cm^2 of WI-38 cell culture has been issued to USAMRIID investigators. Major efforts with WI-38 cells include: human interferon assay, susceptibility to group A arbovirus, susceptibility to Q fever and Rocky Mountain spotted fever rickettsia, susceptibility to selected group B arbovirus strains and California group arboviruses.

A 7-L pilot lot of vaccine against Mayaro virus (Group A arbovirus) has been prepared by Major Robinson of this division. The vaccine was prepared under laboratory conditions rendering it suitable for administration to man.

The use of WI-38 human diploid cells as a model for nonprimary cells for human vaccine has been successful. Efforts are presently being considered to procure other characterized human diploid cell strains. The ability of a cell to support the growth of specific virus to required levels is the limiting circumstance in preparation of required vaccines.

Based upon evidence of varying susceptibility of human diploid cell strains, there is reason to procure and screen strains other than WI-38.

Guidelines for use of human diploid cell strains for oral human vaccines have been presented by the Division of Biological Standards. An application for an oral polio vaccine soon followed and was approved. The precedent for human diploid cells as substrates for vaccine has been set and within the near future a major dependence may exist on diploid cell lines to fill the U.S. vaccine needs.

Summary:

Seed stocks of WI-38 have been and are continuing to be prepared. These cells are available to investigators of USAMRIID in quantities $\geq$ 10,000 cm^2 per day. WI-38 has been examined for its ability to: assay human interferon, produce levels of virus acceptable for vaccines to Q fever, Rocky Mountain spotted fever and selected Group A, Group B, and California Group arbovirus strains. A Mayaro virus pilot vaccine has been produced and is presently being evaluated.

Publication:

None.

ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1W662711A096 02:	Prevention and Treatment of Biological Agent Casualties
Work Unit No. 096 02 010:	Humoral and Cell-Mediated Factors in Immunity to Militarily Important Diseases
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Divisions:	Bacteriology and Pathology
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Authors:	Stanley G. Rabinowitz, Major, MC William H. Adler, Major MC
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OL0903	72 06 30	DD-DRX-(Ar)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISTR INSTR	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	02	010
b. [illegible]	62711A	1B662711A096		
c. [illegible]	CDOG 1212b (9)			

11. TITLE (Precede with security classification code)

(U) Humoral and cell-mediated factors in immunity to militarily important diseases

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical medicine; 004900 Defense; 010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
70 09	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE — EXPIRATION

b. NUMBER: NA

c. TYPE — d. AMOUNT

e. KIND OF AWARD — f. CUM. AMT.

18. RESOURCES ESTIMATE	PROFESSIONAL MAN YRS	FUNDS (In thousands)
PREV. FY 71	2.0	70
CURRENT FY 72	1.0	113

19. RESPONSIBLE DOD ORGANIZATION

NAME: USA Medical Research Institute of Infectious Diseases

ADDRESS: Fort Detrick, MD 21701

RESPONSIBLE INDIVIDUAL

NAME: Crozier, D.

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20. PERFORMING ORGANIZATION

NAME: Bacteriology Division USAMRIID

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PRINCIPAL INVESTIGATOR (List the SSAN if U.S. Academic Institution)

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TELEPHONE: 301 663-7341

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: Dangerfield, H. G. DA

NAME:

21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Precede EACH with security classification code)

(U) Immunoglobulin; (U) Secretory antibody (U) Delayed hypersensitivity; (U) Humoral antibody; (U) Vaccines; (U) Military medicine; (U) Encephalitis, equine (VEE)

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number, precede title of each with security classification code.)

23 (U) To evaluate the contribution of humoral and cell-mediated immune responses to experimental infection. This work unit is an essential element in a comprehensive program for medical defense against BW agents.

24 (U) Develop an animal model for evaluating the contribution of humoral antibody and cell-mediated immunity in resistance to certain infections.

25 (U) 71 07 - 72 06 - Passive transfer of antiserum or spleen cells from VEE immune donors conferred specific adoptive immunity to inbred recipient mice. Thymus-dependent lymphocytes were demonstrated to be primarily responsible for cell-mediated protective activity which became maximal 7-10 days following immunization. Interferon was excluded as a significant factor in cell-mediated aspects of adoptive immunity. In vitro responsiveness of immune cell preparations correlated well with in vivo protective efficacy.

Publications: Clin. Res. 20:54, 535, 1972 (abstracts)

Terminated since FY 1973 funding will be under The Surgeon General, Army. New Project No. 3A062110A834; Accession No. DA 080477.

DD FORM 1498

BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 02: Prevention and Treatment of Biological Agent Casualties

Work Unit No. 096 02 010: Humoral and Cell-Mediated Factors in Immunity to Militarily Important Diseases

Description:

To evaluate the contribution of humoral and cell-mediated immune responses in resistance to experimental infection.

Progress:

As one phase of a program concerned with humoral and cell-mediated aspects of protection against infectious disease, studies were designed to evaluate the protection conferred by passive transfer of antiserum and of immune cell preparations to a susceptible host infected with Venezuelan equine encephalomyelitis (VEE), a group A arbovirus.

Passively transferred antiserum administered by intraperitoneal (IP) injection protected mice of inbred strains C57 and C57 BL/6 against simultaneous subcutaneous (SC) infection with 100 LD_{50} of virulent Trinidad strain VEE virus (Table I). Antiserum therapy was equally effective for intact mice and immunosuppressed mice that were neonatally-thymectomized or pretreated with anti-thymocyte serum (ATS).

TABLE I. EFFICACY OF IMMUNE SERA IN PROTECTION OF NORMAL AND IMMUNOSUPPRESSED MICE AGAINST VEE VIRUS CHALLENGE[a/]

IMMUNOSUPPRESSIVE TREATMENT	SERUM PROPHYLAXIS[b/]	NUMBER/GROUP	RESPONSE TO VEE CHALLENGE %
None	None	40	100
	Normal	40	100
	Immune	40	5
Thymectomy	None	0	100
	Normal	0	100
	Immune	10	0
Anti-thymocyte Serum (ATS)	None	20	100
	Normal	20	100
	Immune	20	0

a. 100 LD_{50} virulent VEE virus inoculated SC at time of prophylaxis.

b. Serum administered IP.

IP injection of 6-9 x 10^7 immune spleen cells that were harvested from donor mice 7 days after immunization with attenuated VEE virus were also capable of preventing infection (Table II). Intact immune cells were required to demonstrate the protective effect; washings from intact cells and sonically-disrupted cells (100 watts, 30 sec) had no protective properties, indicating that preformed antibody was not the active factor.

TABLE II. EFFECT OF INTACT IMMUNE SPLEEN CELLS, SUPERNATANT WASHES OF IMMUNE CELLS OR DISRUPTED IMMUNE SPLEEN CELLS IN MICE INFECTED SC WITH 100 LD_{50} VIRULENT VEE VIRUS AT THE TIME OF ADOPTIVE IMMUNIZATION.

[illegible] TREATMENT	RESPONSE OF RECIPIENTS TO VEE CHALLENGE	
	Death/Total	Mortality %
None	40/40	100
Normal spleen cells (5-9 x 10^7)	40/40	100
Immune spleen cells (6-9 x 10^7)	6/40	15
Washing from immune cells (0.5 ml)	10/10	100
Sonically-disrupted immune cells (5-7 x 10^7)	10/10	100

The capacity of immune spleen cells to confer adoptive immunity was a time-dependent function (Table III). Cells harvested 7-13 days after donor immunization possessed maximum antiviral activity; within 25 days after immunization, essentially no activity was demonstrable.

TABLE III. ANTIVIRAL ACTIVITY OF IMMUNE SPLEEN CELLS[a] OBTAINED AT VARIOUS TIMES FOLLOWING IMMUNIZATION OF MICE WITH ATTENUATED VEE VIRUS

DAY OF CELL HARVEST POSTIMMUNIZATION	RESPONSE OF RECIPIENTS TO VEE CHALLENGE[b]	
	Death/Total	% Mortality
4	6/10	60
[illegible]	6/40	15
8	2/20	10
10	2/15	13
13	2/15	13
25	23/29	79

a. 6-9 x 10^7 spleen cells injected IP at time of challenge with virulent VEE virus.

b. 100 LD_{50} virulent VEE virus inoculated SC.

Protection conferred by adoptive transfer of immune cells was a specific immunologic phenomenon. Spleen cells from mice immunized with attenuated Semliki Forest virus (SF) or with TC-83 protected against only the corresponding virulent strains (Table IV).

TABLE IV. SPECIFICITY OF ADOPTIVE IMMUNITY CONFERRED BY IMMUNE SPLEEN CELLS FROM DONORS IMMUNIZED WITH SF VIRUS OR VEE VIRUS

RECIPIENT TREATMENT	DONOR CELLS[a]	RESPONSE TO VIRULENT FC CHALLENGE	
		SF (100 LD_{50})	VEE (100 LD_{50})
None	--	10/10	10/10
Immune Spleen cells	VEE	9/10	1/10
	SF	2/10	10/10

a. Immune spleen cells harvested 7 days after donor immunization with 3 x 10^3 PFU of attenuated VEE virus or of attenuated SF virus (A-774 strain); 6-9 x 10^7 donor cells administered IP at time of challenge with virulent strains of virus.

Antibody responses in recipients 2, 4, 6 and 8 days following transfer of immune donor cells were not significantly different from those of normal spleen cell recipients or of untreated mice, indicating that a secondary antibody response by donor cells was not responsible for the protective activity (Table V).

TABLE V. ANTIBODY RESPONSES TO SC CHALLENGE WITH 100 LD_{50} VIRULENT VEE VIRUS IN MICE SIMULTANEOUSLY TREATED WITH IMMUNE OR NORMAL SPLEEN CELLS.

INTRAPERITONEAL TREATMENT	RECIPROCAL ANTIBODY TITER[a]							
	2 Days		4 Days		6 Days		8 Days	
	HI	CF	HI	CF	HI	CF	HI	CF
None	ND[b]	<2	10	<2	ND	64	1280	256
Normal spleen cells ($5-6 \times 10^7$ in 0.5 ml)	<10	<2	10	<2	160	32	1280	128
Immune spleen cells ($6-8 \times 10^7$ in 0.5 ml)	10	2	20	4	160	32	640	128

a. HI = Hemagglutination inhibition; CF = complement fixation

b. ND = Not tested

To evaluate the role of interferon in adoptive immunity, mice were treated with interferon (2500 units) or with Poly I:C (100 µg/dose) to induce production of interferon (Table VI). Significant protection against challenge was observed only in mice treated with Poly I:C administered 1 day before and at time of challenge, suggesting that a mechanism other than interferon production was primarily involved in the cell-mediated response. These studies, however, do not exclude a role for interferon in protection.

TABLE VI: EFFECT OF POLY I:C OR PASSIVELY ADMINISTERED INTERFERON ON SURVIVAL OF MICE INFECTED SC WITH 100 LD_{50} VIRULENT VEE VIRUS

TREATMENT	IP DOSE			RESPONSE TO VEE CHALLENGE	
	Day of Treatment[a]				
	-1	0	+1	Dead/Tested	Mortality %
Interferon		2.5×10^3 units		15/15	100
Poly I:C		100 µg		14/18	78
		100 µg		8/12	67
	100 µg		100 µg	15/24	62
	100 µg			2/24[b]	8

a. Day 0 = time of virus challenge.

b. Difference between last 2 treatments significant: $P < 0.001$, t-Test.

That immune spleen cells were specifically sensitized to VEE antigen was demonstrated by an *in vitro* lymphocyte stimulation test. Cells harvested 7-10 days after donor immunization exhibited a maximum response to stimulation with a γ-irradiated preparation of VEE virus (Table VII).

TABLE VII. RESPONSIVENESS OF IMMUNE SPLEEN CELLS TO VEE ANTIGEN AS A FUNCTION OF TIME FOLLOWING DONOR IMMUNIZATION

DAY OF CELL HARVEST FOLLOWING IMMUNIZATION	*IN VITRO* LYMPHOCYTE REACTIVITY (Δ CPM $\times 10^{-3}$)[a]
4	1150
7	3400
10	3250
13	1500
22	375
27	410

a. Difference in CPM between VEE-stimulated lymphocytes and nonstimulated lymphocytes.

The reaction was antigen-specific in that no stimulation occurred upon exposure to a γ-irradiated preparation of SF. It is noteworthy that *in vitro* reactivity correlated closely with *in vivo* antiviral activity of immune spleen cells (Table III).

Further experiments were performed to determine if thymic-derived lymphocytes, known to be correlated with cell-mediated immunity, were responsible for these *in vivo* and *in vitro* findings. In these studies spleen cells were harvested 7 days after immunization with attenuated VEE virus and treated with complement and various cytotoxic antisera. One aliquot was employed for *in vivo* passive transfer experiments and another for *in vitro* lymphocyte stimulation with specific antigen (VEE) or nonspecific mitogens, staphylococcal enterotoxin B (SEB) and phytohemagglutinin (PHA). Tritiated thymidine incorporation was used as an index of proliferative responses to either antigen or mitogen (Table VIII).

TABLE VIII. COMPLEMENT-DEPENDENT EFFECT OF RABBIT ANTI-MOUSE THYMOCYTE SERUM, MOUSE ANTI-θ SERUM OR GOAT ANTI-MOUSE γ-GLOBULIN ON PROTECTIVE CAPACITY AND *IN VITRO* REACTIVITY OF VEE IMMUNE SPLEEN CELLS.

SERUM TREATMENT	PROTECTIVE CAPACITY (Dead/Challenged)	*IN VITRO* REACTIVITY[a] Δ CPM x 10^{-3}		
		VEE	SEB	PHA
Normal Rabbit (1:4)	1/10	1700	56000	40000
Rabbit Anti-Mouse Thymocyte (1:4)	8/10	200	6000	15000
Normal Mouse (1:10)	0/10	2675	30000	29500
Mouse Anti-θ (1:10)	7/10	175	20000	5500
Normal Goat (1:5)	0/10	1350	42500	44000
Goat Anti-Mouse γ-globulin (1:5)	0/10	1275	6000	38000

a. Difference in CPM between stimulated and nonstimulated lymphocytes, stimulation by treatment with VEE antigen and SEB or PHA.

In vivo protective activity and *in vitro* responsiveness to VEE antigen was virtually abolished by treatment with ATS or anti-θ serum. On the other hand, treatment of immune cells with anti-mouse γ-globulin did not alter *in vivo* protective activity or *in vitro* responsiveness to VEE antigen or to

PHA, but SEB reactivity was markedly depressed. Since mitogenic activity of SEB affects both bone-marrow derived lymphocytes (B-cells) and thymus-derived lymphocytes (T-cells) in contrast to PHA which stimulates only T-cells, these findings indicate that the T-cell is primarily responsible for the in vitro immune response to VEE antigen, and for in vivo protection afforded an adoptive host.

Summary:

Passive transfer of antiserum or spleen cells from immune donors conferred specific adoptive immunity to inbred recipient mice. Thymus-dependent lymphocytes were demonstrated to be primarily responsible for cell-mediated protective activity which became maximal 7-10 days following immunization. Interferon was excluded as a significant factor in cell-mediated aspects of adoptive immunity. In vitro reactivity of immune cell preparations correlated well with in vivo protective efficacy.

Presentations:

1. Rabinowitz, S. and W. H. Adler, III. Host defenses during primary Venezuelan equine encephalomyelitis virus infection in mice. Presented to Southern Section, American Federation for Clinical Research, New Orleans, La. 27-29 January 1972.

2. Rabinowitz, S. and W. H. Adler, III. Host defenses during primary Venezuelan equine encephalomyelitis virus infection in mice. Presented at Joint Meeting, American Federation for Clinical Research, American Society for Clinical Investigation and American Association of Physicians, Atlantic City, N.J. 29 April - 1 May 1972.

Publication:

1. Rabinowitz, S., and W. H. Adler, III. [illegible] primary Venezuelan equine encephalomyelitis [illegible] Clin. Res. [illegible]).

ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1W662711A096 02:	Prevention and Treatment of Biological Agent Casualties
Work Unit No. 096 02 011:	Lipid Metabolism and Mechanisms of Host Defense
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Divisions:	Bacteriology, Animal Assessment, and Pathology
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Authors:	Harry G. Dangerfield, Colonel, MC Virginia G. McGann, Ph.D. Michael D. Kastello, Captain, VC Richard A. Proctor, Captain, MC William H. Adler, Major, MC Richard O. Spertzel, Lt. Colonel, VC
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OLO915	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	02	011
b. [illegible]	62711A	1B662711A096		
c. [illegible]	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)

(U) Lipid metabolism and mechanisms of host defense

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical medicine; 004900 Defense; 010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 07	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION

b. NUMBER: NA

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 71	0	0
FISCAL YEAR CURRENT 72	1.0	20

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER

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21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Precede Each with Security Classification Code)

(U) Lipids; (U) Metabolism; (U) Immunoprophylaxis; (U) Hyperlipemia; (U) Military medicine; (U) Encephalitis, equine (VEE)

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Investigate effects of the hypercholesterolemic state upon host resistance to infection and response to immunization. This work unit is an essential element in a comprehensive program for medical defense against BW agents.

24 (U) A variety of immunological parameters and responses to infection will be studied to determine the effects of induced hypercholesterolemia on host resistance.

25 (U) 71 07 - 72 06 - Studies were initiated to evaluate the effect of hypercholesterolemia upon host responses to immunization and/or infection-intoxication. Following infection of rhesus monkeys by a nonfatal viral illness (VEE), clinical responses, e.g. fever, viremia, etc., were similar for control and hypercholesterolemic animals. Phagocytic activity and in vitro metabolic activity (NBT dye reduction) by peripheral granulocytes remained the same throughout the observation period, i.e. unaffected by diet or infection. Bactericidal activity was unaffected by diet; however, evidence of impaired activity following VEE infection was observed in only 1 of 7 hypercholesterolemic animals, but in 5 of 8 monkeys on a control diet and in 3 of 5 on a semisynthetic diet.

Terminated since FY 1973 funding will be under The Surgeon General, Army. New Project No. 3A062110A834; Accession No. DA 0B6418.

*Available to contractors upon originator's approval

DD FORM 1498 1 MAR 68 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A [illegible] AND 1498-1 1 MAR 68 FOR ARMY USE ARE OBSOLETE

BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 02: Prevention and Treatment of Biological Agent Casualties

Work Unit No. 096 02 011: Lipid Metabolism and [illegible] of Host Defense [illegible]

[illegible]

[illegible] the [illegible] cholesterolemic state upon [illegible] and response to immunization [illegible]

Purpose:

[illegible] a recent review, [illegible] and [illegible] the [illegible] of information regarding interrelationships of lipid metabolism and infectious illness. It is apparent that most investigators have been concerned with alterations in lipid metabolism observed during the course of acute or chronic illness. Thus, few reports are available regarding the effect of altered lipid metabolism on resistance to infection or on specific mechanisms of host defense. Investigations in this area appear to be warranted in view of modern dietary habits and the relatively high incidence of hyperlipidemic individuals in the United States, e.g. it has been estimated that approximately 10% of the population have elevated serum cholesterol values.[2]

The few experimental studies in laboratory animals provide little conclusive information regarding interaction between lipid metabolism and host resistance. Most investigators were concerned with biochemical or histopathological parameters of response to infection and employed estimates of mortality or time to death as indicators of resistance. No information is available regarding cellular and humoral defense mechanisms. Some evidence, however, directly implicates lipid metabolism in host defense. Mice maintained on diets sufficient [illegible] which contained unsaturated fatty acids showed increased susceptibility to infection with tuberculosis whereas other mice fed diets containing saturated fatty acids gained no weight but had increased resistance [illegible] In addition, dogs that became obese as a result of [illegible] access to a well balanced ration had increased susceptibility to infection with canine distemper virus, as indicated by severity of clinical response and reduction in survival rate.[3] Similarly, [illegible] et al.[4] reported that, in response to infection with canine hepatitis virus [illegible] on a high [illegible] high fat diet had a more severe disease course and more rapid mortality than dogs on a regular commercial diet. [illegible] recent report indicates that an over-abundant dietary intake of fat [illegible] with [illegible] chronic hypercholesterolemia is associated [illegible] altered immunological defense mechanisms.[5]

Therefore, studies were designed to evaluate the effects in rhesus monkeys of hypercholesterolemia on resistance to viral infection and to sublethal intoxication and on immunogenic responses to living, attenuated bacterial, rickettsial and viral vaccines. The schema for experimental approach is:

(1) Preliminary study phase - Twenty-four rhesus monkeys that had no staphylococcal enterotoxin B (SEB) antibodies were paired according to sex and weight and maintained on Purina monkey diet. One animal in each pair was immunized with SEB, Lot 14-30; the 1st dose (1 μg SEB/kg body weight) was injected intravenously (IV) on day 0 and the 2nd dose (10 μg SEB/kg) on day 7. Within 12 hr after the 10-μg dose, 25% of monkeys died with signs of fatal anaphylaxis. Matched substitutes were then immunized in a like manner. All monkeys developed hemagglutinins (HA) but only 50% had precipitins. Consequently, a 30-μg booster dose was administered intradermally 1-4 mon after the 2nd injection. Severe Arthus reactions occurred at the site of inoculation in all animals, and within 1 week all monkeys developed high HA titers (geometric mean, 1:1400) and precipitin titers (geometric mean, 1:3). To provide the basis for evaluating dietary effect as well as host responses, blood samples for the studies shown in Table I were collected during this interval.

TABLE I. PARAMETERS OF EXPERIMENTAL STUDIES.

CLINICAL	BIOCHEMICAL	IMMUNOLOGICAL
Total white blood cell counts	Blood cholesterol	Phagocytic Index
Differential counts		Leucocyte metabolic activity[a]
Packed cell volume		Immunoglobulin
		Complement
		Beta lysins
		Specific antibodies

a. Measured by nitroblue tetrazolium dye reduction

(2) Diet stabilization phase - Monkeys were assigned to 3 groups of 8 animals each; 4 in each group were immune to SEB. Group I was maintained on a standard primate diet (Wayne, Allied Mills, Chicago, Ill.), and Group II on a semisynthetic control diet[8] to provide an internal control on the effect of atherogenesis. Group III animals were fed the atherogenic test diet formulated by Armstrong[8] (General Biochemicals Corp., Chagrin Falls, Ohio). Within 8 weeks blood cholesterol values became stabilized at 593 ± 96 mg/100 ml for monkeys in Group III and at 193 ± 17 mg/100 ml and 135 ± 6 mg/100 ml for Groups I and II respectively.

(3) Sequential challenge phase -

a) Nonfatal viral infection (Venezuelan equine encephalomyelitis, VEE).

b) Sublethal intoxication (SEB).

c) Response to living attenuated bacterial vaccine and subsequent challenge (Francisella tularensis).

d) Response to living attenuated rickettsial vaccine and subsequent challenge (Coxiella burneti).

e) Response to living attenuated Yellow fever (YF) vaccine (17-D) and subsequent challenge (Asibi strain, YF virus).

All monkeys were infected subcutaneously (SC) with 412 PFU of Trinidad strain VEE; clinical, biochemical and immunological parameters of response were examined prior to challenge and daily for 14 days. During this period, a total dose of 50 mg of supplemental Imferon R_x (Lakeside Laboratories, Inc., Milwaukee, Wisconsin) was injected intramuscularly (IM) to prevent depletion of iron stores by repeated bleedings.

Severity of clinical illness was essentially the same for all groups. All animals were viremic from day 1-4 and most through day 5 postinoculation, all were febrile and the majority exhibited a biphasic response. Total and differential leucocyte counts and packed cell volumes (PCV) showed no significant between-group differences. Blood cholesterol values were maintained at essentially prechallenge concentrations.

The technique of Quie, et al.[9] was employed to measure phagocytic and bactericidal activity of peripheral leucocytes. During preliminary and diet stabilization phases no significant changes in phagocytic activity occurred. bactericidal activity in Group I and II monkeys was likewise unaffected (Table II). Bactericidal activity in Group III animals appeared to decrease when they became hypercholesterolemic; however, the group mean did not differ significantly (t test) from the predietary mean or from the means of control or semisynthetic diet groups. Unusually high day-to-day variability of values for individual animals was noted.

TABLE II. PMN BACTERICIDAL ACTIVITY AGAINST S. AUREUS[a/]

GROUP	MONKEY NUMBER	PRELIMINARY		DIET STABILIZATION	
		% Kill	Mean ± SEM	% Kill	Mean ± SEM
Control Diet (Wayne)	B111	66		70	
	A817	74		70	
	B192	80		63	
	B131	79	71.4 ± 2.4	82	72.6 ± 3.4
	B150	75		85	
	B126	66		71	
	B165	61		58	
	A891	70		82	
Semisynthetic Diet	B175	70		73	
	B179	83		77	
	B188	75		60	
	B152	71	73.6 ± 3.8	70	65.5 ± 2.7
	B164	79		61	
	B135	50		53	
	B185	82		65	
	B151	79		65	
Hypercholesterolemic Diet	B161	83		38	
	B109	71		55	
	B189	86		54	
	B184	62	70.4 ± 4.9	44	56.1 ± 4.0
	B171	70		64	
	B140	42		55	
	B170	72		73	
	B148	77		56	

a. $3.0 - 3.5 \times 10^6$ S. aureus and by 5.0×10^6 PMN/ml; pooled monkey serum opsonin, 1:125 final dilution.

Following VEE challenge, phagocytic capability appeared to be unimpaired. Bactericidal activity for individual monkeys is shown in Table III. It should be noted that typical leukopenia followed VEE challenge; consequently, during the period of maximal illness, a number of samples were inadequate for testing. In addition, approximately 10% of samples could not be evaluated for various technical reasons such as contaminated media, tube breakage, etc. Regarding those animals for which sufficient data were available (at least 2 determinations within 5 days)

TABLE III. BACTERICIDAL ACTIVITY OF PERIPHERAL PMN FOLLOWING INFECTION OF RHESUS MONKEYS WITH VEE VIRUS.

GROUP	MONKEY NUMBER	PRE-CHALLENGE ACTIVITY	% KILL DAY POSTCHALLENGE[b]											
			1	2	3	4	5	6	7	8	9	10	11	12
Control Diet (Wayne)	B111	70[a]	L		68		L		56		76		*	
	A817	70		62		L		61		78		71		44
	B192	63	59		59		70		72		72		*	
	B131	82		73		45		0		45		72		68
	B150	85	79		26		L		76		67		79	
	B126	71		7		18		51[c]		32		61		22
	B165	58	48		28		54		30		60		20	
	A891	82		41		46		37		*		50		76
Semisynthetic Diet	B176	73	L		*		*		69		79		*	
	B179	77		59		65		37		72		67		55
	B188	60	63		61		61		70		61		*	
	B152	70		56		66		65		*		66		53
	B164	61	61		*		*		59		52		45	
	B135	53		46		22[c]		65		8		18		65
	B185	65	40		L		L		56		79		72	
	B151	65		67		58		16		66		69		75
Hypercholesterolemic Diet	B161	38	81		51[c]		78		42		70		*	
	B109	55		L		L		*		37		65		61
	B189	64	65		64		L		58		71		*	
	B184	44		46		57		58		46		63		50
	B171	64	63		74		40		84		54		61	
	B140	55		45		9		87		49		43		12
	B170	73	49		69		59		66		59		75	
	B148	56		68		62		51		40		69		91

a. Indicates % kill of phagocytized S. aureus ($3.0 - 3.5 \times 10^6$) by 5×10^6 PMN/ml; opsonization with 1:125 dilution of pooled monkey serum.

b. L = Leukopenia, insufficient PMN for testing. * = Sample lost.

c. Insufficient PMN for duplicate testing.

TABLE IV. NET REACTION BY PYRIDINE EXTRACTS OF PERIPHERAL GRANULOCYTES BEFORE AND AFTER INFECTION WITH VEE VIRUS.

GROUP	OPTICAL DENSITY AT 515 mμ[a]			
	Preinfection		Postinfection	
	Resting	Stimulated[b]	Resting	Stimulated
Control Diet (Wayne)	.169	.179	.160	.190
	.120	.160	.110	.120
	.099	.140	.100	.120
	.090	.110	.089	.130
	.080	.130	.071	.110
	.069	.090	.071	.091
	.069	.090	.049	.070
	.059	.080	.060	.070
	$\bar{X}_1$ = .096	$\bar{X}_2$ = .120	$\bar{X}_1$ = .086	$\bar{X}_2$ = .110
	$\bar{X}_2 - \bar{X}_1$ = .024		$\bar{X}_2 - \bar{X}_1$ = .024	
Semisynthetic Diet	.220	.260	.201	.225
	.150	.250	.120	.140
	.109	.140	.111	.120
	.090	.140	.100	.110
	.090	.120	.090	.091
	.090	.120	.080	.091
	.079	.120	.070	.081
	.079	.110	.031	.061
	$\bar{X}_1$ = .113	$\bar{X}_2$ = .158	$\bar{X}_1$ = .100	$\bar{X}_2$ = .115
	$\bar{X}_2 - \bar{X}_1$ = .045		$\bar{X}_2 - \bar{X}_1$ = .015	
Hypercholesterolemic Diet	.150	.150	.240	.260
	.110	.150	.140	.150
	.110	.130	.120	.130
	.100	.130	.100	.121
	.100	.120	.090	.121
	.090	.100	.080	.110
	.090	.100	.061	.100
	.080	.090	.030	.041
	$\bar{X}_1$ = .101	$\bar{X}_2$ = .121	$\bar{X}_1$ = .105	$\bar{X}_2$ = .129
	$\bar{X}_2 - \bar{X}_1$ = .020		$\bar{X}_2 - \bar{X}_1$ = .02[illegible]	

[a] Resting: incubated 1 hr with Krebs buffer and R.T.
Stimulated: incubated 1 hr with latex particles, R.T., and Krebs buffer.

5 of 7 monkeys on control diet, 3 of 5 on semisynthetic diet and 1 of 7 hypercholesterolemic animals exhibited impaired bactericidal activity (i.e. less than half of the baseline value).

The nitroblue tetrazolium (NBT) dye reduction test[10] was employed as an in vitro measure of PMN metabolic activity prior to and 4-5 days after VEE challenge (Table IV). No significant differences were noted between dietary groups or within a dietary group pre- or postinfection. Unlike results reported for human peripheral granulocytes,[10] ingestion of latex particles did not increase NBT dye reduction by monkey granulocytes.

Evaluation of serological parameters of response (complement, immunoglobulin, specific antibody, etc.) and exposure to intoxication stage of sequential challenges is in progress.

Summary:

Studies were initiated to evaluate the effect of hypercholesterolemia upon host responses to immunization and/or infection-intoxication. Following infection of rhesus monkeys by a non-fatal viral illness (VEE), clinical responses, e.g. fever, viremia, etc., were similar for control and hypercholesterolemic animals. Phagocytic activity and in vitro metabolic activity (NBT dye reduction) by peripheral granulocytes remained the same throughout the observation period, i.e. unaffected by diet or infection. Bactericidal activity was unaffected by diet; however, evidence of impaired activity following VEE infection was observed in only 1 of 7 hypercholesterolemic animals, but in 5 of 7 monkeys on a control diet and in 3 of 5 on a semisynthetic diet.

Publications:

None.

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ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1W662711A096 02:	Prevention and Treatment of Biological Agent Casualties
Work Unit No. 096 02 102:	Development and Evaluation of an Effective Vaccine Against Plague
Reporting Installation:	U.S. Army Medical Research Institute of Infectious Diseases Forest Glen Section Washington, D.C.
Division:	Microbiology
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Authors:	John D. Marshall, Jr., Colonel, MSC (I,II,III) Daniel N. Harrison, M.S. (III) Dan C. Cavanaugh, Colonel, MSC (WRAIR) (I,II) James E. Williams, Captain, MSC (WRAIR) (II)
Reports Control Symbol:	RCS-MEDDH-288 (R1)
Security Classification:	UNCLASSIFIED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 010833	72 06 30	DD DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISP'N INSTR'N	8B. SPECIAL DATA: CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	02	102
b. CONTRIBUTING	62711A	1B662711A096		
c. CONTRIBUTING	CDOG 12126(9)			

11. TITLE (Precede with Security Classification Code)

(U) Development and evaluation of an effective vaccine against plague (11)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine; 004900 Defense; 010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
62 12		DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER: NA
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PREVIOUS 71	3.0	85
	CURRENT 72	2.0	60

19. RESPONSIBLE DOD ORGANIZATION

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NAME: Harrison, D. N.
NAME: DA

21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Yersinia pestis; (U) Plague; (U) Vaccines; (U) Immunization; (U) Serological tests

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Determine the factors influencing the susceptibility to plague infection and the most appropriate method to prevent the infection.

24 (U) Using standard methods, sera from humans and animals are tested for the presence of Fraction 1 antibody to Y. pestis.

25 (U) 71 07 - 72 06 - Repeated inoculation of plague vaccine resulted in 5.1% local and 2.3% systemic reactions. Approximately 80% of both types were mild and of short duration. Stable antibody levels were observed in 107 of 117 individuals receiving more than 5 booster inoculations. A man attained an antibody plateau which was maintained regardless of subsequent booster inoculations.

Newborn rats of immune mothers had circulating antibody and survived challenge with virulent Y. pestis 195/P. The young of mothers not responding to plague immunization had no evidence of passive immunity.

The IHAT test has been shown to be an economical, simple, and rapid procedure for the detection of Fraction 1 of Y. pestis.

Publications: Infec. Immun. 4:85-87, 1971; Proc. Soc. Exp. Biol. Med. 138:738-741, 1971; J. Infect. Dis. 124:522-526, 527-531, 1971; J. Med. Entomol. 9: 115-116, 1972; J. Wildlife Dis. 8:85-94, 1972; Appl. Microbiol. 23: 721-724, 1972; J. Inf. Dis. 125:556-559, 1972.

Terminated since FY 1973 funding will be under The Surgeon General, Army. New Project No. 3A062110A834; Accession No. DA 060423.

*Available for contractors upon originator's approval

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BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 02: Prevention and Treatment of Biological Agent Casualties

Work Unit No. 096 02 102: Development and Evaluation of an Effective Vaccine Against Plague

Description:

Determine the factors influencing the susceptibility to plague infection and the most appropriate method to prevent the infection.

Progress, Part I:

Studies on the clinical and serological response of man to multiple plague immunizations have been completed.

The immunization records of 1,219 persons who had received from 1 to 51 plague inoculations during a 21-year period were reviewed. Data reflecting the number and amount of vaccines administered, and the occurrence of local and systemic reactions experienced as well as the severity and duration of the reactions were analyzed for each individual. The group received 18,768 inoculations of 3 distinct killed plague vaccines during the period 1950-1971. During this period, 350 (28.7%) individuals had 959 (5.1%) local reactions and 241 (19.8%) experienced 426 (2.3%) systemic reactions. A disproportionately high percentage of persons receiving the vaccine administered during the early 1950's had adverse local (77.2%) and systemic (50.0%) reactions. The respective reaction rates for the presently employed plague vaccine were 14.5% local and 5.2% systemic.

There appeared to be a direct dose-response relationship, with both types of reactions. Reactions occurred approximately 3 times more frequently following the administration of either 1.0 or 0.5 ml of vaccine as compared with booster inoculations of $\leq$ 0.25 ml. Approximately 80% of all reactions were mild and of short duration, $\leq$ 24 hr. Among those individuals experiencing either a single marked reaction or multiple mild reactions, reduction in the volume of vaccine and/or the administration of medication with subsequent inoculations of plague vaccine did not markedly alter the frequency of the severity of additional reactions in those persons.

When the results of this study were compared with the results of foreign studies involving the use of living attenuated plague vaccines, there was no question as to superior acceptability of the killed vaccine. The frequency and severity of both local and systemic reaction resulting from the routine administration of the living attenuated plague vaccine were excessively high, i.e. Aleksandrov et al.[1] reported the occurrence of plague immunization reactions, in 100 individuals, 98% had local reactions lasting 2-7 days and 66%, systemic reactions with loss of working capacity for 1-5 days.

Indirect hemagglutination titers for Fraction 1 antigen of Y. pestis were determined on 5,285 sera collected from 117 individuals receiving multiple plague immunizations during a 20-year period. Three distinct patterns of antibody response were observed. One group of 84 had high titered antibody (1:1,024 - > 1:16,382). A 2nd group of 23 persons had consistent titers ranging from 1:64 to 1:512 and a 3rd group of 10, failed to produce IHA antibody in titers > 1:16.

Every individual regardless of the group to which he belonged attained an antibody plateau after approximately 5 booster inoculations which remained level regardless of subsequent immunizations. Cessation of plague immunization for periods of up to 5 years were not reflected in a decline of antibody titers in those individuals who had attained a stable antibody plateau. Failure to immunize for periods of from 6-12 years resulted in a slow decline in antibody levels in the later years although in no instance did an individual become negative.

There was no correlation between IHA antibody titer and the predisposition to adverse clinical reactions upon immunization. A gross shift in titer could not be associated with the occurrence of a local or systemic reaction to a given inoculation.

On two occasions the appearance of spikes in the titers of small groups of individuals unrelated to immunization indicate the possible, accidental exposure to virulent Y. pestis. These antibody spikes returned to base plateau levels for all involved individuals within 18 mon. Subsequent inoculations of plague vaccine did not induce higher antibody levels in individuals of this group.

Summary, Part I:

Repeated inoculation of plague vaccine resulted in 5.1% local and 2.3% systemic reactions. Approximately 80% of both type reactions were mild and of short duration. Stable antibody levels were observed in 107 of 117 individuals receiving more than 5 booster inoculations of plague vaccine. Each person attained individual antibody plateau which he maintained regardless of the number of subsequent booster inoculations administered.

Progress, Part II:

Levi and Suchkov[2] have demonstrated the transplacental transmission of plague antibodies in Rhombomys opimus (big gerbils) using the IHA test. In a series of studies in this laboratory using rats, it was observed that the young of immunized mothers having demonstrable IHA antibody titers to the Fraction 1 antigen of Y. pestis had circulating IHA antibody and were refractory to challenge with virulent Y. pestis strain 195/P. The surviving young did not show an anamnestic rise in titer due to the challenge. The young of mothers who had not responded to plague immunization procedures with demonstrable IHA antibody did not have circulating antibody and succumbed to challenge. Nonimmune mothers who ate their challenged young died of plague.

Using a foster mother model involving the ofispring of immune and nonimmune mothers showed that passive protection is afforded through nursing. Within a 21-day period the antibody level of the young resembled that of the foster mother rather than the natural mother. Challenge experiments are underway to determine the duration of passive immunity after weaning and the onset of immune competence.

Summary, Part II:

Newborn rats of immune mothers had circulating antibody and survived challenge with virulent Y. pestis 195/P. The young of mothers who did not respond to plague immunization procedures showed no evidence of passive immunity.

Progress, Part III:

Peysakhis and Shmuter[3] reported the use of the indirect hemagglutination inhibition (IHAI) test for the detection of Fraction 1 in cultures of Y. pestis and in the tissues of animals dead of plague. In order to evaluate this procedure and to compare it with the serum agar technique of Albizo and Surgalla[4], a series of experiments were conducted. Preliminary tests to determine the effect of various chemical sterilants on the sensitivity and reproducibility of the IHAI test revealed that 50 mg/liter sodium azide, saturated chloroform saline, 10% neutral formalin, and 10% phenol saline did not adverseely affect the test procedure. Heating to 65 C for 15 min or to 100 C for 1 min completely destroyed the inhibitory activity of purified Fraction 1 and the Fraction 1 associated with whole bacterial suspensions.

Tests using block titration techniques repeatedly detected as little as 0.015 ug of purified Fraction 1. Similar tests using killed plague vaccine, USP, were positive for dilutions containing as few as 5 x 10^2 Y. pestis cells. Cultures containing 5 x 10^7 Y. pestis strain KIM, devoid of Fraction 1, gave negative tests.

Routine screening of suspensions of cultures containing approximately 1 X 10^9 bacteria have demonstrated the practicability of the IHAI test; 100 wild strains of Y. pestis gave positive tests, 8 strains of Y. pestis known to be deficient or lacking in fraction 1 gave negative tests as did 15 strains of Y. pseudotuberculosis. The results obtained to date indicate that the IHAI test for the detection of F-1 antigen of Y. pestis is more rapid and economical of scarce reagents than the serum agar techniques.

Summary, Part III:

The IHAI test has been shown to be a simple, rapid, and economical procedure for the detection of Fraction 1 of Y. pestis.

Presentations:

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2. Marshall, J.D., Jr. Susceptibility of rodents to oral plague infection: A mechanism for the persistence of plague in inter-epidemic periods. Presented at Annual Conference of Wildlife Disease Association, Colorado State University, Fort Collins, Colorado. 25-27 August 1971.

3. Marshall, J.D., Jr. Plague. Presented at meeting of Sigma Xi, Colorado State University, Fort Collins, Colorado. November 1971.

4. Marshall, J.D., Jr. Plague. Presented at Global Medicine Course, Walter Reed Army Institute of Research. January 1972.

Publications:

1. Harrison, D.N., D.C. Cavanaugh, J.H. Rust, Jr., and J.D. Marshall, Jr. 1971. Characteristics of a bacteriophage-infected strain of Pasteurella pestis isolated from a human case of plague. Infec. Immun. 4:85-87.

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1. Aleksandrov, N.I., N. Ye. Gefen, K.G. Gapochko, N.S. Garin, G.G. Koridze, I.N. Markozashvili, N.P. Osipov, M.P. Pischik, I.A. Posobilo, M.S. Smirnov, and V.P. Turov. 1962. Aerosol immunization with dry dust vaccines and toxoids. VIII. A study of the method of aerosol immunization with dust plague vaccines during mass immunization. Zh. Mikrobiol Epidemiol Immunobiol. 33:7:40-50 (Translation no. AD 291 038).

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ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1W662711A096 02:	Prevention and Treatment of Biological Agent Casualties
Work Unit No. 096 02 300:	Immunologic Studies with Rickettsiae of Military Medical Importance
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Virology
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Authors:	David M. Robinson, Major, VC (I) Richard H. Kenyon, Ph.D. (II)
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0L0885	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8b. DISP'N INSTR'N	8c. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	02	300
b. [illegible]	62711A	1B662711A096		
c. [illegible]	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)
(U) Immunologic studies with rickettsiae of military medical importance

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 Clinical medicine; 004900 Defense; 010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
67 07	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER: NA
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 71	2.0	40
FISCAL YEAR CURRENT 72	2.0	102

19. RESPONSIBLE DOD ORGANIZATION
NAME: USA Medical Research Institute of Infectious Diseases
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20. PERFORMING ORGANIZATION
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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
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SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
NAME: Kenyon, R. H.
NAME:

21. GENERAL USE
Foreign intelligence considered

DA

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Immunology; (U) Rickettsial diseases; (U) Spotted fevers; (U)Vaccines; (U) Q fever; (U) Coxiella burneti; (U) Military medicine

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number preceded text of each with Security Classification Code.)

23 (U) Develop vaccines of low reactogenicity for immunoprophylaxis against specific rickettsial diseases. This work unit is an essential element in a comprehensive program for medical defense against BW agents.

24 (U) Propagate representative strains in tissue culture systems. Assess the feasibility of producing rickettsial suspensions of quality and quantity suitable for vaccines for human use.

25 (U) 71 07 - 72 06 - Five lots of RIF-free M strain of Coxiella burneti vaccine were produced and tested according to USPHS regulations. They had a median infectious dose for eggs of 10.7 logs; no fever was produced when 0.5 ml of undiluted material was inoculated subcutaneously into guinea pigs. The median protective dose for guinea pigs was approximately 0.00005 micrograms N; no difference was detected between Phase I and II challenges. The vaccine strain did not cause an increased incidence of hepatic lesions when compared to killed vaccine. A request to administer the material to humans has been approved by AIDRB.

The SS strain of Rickettsia rickettsii was freed of RIF viruses and redesignated SSR strain. Working seeds were propagated; a lot of vaccine suitable for human use is currently being produced.

Terminated since FY 1973 funding will be under The Surgeon General, Army. New Project No. 3A062110A834; Accession No. DA 0B6419.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A [illegible] AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 02: Prevention and Treatment of Biological Agent Casualties

Work Unit No. 096 03 300: Immunologic Studies with Rickettsiae of Military Medical Importance

Description:

Develop vaccines of low reactogenicity for immunoprophylaxis against specific rickettsial diseases.

Progress, Part I:

Testing of 5 lots of R-M strain Q fever vaccine produced last year was completed.

The attenuated R-M strain of Coxiella burneti is intended to be administered by the endermal route; a single dose contains a very small amount of egg protein. However, the problem of significant numbers of local reactions occurring in individuals with and without preexisting antibody appeared to warrant purification of the vaccine. The procedure developed utilized only physical methods, and consisted of 2 cycles of differential centrifugation followed by centrifugation in 10% sucrose onto a 70% sucrose "cushion." A comparison of titers and N concentrations is presented in Table I. The titers were approximately equal in all materials, but the N concentrations decreased greatly. The N concentration given for the vaccine is below the sensitivity of our assay and was determined from the N value of the purified vaccine pool and the dilution factor. The reported human dose is 0.1 ml; and it has been estimated that perhaps 1/10 of this actually enters the skin. Therefore, the N value of a single dose would be about 0.005 mg N. When 0.5 ml of purified undiluted material ($10^{10.1}$ median infective dose for eggs $1DE_{50}$) was injected subcutaneously (SC) into guinea pigs no temperatures $\geq$ 103.8 F were detected. Table II is a compilation of 4 potency assays using the median fever supporession dose (PD_{50}) as described by Ormsbee et al.[1] as an indication of protection. Throughout these 4 assays no fevers were detected when the guinea pigs were vaccinated SI with dilutions of freeze-dried vaccine. There was no significant difference in the protection afforded against either of the 2 levels of phase I or the phase II challenges.

While searching for an improved substrate for the growth of C. burneti we tried several cell cultures which were being produced in the laboratory

for other purposes. Since penicillin had been used in diluents for the production of C. burneti we incorporated 100 units of penicillin per ml in the cell culture media and diluent fluids. Briefly our results showed a progressive number of infected cells up to a maximum at 7-8 days at which time the cultures degenerated. Refeeding decreased the number of infected cells, and of course increased the length of time the cell cultures could be kept without degeneration. When both penicillin and streptomycin were incorporated no rickettsial growth could be detected.

TABLE I. MEDIAN EGG INFECTIVE DOSE AND NITROGEN CONTENT OF R-M STRAIN MATERIALS

MATERIAL	LOG_{10} IDE_{50}	ug N/ml
Master seed	11.6	ND
Working seed	10.9	ND
Yolk sac pool	11.1	620
Purified vaccine pool	11.1	39
Vaccine (1:75)	10.4	0.5

ND = Not done

TABLE II. POTENCY ASSAYS OF R-M SUBSTRAIN VACCINE LOT 1 IN GUINEA PIGS

	CHALLENGE	
Phase	Log_{10} IDE_{50}	Log_{10} PD_{50}
I	10	4.3
	6	5.4
II	10	4.8, 5.3

This fortutious finding was used as the basis for the cell culture testing of the vaccine for adventitious agents.

A list of the testing procedures used in the production of the vaccine is presented in Table III. Many of these tests are prescribed by PHS regulations.[3] All the adventitious agent tests, except the RIF and fluorescent antibody tests for avian leucosis, were conducted on the purified vaccine pool prior to filling and freeze-drying. A single blind pass was conducted 14 days postinoculation with 1/2 of the suckling mice originally inoculated. The confluent cell cultures (WI-38, duck and chick fibroblasts, and VERO cells) were refed with material from the purified pool diluted with an equal volume of maintenance medium containing 200 units of penicillin and 200 μg of streptomycin. As previously stated, these conditions would not support the growth of the rickettsia in any of the cell systems. The cultures were examined daily for cytopathic effect and refed at 4-day intervals. The cell sheets were examined 14 days postinoculation with guinea pig red blood cells for the presence of agents capable of hemadsorption. All tests were negative for the presence of adventitious agents.

Naturally acquired infections with phase I organisms[4] and experimental infections[5] have produced granulomatous hepatic lesions in humans. To determine whether the M strain was capable of producing hepatic lesions, guinea pigs were inoculated with the present killed vaccine, the M strain, the phase II Nine-Mile strain EP-88 strain and the phase I Henzerling EP-2 strain. The experimental results are given in Table IV. The lesions found were either focal or granulomatous necrosis of the hepatocytes. Giant cells or eosinophils were not seen. The high percentage of similar hepatic lesions (10%) in control animals which were inoculated with 0.5 ml of Synder's I buffer indicates the incidence of disease in any group of guinea pigs not derived and maintained under germ-free conditions. The incidence of lesions in the animals given the killed vaccine, and the M strain were the same, and the incidence with the EP-88 strain was midway between the prior strains and the phase I strain. Specimens were collected from the onset of fever through 4 weeks postinoculation. The potential advantages of the attenuated R-M strain as a vaccine over the presently available inactivated phase I and II products are: (1) these presently available products have proven to be effective, but if they are analogous to other inactivated products repeated inoculations of μg amounts of material are necessary to maintain detectable antibody titers. However, with repeated injections the incidence of sterile abscesses increases. A delivered dose of the R-M strain contains protein in the order of hundredths of a μg, and has been used in Europe in persons with or without preexisting antibody with the same percentage of local reactions. These were limited to erythema and induration of a transient nature; (2) a given amount of raw material, be it yolk sac or cell culture, can be used to produce many more doses of live vaccine than inactivated product. In an emergency situation this allows many doses of vaccine to be produced and large lot sizes tested to decrease the lag time in the production process.

TABLE III. TESTING PROCEDURES BY PHS STANDARDS

STERILITY

Bacteria

Fungi and yeasts

Mycobacterium spp

Mycoplasma spp

QUALITY

Nitrogen content

Titer

Potency

Generay safety

Residual moisture

ADVENTITIOUS AGENTS

Mice-suckling and adult

Cell cultures

Embryonated eggs (duck and chick)

Avian leucosis agents

IDENTITY

TABLE IV. INCIDENCE OF HEPATIC LESIONS IN GUINEA PIGS

GROUP	NO./TOTAL	%
Control	2/20	10
Q fever vaccine (killed phase II)	2/10	20
M strain	2/10	20
EP-88	4/10	40
EP-2	6/10	60

In conclusion, we have derived a RIF-free strain of C. burneti from the M strain which we have designated the R-M strain. A series of lots of vaccine suitable for human use have been produced and tested. The dose capable of protecting 50% of the guinea pigs inoculated contains a calculated amount of 0.00005 μg N; the incidence of hepatic lesions was not increased over that observed with the presently available killed phase II vaccine. A request to proceed with testing of this vaccine in humans has been approved by the Army Investigational Drug Review Board.

Summary, Part I:

A series of 5 lots of R-M (RIF-free M) strain of C. burneti vaccine were produced and tested according to PHS regulations. These lots had a mean median infectious dose for eggs of 10^7 logs; no fevers were produced when 0.5 ml of undiluted material was inoculated subcutaneously into guinea pigs. The median protective dose in guinea pigs was approximately 0.00005 μg N; no difference was detected between phase I and II challenges. The R-M strain did not cause an increased incidence of hepatic lesions in guinea pigs when compared to the present killed vaccine. A request to administer the material to humans has been approved by AIDRB.

Progress, Part II:

Work continued on the development of an improved Rocky Mountain spotted fever (RMSF) vaccine for human use. All work for vaccine development has been with the Sheila Smith (SS) strain of RMSF. Since the SS strain had been propagated in eggs not certified to be RIF-free, steps were taken to rid the master seed of any viruses of this group which may have been present. Two methods were tried: (1) passage of the master seed 4 times in duck yolk sacs and then in yolk sac of chicken eggs certified RIF-free (SPAFAS; SPAFAS Inc., Norwich, Conn.); (2) passage of the master seed 2 times in duck embryo cell culture (DEC) and then in SPAFAS chicken eggs. Both of these were freed of rickettsiae by egg passage in the presence of tetracyclines and tested for RIF viruses at the National Institutes of Health (NIH); both were negative. Our working seed has been propagated from that passed 4 times in duck yolk sacs. This working seed has been redesignated SSR (Sheila Smith, RIF-free).

Studies continued on the methods to be used for production of a large lot of vaccine made from rickettsiae propagated in DEC. It was decided that 24-hr-old DEC would be infected and the cells fed with medium containing 2% human serum albumin. The cells and rickettsiae would be harvested 5 days after infection in sucrose-phosphate-glutamate (SPG) buffer and tested for sterility. After pooling into one lot, the mixture would be spun at 150 X g to remove cell debris and the rickettsiae in the supernate inactivated with 0.1% formaldehyde

Problems with contamination have hampered efforts to produce a large lot of vaccine. Repeated contaimination occurred with a gram negative rod identified as Mima polymorpha. The source of this contamination cannot be ascertained, but recent evidence incriminates the calf serum used to grow the DEC. This problem was apparently eliminated by using a new source of calf serum. After 6 L of DEC-grown rickettsiae are produced we will process the mixture for vaccine.

Production of working seeds of the other members of the spotted fever group of rickettsiae has begun. R. siberica, R. conori, R. parkeri, R. akari, and R. australis have been passed 2 times in duck egg yolk sacs and then in SPAFAS eggs in an attempt to rid the seed of any contaminating RIF viruses. These seeds stock will be sent to NIH to ascertain if they are RIF-free. Immunization of guinea pigs with our DEC-grown RMSF vaccine offers some protection against R. conori, R. australis, R. parkeri, and R. siberica, but not against R. akari. Work is continuing on the growth characteristics in eggs and in cell cultures of these members of the spotted fever group. Preliminary characterization of toxin associated with some of the spotted fever rickettsiae will be extended during the next year.

A series of studies was done in rhesus monkeys to determine a lethal challenge dose to use for a planned comparative vaccine efficacy study. This study is being done in collaboration with Captain Ruch of Animal Assessment Division. Three different types of inoculum have been used: a chick-yolk-sac-grown material, blood from infected guinea pigs, and DEC-grown rickettsiae. Of these three, the DEC-grown rickettsiae appears to be the inoculum of choice. Different routes of challenge were also compared using 3 groups of 2 animals each for intraperitoneal, intravenous (IV), and subcutaneous inoculation. The IV challenge was most consistent in producing death. A group of animals was vaccinated in preparation for these comparative vaccine efficacy studies.

Summary, Part II:

The SS strain of R. rickettsii has been freed of RIF viruses and redesignated SSR strain. Working seeds have been propagated and a lot of vaccine suitable for human use in currently being produced.

Presentations:

1. Robinson, D. M. Live Q Fever Vaccine. Presented at Annual Meeting, Commission of Rickettsial Diseases, Washington, D. C., 11-12 Nov 71.

2. Kenyon, R. H. Spotted fever vaccine. Presented at Annual Meeting, Commission of Rickettsial Diseases, Washington, D. C., 11-12 Nov 71.

3. Kenyon, R. H. Spotted fever vaccine. Presented to Commission on Rickettsial Diseases, Washington, D. C., 23 March 1971.

Publications:

1. Robinson, D. M., S. Berman, and J. P. Lowenthal. 1972. Mouse potency assay for Western equine encephalomyelitis vaccines. Appl. Microbiol. 23:104-107.

2. Kenyon, R. H., W. M. Acree, G. G. Wright, and F. W. Melchior, Jr. 1972. Preparation of vaccines for Rocky Mountain spotted fever from rickettsiae propagated in cell culture. J. Infect. Dis. 125:146-152.

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3. Part 73 - Biological Products. 1970. Chapter 1. Public Health Service; Department of Health, Education and Welfare; Federal Register 35: Number 171, 13922-13961.

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ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1W662711A096 02:	Prevention and Treatment of Biological Agent Casualties
Work Unit No. 096 02 407:	Development of Inactivated Aabovirus Vaccines for Disease of Military Importance
Reporting Installation:	U. S. Army MedicalResearch Institute of Infectious Diseases Fort Detrick, Maryland
Divisions:	Virology and Pathology
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Authors:	Francis E. Cole, Jr., Ph.D.(I) Garrett S. Dill, Captain, VC (II) Carl E. Pedersen, Jr. Major, MSC (II) James L. Stookey, Lt Colonel, VC (II)
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 01.0841	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. ORGN INSTN	8B. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NI.	☒ YES ☐ NO	A. WORK UNIT

10. NO. CODES.	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	02	407
b. [illegible]	62711A	1B662711A096		
c. [illegible]	CDOC 1212b(9)			

11. TITLE (Precede with Security Classification Code)

(U) Development of inactivated arbovirus vaccines for diseases of military importance

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical medicine; 004900 Defense; 010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
64 06	CONT	DA	C. In-house

17. CONTRACT GRANT

a. DATES/EFFECTIVE: EXPIRATION

b. NUMBER: NA

c. TYPE: d. AMOUNT

e. KIND OF AWARD: f. CUM AMT

18. RESOURCES ESTIMATE		A. PROFESSIONAL MAN YRS	B. FUNDS (In Thousands)
FISCAL YEAR	PRECEDING 71	1.0	50
	CURRENT 72	1.0	66

19. RESPONSIBLE DOD ORGANIZATION

NAME: USA Medical Research Institute of Infectious Diseases

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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

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SOCIAL SECURITY ACCOUNT NUMBER

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21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Vaccines; (U) Encephalitis, equine (VEE); (U) Mayaro; (U) O'Nyong-nyong; (U) Cell culture; (U) St Louis encephalitis; (U) California encephalitis; (U) Military medicine

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Produce inactivated arbovirus vaccines which may be combined selectively for prophylaxis in specific geographically oriented ways and examine histopathology after use. This work unit is an essential element in a comprehensive program for medical defense against BW agents.

24 (U) Arboviruses are propagated in primary cell culture and inactivated with formalin. Products are tested for safety and potency to animals. Efficacy is determined by subsuquent challenge, or by determination of serological conversion.

25 (U) 71 07 - 72 06 - Thirty small lots of formalin-inactivated TC-83 strain VEE vaccine were prepared in roller bottle cultures of chick embryo cells (CEC). The following parameters for VEE vaccine production are presented: (1) a multiplicity of inoculum (MOI) of 0.0004 may be used yielding maximum titers of virus at 18-20 hr; (2) as little as 300 ml of maintenance medium may be employed without substantially decreasing potency; (3) inactivation by PHS standards at 37 C may be carried out with 0.05% formalin for 30 hr or more or 0.1% formalin for at least 24 hr. Mayaro virus was grown in MK, AG cells for 40 hr and elicited 75% cell destruction. Four small lots of vaccine produced will be potency tested. O'nyong-nyong virus grown in MK, AG cells increased in titer at 3rd passage approaching that required for vaccine production. New strains of California and St. Louis encephalitis viruses were passed 14 times in CEC cultures, and appear to be adapting to these cells.

Commercially prepared VEE virus has been titrated and small numbers of hamsters infected and prepared for examination to check methodology and procedures.

Publications: Infec. Immun. 4:37-43, 1971; Appl. Microbiol. 22:842-845, 1971.

Terminated since FY 1973 funding will be under The Surgeon General, Army. New Project No. 3A062110A834; Accession No. DA 0B6420.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 68 AND 1498 1 MAR 66 FOR ARMY USE ARE OBSOLETE

BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 02: Prevention and Treatment of Biological Agent Casualties

Work Unit No. 096 02 407: Development of Inactivated Arbovirus Vaccines for Disease of Military Importance

Description.

Produce inactivated arbovirus vaccines which may be combined selectively for prophylaxis in specific geographic areas.

Progress, Part I:

Using slight modifications of previously described methods,[1] small lots of formalin-inactivated Venezuelan equine encephalomyelitis (VEE) virus vaccine were produced.

Live, attenuated VEE vaccine[2] (TC-83) was used to produce the killed product described (killed TC-83). Trinidad strain VEE virus[3] was used as challenge virus for potency assays.

Trinidad VEE virus was titrated in 3-week-old white mice (CD-1 strain from Charles River Mouse Farms, Wilmington, Mass.). Virus samples were diluted in cold phosphate buffered saline, pH 7.2, containing 1% normal rabbit serum (PBS). Groups of 5 mice were inoculated intraperitoneally (IP) with 0.3 ml of $\log_{10}$ dilutions of virus-containing fluids and were observed for 14 days for deaths. TC-83 VEE was titrated *via* the intracerebral (IC) route in 1- to 3-day old mice (CD-1 strain). Groups of 6 mice were inoculated IC with 0.03 ml of $\log_{10}$ dilutions of virus-containing fluids in cold PBS. Titration endpoints in all cases were determined by the method of Reed and Muench[4] and were expressed as median lethal dose (LD_{50}) per milliliter.

Rolling bottle cultures of chick embryo cells (CEC) were prepared.[1] Once cell confluency was achieved, the growth medium was decanted and replaced with serum-free medium 199 containing penicillin and streptomycin. The CEC roller cultures were held an additional 20-24 hr at 35 C. Prior to infection, the medium was removed and the residual fluid drained from the cultures.

Formalin-inactivated VEE (killed TC-83) vaccines were assayed using 3-week-old CD-1 white mice. Groups of 10 mice were inoculated IP on day 0 with 0.3 ml of 5-fold dilutions of vaccine. Fourteen days

after this single dose of vaccine, mice were challenged IP with 10^3 mouse 1PLD$_{50}$ of Trinidad VEE. Titration endpoints and median effective dose (ED_{50}) values were determined.[1]

Roller bottle CEC cultures were infected with the TC-83 strain at multiplicities of inoculum (MOI) of 0.04 to 0.00004 and maintained with 200 ml medium 199. Culture fluid samples removed from these bottles 24 hr postinoculation were titrated in suckling mice. As indicated in Table I, good virus yields were obtained at all MOI levels. In all further studies reported here, an MOI of 0.0004 was employed.

TABLE I. EFFECT OF MOI ON PROPAGATION OF TC-83 STRAIN VEE VIRUS IN ROLLER BOTTLE CEC CULTURES

Log_{10} LD_{50}/ml by MOI at 24 hr			
0.04	0.004	0.0004	0.00004
10.3	10.8	10.4	10.5

To determine the effect of maintenance medium volume on virus yield, CEC cultures were inoculated and maintained with 100, 200 or 300 ml of medium 199 containing penicillin, streptomycin and 0.25% human serum albumin (HSA). Summarized in Table II are the results of assays performed on fluids removed from these cultures at various periods postinoculation.

TABLE II. EFFECT OF MAINTENANCE MEDIUM VOLUME ON PROPAGATION OF TC-83 VIRUS IN CEC ROLLER BOTTLE CULTURES (MOI = 0.0004)

HOURS POST-	LOG_{10} LD_{50}/ml BY MAINTENANCE MEDIUM VOLUME		
	100 ml	200 ml	300 ml
6	6.9	5.7	6.1
12	10.5	9.8	10.0
18	10.3	9.8	10.1
20	10.4	10.1	10.3
24	10.3	9.9	9.6

Although adequate virus titers were achieved at all maintenance medium volumes by 12 hr, from a production standpoint the larger volumes would be most advantageous. Cell destruction was moderate by 12 hr in the bottles maintained with 100 ml of medium and increased greatly at 24 hr. In cultures maintained with 200 or 300 ml of medium significant cell destruction was not observed until 24 hr. Thus, high titered virus material with lesser amounts of cellular debris could best be obtained with a harvest at 18-20 hr.

Thirty small lots of killed TC-83 vaccine were prepared using procedures described for the production of Eastern equine encephalitis vaccine[5] with regard to clarification by centrifugation and Millipore filtration, and the addition of formalin to final concentrations of 0.1% and 0.2%. However, inactivation was performed at 37 C in flasks placed in a New Brunswick Reciprocating Water Bath (125 rpm). The fluids were thus kept in gentle, constant movement throughout the inactivation periods of 24 - 96 hr. Typical rates of formalin-inactivation are shown in Table III. The TC-83 strain was inactivated at a rapid rate with both formalin concentrations but was surprisingly stable when subjected to heat only (virus control).

TABLE III. FORMALIN INACTIVATION[a] OF TC-83 STRAIN VEE VIRUS AT 37 C

HOURS POST-FORMALIN	LOG_{10} LD_{50}/0.03 ml BY FORMALIN CONCENTRATION		VIRUS[b] control
	0.05%	0.1%	
0	8.4	8.3	8.3
2	2.0	<1.0	[c]
4	<1.0	<1.0	
6	<1.0	<1.0	
8	<1.0	0	7.5
10	0[d]	0	
12	0	0	
14	0	0	
16	0	0	7.3
18	0	0	
20	0	0	
22	0	0	
24	0	0	7.2

a. Determined by IC inoculation of suckling mice with 0.03 ml of log_{10} dilutions.

b. Virus subjected to 37 C only.

c. Blanks - not tested.

d. 0 indicates no evidence of virus-induced death in mice inoculated; at 8-24 hr ~50 mice were inoculated with undiluted vaccine.

The VEE vaccines were inactivated for extended periods to determine the effect on potency. Table IV is a summary of the results of single dose potency assays performed on 30 lots of vaccine prepared with 0.05 and 0.1% formalin and inactivated for 24 - 96 hr.

TABLE IV. EFFECT OF FORMALIN CONCENTRATION AND LENGTH OF INACTIVATION PERIOD ON POTENCY OF KILLED TC-83 VEE VACCINES

HOURS OF INACTIVATION	0.05 % no. lots	0.05 % ED_{50} ml (range)	0.1% no. lots	0.1% ED_{50} ml (range)
24	3	0.047 (0.022-0.085)	3	0.027 (0.002-0.046)
30	10	0.022 (0.003-0.120)	4	0.011 (0.001-0.029)
48	3	0.023 (0.019-0.028)	3	0.069 (0.038-0.110)
72	1	0.042	1	0.038
96	1	0.060	1	0.034

These data indicate that the killed TC-83 strain is stable antigenically, even after inactivation for periods as long as 96 hr. The volume of maintenance medium and preinactivation titers of the fluids used to prepare these vaccines varied from 100 - 300 ml and $10^{9.5}$ to $10^{10.8}$ suckling mouse (SM) $1CLD_{50}$/ml, respectively. There is no apparent correlation between these values and the potencies observed. One must conclude, therefore, that formalin-inactivated, TC-83 VEE vaccine may be made with larger volumes of maintenance medium and with great latitude with regard to period of inactivation.

During the past year attempts were made to develop inactivated vaccines for the following viruses: Mayaro (MAY), O'nyong-nyong (ONY), California encephalitis (CE) and St. Louis encephalitis (SLE). As reported previously[1] the 4 strains shown in Table V were used for initial studies which were carried out for 2 purposes: (1) to determine if the viruses would grow in cell culture to any appreciable titer, and (2) to determine if passage (subculture) of the viruses in the cells shown would result in an adaption to the cells, and, hopefully, higher titers. It should be noted that all of these viruses were received as suckling mouse brain preparations, and that serial subculture would also reduce the level of mouse brain antigen(s) to an insignificant level. Thus, such passage

would satisfy PHS regulations which demand that foreign proteins must not exceed a concentration of 1:1,000,000 in the final product.

TABLE V. GROWTH OF FOUR VIRUSES IN VARIOUS CELL CULTURES

VIRUS[a] (Strain)	PASSAGE NUMBER	LOG_{10} SMICLD$_{50}$/ml BY CELL 24 HR POSTINOCULATION:						
		Kidney				Embryo		WI-38
		Canine	Rabbit	Hamster	MK,AG	Chick	Duck	
Mayaro (vr-1)	1	≤6.0	≤6.0	7.0	9.2	≤6.2	6.7	[b]
	2	6.9		8.7	8.2		7.5	
	3	≤6.0		8.2	7.8		7.1	
O'nyong-nyong (osege)	1	≤6.0	≤6.0	7.0	≤6.0	≤6.0	≤6.0	≤6.3
	2	≤6.0		7.0	≤6.0		≤6.0	
	3	≤6.0		≤6.0	≤4.0		≤4.0	
CE (Bfs-283)	1	≤6.0	≤6.0	≤6.0	≤6.0	6.3	≤6.0	≤6.0
	2	≤6.0		≤6.0	≤6.0		≤6.0	
	3	≤6.0		≤6.0	≤4.7		5.0	
SLE (Hubbard)	1	≤6.0	≤6.0	≤6.0	≤6.0	6.3	≤6.0	≤6.0
	2	≤6.0		≤6.0	≤6.0		≤6.0	
	3	≤6.0		≤6.0	≤4.0		≤4.0	

a. 10^{-3} input usually represented an MOI of 0.001 - 0.0001.

b. Blank - not tested.

All viruses (Table V) were inoculated at an empirical dilution of 10^{-3} into the indicated cell cultures, all of which are of the primary type, with the exception of the human diploid cell, WI-38. After inoculation, maintenance medium (199 + HSA and antibiotics) was added and the cultures incubated at 37 C for 24 hr. Replicate samples of culture fluid were removed at 18 and 24 hr. These samples were frozen and subsequently used as inoculum for the next passage and were also titrated insuckling mice via the IC route.

The results shown in Table V are for 24-hr samples only, but are representative. Little or no viral growth occurred in canine or rabbit kidney or WI-38. For Mayaro monkey kidney (MK, AG) appeared to be the cell of choice. For ONY virus minimal titers were obtained in hamster kidney through the 2nd passage only. CE and SLE viruses were the most difficult to propagate, with only minimal titers being achieved in CEC culture.

Since no other acceptable cell lines were available, these studies were repeated, using a higher MOI, as well as incubation periods up to 96 hr. MK, AC and CEC cultures were selected for these studies, principally on the basis of the data in Table V.

TABLE VI. EFFECT OF VIRUS INPUT AND INCUBATION PERIOD ON YIELD ON MAYARO[a/] VIRUS DURING 3 PASSAGES IN CELL CULTURE

PASSAGE NUMBER	HR POST INOC.	LOG_{10} $SMICLD_{50}$ ml BY CELL CULTURE AND VIRUS INPUT:			
		MK, AG		CEC	
		10^{-1}	10^{-3}	10^{-1}	10^{-3}
1	24	≥8.0	≥6.0	6.9	≥6.0
	48	8.7	≥8.0	6.1	6.3
	72	8.3	8.3	≤6.0	≤6.0
	96	7.8	8.1	≤6.0	≤6.0
2	24	≥8.0	5.7	5.7	5.3
	48	≥9.0	≥8.0	≤5.0	7.1
	72	8.3	9.1	≤6.0	7.1
	96	8.5	9 0	≤6.0	≤6.0
3	24	≥8.0	5.9	6.0	6.0
	48	≥9.0	≥8.0	6.7	7.7
	72	9.7	9.3	≤6.0	7.9
	96	9.0	9.1	≤6.0	7.3

a. TRVL 15537, strain

Table VI is a summary of the results with a different strain of MAY. These data suggest that this strain can be grown in MK, AG with a 72-96-hr incubation period to titers approaching those seen with other group A arboviruses used for vaccine production. As shown these titers persisted after 3 cell culture passages using as virus input either a 10^{-1} or 10^{-3} dilution of seed virus. Titers in CEC cultures were not satisfactory even after 3 passages. Based on these data small lots of MAY vaccine have been produced in commercially obtained MK, AG cell cultures. Static MK, AG cultures were inoculated with the TRVL 15537 strain of MAY virus. Culture fluids were harvested 40 hr postinoculation, a time at which 75% cell destruction was observed. After clarification by centrifugation and membrane (0.45 μ) filtration, formalin was added to final concentrations of 0.05 or 0.1%. Inactivation was at 37 C for 30 or 48 hr. Four laboratory-scale lots were made in this manner. Preinactivation titers ranged from $10^{8.8}$ to $10^{9.3}$ LD_{50}/ml as tested in SM. Potency tests on these lots of vaccine will be conducted in the near future.

Results with ONY virus (Table VII) were somewhat encouraging. By the 3rd passage in MK, AG culture titers were obtained after 72-96 hr incubation that approach those desirable for vaccine production. Virus yield in CEC culture was unsatisfactory.

With CE virus (Table VIII) virtually no growth occurred in MK, AG. In CEC culture the titers were marginal at the 1st and 2nd passage and were certainly not acceptable at the 3rd passage, regardless of virus input.

SLE virus (Table IX) exhibited inadequate growth in MK, AG cells, regardless of virus input or period of incubation. Further, with the possible exception of the 1st passage in CEC culture, virus growth in this host cell must also be considered inadequate for vaccine production.

Since the strains of CE and SLE viruses employed in the preceding studies did not replicate to significantly high titers in the cell systems employed, new strains were obtained from the Center for Disease Control in December 1971. Master seed preparations (suckling mouse brain) have been made for the La Cross and Snowshoe Hare strains of CE virus and for the P-15 strain of SLE virus. Rapid passage of these 3 strains are being made in primary CEC culture in an attempt to increase virus yield by adapting the viruses to the cells. At the time of writing 14 passages had been made. Preliminary titrations of passages 3, 5, and 8 in SM and by the plaque technique indicate that the viruses are adapting to the cells, and that additional passages may result in virus yields sufficiently high for vaccine production. These studies are continuing.

TABLE VII. EFFECT OF VIRUS INPUT AND INCUBATION PERIOD ON YIELD OF O'NYONG-NYONG VIRUS DURING 3 PASSAGES IN CELL CULTURE

PASSAGE NUMBER	HR PAST INOC.	LOG_{10} SMICLD$_{50}$/ml BY CELL CULTURE AND VIRUS INPUT:			
		MK,AG		CEC	
		10^{-1}	10^{-3}	10^{-1}	10^{-3}
1	24	6.7	5.9	6.1	≤6.0
	48	7.9	7.0	6.1	6.3
	72	7.5	7.2	≤6.0	≤6.0
	96	≤6.0	≤6.0	≤6.0	≤6.0
2	24	5.7	3.8	5.0	3.3
	48	6.9	5.5	6.7	6.8
	72	7.7	≤6.0	≤6.0	≤6.0
	96	≤6.0	≤6.0	≤6.0	≤6.0
3	24	4.7	≤4.0	5.0	3.7
	48	7.0	6.0	6.2	7.2
	72	7.9	6.9	≤6.0	7.0
	96	8.7	8.7	≤6.0	7.1

TABLE VIII. EFFECT OF VIRUS INPUT AND INCUBATION PERIOD ON YIELD OF CE VIRUS DURING 3 PASSAGES IN CELL CULTURE

PASSAGE NUMBER	HR POST INOC.	LOG_{10} $SMICLD_{50}$/ml BY CELL CULTURE AND VIRUS INPUT:			
		MK, AG		CEC	
		10^{-1}	10^{-3}	10^{-1}	10^{-3}
1	24	≤5.0	5.0	7.3	≥6.0
	48	≤5.0	5.7	7.3	≥8.0
	72	≤6.0	≤6.0	≤6.0	7.3
	96	≤6.0	≤6.0	≤6.0	7.0
2	24	≤4.0	≤4.0	6.8	5.0
	48	≤4.0	≤4.0	7.7	7.9
	72	≤6.0	≤6.0	7.2	7.1
	96	≤6.0	≤6.0	6.9	7.3
3	24	≤4.0	≤4.0	6.7	4.7
	48	≤4.0	≤4.0	7.0	7.2
	72	≤6.0	≤6.0	7.7	7.5
	96	≤6.0	≤6.0	7.5	7.2

TABLE IX. EFFECT OF VIRUS INPUT AND INCUBATION PERIOD ON YIELD OF SLE VIRUS DURING 3 PASSAGES IN CELL CULTURE

PASSAGE NUMBER	HR POST INOC.	LOG_{10} $SMICLD_{50}$/ml BY CELL CULTURE AND VIRUS INPUT:			
		MK, AG		CEC	
		10^{-1}	10^{-3}	10^{-1}	10^{-3}
1	24	5.8	5.0	≤8.0	≤6.0
	48	6.1	5.7	7.9	7.3
	72	≤6.0	≤6.0	7.1	7.0
	96	≤6.0	≤6.0	7.1	6.8
2	24	≤4.0	≤4.0	6.8	5.2
	48	≤4.0	≤4.0	6.3	7.3
	72	≤6.0	≤6.0	≤6.0	7.0
	96	≤6.0	≤6.0	≤6.0	≤6.0
3	24	≤4.0	≤4.0	4.9	2.8
	48	≤4.0	≤4.0	6.1	6.5
	72	≤6.0	≤6.0	≤6.0	≤6.0
	96	≤6.0	≤6.0	≤6.0	≤6.0

In ancillary studies, attempts have been made to produce a more efficacious product, due to the poor serological responses of National Drug Co. personnel to the present Chikungunya (CHIK) vaccine. Three strains of CHIK virus (Ross, CHIK-168, and #15561) were passed 3 times in static cultures of WI-38 cells. A total of 9 laboratory-scale lots were prepared in rolling bottle cultures of WI-38 cells using 3rd cell culture passage material as inoculum. Inactivation was at 37 C for 30 hr using 0.05 and 0.1% formalin. Preinactivation titers in SM ranged from $10^{6.7}$ to $10^{9.0}$ LD_{50}/ml. However, potency assays in mice revealed no significant degree of protection. No reason for this failure is apparent. These studies were discontinued when it was found that a contractor was presently conducting such studies.

Summary, Part I:

Thirty small lots of formalin-inactivated TC-83 strain VEE vaccine were prepared in roller bottle cultures of chick embryo cells (CEC). The following parameters for VEE vaccine production are presented: (1) a multiplicity of inoculum (MOI) of 0.0004 may be used yielding maximum titers of virus 18 - 20 hr postinoculation; (2) $\leq$300 ml of maintenance medium may be employed without decreasing final potency to any substantial degree; (3) inactivation by PHS standards at 37 C may be carried out with 0.05% formalin for $\geq$30 hr or 0.1% formalin for $\geq$24 hr.

Mayaro (MAY) virus (strain TRVL #15537), after adaption by serial passage, grown in MK, AG cells for 40 hr, elicited 75% cell destruction. Four small lots of vaccine have been produced and await potency testing.

By the 3rd passage in MK, AG cells O'nyong-nyong virus achieved a substantial increase in titer, approaching that required for vaccine production.

New strains of California and St. Louis encephalitis viruses have undergone 14 rapid passages in chick embryo cell cultures, and appear to be adapting to these cells. Further passage is in progress in an attempt to further increase the titer of these viruses.

Progress and Summary, Part II:

Hamsters histopathologically examined at this institute after infection with TC-83 have had a consistent vasculitis, neuronal necrosis, and nonsuppurative meningoencephalitis in the olfactory bulbs and ventral cerebrum of the brain. Austin and Scherer examined histopathology of TC-83 in hamsters they saw no unequivocal lesions in the brain.[1] Because of this variation in results, it was decided to serially sacrifice hamsters in significant numbers with a known dose of virus to clarify the histopathology, and at the same time to compare it with another strain of VEE with lower virulence (Florida Fe 3-7c).

Commercial TC-83 vaccine (National Drug Co.) has been obtained and titrated. A small number of hamsters have been infected with 500 PFU of TC-83 and 500 PFU of the small plaque variant of the Florida strain. These animals have been killed, necropsied and are in the histopathological lab being processed for examination.

Publications:

1. Cole, F. E. Jr. and R. W. McKinney. 1971. Cross-protection in hamsters immunized with group Aarbovirus vaccines. Infec. Immun. 4:37-43.

2. Cole, F. E. Jr. 1971. Inactivated Eastern equine encephalomyelitis vaccine propagated in rolling-bottle cultures of chick embryo cells. Appl. Microbiol. 22:842-845.

3. Pedersen, C. E. Jr., D. M. Robinson, and F. E. Cole, Jr. 1972. Isolation of the vaccine strain of Venezuelan equine encephalitis virus from mosquitoes in Louisiana. Amer. J. Epidemiol. 95:490-496.

4. Cole, F. E. Jr., C. E. Pedersen, Jr., and D. M. Robinson. 1972. Early protection in hamsters immunized with attenuated Venezuelan equine encephalomyelitis vaccine. Infec. Immun. (In press).

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2. Berge, T. O., I S. Banks, and W. D. Tigertt. 1961. Attenuation of Venezuelan equine encephalomyelitis virus by in vitro cultivation in guinea pig heart cells. Amer. J. Hyg. 73:209-218.

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5. Cole, F. E. Jr. 1971. Inactivated Eastern equine encephalomyelitis vaccine propagation in rolling-bottle cultures of chick embryo cells. Appl. Microbiol. 22:842-845.

6. Austin, F. J., and W. F. Scherer. 1971. Studies of viral virulence I. Growth and histopathology of virulent and attenuated strains of Venezuelan encephalitis virus in hamsters. Amer. J. Path. 62:195-219.

ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	**Medical Defense Aspects of Biological Agents (U)**
Task No. 1W662711A096 02:	**Prevention and Treatment of Biological Agent Casualties**
Work Unit No. 096 02 408:	Role of Antibody in the Clinical Manifestations of Venezuelan Equine Encephalomyelitis
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Medical
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Authors:	Peter J. Bartelloni, Lt Colonel, MC
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OL0811	72 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	02	408
b. CONTRIBUTING	62711A	1B662711A096		
c. CONTRIBUTING	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)
(U) Role of antibody in the clinical manifestations of Venezuelan equine encephalomyelitis

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 Clinical medicine; 004900 Defense; 002300 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
62 02	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER: NA
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
PRECEDING FISCAL YEAR 71	1.0	0
CURRENT FISCAL YEAR 72	1.0	10

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME:
NAME:

21. GENERAL USE
Foreign intelligence considered

DA

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Prophylaxis; (U) Encephalitis, equine (VEE); (U) Virus; (U) Virus diseases; (U) Immune serum; (U) Military medicine

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Study the role of antibody as it relates to the clinical manifestations of Venezuelan equine encephalomyelitis (VEE) virus infection, and the usefulness of immune serum in prophylaxis and treatment of this infection. This work unit is an essential element in a comprehensive program for defense against BW agents.

24 (U) Animals are inoculated with either attenuated or virulent VEE virus. The efficacy of antiserum in preventing undesirable reactions to these viruses is evaluated. The resulting immune response and its dependency on the relationship of the quantity of antiserum given to time of its administration is investigated.

25 (U) 71 07 - 72 06 - During the year, no tests were performed. This is a work unit needed for future work as required.

Terminated since FY 1973 funding will be under The Surgeon General, Army. New Project No. 3A062110A834; Accession No. DA 0B6421.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, NOV 68 AND 1498-1, 1 MAR 68, FOR ARMY USE, ARE OBSOLETE

BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 02: Prevention and Treatment of Biological Agent Casualties

[illegible] 096 02 408: Role of Antibody in the Clinical Manifestations of Venezuelan Equine Encephalomyelitis

Description:

Study the role of antibody as it relates to the clinical manifestations of Venezuelan equine encephalomyelitis (VEE) virus infection, and the usefulness of immune serum in prophylaxis and treatment of this infection.

Progress and Summary:

During the year, no tests were performed.

Publications:

None.

ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1W662711A096 02:	Prevention and Treatment of Biological Agent Casualties
Work Unit No. 096 02 410:	Pathophysiology, Pathogenesis and Therapy of Yellow Fever
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Divisions:	Animal Assessment and Pathology
Professional Authors:	Richard O. Spertzel, Lt Colonel, VC (I) James L. Stookey, Lt Colonel, VC (I) Philip C. Kosch, Captain, VC (I) Frank E. Chapple, III, Captain, VC (I) Steven H. Gilbertson, Captain, VC (II)
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OL0877	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. GP/P INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	02	410
b. [illegible]	62711A	1B662711A096		
c. [illegible]	CDGO 1212b(9)			

11. TITLE (Precede with Security Classification Code)
(U) Pathophysiology, pathogenesis and therapy of yellow fever

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 Clinical medicine; 004900 Defense; 002300 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
66 12	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER: NA
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 71	2.0	45
FISCAL YEAR CURRENT 72	2.0	59

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS:
NAME: Kosch, P. C.
NAME: Gilbertson, S. H. DA

21. GENERAL USE
Foreign intelligence considered

22. KEYWORDS (Precede each with Security Classification Code)
(U) Yellow fever; (U) Pathology; (U) Therapy; (U) Physiology, medical; (U) Military medicine

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) A multidisciplinary approach is used for the study of the pathogenesis and pathophysiology of yellow fever.

24 (U) Inoculate laboratory animals with yellow fever virus, Asibi and 17-D vaccine strains. Study changes in blood and tissues and alterations in organ function during the course of the disease. Such fundamental knowledge is needed prior to testing methods of prevention and treatment.

25 (U) 71 07 - 72 06 - I: Current evidence suggests that incubation is the only dose-dependent feature of the disease. The initial outward clinical sign of the disease in the rhesus monkey is a rapid and marked elevation in body temperature. In general, monkeys are viremic 0-24 hr preceding onset of fever, as well as developing a significant progressive leukopenia. Both the fall in hematocrit and elevation in serum LDH activity occur late in the clinical illness. One monkey median lethal dose (LD-50) appears to be approximately equivalent to 0.01 median mouse intracerebral lethal dose. Furthermore, our evidence suggests that 1 LD-50 in the rhesus monkey may be the minimal infective dose as well.

II: Various techniques for sample collection, histopathology, fluorescence microscopy, and electron microscopy have been investigated and employed in studies of yellow fever infection of mouse central nervous system. Histopathological lesions were most consistent in cerebral gray matter, hippocampal neurons, and their accompanying vessels. Immunofluorescent staining was positive for yellow fever antigen in the same structures. Ultrastructural studies are continuing, but are inconclusive thus far.

Publication: Lab. Anim. Sci. 21:610-612, 1971.
Terminated since FY 1973 funding will be under The Surgeon General, Army.
New Project No. 3A062110A834; Accession No. DA 0B6422.

*Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1, 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE

BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 02: Prevention and Treatment of Biological Agent Casualties

Work Unit No. 096 02 410: Pathophysiology, Pathogenesis and Therapy of Yellow Fever

Brief Start:

A multidisciplinary approach is used for the study of the pathogenesis and pathophysiology of yellow fever.

Progress, Part 1:

As part of the overall research plan to study the pathophysiology, pathogenesis and therapy of yellow fever, we have attempted to establish the yellow fever-infected rhesus monkey as an animal model for studies of trace metal analysis, free amino acids, renal and hepatic function, sites of tissue damage and effects on various organ systems. In support of and correlated with these studies, a set of standardized clinical and laboratory test evaluations are being performed on each control and infected animal in order to adequately relate the various findings to the infectious model.

Studies have been conducted to characterize the illness occurring in yellow fever-infected rhesus monkeys relative to the relationship of inoculum dose to incubation period, early onset of clinical signs and length of illness. All monkeys were bled for preinoculation serum neutralization indices for preexisting antibody titers for yellow fever, and only those monkeys with insignificant titers were utilized. A range of yellow fever virus (Asibi strain) quantities, 0.001-1000 median mouse intracerebral lethal doses ($MiCiD_{50}$), was utilized in 28 monkeys. The titration is shown in Table 1.

Incubation is that period of time from subcutaneous (SC) inoculation to the initial upward deviation from the normal daily temperature pattern of each monkey. Length of illness is that period of time from the end of the incubation period to time of death.

The incubation and length of illness of the monkeys inoculated with 10 $MiCiD_{50}$ were not markedly different from those monkeys given 1000 $MiCiD_{50}$. However, when monkeys were inoculated with 1 $MiCiD_{50}$, the average incubation period was significantly longer than those above. The length of illness remained quite constant. With 0.1 $MiCiD_{50}$, the incubation period was lengthened still further; however, again the length of illness remained constant. Of the 3 monkeys inoculated with 0.01 $MiCiD_{50}$, 2 developed signs of disease with an average incubation period quite similar to that at the next higher dosage,

TABLE I. YELLOW FEVER IN THE RHESUS MONKEY

DOSE $MICLD_{50}$	NO. DEAD/ TOTAL	HOURS (RANGE) Incubation Period	Length of Illness	Time of Death
1000.0	3/3	60 (60)	40 (34-42)	100 (94-104)
10.0	9/9	67 (54-76)	44 (38-50)	111 (94-124)
1.0	4/4	90 (76-100)	40 (30-45)	130 (120-138)
0.1	4/4	186 (132-234)	43 (33-66)	224 (198-273)
0.01	2/3	182 (100-264)	39.5 (33-46)	222 (133-310)
0.001	0/5			

as well as the length of illness. The surviving monkey did not show clinical signs of the disease or any abnormal laboratory findings. We were unable to demonstrate any circulating virus or specific neutralizing antibody up to 44 days postinoculation. All 5 monkeys inoculated with 0.001 $MICLD_{50}$ remained clinically healthy. There was no evidence of viremia or neutralizing antibody.

From these data, 1 monkey $SCLD_{50}$ appears to be approximately equivalent to 0.01 $MICLD_{50}$. The lack of clinical signs or abnormal laboratory findings with no evidence of virus multiplication suggests that 1 LD_{50} in the rhesus monkey may be the minimal infective dose as well. Considering the limited sensitivity of the mouse inoculation assay of yellow fever virus, we must recognize the possibility of the absence of virus in the inocula at and below 0.01 $MICLD_{50}$. Also from these data, the incubation period in monkeys inoculated with small quantities of virus tends to be longer in duration than that observed in monkeys inoculated with larger doses. However, the disease is equally as fulminating as that occurring in animals given large infective doses.

The clinical illness of approximately 48 hr in duration in all infected monkeys appeared very similar in regard to all parameters measured, regardless of dosage. Since only the length of incubation was dose-dependent, we combined the data compiled during the clinical illness for the 22 monkeys

with disease. The results were evaluated at a number of hr preceding death rather than postinoculation. The average temperature curve of all 22 monkeys showed a rapid and marked elevation from normal, occurring at approximately 48 hr preceding death and reaching a peak fever temperature of about 105 F at 20 hr before death. A precipitous fall in body temperature occurred terminally in all infected monkeys, resulting in coma and death. Viremia was measured in 11 of the monkeys encompassing the entire range of dosages; 10 of 11 of these monkeys were viremic within 24 hr preceding the onset of fever. Thus, these results suggest that a majority of the monkeys were viremic at or before onset [illegible] the fever.

Total white blood cell (WBC) and differential counts were performed; these data showed a progressive fall in total count, beginning about 24 hr before the onset of fever. In mild cases reported in man, the leukopenia disappears rapidly, returning to normal; in severe cases, some undergo a transition to leukocytosis, with neutrophilia.[1] It is also reported that in the rhesus monkey, prior to death, some WBC counts rise to a higher level than can be explained by hemoconcentration, and show a predominance of neutrophils.[2] This terminal rebound of WBC was observed in 8 of 22 monkeys studied. The absolute lymphopenia and resultant relative neutrophilia did not become evident until well into the febrile stage of the disease. A gradual fall in hematocrit due to serial bloodletting was observed in all animals. An additional marked decrease in hematocrit was observed during the febrile stage of the disease; monkeys not developing disease did not show this marked fall, but continued the gradual decline in hematocrit, eventually stabilizing within a week postinoculation. Although occurring late in the clinical illness, 13-24 hr preceding death, a rise in lactic dehydrogenase (LDH) activity was dramatic.

The technique of cellulose acetate zone electrophoresis has been instituted in our laboratory recently and samples for LDH isoenzyme assay will be run in the near future. It is hoped that this technique will better elucidate sites of tissue damage and the effects on various organ systems. In addition to both serum and urine isoenzyme patterns, serum glycoprotein, serum and urine protein electrophoretic patterns will be studied.

Efforts are under way to infect rhesus monkeys with the virulent Asibi strain of the yellow fever virus at a standard challenge level of 10.0 $MICLD_{50}$ and to obtain prospective information concerning serial renal function studies and measurements of cardiac output and fluid volume spaces. Due to changes in experimental design and animal instrumentation, little definitive data have been produced as yet.

Summary, Part I:

Current evidence suggests that incubation is the only dose-dependent feature of yellow fever. The initial outward clinical sign of the disease in the rhesus monkey is a rapid and marked elevation in body temperature. In general, monkeys are viremic 0-24 hr preceding onset of fever and develop a significant progressive leukopenia. Both the fall in hematocrit and elevation in serum LDH activity occur late in the clinical illness. It appears that

1 monkey LD_{50} is approximately equal to 0.01 mouse $ICLD_{50}$. Furthermore, our evidence suggests that 1 LD_{50} in the rhesus monkey may be the minimal infective dose as well.

Progress, Part II:

There have been only a few electron microscopic studies of yellow fever virus in the literature with numerous conflicting results. In 1953,[3] the first identification of the virus with the electron microscope was done in [illegible] fluid suspension from 17-D vaccine strain-infected mouse brains and spinal cords. Spherical particles 50-55 mμ in diameter, without internal structure, were reported. In 1960,[4,5] extensive studies were carried out on the histopathology and ultrastructural changes in Asibi strain-infected monkey hepatocytes. Spherical particles 55-61 mμ in diameter, without internal structure, were described with concomitant ultrastructural changes in liver cells. In another study,[6] KB tissue culture cells, infected with Asibi strain yellow fever, contained spherical particles, without internal structure, 25-27 mμ in diameter. In studies done on 17-D-infected mouse brains[7] and spinal cords,[8] 38-mμ spherical particles with dense cores surrounded by a membrane were described. The particles were found only in astrocytes in brain tissue, and in neurons and microglial cells in spinal cord. In a Fort Detrick study,[9] immunofluorescence and electron microscopy were used to study tissue culture cells and cynmologous monkey liver cells infected with the Asibi strain. No viral particles or ultrastructural changes could be visualized in monkey hepatocytes; however, some viral antigen was present in low titer. Spherical particles 42 mμ in diameter, with dense cores and limiting membranes, were noted within endoplasmic reticulum of tissue culture cells.

At USAMRIID, Swiss white mice and 17-D vaccine strain yellow fever virus were used as the experimental animal and agent respectively. Groups of mice at varying ages were inoculated by several different routes; it was determined that 18-21-day-old suckling mice had the most consistent mortality, maximum virus titers, and consistent pathological lesions. The IC route of inoculation was the only one resulting in consistent mortality.

Simple, useful techniques for collecting samples and subsequently processing them were developed. A rapid, relatively artifact-free procedure for removal of mouse brain and spinal cord was perfected. Several different fixatives and buffer systems were tested, and it was found that 3% glutaraldehyde and phosphate buffer resulted in the best fixation for electron microscopy. Standard epoxy resin embedding and sectioning were done for electron microscope specimens. Immunofluorescence studies were conducted according to standard procedures. Yellow fever antiserum was produced and conjugated by usual techniques in conjunction with the Virology Division, USAMRIID.

Brain, spinal cord and adrenal glands were collected from representative infected and control mice. The samples were fixed and processed for histopathology, electron and fluorescence microscopy, and virus titration. Virus titration was performed by animal inoculation and titers of 10^4-10^7 were found in brain and cord tissue, there being no significant difference between

them. No virus was found in adrenal glands; they were subsequently eliminated from the study.

Several important histological alterations have been observed consistently in infected mice. The most prominent is a diffuse to multifocal neuronal and glial cell necrosis in all layers of the cerebrum, but especially in deep laminar areas of cerebral cortex. Accompanying this is a diffuse to multifocal vasculitis of the cerebrum and leptomeninges. Vasculitis is nearly always noted in areas of necrosis. In hippocampal neurons, so-called "classical segmental necrosis" is often seen, suggestive of ischemic necrosis of vascular origin. Twenty per cent of spinal cords showed vasculitis and necrosis. No inclusion bodies were seen.

Immunofluorescent studies were conducted on central nervous tissue of infected and control mice. Monkey yellow fever antisera were conjugated with fluorescein and tested for specificity on yellow fever-infected tissue culture cells. Positive staining of frozen brain and cord tissue was most prevalent in the areas of pathological change. Hippocampal neurons and deep cerebral gray matter were consistently positive for yellow fever antigen. Vessels were occasionally stained, especially in deep cerebral laminae. Spinal cord occasionally stained in neurons and small vessels. Cerebellum and white matter very rarely stained.

Despite extensive examination of brain and cord tissue, yellow fever virus has not been visualized with the electron microscope. The above retrospective immunofluorescent studies have shown that this was probably due to errors in sampling of tissue. With the knowledge of actual location of viral antigen, studies are now in progress attempting to visualize yellow fever virus. Ultrastructural changes in infected mice have been observed, but results are preliminary thus far.

Summary, Part II:

Various techniques for sample collection, histopathology, fluorescence and electron microscopy were investigated and employed in studies of yellow fever infection of mouse central nervous system. Histopathological lesions were prominent and consistent in cerebral gray matter, hippocampal neurons, and their accompanying vessells. Immunofluorescence staining was positive for yellow fever antigen in hippocampal neurons, cerebral gray matter, and occasional vessels. Electron microscopic studies are continuing, but thus far are inconclusive.

Publications:

Chapple, F. E., III, J. M. Crosbie, and B. E. Reisberg. 1971. Surgical technic for cross-circulation of rhesus monkeys. Lab. Anim. Sci. 21:610-612.

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ANNUAL REPORT

Project No. 1W662711A096:	**Medical Defense Aspects of Biological Agents (U)**
Task No. 1W662711A096 02:	**Prevention and Treatment of Biological Agent Casualties**
Work Unit No. 096 02 411:	**Evaluation of Promising Compounds for Antiviral Use Against Diseases of Medical Importance to the Military**
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Animal Assessment
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Authors:	**Richard O. Spertzel, Lt Colonel, VC** **Gerald L. Ruch, Captain, VC**
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OL0907	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA: CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	02	411
b. COMPETITIVE	62711A	1B662711A096		
c. COMPETITIVE	CDOG 1212b(9)			

11. (U) Evaluation of promising compounds for antiviral use against diseases of medical importance to the military

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical medicine; 004900 Defense; 010100 Microbiology; 001700 Animal husbandry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
70 12	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER: NA
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	h. PROFESSIONAL MAN YRS	h. FUNDS (In thousands)
FISCAL YEAR PRECEDING 71	1.5	10
FISCAL YEAR CURRENT 72	1.0	62

19. RESPONSIBLE DOD ORGANIZATION
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SOCIAL SECURITY ACCOUNT NUMBER:

21. GENERAL USE
Foreign intelligence considered

ASSOCIATE INVESTIGATORS
NAME: Ruch, G. L.
NAME: DA

22. KEYWORDS (Optional Keywords Followed by Security Classification Code)
(U) Therapy; (U) Virus diseases; (U) Tissue culture; (U) Laboratory animals; (U) Military medicine

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text or N with Security Classification Code.)

23 (U) Evaluate chemical compounds for treatment and control of virus diseases of importance to the military. This work unit is an essential element in a comprehensive program for medical defense against BW agents.

24 (U) Test chemicals in tissue culture and laboratory animals against selected viruses.

25 (U) 71 07 - 72 06 - A synthetic poly I-poly C, when inoculated IV into rhesus monkeys which were later infected with Asibi strain of yellow fever, produced an effect similar to lowering the infecting dose of the virus. Incubation period was lengthened, while the clinical illness remained unchanged. The microtitration technique to run serum and plaque neutralization tests is now a useful laboratory tool to test serial samples, as well as evaluating antiviral chemical agents for their possible antiarbovirus activities.

Isatin beta thiosemicarbazone was shown to be ineffective as a chemotherapeutic agent for Venezuelan equine encephalomyelitis in the dose tried, using the plaque-neutralization technique. Additional antiviral compounds will be evaluated in the near future, as well as some additional studies with poly I-poly C.

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BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 02: Prevention and Treatment of Biological Agent Casualties

Work Unit No. 096 02 411: Evaluation of Promising Compounds for Antiviral Use Against Diseases of Medical Importance to the Military

Description:

Evaluate chemical compounds for treatment and control of virus diseases.

Progress:

During the past year, limited progress has been made in this area due to the change-over in investigators. Much time has been devoted to becoming familiar with the research plan as it exists and the techniques needed to carry it out.

The ability of a synthetic polyribonucleotide (poly I:C) to alter the course of Simian yellow fever was investigated. Initially, 2 monkeys were inoculated intravenously (IV) with 0.5 mg/μg of poly I:C; 2 doses, spaced 24 hr apart, were given. Transient hyperthemia followed each administration of poly I:C although it was more pronounced after the 2nd inoculation. Within 1½ hr of the 2nd dose of poly I:C, the body temperature of one monkey increased from 101.2 to 104.8 F; the temperature of the 2nd animal rose from 101.5 to 104.0 F. These temperatures were within normal range 6 hr later.

Two monkeys were treated with 0.5 mg/μg poly I:C, IV, ½ hr before they were administered a subcutaneous inoculation of 10 median mouse intracerebral lethal doses ($MICLD_{50}$) of Asibi yellow fever virus. A 2nd dose of poly I:C was given 24 hr following virus inoculation. One of the monkeys (B-115) had been dosed with poly I:C on days -6 and -5 before virus inoculation. The other (A-964) had not received poly I:C until the day of virus inoculation.

Two untreated monkeys died between 132 and 144 hr postinoculation with 10 $MICLD_{50}$ of Asibi virus. [A mean survival time of 138 hr has been observed in 8 other monkeys receiving similar doses of virus (Work Unit 096 02 410).] One of the poly I:C-treated animals (B-115) died 146 hr after virus inoculation. The 2nd (A-964) survived for 216 hr.

Poly I:C administration appears to have the same effect as lowering the virus dose, i.e., prolongation of the incubation period of the disease. The length and severity of the clinical illness are unaffected.

Further experimentation seems warranted to ascertain the poly I:C dosage scheme producing the most beneficial results. Poly I:C and perhaps other synthetic interferon inducers could play a role in antiviral chemotherapy by prolonging the period of incubation and reducing the number of virus infectious units for a period of time so that a chemotherapeutic agent may effectively limit or stop the clinical disease.

Microtitration methods for serum neutralization tests by plaque neutralization have been standardized. The method employs a constant-virus varying-serum dilution technique. We have been able to obtain consistent plaque formation with known concentrations of live, attenuated Venezuelan equine encephalomyelitis (VEE) virus (TC-83) vaccine strain on VERO cells. In addition, we can accurately detect serum antibody levels by the plaque neutralization technique (PNT). This test was necessary if serial antibody responses of laboratory animals were to be monitored. It may also be a helpful tool in in vitro studies of chemotherapeutic agents to evaluate their direct effects on certain viruses by measuring any plaque-reducing capabilities of the drug.

Several of the evaluation studies were conducted to determine if Isatin β-thiosemicarbazone (IBT) had any effect on TC-83 infection as measured by PNT. These studies were done primarily to gain familiarity with study techniques. The VERO cell cultures were inoculated with 100 plaque-forming units (PFU) per well and various dilutions of IBT in different combinations and time sequences. The doses of IBT were taken from Appleyard et al.'s study[1] of the effects of IBT on pox virus. IBT, in doses of 0.5, 2, 4, and 8 mg/ml, dissolved in acetone and diluted in Earle's Medium 199, was inoculated into VERO cell cultures; 4 groups of wells were used. In Group 1, the IBT dilutions were added, followed, after 1 hr incubation at 35 F, by TC-83 at 100 PFU/well; in Group 2, IBT dilutions were followed by TC-83 immediately at 100 PFU/well; in Group 3, 100 PFU/well of TC-83 were followed immediately by IBT dilutions; and in Group 4, 100 PFU/well of TC-83 were followed, after 3 hr of incubation at 35 F, by IBT dilutions. There was no significant reduction of plaques in any of the wells of the 4 groups. Another study using 25 and 100 mg/ml of IBT was run with no significant reduction of plaques in any of the wells.

It appears that IBT has no effect on TC-83 virus replication in VERO cells, at least at the doses used.

PNT is a useful tool for in-vitro evaluation of chemical agents for their possible antiviral activity, and additional compounds will be tested using this method.

Summary:

A synthetic poly I:C, when inoculated IV into rhesus monkeys which were later infected with Asibi strain of yellow fever, produced an effect similar to lowering the infecting dose of the virus. The incubation period was lengthened, while the clinical illness remained unchanged.

The microtitration technique to run serum and plaque neutralization is useful to test serial serum samples for antibody titers, as well as evaluating antiviral chemical agents for their possible antiarbovirus activities. IBT was shown to be ineffective as a chemotherapeutic agent for VEE in the doses tried, using the plaque-neutralization technique.

More chemical agents will be tested in the near future, as well as some additional studies with poly I:C to determine approximately how much virus can be eliminated by its use.

Attempts to plaque our Asibi strain of yellow fever virus are now in progress to expand the evaluation of promising antiviral agents.

Publications:

None.

LITERATURE CITED

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ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1W662711A096 02:	Prevention and Treatment of Biological Agent Casualties
Work Unit No. 096 02 412:	Develop Serological Methods for Military Vaccine Evaluation
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Virology
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Author:	Helen H. Ramsburg
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 01.0913	72 06 30	DD DRAG AN-R-16

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DOD'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	02	412
b. [illegible]	62711A	1B662711A096		
c. [illegible]	CDOG 1212b(9)			

11. TITLE (Provide with Security Classification Code)

(U) Develop serological methods for military vaccine evaluation

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical medicine; 004900 Defense; 010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 05	CONT	DA	C. In-house

17. CONTRACT GRANT

a. DATES/EFFECTIVE: EXPIRATION

b. NUMBER: NA

c. TYPE: d. AMOUNT

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 71	0.5	10
FISCAL YEAR CURRENT 72	1.0	110

19. RESPONSIBLE DOD ORGANIZATION

NAME: USA Medical Research Institute of Infectious Diseases

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RESPONSIBLE INDIVIDUAL

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20. PERFORMING ORGANIZATION

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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

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SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME:

NAME: DA

21. GENERAL USE

Foreign intelligence considered

22. KEY WORDS (Provide EACH with Security Classification Code)

(U) Immunity; (U) Serology; (U) Tissue culture; (U) Plaque reduction; (U) Military medicine

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with security classification code.)

23 (U) Develop a neutralization test in tissue culture which would be more sensitive and less costly to perform than the current mouse test. This work unit is an essential element in a comprehensive program for medical defense against BW agents.

24 (U) After selecting a cell-line, tests will be performed with various viruses to determine the optimal conditions for each.

25 (U) 71 07 - 72 06 - Tests were carried out using the VERO cell line to determine the optimal conditions for performing plaque reduction (PR) serum neutralization (SN) tests. Two PR-SN tests were developed and compared to the mouse SN test. The PR-SN tests were found to be less costly, less time consuming, and more sensitive than the current mouse SN test. The serum dilution method was found to afford more advantages than the undiluted serum method. An evaluation of certain of these advantages is still in progress.

Terminated since FY 1973 funding will be under The Surgeon General, Army. New Project No. 3A062110A834; Accession No. DA 0B6424.

*Available to contractors only when originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE AND MAY BE USED UNTIL EXHAUSTED

BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 02: Prevention and Treatment of Biological Agent Casualties

Work Unit No. 096 02 412: Develop Serological Methods for Military Vaccine Evaluation

Description:

Develop neutralization tests in tissue culture which would be more sensitive and less costly to perform than the current mouse test.

Progress:

Studies were initiated to standardize a plaque reduction (PR) test for the detection of neutralizing antibodies formed in response to the administration of arbovirus vaccines.

Each of the 6 dishes in 35 mm plastic disposo-trays (Linbro Chemical Co., New Haven, Conn.) was seeded with 2.0 ml of a suspension of VERO cells[1] (250,000 cells/ml) made up in medium 199-Earle's base (199-E) containing 5% fetal bovine serum and 100 units of penicillin (P) and 50 µg of streptomycin (S) per ml. The monolayers were confluent by 3 days.

Five diluents were compared for their effect on the monolayers and on the virus titer: (1) phosphate buffered saline, pH 7.4, containing 1% inactivated normal rabbit serum and P and S; (2) 2% beef heart infusion broth; (3) medium 199 - Hank's base (199-H) containing P and S and 0.25% human serum albumin (HSA); (4) medium 199-E containing P and S and HSA; and (5) balanced salt solution - Hank's base containing P and S and HSA buffered with Hepes (10 mM), pH 7.3 (BSS-He). No. 1 was cytotoxic, whereas the other diluents were not. Plaques were uniform in size and shape for all diluents except no. 2. A fluctuation in pH occurred using 199-E and 199-H which was reflected in a variation in the virus titers. Results showed that BSS-He was the most satisfactory of the diluents.

Three eastern equine encephalitis (EEE) virus preparations were selected for testing: (1) a 20% mouse brain suspension of the Cambridge strain used in performing the serum neutralization test in suckling mice; (2) a 50% chick embryo suspension of the PE-6 strain used for vaccine production; and (3) a suspension of the PE-6 strain prepared in VERO cells. Serial $\log_{10}$ dilutions of the virus preparations were made and titrated. The mouse brain suspension was cytotoxic when lower dilutions were tested in the presence of immune serum. The suspension prepared in VERO cells

varied in titer from test to test with a significant drop over a period of 6 mon. The chick embryo preparation has remained stable over a period of 4 yr and is not cytotoxic.

Established procedures were used for inoculating the monolayers and adsorbing the virus: 3 replicate dishes were inoculated with 0.2 ml of each preparation tested, followed by adsorption for 1 hr at 36 C.

Three different volumes of overlay were compared for their effect on the monolayers and on the virus titer. Results of tests using 2.0, 3.0, and 4.0 ml overlay showed that maximum virus titer was obtained with 3.0 ml. There were no differences in the effect on the monolayers. Three separate plaquing conditions were tested: (1) 1% agarose in medium 199 containing P and S and in a 5% CO_2 atmosphere; (2) the same medium in a standard incubator utilizing sealed trays; and (3) 1% agarose in L-15 medium containing P and S in a standard incubator. In each of the procedures described, 1-mm plaques formed in 3 days under 3.0 mlof overlay at a temperature of 36 C and a pH of 7.3.

The counting of plaques was facilitated by staining the viable cells in the monolayers with a 1:7,800 dilution of neutral red prepared in BSS-He. This diluent was more satisfactory than either Saline A or BSS-Earle's base because its pH remained constant. A 1:9,000 dilution of neutral red was found to be less effective. Two-ml volumes were used as a matter of convenience and placed in each dish of the trays which were incubated for several hours at 36 C, after which time the stain was poured off. The trays were then placed in a 4 C refrigerator overnight; plaques were counted the following day. Refrigeration not only prevented an increase in plaque size but it also allowed maximum staining to take place providing well defined plaques that could be quickly and easily counted.

Plaque reduction serum neutralization (PR-SN) tests were performed using the test procedures indicated above. The serum samples tested were obtained from an EEE-vaccine study project in volunteers (Medical Division Protocol No. 71-5). Sixteen individuals were bled prior to and on days 7, 14, 28, 35, 42, 56, and 90 after vaccination. Serum pools from each day's bleeding except day 90, were prepared using 0.5 ml of serum from each volunteer. The remainder of each serum sample was stored at -30 C for future testing. Each pool was tested for its ability to reduce plaque formation in the fresh state, after freezing and thawing, and following heat-inactivation at 60 C for 30 min. Serial $\log_{10}$ dilutions of virus were prepared and added to equal volumes of each serum pool. Incubation of the virus-serum mixtures was carried out at 37 C for 1 hr followed by inoculation onto the monolayers. The results of these tests are presented in Table 1. No significant differences in titers between the fresh and frozen serum pools are seen. However, significant differences are seen in titers when the heat-inactivated serum pools are compared to the fresh and frozen serum pools. These differences occur beginning on day 14 postvaccination at the time antibody formation begins to appear.

The PR tests on the individual serum samples were done in duplicate using the constant serum-varying virus method noted above and using the constant virus-varying serum method performed as follows: 12 serial 2-fold dilutions of each serum sample were prepared starting with a 1:10 dilution. An equal volume of virus suspension diluted to contain approximately 100 PFU was added to each serum dilution. The remaining test procedures performed were the same as for the constant serum method.

Sera were inactivated with heat for the constant serum method since results using serum that was not so treated were inconsistent. Heat treatment was unnecessary for the varying serum method. The titers obtained using these 2 methods were compared to the titers obtained using the suckling mouse serum neutralization (SM-SN) test. SM-SN tests were performed with heat-inactivated sera using the constant serum-varying virus routine procedures developed in this laboratory. One of these procedures, the incubation of serum-virus mixtures overnight at 4 C in the presence of complement (C'), is a matter of convenience. Results do not differ from those obtained with serum-virus mixtures incubated at 37 C for 1 hr without C'. In the routine performance of PR-SN tests, the serum-virus mixtures are incubated at 37 C for 1 hr, again, a matter of convenience. Results obtained with serum-virus mixtures incubated overnight at 4 C with or without C' were comparable.

The SN indices, expressed as $\log_{10}$ values, represent the difference in titer between the pre- and postvaccination serum samples using the constant serum method. SN titers, expressed as $\log_{10}$ values, represent that dilution of serum causing a 50% reduction in the number of plaque forming units used in that particular test. The results of these tests are presented in Table II. The indices and titers differ for each test, which is to be expected since 3 different SN tests were used. The highest titers were obtained using the varying serum PR test. Indices 0.3 of a log lower were obtained using the mouse test. Indices using the constant serum PR test were 1 log lower than the titers obtained with the varying serum PR test. Nevertheless, these results may be used to compare the mouse test with the 2 PR tests since they were found by statistical analysis to be well correlated. A linear regression was calculated relating the mouse SN index (X) to the constant serum PR-SN index (Y) of 128 sera, using the method of least squares a regression line was also calculated relating the SM-SN index (X) to the varying serum PR titer (Y) of the same 128 sera. Based on linear regression, an X value of 1.7 (the accepted lower limit for a "positive" serum) correlated with a value of 1.0 for Y, using the constant serum method and a value of 2.0, using the varying serum method. For a value of 1.7 for X, the 95% confidence limits for Y = 1.0158 were 0.9757 and 1.0559 and for Y = 2.0257 were 1.8984 and 2.1530. The relationship between the indices and titers for these 3 tests is depicted in Table III. The values shown for the PR-SN tests represent averages; therefore, the scale shown should not be used for direct extrapolation.

TABLE I. COMPARISON OF RESULTS OF PLAQUE REDUCTION TESTS USING FRESH, FROZEN, AND HEAT-INACTIVATED SERUM POOLS

DAY POST VACCINATION	TITER LOG_{10}			
	No serum	Serum		
		Fresh	Frozen	Heat-Inactivated
0	8.6	n.d.	7.0	7.3
7	8.4	7.3	7.3	7.4
14	8.6	6.0	6.0	7.1
28	8.5	4.8	5.1	6.8
35	8.6	4.5	4.4	6.4
42	9.1	4.6	4.5	6.6
56	9.1	4.4	4.4	6.7

n.d. = not done

TABLE II. COMPARISON OF RESULTS USING TWO METHODS FOR PERFORMING PR-SN TESTS AND THE SM-SN TEST

DAY POSTVACCINATION	INDEX[a]		TITER[b]
	SM-SN log_{10}	Constant Serum PR-SN log_{10}	Varying Serum PR-SN log_{10}
0	0.0	0.0	0.5
7	0.3	0.1	0.6
14	0.8	0.7	1.5
28	0.9	0.7	1.4
35	1.9	1.2	2.3
42	2.1	1.2	2.5
56	1.9	1.1	2.2
90	1.6	0.9	1.8

a. Difference in titer between the pre- and postvaccination serum samples.
b. 50% reduction in the number of PFU used in the test.

TABLE III. SCALE OF PR-SN INDICES AND TITERS IN RELATION TO SM-SN INDICES

SCALE LOG_{10}

PR SN Index	Mouse SN Index	PR SN Titer
1.9	3.5	3.9
		3.5
	3.0	
1.6		
		3.0
1.4	2.5	
		2.5
1.2		
	2.0	
1.0	1.7	2.0
	1.5	
0.8		
		1.5
	1.0	
0.6		
		1.0
0.4	0.5	
		0.5
0.2		
	0.0	
0.0		0.0

The SN indices of 1.9 and 3.5 logs, and the SN titer of 3.9 logs, represent the actual peak values obtained for each of the 3 SN tests. The 0.0 levels for both PR-SN tests were found to be lower than the 0.0 level for the SM-SN test. This finding was reflected in the fact that low levels of antibody which were not detected using the SM-SN test were shown to be present using both of the PR-SN tests.

Summary:

Two PR-SN tests were compared to the SM-SN test. These PR tests were performed for 1/6 the cost and in 1/2 the time of the mouse test. These same 2 tests were also found to be more sensitive, in that low levels of specific antibody were detected with them which were not detected in the SM-SN test.

Of the 2 PR-SN tests, the varying serum method affords more advantages, most importantly, sera may be tested individually instead of in pairs or groups. This is possible since the serum titer is determined by the ability of the dilutions of serum to reduce 50% of the viral plaques present in the control monolayers. Other advantages which became apparent in the course of these are currently being evaluated.

Publications:

None

LITERATURE CITED

1. Stem, T. 1969. Arbovirus plaquing in two simian kidney cell lines. J. Gen. Virol. 5:329-338.

ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1W662711A096 02:	Prevention and Treatment of Biological Warfare Casualties
Work Unit No. 096 02 800:	Development of a Polyvalent Staphylococcus aureus Toxoid
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland 21701
Division:	Pathology
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Authors:	Joseph F. Metzger, Colonel, MC Anna D. Johnson
Reports Control Symbol:	RCS-MEDDH-288 (R1)
Security Classification:	UNCLASSIFIED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 01.0895	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA: CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO /CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	02	800
b. COMPETITIVE	62711A	1B662711A096		
c. COMPETITIVE	GDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)

(U) Development of a polyvalent staphylococcus aureus toxoid for protection against an illness of military importance

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical medicine; 004900 Defense; 016800 Toxicology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
70 04	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER: NA

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 71	1.0	50
FISCAL YEAR CURRENT 72	1.0	39

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Johnson, A. D. DA

NAME:

21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Enterotoxin; (U) Staphylococcus aureus; (U) Toxoid; (U) Military medicine

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Develop a polyvalent toxoid which would include Staphylococcus aureus enterotoxins A, B, C, and D and other exoproteins. This work unit is an essential element in a comprehensive program for medical defense against BW agents.

24 (U) Preparation of a polyvalent toxoid of enterotoxins A, B, C, and D at a concentration of 500 micrograms per milliliter of each toxoid.

25 (U) 71 07 - 72 06 - Gram amounts of enterotoxins A, B, and C (SEA, SEB, and SEC) have been prepared. Each toxin will be formalin toxoided and mixed only after each toxoid preparation passes all safety tests. SEA utilized in previous studies was prepared by Schantz and had an oral median illness dose (ID-50) of 40 micrograms/kg. This material was impure by electrofocusing and electrophoresis. Modification of Scantz's procedure has resulted in preparation of a highly purified enterotoxin A which has an oral ID-50 of 4 micrograms/kg. A D enterotoxin has been prepared and purified.

Publication: Biochim. Biophys. Acta 254:183-186, 1972.

Terminated since FY 1973 funding will be under The Surgeon General, Army. New Project No. 3A062110A834; Accession No. DA OB6425.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498.1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

BODY OF REPORT

Project No. 1W662706A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662706A096 02: Prevention and Treatment of Biological Warfare Casualties

Work Unit No. 096 [illegible]: Development of a Polyvalent Staphylococcal Enterotoxin Toxoid

Objective:

To produce a polyvalent toxoid which would include Staphylococcal enterotoxins B, C and D and other exoproteins.

Progress:

The production of highly purified staphylococcal enterotoxin B (SEB) was facilitated by alteration of the pH of the eluting buffer of the first CG-50 chromatography. This modification procedure of Schantz et al.[1] increases both yield and purity of the SEB preparation. Approximately 10 gm can be prepared from a single 50-L fermenter run utilizing NAK-yeast extract media with 0.2% glucose. No CO_2 aeration is necessary.

A method for production of enterotoxin C_1 in gram amounts was developed. Complete immunological cross reactivity was demonstrated between C_1, C_2, and C_3 and, therefore, only C_1 will be included in the polyvalent toxoid.

A method for production of enterotoxin A (SEA) was developed yielding 60-100 µg/ml of crude culture filtrate from mixed-bed resin permitted better attachment to CG-50. CM cellulose chromatography and hydroxyapatite were performed by the method of Schantz et al.[1] with slight modification. The purity of the SEA was considerably better than the original material, which had some enterotoxin present, the median illness dose was 4 µg/kg vs. 40 µg/kg for the original material.

Enterotoxin D (SED) was prepared from strain 07-0-0 since the original strain was found to contain an enterotoxin which we have designated C_3. SED was prepared in small amounts by desalting the crude broth supernate and absorbing the toxin on CG-50 at pH 6.2. This material was dialyzed against H_2O and electrofocused on a pH 3-10 ampholine [illegible] protein peak at pH 9.2-9.5 reacts only with SED antiserum (supplied by Dr. Bennett, Food and Drug Administration).

Summary:

Gram amounts of staphylococcal enterotoxins A, B, and C_1 have been prepared. Toxoiding with formalin will be accomplished in the near future. Definitive identification of enterotoxin D has been accomplished. A new enterotoxin C_3 was found to be produced by the prototype strain of D (ATCC 23235).

Metzger, J. F., A. D. Johnson, and W. S. Collins, II. 1972. Fractionation and purification of Staphylococcus aureus enterotoxin B by isoelectrofocusing. Biochim. Biophys. Acta 257:183-186.

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1. Schantz, E. J., W. G. Roessler, J. Wagman, L. Spero, D. A. Dunnery, and M. S. Bergdoll. 1965. Purification of staphylococcal enterotoxin B. Biochemistry 4:1011-1016.

2. Schantz, E. J., W. G. Roessler, M. J. Woodburn, J. M. Lynch, H. M. Jacoby, S. J. Silverman, J. C. Gorman, and L. Spero. 1972. Purification and some chemical and physical properties of staphylococcal enterotoxin A. Biochemistry 11:360-366.

3. Casman, E. P., R. W. Bennett, A. E. Dorsey, and J. A. Issa. 1967. Identification of a fourth staphylococcal enterotoxin D. J. Bacteriol. 94:1875-1882.

ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1W662711A096 02:	Prevention and Treatment of Biological Warfare Casualties
Work Unit No. 096 02 801:	Effects of Staphylococcal Enterotoxin B on Lymphoid Cells *in vitro*
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland 21701
Division:	Pathology
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Author:	William H. Adler, Major, MC
Reports Control Symbol:	RCS-MEDDH-288 (R1)
Security Classification:	UNCLASSIFIED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OL0908	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. SMRY'N MNTH'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	02	801
b. CONTRIBUTING	62711A	1B662711A096		
c. CONTRIBUTING	CDOG 1212b(5)			

11. TITLE (Precede with Security Classification Code)

(U) Effects of staphylococcal enterotoxin B on lymphoid cells in vitro in BW defense

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical medicine; 004900 Defense; 010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
70 12	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA — EXPIRATION:
b. NUMBER:
c. TYPE: — d. AMOUNT:
e. KIND OF AWARD: — f. CUM. AMT.

18. RESOURCES ESTIMATE	g. PROFESSIONAL MAN YRS	h. FUNDS (In thousands)
FISCAL YEAR — PRECEDING 71	1.0	55
FISCAL YEAR — CURRENT 72	1.0	68

19. RESPONSIBLE DOD ORGANIZATION

NAME: USA Medical Research Institute of Infectious Diseases
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20. PERFORMING ORGANIZATION

NAME: Pathology Division USAMRIID
ADDRESS: Fort Detrick, MD 21701

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME:
NAME:

21. GENERAL USE

Foreign intelligence considered — DA

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Enterotoxin; (U) Staphylococcus aureus; (U) Lymphoid cells; (U) Pathogenesis; (U) Cell culture; (U) Military medicine

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Investigate interaction of staphylococcal enterotoxin B (SEB) with lymphoid cells and determine its significance. This work unit is an essential element in a comprehensive program for medical defense against BW agents.

24 (U) Short term lymphoid cell culture allows the study of the action of SEB on isolated cell population and a detailed analysis of the intracellular events triggered by SEB.

25 (U) 71 07 - 72 06 - The types of cells which can be induced into mitotic cycles by SEB have been determined. The initial events of its interaction with the cell membrane have been studied both by following the uptake of SEB by the cells and by studying membrane enzyme changes after exposure of cells to SEB. No direct evidence exists to show that SEB enters the cell which it then can cause to divide, nor is there evidence that SEB can cause changes in membrane enzyme activity as has been reported to occur with cholera enterotoxin.

The specific mitogenic effects of SEB are probably not related to its toxicity, but its rapid interaction with cell membranes may be essential to our understanding of its toxic effects.

Terminated since FY 1973 funding will be under The Surgeon General, Army. New Project No. 3A062110A834; Accession No. DA 0B6426.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 — PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A (1 NOV 68) AND 1498-1 (1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 02: Prevention and Treatment of Biological Warfare Casualties

Work Unit No. 096 02 801: Effects of Staphylococcal Enterotoxin B on Lymphoid Cells *in vitro*

[illegible].

[illegible] the action of staphylococcal enterotoxin B with lymphoid [illegible] and determine its significance.

[illegible].

The actions of the staphylococcal enterotoxins on the whole animal have been well established.[1] The localization of enterotoxin B in experimental animals has been described[1] but the localization has not been firmly linked to the mechanism of toxicity.[2] The studies that were carried out with the staphylococcal enterotoxins, in particular enterotoxin B (SEB), were designed to trace the effect of the toxins on isolated cell population using *in vitro* tissue culture techniques.

It was found, that a variety of lymphoid cells *in vitro*, mouse spleen, thymus and bone marrow cells and human peripheral blood lymphocytes would be stimulated to undergo division cycles after a brief exposure to SEB. It appeared from the types and numbers of cells stimulated by SEB that it was causing a mitogenic stimulation in some nonspecific fashion. Besides the mitogenic effect of SEB, it was found that enterotoxin A and to a lesser extent enterotoxin C were also mitogenic stimuli. Even very purified preparations of these toxins were able to stimulate the cells. The ability of the toxins to stimulate cells did not depend on a previous exposure of the donor animal to the toxin. Immunization of the cell donor animal did not result in a change in *in vitro* reactivity of the animals' cells of SEB. Furthermore, the amount of anti-SEB antibody in the serum of 90 humans did not correlate with the extent of SEB induced *in vitro* stimulation of their peripheral blood lymphocytes. In several experiments, it was found that a nontoxic toxoid of SEB which retained antigenic characteristics similar to the toxin could also act as a cell mitogen.

Besides the ability of the enterotoxins to induce lymphoid cell mitosis, it was seen that the toxins could induce the cells to produce various substances which in turn could affect other types of cells which were not affected directly by the toxin. For example, L cells or mouse fibroblasts showed no cytopathic effects when exposed to enterotoxin B. However, if lymphoid cells plus SEB were incubated with L cells or fibroblasts for 24 hr, the L cells and fibroblasts were destroyed. Furthermore, if the tissue

culture media from lymphoid cell cultures stimulated with SEB was put on the L cells or fibroblasts, the same pattern of destruction was seen.

The mitogenic effect of the SEB was found to require only a brief exposure of the lymphocytes to SEB, about 10 min in some experiments. After 10 min the cells could be washed and all traces of SEB removed, but the cells still entered mitotic cycles. Using radiolabeled SEB and the techniques of autoradiography, it was impossible to show that the SEB was incorporated into the lymphoid cells which it affected. Other cell mitogens have been shown to be incorporated into the affected cells, and that a specific membrane [illegible] port system exists which allows this interaction. Other techniques were employed to investigate this latter observation. Using a sensitive assay system to detect SEB in cell culture supernates, it was found that after exposure of SEB to lymphoid cells there was a rapid drop in the detectable SEB in the supernate of the culture; however, after another brief time period the detectable SEB increased in the tissue culture media; at the end of 2 hr incubation the original amount of SEB which had been added to the culture could again be found present in the media and not cell bound. Therefore, it appears that SEB acts somewhat like an enzyme, rapidly attaching to the substrate on the cell surface and after interacting with the surface, detaching; it can then be found in the media.

A series of experiments were performed in an attempt to elucidate the connection between the cell surface effect of SEB and the intracellular events initiated by SEB which result in cell division. These studies, with Major DeRubertis, centered on the measurement of the adenyl cyclase activity in a cell population which was exposed to various substances. The assay system was shown to be sensitive enough to pick up the cyclase activity changes induced by low levels of prostaglandins. As yet no significant effect on adenyl cyclase activity or cyclic AMP levels has been shown to be a property of SEB. It may be that the SEB will be shown to decrease the cyclase activity, but the significance of these preliminary results is unknown at present and is the subject for future investigations.

Summary:

Staphylococcal enterotoxins are potent lymphocyte mitogens. Their other effects on lymphocytes are to induce the release of toxic substances and allow the lymphocyte to destroy other tissue culture cell lines. However, these effects of enterotoxins on lymphoid tissue are probably not the mechanism of toxicity in the whole animal, because (1) the amount of SEB needed to effect the lymphoid tissue is more than that needed to produce toxic effects *in vivo*, (2) the time needed to produce effects on lymphoid tissue is more than that needed to produce toxic effects *in vivo*, and (3) toxoid also serves as a mitogenic stimulus but is nontoxic *in vivo*. SEB exerts its effects on lymphocytes by a rapid interaction with the cell membrane, analogous to an enzyme effect on a substrate. The intracellular events triggered by this membrane interaction are not yet known.

Publications:

None.

LITERATURE CITED

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ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1W662711A096 02:	Prevention and Treatment of Biological Warfare Casualties
Work Unit No. 096 02 802:	Chemical Modification of Microbial Protein Antigens
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland 21701
Division:	Pathology
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Authors:	John R. Warren, Major, MC
Reports Control Symbol:	RCS-MEDDH-288 (R1)
Security Classification:	UNCLASSIFIED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0L0917	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. PRGM INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
72 02 02	H. TERMINATION	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	02	802
b. [illegible]	62711A	1B662711A096		
c. [illegible]	GDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)

(U) Chemical modification of microbial protein antigens

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical medicine; 004900 Defense; 002300 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 07	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER: NA

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	PRECEDING FISCAL YEAR	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
	71	0	0
	CURRENT 72	2.0	20

19. RESPONSIBLE DOD ORGANIZATION

NAME: USA Medical Research Institute of Infectious Diseases

ADDRESS: Fort Detrick, MD 21701

RESPONSIBLE INDIVIDUAL

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20. PERFORMING ORGANIZATION

NAME: Pathology Division USAMRIID

ADDRESS: Fort Detrick, MD 21701

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. academic institution)

NAME: Warren, J. R.

TELEPHONE: 301 663-7211

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Metzger, J. F.

NAME: Spero, L. DA

21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Precede each with Security Classification Code)

(U) Toxins; (U) Staphylococcus aureus; (U) Antigens; (U) Military medicine

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with security Classification Code.)

23 (U) Improve efficiency and specificity of methods used for chemical treatment of antigens. This work unit is an essential element in a comprehensive program for medical defense against BW agents.

24 (U) Using staphylococcal enterotoxins as a model, try various methods of inactivation to produce better antigens.

25 (U) 71 07 - 72 06 - Staphylococcal enterotoxin B (SEB) was formalinized in aqueous 0.9% formalin at 37 C and pH 5.0, 7.5 or 9.5 for 30 days. It was demonstrated by Sephadex gel filtration and polyacrylamide gel electrophoresis that toxin treated with acid or neutral formalin is cross-linked into high molecular weight polymers by intermolecular covalent bonds. Denatured enterotoxin monomer only was obtained from formalin treatment at pH 9.5. In preliminary experiments each of the 3 formalinized preparations was nontoxic for rhesus monkeys. However, only polymeric enterotoxoid induced sustained high levels of SEB hemagglutinins in these animals. Thus antigenic sites, but not toxic sites, were protected against formaldehyde denaturation during cross-linking of SEB into polymeric species.

In other studies the effect of guanidine hydrochloride and acid pH upon the conformation of SEB was determined by viscometric and direct ultraviolet spectrophotometry. Preliminary work suggests that the toxin possesses an unusually stable native conformation. If confirmed, such structural stability would permit interaction of the toxin with primary alimentary receptor sites despite the extreme intraluminal pH of the gastrointestinal tract.

Terminated since FY 1973 funding will be under The Surgeon General, Army. New Project No. 3A06211QA834; Accession No. DA 0B6427.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 02: Prevention and Treatment of Biological Warfare Casualties

Work Unit No. 096 02 802: Chemical Modification of Microbial Protein Antigens

Description:

Improve efficiency and specificity of methods used for chemical treatment of antigens.

Progress:

This study consists of 3 phases. Phase I will establish the effect of formaldehyde upon the toxicity, in vivo antigenicity, and physical-chemical properties of staphylococcal enterotoxin. Phase II is a study of the conformational stability of the enterotoxins towards chemical denaturants, extreme acid or alkaline pH, and elevated temperatures. Phase III will deal with the interaction of highly specific amino acid modifying reagents with enterotoxin.

Efforts in the first phase have concentrated on the effect of formalinization upon the size, shape, charge, and free amino content of the staphylococcal enterotoxin B (SEB) molecule. SEB was added at a concentration of 2 mg/ml to 0.15 M phosphate buffer at pH 5.0 or 7.5 or to 0.05 M bicarbonate buffer at pH 9.5. Formaldehyde was then dialyzed into each enterotoxin-buffer solution to a final concentration of 0.9% (v/v) over a 16-hr period. Each of the solutions was maintained for 30 days at 37C in a slowly-shaking water bath. At frequent intervals aliquots were removed and subjected to cellulose acetate electrophoresis to ascertain changes in charge, to Sephadex G-100 gel filtration to characterize size and/or shape of the reaction products, polyacrylamide gel electrophoresis in sodium dodecyl sulfate for a precise estimate of molecular weights and ninhydrin determination of free amino groups. Within 24-48 hr of exposure to formaldehyde at the 3 pHs, SEB, a basic protein in its native state,[1] demonstrated the anodal mobility of an acid protein upon cellulose acetate electrophoresis. This observation confirms the ability of formaldehyde to react with the basic amino acid residues of proteins.[2] For the protein formalinized at pH 5 no material was detected in the void of Sephadex G-100 gel until days 2-3 of treatment, a progressively larger peak then occupying the void volume position in G-100 chromatograms from days 3-10. By the 10th day most of the enterotoxin formalinized at low pH eluted from columns in the void volume, 2 smaller peaks observed after the void representing heterogeneous material retarded by the gel. A similar G-100 chromatogram was [illegible] exposed to formaldehyde in the pH 7.5 phosphate buffer with a [illegible] major void volume peak was observed within 1 hr following the addition of formaldehyde to the aqueous solution. In contrast, the major

formaldehyde reaction product at pH 9.5 was mostly retarded by Sephadex G-100, only a very small amount of protein appearing in the void. The column was calibrated with 9 separate marker proteins, thus enabling an estimate of the molecular weight of the different filtration fractions at each pH as follows: (1) pH 5 derivative consisted mostly of polymeric material of > 150,000 m.w., small amounts of a formaldehyde-denatured monomer (apparent m.w. 38,000 ± 2,000) and dimer (84,000 ± 4,000); (2) pH 7.5, derivative mostly polymer(s) > 150,000, some denatured monomer or dimer (55,000 ± 9,000) and dimer or tetramer (100,000); (3) pH 9.5 formalinized enterotoxin almost totally formaldehyde-denatured monomer or native dimer (55,000 ± 6,000) and some dimer or tetramer (120,000). The presence of formaldehyde-denatured enterotoxin monomer in each of the 3 derivatives and polymers of this monomer with the pH 5 and 7.5 treated protein was confirmed by polyacrylamide gel electrophoresis in sodium dodecyl sulfate, utilizing 4 standard marker proteins for calibration. Finally, ninhydrin analysis revealed that at pH 9.5, 65% of amino groups of SEB were blocked by formaldehyde, at pH 7.5, 60%, and at pH 5.0, 50%.

After 30 days, enterotoxin preparations from each pH were dialyzed free of formaldehyde and examined for antigenicity and toxicity. The enterotoxin treated with acid (pH 5) formalin demonstrated a clear reaction of partial identity with native enterotoxin on Ouchterlony double-diffusion plates. No precipitin lines were detected on double-diffusion plates with the protein formalinized at pH 7.5 or 9.5. Finally, each derivative was injected into a rhesus monkey at a subcutaneous dose 70 times greater than that lethal for native protein. No toxic manifestations (emesis, diarrhea, death) occurred in any of the monkeys. SEB hemagglutinins were then determined at 2-week intervals in each monkey. Enterotoxin toxoided with acid formalin induced the highest hemagglutinin titer, 1:1280 at 18 weeks postinjection; with protein detoxified with neutral formalin inducing a more sluggish response, i.e., 1:320. The formaldehyde-denatured monomer derived at pH 9.5 caused no antibody formation following subcutaneous injection.

During the next year, effort in this phase will be directed towards the following goals: (1) confirmation of *in vivo* toxicity and antigenicity data in a larger group of rhesus monkeys; (2) examination of the other antigenic types of staphylococcal enterotoxins, i.e., toxins A and C_1; (3) the use of specific gel filtration fractions to reduce time needed for toxoiding and/or to eliminate residual toxicity, if observed in future studies.

Work has recently been initiated on this phase II. As determined by viscometry, enterotoxin B completely unfolds between 2 M and 4.5 M aqueous guanidine hydrochloride. However, it was found by direct spectroscopy in the near ultraviolet spectrum that this unfolding is very slow, taking days at the lower concentrations of guanidine hydrochloride. This is most unusual behavior, other proteins studied by these techniques unfolded at a much faster rate.[3] The high degree of conformational stability of SEB was further confirmed by its failure to unfold except at very acid pH (> 2.2) as demonstrated by viscometry. If confirmed by further work, such structural stability would allow interaction of native enterotoxin with primary alimentary receptor sites even at the extreme intraluminal pHs of the gastrointestinal tract.[4]

During the next year the behavior of enterotoxin B in aqueous guanidine hydrochloride and acid solution will be exactly quantitated by viscometry, difference spectroscopy, and disulfide bond accessibility studies.[5,6] Other antigenic types of staphylococcal enterotoxins will also be examined.

Summary:

The extent to which SEB is cross-linked into large polymers is determined by the pH at which formalinization is performed. Native antigenicity appears to be partially preserved during intermolecular cross-linking of this protein. [illegible] substitution of sephadex gel filtration for tedious and costly bioassay techniques in the monitoring of formaldehyde toxoiding is suggested.

Preliminary data suggest an extreme conformational stability of native SEB.

Publications:

None.

LITERATURE CITED

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ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1W662711A096 02:	Prevention and Treatment of Biological Agent Casualties
Work Unit No. 096 02 805:	Physical and Chemical Characterization of Proteins of Microbiological Origin
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland 21701
Division:	Pathology
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Author:	Leonard Spero, Ph.D.
Reports Control Symbol:	RCS-MEDDH-288 (RI)
Security Classification:	UNCLASSIFIED

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0L0918	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
72 03 10	H. TERMINATION	U	U	NA	NL	YES NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	02	803
b. CONTRIBUTING	62711A	1B662711A096		
c. CONTRIBUTING	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)

(U) Physical and chemical characterization of proteins of microbial origin

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical medicine; 004900 Defense; 002300 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 08	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER: NA

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
PRECEDING FISCAL YEAR 71	0	0
CURRENT FISCAL YEAR 72	1.0	5

19. RESPONSIBLE DOD ORGANIZATION

NAME: USA Medical Research Institute of Infectious Diseases

ADDRESS: Fort Detrick, MD 21701

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TELEPHONE: 301 663-2833

20. PERFORMING ORGANIZATION

NAME: Pathology Division USAMRIID

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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

NAME: Spero, L.

TELEPHONE: 301 663-7211

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Metzger, J. F.

NAME: Warren, J. R. DA

21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Proteins; (U) Physical chemistry; (U) Staphylococcus; (U) Bacterial toxins; (U) Toxoids; (U) Vaccines

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with security Classification Code.)

23 (U) Demonstrate homogeneity of proteins isolated from microorganisms and determine their physicochemical parameters for production of toxoids or vaccines. This work unit is an essential element in a comprehensive program for medical defense against BW agents.

24 (U) Apply electrofocusing techniques to homogenous components of enterotoxin (SEB) and perform amino acid analysis, amide determinations and peptide mapping on such components.

25 (U) 71 08 - 72 06 - SEB was unfolded in 6 M guanidine hydrochloride and dialyzed against the same solvent. After removal of the guanidine and gel filtration on Sephadex G-75, the enterotoxin was focused isoelectrically. The pattern was virtually identical to that obtained before treatment. This provides a clear demonstration that the differences in isoelectric points of the components of SEB are not due to bound ligand and suggests that these components are not stable conformation variants.

Terminated since FY 1973 funding will be under The Surgeon General, Army. New Project No. 3A062110A834; Accession No. DA 0B6428.

*Available to contractors upon originator's approval

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BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 02: Prevention and Treatment of Biological Agent Casualties

Work Unit No. 096 02 803: Physical and Chemical Characterization of Proteins of Microbiological Origin

Description:

Demonstrate homogeneity of proteins isolated from microorganisms and determine their physiochemical parameters for production of toxoids or vaccines.

Progress:

The origin of the several components found on isoelectric focusing of staphylococcal enterotoxins A and B (SEA and SEB) may be attributed to genetic or nongenetic variants. In the latter group 3 general types may be distinguished: artifacts, aggregates, and conformers.[1] Extensive experimentation eliminates aggregation as a significant phenomenon with SEB.[2] Conformers are stable conformational variants of the same proteins which have the same amino acid sequence and molecular weight but different electrophoretic mobility. They differ structurally only in the way the polypeptide chains are folded. The folding may be influenced by the presence of bound ligands.

It has been demonstrated that SEB may be reversibly denatured.[3] Exposure to high concentrations of guanidine completely unfolds the molecule; when the guanidine is removed fully native material is obtained. Thus if SEB is put through this cycle, some insight should be gained as to whether the different isoelectric components are stable conformers.[4] Accordingly, SEB was dissolved in 6 M guanidine hydrochloride containing 0.05 M Tris buffer at pH 8.5 at a concentration of 5 mg/ml. This solution was dialyzed overnight against a large excess of the same buffer in the cold. The guanidine was then removed by dialysis against Tris buffer and the toxin-solution run through a column of Sephadex G-75. A small amount of material appeared at the void volume but the remainder emerged as a symmetrical peak. The SEB peak was concentrated by ultrafiltration, dialyzed against water, and the isoelectric focusing pattern determined on a pH 7-10 gradient. Four components were obtained whose pI values and relative concentrations were virtually identical to the original material.

The dialysis against guanidine would have removed any possible bound ligand and would appear to eliminate this as a cause of the isoelectric paucidispersity. Since the refolding occurred at a different concentration and temperature than originally prevailed in the synthesizing organism, it is unlikely that the refolding would yield the 4 components in the same ratios. These results suggest that stable conformers are not involved; however, it will be necessary to repeat the experiment with homogeneous isolated components for definitive proof.

Summary:

SEB was unfolded in 6 M guanidine hydrochloride and dialyzed against the same solvent. After removal of the guanidine and gel filtration on Sephadex G-75, the enterotoxin was focused isoelectrically. The pattern was virtually identical to that obtained before treatment. This provides a clear demonstration that the differences in isoelectric points of the components of SEB are not due to bound ligand and suggests that these components are not stable conformation variants.

Publications:

None.

LITERATURE CITED

1. Kaplan, N. O. 1968. Nature of multiple molecular forms of enzymes. Ann. N. Y. Acad. Sci. 151:382-399.

2. Wagman, J., R. C. Edwards, and E. J. Schantz. 1965. Molecular size, homogeneity, and hydrodynamic properties of purified staphylococcal enterotoxin B. Biochemistry 4:1017-1023.

3. Dalidowicz, J. E., S. J. Silverman, E. J. Schantz, D. Stefanye, and I. Spero. 1966. Chemical and biological properties of reduced and alkylated staphylococcal enterotoxin B. Biochemistry 5:2375-2381.

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ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	**Medical Defense Aspects of Biological Agents (U)**
Task No. 1W662711A096 02:	**Prevention and Treatment of Biological Agent Casualties**
Work Unit No. 096 02 804:	Controlled Enzymatic and Chemical Alteration of Proteins of Microbiological Origin
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland 21701
Division:	Pathology
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Author:	**Leonard Spero, Ph.D.**
Reports Control Symbol:	**RCS-MEDDH-288 (R1)**
Security Classification:	**UNCLASSIFIED**

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	3. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OL0919	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
72 03 10	H. TERMINATION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	02	804
b. [illegible]	62711A	1B662711A096		
c. [illegible]	CDOG 1212b(S)			

11. TITLE (Precede with Security Classification Code)
(U) Controlled enzymatic and chemical alteration of microbial proteins

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 Clinical medicine; 004900 Defense; 002300 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 08	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER: NA
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 71	0	0
FISCAL YEAR CURRENT 72	1.0	5

19. RESPONSIBLE DOD ORGANIZATION
NAME: USA Medical Research Institute of Infectious Diseases
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RESPONSIBLE INDIVIDUAL
NAME: Crozier, D.
TELEPHONE: 301 663-2833

20. PERFORMING ORGANIZATION
NAME: Pathology Division USAMRIID
ADDRESS: Fort Detrick, MD 21701

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Spero, L.
TELEPHONE: 301 663-7211
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Metzger, J. F.
NAME: Warren, J. R. DA

21. GENERAL USE
Foreign intelligence considered

22. KEY WORDS (Precede each with Security Classification Code)
(U) Bacterial toxins; (U) Amino acids; (U) Enzymes; (U) Toxoids; (U) Vaccines; (U) Staphylococcus

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number, precede text of each with Security Classification Code.)

23 (U) Modify proteins of microbial origin that play roles in their biological effects in order to prepare more effective immunogens against militarily important diseases. This work unit is an essential element in a comprehensive program for medical defense against BW agents.

24 (U) After determination of optimal enzyme reaction parameters, purified materials will be tested for their serological and emetic activities.

25 (U) 71 08 - 72 06 - Only threonine has been identified as a new amino terminus in the digestion of staphylococcal enterotoxin B by trypsin. The cleavage occurs in the region of the molecule between the half-cystines of the disulfide bridge and the molecular size appears to be unchanged in the ultracentrifuge. Reduction in the presence of sodium dodecyl sulfate permits the demonstration by disc electrophoresis of 2 polypeptides of about 12,000 and 15,000 molecular weight. Reduction in the absence of denaturant does not bring about separation of these 2 fragments. The product retains complete emetic and serological activities.

Terminated since FY 1973 funding will be under The Surgeon General, Army. New Project No. 3A062110A834; Accession No. DA 0B6429.

*Available to contractors upon [illegible] approval

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BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 02: Prevention and Treatment of Biological Agent Casualties

Work Unit No. 096 02 804: Controlled Enzymatic and Chemical Alteration of Proteins of Microbiological Origin

Description:

Modify proteins of microbial origin that play roles in their biological effects in order to prepare more effective immunogens against militarily important diseases.

Progress:

The digestion of staphylococcal enterotoxin B (SEB) by trypsin has been followed by reaction in a pH stat. Calculation of the extent of the initial rapid reaction at pH 8.0, 8.5 and 9.0 assuming a pK_a of the liberated amino groups of 7.8[1] gave values of 1.56, 1.83, and 1.87 bonds cleaved per molecule, respectively. If these values are accurate, it would be expected that the molecule would be broken into 3 fragments. When the product trypsin-treated SEB (SEB-T) was examined in the ultracentrifuge and by Sephadex gel filtration, only one component was found; it had the same molecular size as untreated SEB.

That a specific reaction actually took place was verified by the Sanger fluorodinitrobenzene technique.[2] Only DNP-threonine was found in significant amounts in addition to the original N-terminus of DNP-glutamic acid. Examination of the amino acid sequence of SEB[3] disclosed that there were only 2 Lys-Thr sequences (no Arg-Thr sequences are present) in the entire molecule and they both occur within the disulfide loop at positions 97 and 110. This explains why no reduction in size was observed by physical methods.

Confirmation was obtained by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS).[4] SEB-T gave only a single line at the same position as SEB. However, when SEB-T was preincubated with β-mercaptoethanol in order to reduce the disulfide bond, this line virtually disappeared and 2 new lines corresponding to polypeptides with molecular weight of about 12,000 and 15,000 appeared. When the reduction was carried out in the absence of SDS and the product was ran through a column of Sephadex G-50, only one peak was found. It emerged at the same position as SEB indicating that the 2 fragments were held together by noncovalent forces in the absence of denaturant.

The serological activity of SEB-T was determined by the quantitative precipitin test against rabbit anti-SEB. A curve identical with SEB was

obtained. Reduced SEB-T also gave a similar curve. Finally Ouchterlony immunodiffusion gave a reaction of identity between SEB and SEB-T.

The emetic activity of SEB-T was measured in rhesus monkeys. Two of 3 monkeys became ill at a dose of 0.1 μg/kg, 2 of 3 at 0.3 μg/kg, and all 3 at 1.0 μg/kg. Since the median effective dose of SEB is estimated to be 0.1 μg/kg,[5] SEB-T is fully active.

Summary:

SEB undergoes a rapid limited digestion by trypsin. Only threonine has been identified as a new amino terminus. The cleavage occurs in the region of the molecule between the half-cystines of the disulfide bridge and the molecular size appears to be unchanged in the ultracentrifuge. Reduction in the presence of sodium dodecyl sulfate permits the demonstration by disc electrophoresis of 2 polypeptides of about 12,000 and 15,000 molecular weight. Reduction in the absence of denaturant does not bring about separation of these 2 fragments. The product retains full emetic and serological activity.

Publications:

None.

LITERATURE CITED

1. Cohn, E. J. and J. T. Edsall. 1943. Proteins, amino acids and peptides as dipolar ions. New York, N. Y., p. 445.

2. Levy, D. L. 1955. The fluorodinitrobenzene method for amino end group analysis. Methods Biochem. Anal. 2:360-383.

3. Huang, I. Y., and M. S. Bergdoll. 1970. The primary structure of staphylococcal enterotoxin B. III. The cyanogen bromide peptides of reduced and aminoethylated enterotoxin B, and the complete amino acid sequence. J. Biol. Chem. 245:3518-3525.

4. Weber, K. and M. Osborn. 1969. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. 244:4406-4412.

5. Schantz, E. J., W. G. Roessler, J. Wagman, L. Spero, D. A. Dunnery and M. S. Bergdoll. 1965. Purification of staphylococcal enterotoxin B. Biochemistry 4:1011-1016.

ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	**Medical Defense Aspects of Biological Agents (U)**
Task No. 1W662711A096 03:	**Laboratory Identification of Biological Agents**
Work Unit No. 096 03 006:	**Early Immune Response In Infectious Disease and Toxemia**
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Bacteriology
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Authors:	**Joseph Kaplan, Major, MC (I, II, III, IV)** **William J. Caspary, Captain, MSC (III)**
Reports Control Symbol:	**RCS-MEDDH-288(R1)**
Security Classification:	**UNCLASSIFIED**

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OL0870	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. GRP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	03	006
b. CONTRIBUTING	62711A	1B662711A096		
c. CONTRIBUTING	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code): (U) Early immune response in infectious disease and toxemia of significance in BW defense

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical medicine; 004900 Defense; 016800 Toxicology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
66 08	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER: NA
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 71	1.0	60
FISCAL YEAR CURRENT 72	1.0	90

19. RESPONSIBLE DOD ORGANIZATION
NAME: USA Medical Research Institute of Infectious Diseases
ADDRESS: Fort Detrick, MD 21701

RESPONSIBLE INDIVIDUAL
NAME: Crozier, D.
TELEPHONE: 301 663-2833

20. PERFORMING ORGANIZATION
NAME: Bacteriology Division USAMRIID
ADDRESS: Fort Detrick, MD 21701

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Kaplan, J.
TELEPHONE: 301 663-7341
SOCIAL SECURITY ACCOUNT NUMBER:

21. GENERAL USE: Foreign intelligence considered

ASSOCIATE INVESTIGATORS
NAME:
NAME: DA

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Immunology; (U) Antibody formation; (U) Chemotaxis; (U) Hemagglutination; (U) Complement; (U) Macrophage; (U) Enterotoxin; (U) Staphylococcus; (U) Military med.

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Develop and employ in vitro methods of studying lymphocyte activation by antigens. This work unit is an essential element in a comprehensive program for medical defense against BW agents.

24 (U) Electron spin resonance spin label probes are used to study lymphocyte membrane changes that occur with antigen stimulation. Lymphocyte lysates are prepared and tested for their ability to transfer immunity against intracellular pathogens.

25 (U) 71 07 - 72 06 - Studies of migration inhibition were completed and a report prepared for publication. Techniques have been developed for incorporating spin labels into lymphocyte membranes and for detecting surface membrane signals distinct from internal signals. Factors affecting the stability of cell signal have been established. The viability and continued biological activity of spin labeled lymphocytes have been demonstrated. The ability of various spin labels to reflect membrane changes associated with surface receptor-ligand interaction were studied and indicated that spin labels specifically located at the sites of interaction are required.

Publication: Cell. Immunol. 3:245-252, 1972.

Terminated since FY 1973 funding will be under The Surgeon General, Army. New Project No. 3A062110A834; Accession No. DA 0C6410.

*Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498 (PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE

BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 03: Laboratory Identification of Biological Agents

Work Unit No. 096 03 006: Early Immune Response In Infectious Disease and Toxemia

Description:

Develop and employ *in vitro* methods of studying lymphocyte activation by antigens.

Progress and Summary, Part I:

Investigations were completed on induction of migration inhibition factor production by staphylococcal enterotoxin B (SEB). Results were published in Cellular Immunology 3:245, 1972.

Progress and Summary, Part II:

Studies of the effect of X-irradiation on delayed hypersensitivity were continued. Methods were developed to separate lymph node and peritoneal exudate lymphocytes from macrophages but studies were interrupted by mechanical breakdown of the radiation source.

Progress, Part III:

Evidence suggests that lymphocyte activation is triggered by a conformational change in the surface membrane induced by interaction between antigen and surface receptor.[1] To study this question, we applied electron spin resonance (ESR) spectroscopy and spin label techniques.[2]

Prior to evaluating conformational changes in surface membranes of antigen-sensitive lymphocytes, a number of technical capabilities had to be developed: (1) techniques for labeling membranes, (2) determination and selection of specific labels for protein-membrane interactions, (3) differentiation between surface and intracellularly incorporated label, and (4) definition of conditions required to maintain viability and biological function in labeled cells.

To gain familiarity with spin labeling techniques, as well as to obtain information regarding the nature and magnitude of changes that might occur, intact sheep erythrocytes (RBC) and erythrocyte stroma were employed in initial studies (Table 1). Drastic chemical or physical treatment was required to affect mobility of incorporated label.

TABLE I: EFFECT OF VARIOUS TREATMENTS ON RATE OF TUMBLING (MOBILITY) OF SPIN LABELS INCORPORATED INTO MEMBRANES OF SHEEP ERYTHROCYTES OR STROMA.

SPIN LABEL	AFFINITY OF LABEL	ERYTHROCYTE PREPARATION	TREATMENT OF LABELED PREPARATION	MOBILITY OF INCORPORATED LABEL
Iodoacetamide	Protein	Whole cells	Anti-RBC serum	No change
		Stroma	Anti-RBC serum	No change
		Stroma	80 C, 10 min	Markedly increased (irreversible)
Androstane	Lipid	Whole cells	Anti-RBC serum	No change
		Whole cells	Lidocane	Increased
		Stroma	Anti-RBC serum	No change
		Stroma	38% HCHO	Decreased

Lipid-soluble spin labels, e.g. stearic acid, were readily incorporated into viable intact nucleated cells. Subcellular distribution of this spin label is shown in Table II. Lipid-soluble labels became incorporated into all membrane-containing subcellular fractions.

TABLE II: DISTRIBUTION OF STEARIC ACID SPIN LABEL (ESR ACTIVITY) IN LABELED MOUSE L-CELLS AND HUMAN LYMPHOCYTES AFTER FRACTIONATION BY DIFFERENTIAL CENTRIFUGATION.

CELL FRACTION	ESR ACTIVITY
Nuclei and cell membranes	Strong
Heavy mitochondria	Strong
Light mitochondria	Strong
Microsomes	Weak
Cell sap	None

The functional competence of nucleated mammalian cells appeared to be unaffected by incorporation of the androstane spin label. Labeled guinea pig lymph node lymphocytes cultured for 72 hr with SEB, a nonspecific mitogen, responded to the same degree as unlabeled lymphocytes as measured by uptake of tritiated thymidine.

Decay of ESR signal from androstane or stearic acid labeled cells was observed within 30 min at 37 C and at lower temperatures after longer periods

of time. Exposure of labeled inactivated cells to aeration or the oxidizing agent $K_3Fe(CN)_6$ rapidly restored original signal strength, suggesting that decay resulted from reversible reduction of the nitroxide radical by cellular components. Cell signal disappeared when $K_3Fe(CN)_6$ was removed by washing but reappeared immediately after reexposure, suggesting that ferricyanide had not entered the cell but had reactivated the surface membrane signal. To investigate this possibility, stearic acid labeled L-cells were incubated at 37 C for 30 min to inactivate ESR signal and then exposed to 0.001 molar $K_3Fe(CN)_6$, the lowest concentration capable of reactivating decayed cell signal. Cells were washed repeatedly to remove any unattached $K_3Fe(CN)_6$, lysed by suspension in distilled water and freeze-thawed; lysates were clarified by centrifugation. Ferricyanide and ferrocyanide in washes and cell lysate were determined by a colorimetric method. While both cyanides were detected in the initial washings, neither was detected in the cell lysate, indicating that $K_3Fe(CN)_6$ did not enter the cell. Should this postulate be true, reactivation of cell signal by $K_3Fe(CN)_6$ would allow differentiation between cell surface and intracellular signals.

As was true with erythrocytes, signals from nucleated cells with lipid soluble or nonspecific protein spin labels, e.g. iodoacetamide, were insensitive to structural changes associated with antibody attachment to cell surface. These findings suggest that conformational changes caused by surface receptor-ligand interaction are local rather than general membrane events and only spin labels specifically incorporated at these local sites will reflect such changes. It is anticipated that use of spin-labeled haptens as specific probes of surface-antibody combining sites will provide a tool for detecting alterations at the sites.

Summary, Part III:

ESR spectroscopy was applied to study early membrane changes during lymphocyte activation by foreign antigens. Techniques were developed for spin labeling intact lymphocytes and detecting surface membrane signals distinct from internal signals. Factors affecting the stability of cell signal were established. Viability and biological activity of spin labeled lymphocytes was demonstrated. The ability of various spin labels to reflect membrane changes associated with surface receptor-ligand interaction was examined and indicated that spin labels located at specific sites of interaction are required.

Progress and Summary, Part IV:

An important mechanism of resistance to intracellular infection is cell-mediated immunity (CMI) involving the interaction of lymphocytes and macrophages. The same thymus-dependent lymphocytes responsible for CMI are involved in delayed hypersensitivity responses. Transfer factor (TF), a dialyzable substance obtained from white blood cell lysates, transfers delayed hypersensitivity from a sensitive individual to a nonsensitive individual.[3] From clinical studies with human transfer factor it appears,

but has not been proven, that cell-mediated immunity can also be transferred with TF. Studies have been initiated in rhesus monkeys to determine whether TF can transfer immunity against tularemia from one monkey to another. Monkeys were vaccinated with tularemia (LVS) and also given ovalbumin in complete Freund's adjuvant. Unequivocal delayed hypersensitivity to ovalbumin was induced but only weak delayed skin reactions to Foshay tularemia antigen were demonstrated. Transfer factor was prepared from peripheral leucocytes of these monkeys and given subcutaneously to a group of normal monkeys. Transfer of sensitivity to either ovalbumin or tularemia could not be demonstrated. Further work is in progress.

Publications:

1. Kaplan, J. 1972. Staphylococcal enterotoxin B induced release of macrophage migration inhibition factor from normal lymphocytes. Cell. Immunol. 3:245-252.

2. Kaplan, J. 1972. Effect of X-irradiation on induction of delayed hypersensitivity as measured by *in vitro* macrophage migration inhibition. J. Reticuloendothel. Soc. In press.

LITERATURE CITED

1. Coulson, A. J. 1969. Recognition pathways in lymphocytes. J. Theor. Biol. 25:127-136.

2. Hamilton, C. L., and H. M. McConnell. 1968. Spin labels. p. 115 to 149. In Structural Chemistry and Molecular Biology, (A. Rich and N. Davidson, ed.) W. H. Freeman and Co., San Francisco.

3. Lawrence, H. S. 1955. The transfer in humans of delayed skin sensitivity to streptococcal M substance and to tuberculin with disrupted leucocytes. J. Clin. Invest. 34:219-230.

ANNUAL PROGRESS REPORT

Project No. 3A061101A91C:	In-house Laboratory Independent Research (U)
(1W662711A096):	(Medical Defense Aspects of Biological Agents) (U)
Task No. 3A061101A91C 00:	
(1W662711A096 03):	(Laboratory Identification of Biological Agents)
Work Unit No. 91C 00 131:	Mathematical and Computer Applications in
(096 03 008):	Infectious Disease Research
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Administrative
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Author:	Alan T. Rowberg, Captain, MC
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OL0897	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISP'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NI.	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A061101A91C	00	131
b. [illegible]	62711A	1W662711A096	03/008	
c. [illegible]	CDOG 1212b(9); 1412a(2)			

11. TITLE (Precede with Security Classification Code)

(U) Mathematical and computer applications in infectious disease research

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical medicine; 004900 Defense; 009700 Mathematics and Statistics

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
69 11	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION

b. NUMBER: NA

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	A. PROFESSIONAL MAN YRS	B. FUNDS (In Thousands)
PRECEDING FISCAL YEAR 71	1.0	16
CURRENT FISCAL YEAR 72	1.0	16

19. RESPONSIBLE DOD ORGANIZATION

NAME: USA Medical Research Institute of Infectious Diseases

ADDRESS: Fort Detrick, MD 21701

RESPONSIBLE INDIVIDUAL

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TELEPHONE: 301 663-2833

20. PERFORMING ORGANIZATION

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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

NAME: Rowberg, A. H.

TELEPHONE: 301 663-7196

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME:

NAME:

21. GENERAL USE

Foreign intelligence considered

DA

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Diagnosis; (U) Computers; (U) Medicine; (U) Military medicine

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Develop and apply techniques for utilization of computers, statistics and mathematics to process and interpret scientific data. This work unit is an essential element in a comprehensive program for medical defense against BW agents.

24 (U) Automation of data analysis currently accomplished with a computer is being expanded with a data acquisition system. Theories and disciplines of numerical analysis, differential equations, statistical tests of hypotheses, experimental design, information storage and retrieval, and pattern recognition are utilized to analyze and interpret data gathered by investigators.

25 (U) 71 07 - 72 06 - Mathematical, statistical and computer techniques have been applied to data emanating from the Institute.

A series of statistical routines to analyze data from experiments with paired and unpaired controls was developed and applied to data from a sandfly fever experiment. These techniques may be an aid to the early diagnosis of infection in man.

Many forms of automatic data acquisition were explored and applied to projects. Expansion of facilities continues in the direction shown to be most productive.

Terminated because the accession number is changed starting FY 1973. New accession number will be DA 0C0411.

*Available to contractors upon originator's approval

DD FORM 1498 (MAR 68) PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. AF FORMS 1498A, 1 NOV 68 AND 1498B, 1 MAR 68, WHICH ARE USED, ARE OBSOLETE.

BODY OF REPORT

Project No. 3A061101A91C: In-house Laboratory Independent Research (U)
(1W662711A096): (Medical Defense Aspects of Biological Agents) (U)

Task No. 3A061101A91C 00:
(1W662711A096 03): (Laboratory Identification of Biological Agents)

Work Unit No. 91C 00 131: Mathematical and Computer Applications in
(096 03 008): Infectious Disease Research

Description:

Develop and apply techniques for utilization of computers, statistics and mathematics to process and interpret scientific data.

Progress:

The wide scope of this work unit results in progress along many different lines as guided by the needs of the Institute. Work described here is in addition to numerous other projects, some of which were of a routine nature.

Statistical consultation was obtained and utilized to develop a data analysis plan that can be applied to experiments with paired and unpaired control subjects. Use of these techniques results in maximum sensitivity to the effects of the experimental "treatment." The data from the same subject during the pretreatment time period is considered together with data from control subjects who did not undergo the experimental treatment in producing a test for significant changes in measured variables, presumably changes induced by the treatment. Allowance is made for individual and group variations, as well as changes in both control and treated groups due to the nature of the experimental regimen. Variables found to be significantly effected by the treatment are subjected to discriminant analysis for successive time segments in the treatment time period. These techniques may be of aid in the early diagnosis of infection in man by sensing small changes in measured variables (clinical and laboratory data) and calculating a probability that the individual is infected.

Several techniques of automated data acquisition were explored and applied. A high-speed paper tape punch was interfaced with an electron spin resonance spectrograph through a computer of average transient. The resultant data were delivered to a full-sized computer via paper tape. Time and frequency domain techniques were applied to the spectra and yielded a spectral resolution enhancement not possible without computer techniques, thus extending the useful range of the instrument.

Teletypes were connected to β and γ scintillation counters and used to produce paper tape images of the counter data. The tape could then be processed by a computer with fully automatic corrections for background and quench. Many subsystems were developed for investigators, to produce results in final form without data transcription or manual calculation. For instance, the calculations for simultaneous radioimmunoassay for growth hormone and insulin are accomplished automatically, including the calculation and display of the standard curve used for the set of assays.

A high-speed paper tape punch was interfaced with 2 amino acid analyzers through a programmable calculator. The calculator is used to control timing and perform some data reduction before transferring the data onto paper tape. The tape is then read by a computer, which calculates amino acid concentration. The system is designed to function unattended for periods of days, with automatic sample handling. The computerization of the curves will allow calculation of areas under the curves rather than measurement of peak height by manual means.

Data from many projects were transcribed to computer compatible form and results analyzed. An example is the study of about 90 persons who have been subjected to immunization with a variety of antigens over a long period of time. History and clinical and laboratory data from these persons were entered into a computerized data-bank for analysis. Many types of comparisons will be performed that could not be done readily by manual techniques.

Summary:

Progress was made in the areas of data acquisition and data reduction, utilizing both standard and new techniques.

Publications:

None.

ANNUAL PROGRESS REPORT

Project No. 3A061101A91C: (1W662711A096):	**In-House Laboratory Independent Research (U)**
Task No. 3A061101A91C 00: (1W662711A096 03):	**(Laboratory Identification of Biological Agents)**
Work Unit No. 91C 001132: (096 03 009):	**Application of Electron Spin Resonance Spectroscopy to Infectious Disease Research**
Reporting Installation:	**U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland**
Division:	**Bacteriology**
Period Covered by Report:	**1 July 1971 to 30 June 1972**
Professional Author:	**William J. Caspary, Captain, MSC**
Reports Control Symbol:	**RCS-MEDDH-288(R1)**
Security Classification:	**UNCLASSIFIED**

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OL0898	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISPN INSTR	8b. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NL	YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A061101A91C	00	132
b. [illegible]	62711A	1W662711A096	03/009	
c. [illegible]	CDOG 1212b(9); 1412a(2)			

11. (U) Application of electron spin resonance spectrometry to infectious disease research

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical medicine; 004900 Defense; 003200 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
69 11	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER: NA
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT:

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 71	1.0	44
	CURRENT 72	1.0	44

19. RESPONSIBLE DOD ORGANIZATION
NAME: USA Medical Research Institute of Infectious Diseases
ADDRESS: Fort Detrick, MD 21701

RESPONSIBLE INDIVIDUAL
NAME: Crozier, D.
TELEPHONE: 301 663-2833

20. PERFORMING ORGANIZATION
NAME: Bacteriology Division USAMRIID
ADDRESS: Fort Detrick, MD 21701

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Caspary, W. J.
TELEPHONE: 301 663-7341
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: DA
NAME:

21. GENERAL USE: Foreign intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Spectroscopy; (U) Biochemistry; (U) Electron spin resonance; (U) Military medicine; (U) Spin labels

23. TECHNICAL OBJECTIVE; 24. APPROACH; 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Establish usefulness of electron spin resonance spectroscopy in solving problems related to infectious diseases. This work unit is an essential element in a comprehensive program for medical defense against BW agents.

24 (U) Search for the effect of infectious agents and toxins on biochemical reactions involving free radicals. Spin label compounds involved in infectious or immunologic processes.

25 (U) 71 07 - 72 06 - Initial attempts at spin labeling nucleic acid of tobacco mosaic virus for ESR spectroscopy were unsuccessful. Other conditions are being sought so that the fate of viral nucleic acid in an infected host can be determined. In other studies carried out with Major Joseph Kaplan on conformational changes in antigen sensitive lymphocyte membranes, factors affecting the stability of cell signals were established. Spin labels had the ability to reflect changes associated with surface-ligand interactions. The labels must be specifically located at the interaction sites.

Publications: Program, American Chemical Society, Abstracts 26 and 131, 1971.

Terminated because the Accession Number is changed starting FY 1973.
New Accession Number DA OC6412.

*Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1 1 MAR 66 FOR ARMY USE ARE OBSOLETE

BODY OF REPORT

Project No. 3A061101A91C: (1W662711A096): In-House Laboratory Independent Research (U)

Task No. 3A061101A91C 00: (1W662711A096 03): (Laboratory Identification of Biological Agents)

Work Unit No. 91C 001132: (096 03 009): Application of Electron Spin Resonance Spectroscopy to Infectious Disease Research

Description:

Establish the usefulness of electron spin resonance spectroscopy in solving problems related to infectious diseases.

Progress, Part I:

Conditions for incorporation of spin label into molecules of nucleic acid were described previously.[1] Based upon these findings, it was postulated that nucleic acids from an infectious virus could be spin labeled and employed as electronic probes to obtain basic information regarding replication of viral nucleic acids in cells of infected hosts.

Tobacco mosaic virus (TMV) was selected as a model. Preparations of RNA core, free of TMV capsid, were supplied by Dr. Milton Gordon, University of Washington, Seattle, Washington. Previously described techniques were employed to label the RNA and record its electron spin resonance (ESR) spectrum.[1] Two special components were detected, a large mobility spectrum superimposed upon a strongly immobilized spectrum. Strong binding of label to RNA was indicated by the absence of label in the last wash solution. The sedimentation coefficient of the RNA sample, however, indicated that the nucleic acid had been degraded.

In an attempt to mimic the effects of labeled virus, Dr. Gordon tried to encapsulate labeled polyadenylic acid (Poly A) with TMV protein capsid. The ESR spectrum of this material was also highly mobile, again indicating degradation.

As another approach, it was assumed that under appropriate conditions the nucleic acid core of intact virus could be labeled. However, addition of label at pH 5.5 to intact TMV virus (0.01 M phosphate in 60% alcohol) also caused RNA denaturation.

Summary, Part I:

ESR spectra of spin labeled preparations indicated that RNA in intact TMV, the RNA component of TMV and Poly A encapsulated with TMV protein were degraded during treatment or shipment.

Progress and Summary, Part II:

Studies on conformational changes in antigen sensitive lymphocyte membranes are reported under Work Unit No. 096 03 006.

Presentations:

1. Caspary, W. J. The in vitro reaction of 6-hydroxylbenzo(a)pyrene with DNA. Presented at American Chemical Society Meeting, Washington, D. C., 12-17 September 1971.

2. Caspary, W. J. Mechanism for the covalent linkage of carcinogenic polycyclic hydrocarbons (HC) to DNA. Presented at American Chemical Society Meeting, Washington, D. C., 12-17 September 1971.

Publications:

1. Lorentzen, K., W. Caspary, and P. O. P. Ts'o. 1971. In vitro chemical reaction of 6-hydroxylbenzo(a)pyrene with DNA. In Program, American Chemical Society. Abstract 26.

2. Cohen, B. I., W. J. Caspary, S. A. Lesko, and P. O. P. Ts'o. 1971 Mechanism for the covalent linkage of carcinogenic polycyclic hydrocarbons (HC) to DNA. In Program, American Chemical Society. Abstract 131.

LITERATURE CITED

1. U. S. Army Medical Research Institute of Infectious Diseases. 1 July 1971. Annual Progress Report, FY 1971. p. 275 to 281. Fort Detrick, Maryland.

ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1W662711A096 03:	Laboratory Identification of Biological Agents
Work Unit No. 096 03 010:	Trace Metal Metabolism During Infectious Disease of Military Medical Importance
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Frederick, Maryland
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Authors:	Robert S. Pekarek, Ph.D. (I, II,III, IV) Robert W. Wannemacher, Jr. Ph.D. (I, II, III) William R. Beisel, M.D. (I, III) Edward C. Hauer, B.S. (IV)
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OL0899	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9a. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	03	010
b.	62711A	1B662711A096		
c.	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)
(U) Trace metal metabolism during infectious disease of military medical importance

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 Clinical medicine; 004900 Defense; 002300 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
67 01	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER: NA
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 71	2.0	70
	CURRENT 72	2.0	88

19. RESPONSIBLE DOD ORGANIZATION
NAME: USA Medical Research Institute of Infectious Diseases
ADDRESS: Fort Detrick, MD 21701
RESPONSIBLE INDIVIDUAL
NAME: Crozier, D.
TELEPHONE: 301 663-2833

20. PERFORMING ORGANIZATION
NAME: Physical Sciences Division USAMRIID
ADDRESS: Fort Detrick, MD 21701
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Pekarek, R. S.
TELEPHONE: 301 663-7181
SOCIAL SECURITY ACCOUNT NUMBER
ASSOCIATE INVESTIGATORS
NAME: Wannemacher, Jr., R. W.
NAME: Hauer, E. C.
DA

21. GENERAL USE
Foreign intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Trace metals; (U) Leukocytic endogenous mediator (LEM); (U) Bacterial and viral diseases; (U) Toxemia; (U) Human volunteers; (U) Animal models

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Assess trace metalchanges during infectious illness as a possible aid in early identification of disease etiology. This work unit is an essential element in a comprehensive program for medical defense against BW agents.

24 (U) Measure serum and tissue trace metals during infectious illness of laboratory animals and man and determine the mechanisms responsible for the observed changes.

25 (U) 71 07 - 72 06 - Leukocytic endogenous mediator (LEM), a depressant of serum Zn and Fe, was shown to be present in sera of patients with infectious bacterial disease. Considerable effort has been made to characterize, isolate and purify LEM. It was found to be heat labile, nondialyzable, low molecular weight protein and soluble to some extent in organic solvents. Some difficulties have been encountered in obtaining sufficient PMN leukocytes to produce the amount of LEM necessary for purification procedures.

In other work on trace metals a graphite furnace for atomic absorption spectrophotometry was purchased, with which it is possible to measure other metals, namely nickel, chromium, aluminum, cobalt and cadmium. Initial work has been done on the first two of these trace metals using serum samples of only 50 microliters.

Publications: Program, Western Hemisphere Nutrition Congress III, p. 43, 1971; Proc. Soc Exp. Biol. Med. 138:728-732, 1971, 139:128-132, 1972, 140:685-688, 1972; Appl. Microbiol. 22:1096-1099, 1971; Metabolism 21:67-76, 1972; Amer. J. Clin. Path. 57:506-510, 1972; Amer. J. Clin. Nutr. 25:461, 1972 (abstract); Fed. Proc. 31:667, 700, 1972 (abstracts).

Terminated since FY 1973 funding will be under The Surgeon General, Army. New Project No. 3A062110A834; Accession No. DA OC6413.

*Available to contractors upon originator's approval

BODY OF REPORT

Project No: 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 03: Laboratory Identification of Biological Agents

Work Unit No. 096 03 010: Trace Metal Metabolism During Infectious Disease of Military Medical Importance

Description:

Assess trace metal changes during infectious illness as a possible aid in early identification of disease etiology.

Progress, Part I:

Significant alterations in Fe, Zn and Cu metabolism have been demonstrated in man by this laboratory following either acute bacterial (Francisella tularensis - Medical Division Project FY 68-4 and Salmonella typhi - Contract No. DA-49-193-MD-2867) or viral (attenuated Venezuelan equine encephalomyelitis vaccine - Medical Division Protocol FY 69-1 and sandfly fever virus, Sicilian type, Medical Division Protocol FY 70-1) infections.[1-5]

Similar alterations in trace metal metabolism were observed in a variety of laboratory animals following experimentally induced infections or the administration of endotoxin or synthetic double-stranded RNA compounds.[6-9] Recent studies in animal models have shown that the infection and stress-induced alterations in Fe, Zn and Cu metabolism were mediated by an endogenous hormone-like substance released in part by PMN leukocytes.[10-12] Leukocytic endogenous mediator (LEM) obtained from peritoneal leukocytes of various laboratory animals were shown to produce significant dose-related depressions in serum Fe and Zn in normal, endotoxin tolerant, hypophysectomized, or adrenalectomized animals.[5,7,9,12]

Recently, we have reported that this endogenous Zn-depressing factor was present in the serum of febrile individuals (volunteers) with experimentally-induced typhoid fever.[4,9] To further substantiate this finding, acute serum obtained from Dr. Klainer, (Contract No. DADA 17-68-C-8080) from febrile patients with documented Diplococcus pneumoniae infections and patients with either Staphylococcus aureus abscesses, cellulitis, or mixed bacterial infections was millipored filtered and injected intraperitoneally (IP) into respective groups of normal recipient rats. These sera also induced significant serum Zn

depressions in the animals when compared to rats given normal human serum. When the infected (filtered) serum was heated at 90 C for 30 min the serum Zn-depressing factor was inactivated; thus, it was shown that a heat labile endogenous factor was present in these acute serums. The fact that the effect of the endogenous mediator from man can be transferred to a laboratory animal may prove to have potential diagnostic value.

Summary, Part I:

Significant alterations in Fe, Zn and Cu metabolism have been shown to occur in man during a variety of acute bacterial and viral infections. Studies in various laboratory animals have demonstrated that these infection- and stress-induced alterations in trace metal metabolism are mediated by a heat labile endogenous factor released, in part, by PMN leukocytes. Further this endogenous mediator of altered trace metal metabolism has been shown to be present in human serum during febrile illnesses caused by a variety of bacterial infections.

Progress, Part II:

Studies from our laboratory have initially characterized partially purified leukocytic endogenous mediator (LEM) to be a heat labile, non-dialyzable, low m.w. protein, which is soluble to some extent in organic solvents.[5,12] Experiments have been in progress to isolate and purify the LEM of serum Zn and Fe depression. Recently, crude preparations of LEM obtained from PMN leukocytes were pooled from rabbit peritoneal exudates. This pooled material was concentrated and fractionated on a G-200 Sephadex column, with the absorbance of the effluent being measured at 280 mμ with an ultraviolet analyzer and recorder. The various fractions were compared to standards of known molecular weights. Each major fraction was concentrated and tested for its Zn and Fe depressing effects as well as for other biochemical parameters in the rat. The fraction having the highest specific activity for depression of these 2 serum metals was in the molecular weight range of between 10,000 and 20,000. However, activity was also found in a high m.w. fraction, >200,000. Studies employing Cm and DEAE ion exchange resins on both crude LEM preparations and fractions from Sephadex columns indicate that the activity seen in the high molecular weight fraction represents aggregates of the lower molecular weight material.

The effect of the proteolytic enzymes, pronase and trypsin, on LEM have been tested, as well as the effect of varying pH on LEM activity. These studies indicate LEM to be a protein which is active in a pH range between 4.5-8.0.

Since the endogenous mediator represents only a small percentage of the proteins released by the PMN leukocyte, attempts were made to find a suitable animal species for obtaining large quantities of PMN leukocytes

from peritoneal exudates in order to have sufficient material for purification of the mediator. Therefore, leukocytic extracts were obtained from peritoneal exudates of goat, burro and dog. When the crude extracts were tested for serum Zn and Fe depressing activity in the rat assay model, only the dog leukocytic extract had activity. However, these preparations have proven to be inadequate in quantity for supplying enough starting material for purification at the present. Studies are now in progress to increase both cell yield and LEM secretion. Furthermore, since monocytic cells have been shown to produce in vitro endogenous pyrogen (a protein closely related to LEM), an attempt is now in progress to design a cell culture system for the production of enough material needed for the purification of the mediator.

Summary, Part II:

Initial studies on the purification of the mediator of altered trace metal metabolism characterize LEM to be a heat labile, low molecular weight (10,000-20,000 m.w.) protein that can result in higher molecular weight aggregates. Since LEM represents only a small percentage of the proteins released by PMN leukocytes, attempts have been made to find a suitable method and animal species for obtaining enough starting material for its purification.

Progress, Part III:

Studies from our laboratory, as well as those reported in the literature, indicated that the very early infection-induced decreases in serum Zn and Fe concentrations were not the result of decreased gastro-intestinal absorption or increased excretion, but were due to a rapid redistribution of the 2 metals within the host. To support this hypothesis, rats were pulse labeled with ^{65}Zn (5 μCi/100 gm body weight, IP) and then infected with virulent D. pneumoniae organisms. After 8 hr both infected and NaCl sham-inoculated controls were killed and the concentration of the isotope in the plasma and various tissues was determined and compared. Infected animals demonstrated significant decreases of ^{65}Zn in the plasma with significant increase in the liver when compared to controls.

Furthermore, if LEM is truly an intermediate released during the infectious or inflammatory process, then it too should stimulate a redistribution of the metals when administered to a normal rat. Rats were then pulse labeled in the same manner with either ^{65}Zn or ^{59}Fe and administered LEM (150 μg protein IP). As predicted LEM produced significant decreases in the plasma levels of ^{65}Zn and ^{59}Fe with significant liver uptake of the 2 isotopes when compared to controls. These data suggest that infection induces a rapid redistribution of Zn and Fe within the host, and this redistribution is mediated by an endogenous factor (LEM).

Summary, Part III:

Isotopic studies have demonstrated that infection produces a rapid redistribution of Zn and Fe in the tissues of the host. The same redistribution can be demonstrated when LEM is administered to the normal host, which adds further support to the role of LEM as an early intermediate in altered host metabolism during the inflammatory process.

Progress, Part IV:

Two elements of recent biological interest are Cr and Ni. Cr has been shown to be an essential micronutrient with its deficiency being characterized by impaired growth and disturbances in glucose, lipid and protein metabolism.[13] Recently, increased serum Ni concentrations have been reported in patients following acute myocardial infarction.[14] However, in order to study the effect of acute infection and other inflammatory stresses on Cr and Ni metabolism, large numbers of samples from various fluids and tissues have to be examined. Although Cr and Ni have been measured in biological materials by a variety of analytical methods, the techniques required tedious extraction and ashing procedures, were time consuming, and offered considerable opportunities for sample contamination from exogenous sources.

What was needed was a method, whereby Cr and Ni in biological samples could be measured directly in a rapid, accurate and reproducible manner. Recently, a new Perkin-Elmer Model 403 spectrophotometer with a heated graphite atomizer was obtained and installed. This new equipment has allowed for the development and employment of newer techniques to measure and analyze such trace metals as Ni, Cr, Al, Co, and Cd in biological samples.

By the use of the new heated graphite furnace serum Cr and Ni concentrations can now be measured directly by atomic absorption spectrophotometry in 50-µl serum samples. Pretreatment or tedious extraction procedures on large serum samples can be avoided by this simple, rapid and reproducible method. A 50-µl serum sample is inserted into the graphite cell where it is dried, ashed and atomized in less than 15 min. Interference by other trace metals or electrolytes is eliminated by proper program selection. When serum Cr and Ni concentrations were measured in a group of 20 healthy young adults (Medical Division Protocol FY 72-2) mean serum concentrations of 0.17 µg/100 ml (SD = ± 0.06) and 1.5 µg/100 ml (SD = ± 0.5) were obtained for Cr and Ni respectively. The coefficients of variability for 15 determinations on a single pooled serum sample were 6% for Cr and 5% for Ni. The human serum Ni concentrations in this study either correspond to or are slightly lower than those reported in the literature. The serum Cr concentrations reported herein are at least one log lower than those previously reported. These lower results can be attributed to both the sensitivity of the system and the reduction of possible sources of exogenous contamination.

Summary, Part IV:

By use of a heated graphite atomizer, serum Cr and Ni can now be measured directly by an atomic absorption spectrophotometric technique in samples as small as 50 µl of serum. Pretreatment or tedious extraction procedures on large serum samples can be avoided by this simple, rapid, and reproducible method.

Presentations:

1. Pekarek, R. S., and W. R. Beisel. Metabolic losses of zinc and other trace elements during acute infection. Presented at, Western Hemisphere Nutrition Congress III, Bal Harbour, Miami, Florida, 30 Aug-2 Sept 1971.

2. Pekarek, R. S., and E. C. Hauer. Direct determination of serum Cr and Ni by an atomic absorption spectrophotometer with a heated graphite furnace. Presented at Federation of American Societies for Experimental Biology, Atlantic City, New Jersey 9-14 April 1972.

3. Beisel, W. R., R. S. Pekarek, and R. W. Wannemacher, Jr. Effects of leukocytic endogenous mediator (LEM) on the distribution in tissues of zinc and iron. Presented at Federation of American Societies for Experimental Biology, Atlantic City, New Jersey 9-14 April 1972.

Publications:

1. Pekarek, R. S., and W. R. Beisel. 1971. Characterization of the endogenous mediator(s) of serum zinc and iron depression during infection and other stresses. Proc. Soc. Exp. Biol. Med. 138:728-732.

2. Squibb, R. L., W. R. Beisel, and K. A. Bostian. 1971. Effect of Newcastle disease on serum copper, zinc, cholesterol, and carotenoid values in the chick. Appl. Microbiol. 22:1096-1099.

3. Wannemacher, R. W., Jr., R. S. Pekarek and W. R. Beisel. 1972. Mediator of hepatic amino acid flux in infected rats. Proc. Soc. Exp. Biol. Med. 139:128-132.

4. Wannemacher, Jr., R. W., R. S. Pekarek, P. J. Bartelloni, R. T. Vollmer and W. R. Beisel. 1972. Changes in individual plasma amino acids following experimentally induced sand fly fever virus infection. Metabolism 21:67-76.

5. Pekarek, R. S., W. R. Beisel, P. J. Bartelloni, and K. A. Bostian. 1972. Determination of serum zinc concentration in normal adult subjects by atomic absorption spectrophotometry. Amer. J. Clin. Path. 57:506-510.

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8. Beisel, W. R., R. S. Pekarek, and R. W. Wannemacher, Jr. 1972. Effects of leukocytic endogenous mediator (LEM) on the distribution in tissues of zinc and iron. Fed. Proc. 31:667 (abstract).

9. Wannemacher, Jr., R. W., R. S. Pekarek, and W. R. Beisel. 1972. An endogenous mediator(s) of plasma amino acid flux and trace metal depression during experimentally induced infection in man. Amer. J. Clin. Nutr. 25:464 (abstract).

10. Wannemacher, Jr., R. W., R. S. Pekarek, M. C. Powanda, W. R. Beisel, R. L. DuPont, A. Schwartz, and R. B. Hornick. 1972. An endogenous mediator of serum amino acid and trace metal depression during typhoid fever. J. Infect. Dis. (in press).

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1. Pekarek, R. S., K. A. Bostian, P. J. Bartelloni, F. M. Calia and W. R. Beisel. 1969. The effects of Francisella tularensis infection on iron metabolism in man. Amer. J. Med. Sci. 258:14-25.

2. Pekarek, R. S., G. A. Burghen, P. J. Bartelloni, F. M. Calia, K. A. Bostian, and W. R. Beisel. 1970. The effect of live attenuated Venezuelan equine encephalomyelitis virus vaccine on serum iron, zinc and copper concentrations in man. J. Lab. Clin. Med. 76:293-303.

3. Wannemacher, Jr., R. W., R. S. Pekarek, P. J. Bartelloni, R. T. Vollmer, and W. R. Beisel. 1972. Changes in individual plasma amino acids following experimentally induced sand fly fever virus infection. Metabolism 21:67-76.

4. Wannemacher, Jr., R. W., R. S. Pekarek, M. C. Powanda, W. R. Beisel, R. L. DuPont, A. Schwartz, and R. B. Hornick. 1972. An endogenous mediator of serum amino acid and trace metal depression during typhoid fever. J. Infect. Dis. (in press).

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11. Kampschmidt, R. F., and H. F. Upchurch. 1969. Lowering of plasma iron concentration in the rat with leukocytic extracts. Amer. J. Physiol. 216:1287-1291.

12. Pekarek, R. S., and W. R. Beisel. 1971. Characterization of the endogenous mediator(s) of serum zinc and iron depression during infection and other stresses. Proc. Soc. Exp. Biol. Med. 138:728-732.

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ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1W662711A096 03:	Laboratory Identification of Biological Agents
Work Unit No. 096 03 011:	Chemical Mediators of Infection of Military Medical Importance
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Physical Sciences
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Author:	Jean B. DuBuy, Major, MC
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OL0910	72 06 30	DD-[illegible](AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISTR INSTR'N	9b. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NL	YES / NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	03	011
b. [illegible]	62711A	1B662711A096		
c. [illegible]	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)

(U) Chemical mediators of infection of military medical importance

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical medicine; 004900 Defense; 002300 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 02	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER: NA

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	A. PROFESSIONAL MAN YRS	B. FUNDS (In thousands)
FISCAL YEAR — PRECEDING 71	2.0	60
FISCAL YEAR — CURRENT 72	1.0	84

19. RESPONSIBLE DOD ORGANIZATION

NAME: USA Medical Research Institute of Infectious Diseases

ADDRESS: Fort Detrick, MD 21701

RESPONSIBLE INDIVIDUAL

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TELEPHONE: 301 663-2833

20. PERFORMING ORGANIZATION

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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

NAME: DuBuy, J. B.

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Beisel, W. R.

NAME: DA

21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Purification; (U) Electrophoresis; (U) Endogenous pyrogen; (U) Military medicine

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Purify and characterize several chemical mediators of infection. This work unit is an essential element in a comprehensive program for medical defense against BW agents.

24 (U) Polyacrylamide gel electrophoresis is applied to various specimens; resulting fractions are characterized by various applicable methods.

25 (U) 71 07 - 72 06 - The major problem in purification of endogenous pyrogen has proven to be the small amounts of material available after purification procedures, due to both the high specific activity of the material and procedural losses. Membrane ultrafiltration has been resorted to in order to overcome these difficulties. Preliminary results suggest this to be a satisfactory pre-purification procedure.

Terminated since FY 1973 funding will be under The Surgeon General, Army. New Project No. 3A062110A834; Accession No. DA 0C6414.

* Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, NO. 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 03: Laboratory Identification of Biological Agents

Work Unit No. 096 03 011: Chemical Mediators of Infection of Military Medical Importance

Description:

Purify and characterize several chemical mediators of infection.

Progress:

Work on the purification of the endogenous mediator of fever, endogenous pyrogen (EP) was continued. The use of preparative acrylamide gel electrophoresis appeared promising; due to the small quantity of starting material that can be used with this technique, very little or no material was available for further characterization after necessary assays and controls to establish the presence of EP had been performed. These preliminary studies suggested a molecular weight considerably higher than that obtained by other methods, which may suggest that EP is capable of polymer formation.

In order to obtain sufficient amounts of unpurified EP material for more definitive study, it thus became necessary to utilize a preliminary purification technique. The requirements for this technique were: (1) Minimal loss of activity due to degradation or non-specific adsorption, (2) Ability to handle large volumes of starting material, (3) Removal of significant amounts of non-active material, and (4) Potential for concentration and/or desalting. A survey of large volume preliminary purification techniques including zonal centrifugation, zone electrophoresis, preparative precipitation, ion exchange and molecular seive chromatography, and membrane ultrafiltration suggested that the last best satisfied the above requirements. Accordingly preliminary studies were undertaken using the Amicon stirred pressure cell apparatus already available. It was found that the apparatus could be rendered pyrogen-free by extensive washing, and that large amounts of EP activity could be obtained without apparent degradation, although with some losses presumably due to non-specific adsorption. Therefore, an improved version of this apparatus permitting more rapid processing with less possibility of non-specific adsorption was ordered and is presently being evaluated.

Recently, a report in the literature[1] of significant purification of EP has appeared. The authors used membrane ultrafiltration as the starting step, followed by 2 column steps and finally electrofocusing and report final specific activity in the nanogram range, but unfortunately also have insufficient material for further definitive characterization. The major losses of activity seem to be in the 2 column steps.

Summary:

The major problem in purification of endogenous pyrogen has proven to be the small amounts of material available after purification procedures, due to both the high specific activity of the material and procedural losses. Membrane ultrafiltration has been used to overcome these difficulties. Preliminary results suggest this to be a satisfactory pre-purification procedure.

Publications:

None.

LITERATURE CITED

1. Murphy, P. A., P. J. Chesney, and W. B. Wood, Jr. 1971. Purification of an endogenous pyrogen, with an appendix on assay methods. In Pyrogens and Fever, (G. E. W. Wolstenholme and J. Birch, ed.) p. 59 to 79. Churchill Livingstone, Edinburgh.

ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1W662711A096 03:	Laboratory Identification of Biological Agents
Work Unit No. 096 03 012:	Evaluation of Serum Glycoprotein Changes in Early Diagnosis of Infectious Illness of Medical Importance
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Physical Sciences
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Author:	Gary L. Cockerell, Captain, VC
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OL0861	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. ERD'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	03	012
b. CONTRIBUTING	62711A	1B662711AC96		
c. CONTRIBUTING	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code) (U) Evaluation of serum glycoprotein changes in early diagnosis of infectious illness of medical importance

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 Clinical medicine; 004900 Defense; 003200 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
61 07	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION
b. NUMBER: NA
c. TYPE: d. AMOUNT
e. KIND OF AWARD f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR PRECEDING 71	1.0	50
FISCAL YEAR CURRENT 72	1.0	70

19. RESPONSIBLE DOD ORGANIZATION
NAME: USA Medical Research Institute of Infectious Diseases
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SOCIAL SECURITY ACCOUNT NUMBER

21. GENERAL USE
Foreign intelligence considered

ASSOCIATE INVESTIGATORS
NAME:
NAME: DA

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Glycoprotein; (U) Electrophoresis; (U) Infectious diseases; (U) Military medicine; (U) Protein-bound carbohydrate

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code)

23 (U) Evaluate biological activity, role and significance of serum glycoproteins in the early diagnosis of infectious diseases. This work unit is an essential element in a comprehensive program for medical defense against BW agents.

24 (U) Determine serum glycoprotein and protein electrophoretic patterns in normal and infected states using improved techniques, in order to understand their significance.

25 (U) 71 07 - 72 06 - Methodology has been standardized for a more complete and accurate characterization of serum proteins and glycoproteins. Normal baseline values have been collected for man, rhesus monkey and rat. Changes which occur in these substances in disease conditions appear (1) to be nonspecific with regard to inciting agent, (2) have a high priority in the host response, and (3) may be triggered by the release of host endogenous mediator(s).

Publications: Amer. J. Vet. Res. 33:323-327, 1972
Clin. Res. 19:675, 1971 (abstract)
Fed. Proc. 31:710, 1972 (abstract)

Terminated since FY 1973 funding will be under The Surgeon General, Army. New Project No. 3A062110A834; Accession No. DA 0C6415.

*Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE AND WILL NOT BE FURNISHED FOR ARMY USE ARE OBSOLETE

BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 03: Laboratory Identification of Biological Agents

Work Unit No. 096 03 012: Evaluation of Serum Glycoprotein Changes in Early Diagnosis of Infectious Illness of Medical Importance

Description:

Evaluate biological activity, role and significance of serum glycoproteins in the early diagnosis of infectious diseases.

Progress:

During the year we have been able to standardize techniques and procedures for a more complete and accurate characterization of serum glycoproteins.

To measure total glycoprotein concentration we have standardized procedures for analyzing 4 major carbohydrate moieties contributing to the sugar portion of serum glycoprotein concentration. These sugar moieties are hexose, hexosamine, sialic acid and methylpentose; the sum of their concentrations in serum represents total serum protein-bound carbohydrate and is thus an index of total glycoprotein concentration. This value, when combined with cellulose polyacetate electrophoresis of serum and staining for glycoprotein with periodic acid-Schiff reagent, can then be used to compute absolute and relative amounts of glycoprotein in each of the electrophoretic fractions. Likewise, using total serum protein determined by a biuret method and electrophoresis followed by staining for protein, similar parameters can be quantitated for serum proteins. Computer programs have been written to derive all the above mentioned values from raw laboratory data.

The Farrand Chromatogram Analyzer has been adapted for scanning cellulose acetate electrophoretograms stained for protein and glycoproteins. This machine provides a more accurate and less laborious method of scanning our electrophoretograms than the Beckman Analytrol. Compared to the Farrand Chromatogram Analyzer the Beckman Analytrol gives an erroneously high percentage of albumin and low percentages of all globulin fractions.

Employing the above described techniques and procedures we first established baseline values. At this time we have collected normal values for man (Medical Division Protocol FY72-2), rhesus monkey, and rat. The values for the first two are relatively similar but significant differences are found when these 2 species are compared to the rat. For example, man has a total protein-bound carbohydrate concentration of 220.07 ± 4.88 mg/100 ml (mean ± SEM) compared to 392.16 ± 16.20 mg/100 ml for the rat. In man 14.7 ± 0.8% of this total glycoprotein falls in the α_1 glycoglobulin fraction and 28.1 ± 0.7% in the α_2 fraction. Whereas in the rat the percentage distribution of these 2 fractions is 53.4 ± 1.6 and 15.7 ± 0.9 respectively. Differences of this magnitude must be taken into consideration when interpreting experimental results from different species.

Secondly, we measured changes in protein and glycoprotein patterns of rats infected with a systemic febrile disease induced by Diplococcus pneumoniae, or a localized afebrile inflammatory reaction elicited by the subcutaneous injection of sterile turpentine.[4] In both instances, changes in patterns of plasma proteins and glycoproteins were similar and characterized by: an absolute decrease in albumin, increased α_2 and β globulins, an even more dramatic absolute increase in α_1, α_2 and β glycoglobulins and total plasma protein-bound carbohydrate including elevations in each of the 4 individual carbohydrate moieties measured. Furthermore, these same patterns of change were seen in rats which were starved to a 25% body-weight loss prior to being stressed with either pneumococci or turpentine. It was concluded therefore: (1) the changes noted in plasma proteins and glycoproteins were nonspecific with respect to inciting agent; and (2) these changes occupy a high priority in the host response, in that they occur despite drastic reductions in nutritionally derived plasma precursor pools.

Lastly, we began an investigation to determine if leukocytic endogenous mediator (LEM), similar to that described by Pekarek,[5] may be responsible for these changes. Amino acid, Zn and Fe movement into liver found to occur following LEM administration to normal rats,[3] has been also found to occur in pneumococcal infected rats.[6] Preliminary data in this laboratory also document such a flux in turpentine-stressed rats. Injection of LEM into normal rats produces an absolute decrease in albumin, increases in α_2 globulin and β glycoglobulin fractions and total protein-bound carbohydrates. These data are within the pattern of change seen with pneumococcal infected or turpentine inflamed rats, therefore suggesting that some such mediator(s) may be involved in triggering these changes.

Summary:

Methodology has been standardized for a more complete and accurate characterization of serum proteins and glycoproteins. Normal baseline values have been collected for man, rhesus monkey and rat. Changes which occur in these substances in disease conditions appear (1) to be nonspecific with regard to inciting agent, (2) have a high priority in the host-response, and, (3) may be triggered by the release of host endogenous mediator(s).

Presentation:

Cockerell, G. L. Plasma protein and glycoprotein changes in inflammation, infection and/or starvation. Presented at Annual Meeting of Federation of American Societies for Experimental Biology, Atlantic City, New Jersey, 9-14 April 1972.

Publications:

1. Klainer, A. S., E. Dixon, and W. R. Beisel. 1971. Serum glycoproteins in acute infection. Clin. Res. 19:675 (abstract).

2. Rollins, J. B., T. D. Shultz, and R. H. Fiser. 1972. Serial measurements of serum protein, glycoprotein, and lipoprotein fractions in normal and Venezuelan equine encephalomyelitis-vaccinated ponies and burros. Amer. J. Vet. Res. 33:323-327.

3. Cockerell, G. L. 1972. Plasma protein and glycoprotein changes in inflammation, infection and/or starvation. Fed. Proc. 31:710 (abstract).

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1. Cockerell, G. L. 1972. Plasma protein and glycoprotein changes in inflammation, infection and/or starvation. Fed. Proc. 31: 710 (abstract).

2. Pekarek, R. S., and W. R. Beisel. 1971. Characterization of the endogenous mediator(s) of serum, zinc and iron depression during infection and other stresses. Proc. Soc. Exp. Biol. Med. 138:728-732.

3. Wannemacher, Jr., R. W., R. S. Pekarek and W. R. Beisel. 1972. Mediator of hepatic amino acid flux in infected rats. Proc. Soc. Exp. Biol. Med. 139:128-132.

4. Wannemacher, Jr., R. W., M. C. Powanda, R. S. Pekarek, and W. R. Beisel. 1971. Tissue amino acid flux after exposure of rats to Diplococcus pneumoniae. Infec. Immun. 4:556-562.

ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1W662711A096 03:	Laboratory Identification of Biological Agents
Work Unit No. 096 03 013:	Serum Protein and Enzyme Changes for Diagnosis of Militarily Important Infections
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Physical Sciences
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Authors:	Michael C. Powanda, Captain, MSC Robert W. Wannemacher, Jr., Ph.D.
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OLO912	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISP'N INSTR'N	8B. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	03	013
b. ~~SECONDARY~~	62711A	1B662711A096		
c. ~~SECONDARY~~	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code): (U) Serum protein and enzyme changes for diagnosis of militarily important infections

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical medicine; 004900 Defense; 002300 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 04	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER: NA
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 71	2.0	8
FISCAL YEAR CURRENT 72	2.0	33

19. RESPONSIBLE DOD ORGANIZATION
NAME: USA Medical Research Institute of Infectious Diseases
ADDRESS: Fort Detrick, MD 21701

RESPONSIBLE INDIVIDUAL
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TELEPHONE: 301 663-2833

20. PERFORMING ORGANIZATION
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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
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TELEPHONE: 301 663-7181
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Wannemacher, R. W.
NAME: DA

21. GENERAL USE
Foreign intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Proteins; (U) Enzymes; (U) Infectious disease; (U) Military medicine; (U) Electrophoresis

23. TECHNICAL OBJECTIVE * 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Evaluate changes in serum proteins and isoenzymes occurring during infectious disease and elucidate the kinetics of such changes as an aid to early diagnosis. This work unit is an essential element in a comprehensive program for medical defense against BW agents.

24 (U) Using polyacrylamide gel electrophoresis, measure changes in serum proteins and enzymes during infections.

25 (U) 71 07 - 72 06 - Increases in lactate dehydrogenase isoenzyme forms 1, 2 and 3 have been demonstrated to occur during the incubation period of typhoid fever, long before clinical signs of illness.

Terminated since FY 1973 funding will be under The Surgeon General, Army. New Project No. 3A062110A834; Accession No. DA OC6416.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 68 AND 1498 1 MAR 68 FOR ARMY USE ARE OBSOLETE.

BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 03: Laboratory Identification of Biological Agents

Work Unit No. 096 03 013: Serum Protein and Enzyme Changes for Diagnosis of Militarily Important Infections

Description:

Evaluate changes in serum proteins and isoenzymes occurring during infectious disease and elucidate the kinetics of such changes as an aid to early diagnosis.

Progress:

In a cooperative study with a contractor (University of Maryland, DA49-193-MD-2867), sera of typhoid fever patients were examined for lactate dehydrogenase (LDH) activity. Eleven volunteers with experimentally-induced typhoid fever had significant rises in LDH levels day 1 post-infection. Analysis of these sera by polyacrylamide gel electrophoresis indicated that LDH isoenzymes 1, 2 and 3 were noticeably increased during the incubation period, in some instances as much as a week before clinical evidence of illness[1] was seen. A possible source of the increased concentration of these isoenzymes may be the leukocytes which contain primarily these LDH forms[2] and release them during phagocytosis.[3]

Experimentation regarding staining techniques for glycoproteins and ceruloplasmin was carried out during this period. Marked qualitative changes in serum glycoprotein patterns in rats either infected with Diplococcus pneumoniae or subjected to turpentine abscess were seen (see Work Unit 096 03 012).

The Ortec slab acrylamide gel electrophoresis system has also been used to monitor progress in the purification of the endogenous mediator(s) of the alterations in serum trace metal concentration, in amino acid movement and in serum protein synthesis (see also Work Units 096 01 009 and 096 03 010).

Summary:

Qualitative increases in LDH isoenzymes forms 1, 2 and 3 were shown to occur in the sera of volunteers exposed to typhoid fever during the incubation period, long before clinical signs of illness.

Stains for other enzymes possessing multiple active forms as well as for glycoproteins are being evaluated.

Publications:

Wannemacher, R. W., Jr., H. L. DuPont, R. S. Pekarek, M. C. Powanda, A. Schwartz, R. B. Hornick, and W. R. Beisel. 1972. Evidence for an endogenous mediator of serum amino acid and trace metal depression following experimentally-induced typhoid fever in man. J. Infect. Dis. In press.

LITERATURE CITED

1. Wannemacher, R. W., Jr., H. L. DuPont, R. S. Pekarek, M. C. Powanda, A. Schwartz, R. B. Hornick, and W. R. Beisel. 1972. Evidence for an endogenous mediator of serum amino acid and trace metal depression following experimentally-induced typhoid fever in man. J. Infect. Dis. In press.

2. Vesell, E. S., and A. G. Bern. 1961. Isozymes of lactate dehydrogenase in human tissues. J. Clin. Invest. 40:586-591.

3. Hale, T. L., and J. M. Woodward. 1971. Effect of infection and endotoxicosis on plasma lactate dehydrogenase isozymes in white rats. Infec. Immun. 3:468-472.

ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	**Medical Defense Aspects of Biological Agents (U)**
Task No. 1W662711A096 03:	**Laboratory Identification of Biological Agents**
Work Unit No. 096 03 402:	**Development of Methods for Detection and Assay of Interferon for Rapid Identification of Illnesses of Military Importance**
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Virology
Period Covered by Report:	1 July 1972 to 30 June 1972
Professional Authors:	Bruno J. Luscri, Ph.D. (I) George W. Jordan, Major, MC (II)
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	**UNCLASSIFIED**

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OL0855	72 06 30	DD DR&E-AR(6)6

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISPN INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	03	402
b. [illegible]	62711A	1B662711A096		
c. [illegible]	CDOG 1212b(9)			

11. (U) Development of methods for detection and assay of interferon for rapid identification of illnesses of military importance

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 Clinical medicine; 004500 Defense; 010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
61 08	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE — EXPIRATION
b. NUMBER: NA
c. TYPE — d. AMOUNT
e. KIND OF AWARD — f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 71	2.0	100
FISCAL YEAR CURRENT 72	2.0	100

19. RESPONSIBLE DOD ORGANIZATION
NAME: USA Medical Research Institute of Infectious Diseases
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TELEPHONE: 301 663-7241
SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
NAME: Jordan G. W.
NAME: DA

31. GENERAL USE
Foreign intelligence considered

32. KEYWORDS (Precede EACH with Security Classification Code)
(U) Interferon; (U) Viral interference; (U) Sindbis virus; (U) Semliki Forest virus; (U) Chikungunya virus; (U) Military medicine

33. TECHNICAL OBJECTIVE. 34. APPROACH. 35. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Develop tests for use in rapid diagnosis of viral diseases. This work unit is an essential element in a comprehensive program for medical defense against BW agents.

24 (U) An interferon of tissue culture origin will be produced and characterized. Standard methods for bioassay will be applied. Interferon is to be produced in quantity and partially purified. Antisera to partially purified interferon are to be tested for possible use in an immunological assay for interferon.

25 (U) 71 07 - 72 06 - I. A human interferon preparation from human embryo cell cultures convenient for use as an internal laboratory standard has been produced. Interferons from a human foreskin, WI-38, and MA-160 cell cultures have been produced in serum-free media, are suitable for study of their chemical and physical characteristics and are convenient for use for immunization of experimental animals.

II. Antisera which are active in neutralizing the biologic activity of mouse interferon have been produced. The application of an immunological technique for the assay of interferon must await further developments in the labeling and purification of the interferons. A study of virulent and attenuated strains of VEE virus indicates that the interferon system is important in determining the yield of virus from tissue culture. Interferon sensitivity may be one of the factors one should examine when attenuating a virus strain for vaccine production.

Publications: Abst. Annual Meeting ASM 1972, p. 196.
J. Gen Virol. 14:49-61, 1972.
Virology 48:425-432, 1972.

Terminated since FY 1973 funding will be under The Surgeon General, Army. New Project No. 3A062110A834; Accession No. DA 0C6417.

*Available [illegible] approval.

DD FORM 1498 [illegible]

BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 03: Laboratory Identification of Biological Agents

Work Unit No. 096 03 402: Development of Methods for Detection and Assay of Interferon for Rapid Identification of Illnesses of Military Importance

Description:

Develop methods for the detection and bioassay of interferon.

Progress, Part I:

Interferon (IF) from several human cell cultures were prepared and assayed. The IF preparations were processed by aseptic dialysis against buffers of pH 2 and 7.4. Their characterization included the following criteria: (1) noninfective for embryonated eggs, (2) not antiviral in chick embryo cell cultures, (3) unable to neutralize the challenge viruses, and (4) retained antiviral activity after centrifugation at 100,000 X g for 3 hr.

One source from human embryo cell cultures was produced by exposure of these cells to live Newcastle disease virus, strain B1 (NDV B1), and collecting the interferon in medium 199 containing 2% fetal bovine serum. The methods of induction were suggested in literature references using the CG strain of NDV[1] and a UV-irradiated strain of NDV[2]. These antiviral fluids were assayed on human foreskin (CCL54, Detroit 532)[3] and HeLa cells using Venezuelan equine encephalomyelitis (VEE) virus for challenge, with the results shown in Table I.

TABLE I. RESPONSE OF HUMAN FORESKIN (CCL54) AND HELA CELLS TO INTERFERON PREPARED FROM PRIMARY HUMAN EMBRYO (WHOLE) CELL CULTURES WITH NDV B1

BIOASSAY CELL TYPE	IF TITER[a]/ PR_{50}/3 ml
Human foreskin	5000-8000
HeLa	126

a. Plaque-reduction titer, 50% reduction.

The results indicate that the interferon serves conveniently as an internal standard for the bioassay of interferons of human origin.

An interferon was produced from human foreskin cells (ATCC, CCL54) in response to infection with live NDV B1. The interferon was produced in serum-free medium 199, and assayed in homologous cells by a yield reduction method[4]. The antiviral spectrum spectrum of this interferon can be seen in Table II. These levels of interferon supercede those plaque reduction values presented previously for this interferon[5].

TABLE II. LEVELS OF A HOMOLOGOUS HUMAN FORESKIN (CCL54) INTERFERON AS ASSAYED BY A YIELD-INHIBITION METHOD AGAINST 5 VIRUSES

CHALLENGE VIRUSES	INTERFERON LEVELS UNITS/3 ml
Sindbis	1000
Encephalomyocarditis	320-800
Vesicular stomatitis	320-500
Western equine encephalitis	320
VEE	160-200

Evidence was obtained that the addition of 2% fetal bovine serum to the human foreskin interferon amplifies its antiviral activity in a yield-reduction assay against the virus of Western equine encephalitis.

Interferons of human cell culture origins were also produced in 2 additional cell strains as shown in Table III.

TABLE III. ANTIVIRAL TITERS OF WI-38 AND MA-160 CELL INTERFERONS BY PLAQUE REDUCTION OF VEE IN HUMAN AMNION CELLS (AV_3)

INTERFERON SOURCE CELLS	INDUCING VIRUS	IF TITER PR_{50}/3 ml
WI-38, human embryonic lung	Sendai	8
	NDV B1	6
	NDV (Herts)	2.5
	NDV B1 UV-irradiated	6.3
MA-160, human prostatic adenoma	Sendai	16
	Sendai (37 C, 18 hr)	2

These titers are not elevated, and higher IF titers were found when the interferon produced in WI-38 cells in response to Sendai virus was assayed in homologous and HeLa cells.[5] Thus, Sendai virus was shown to induce interferon in WI-38 cells, and joins Chikungunya,[6] respiratory syncytial,[7] and NDV[8] as known interferon inducers in this cell strain.

Previous procedures had indicated that when guines pigs were injected[9] with attenuated VEE vaccine, strain TC-83, and a L-cell interferon,[9] a neutralizing antibody of about 1/8 for the mouse interferon was demonstrable. Immunization schedules are being planned for inoculation of experimental animals with selected human interferons harvested in serum-free media.

Summary, Part I:

A human interferon preparation from human embryo cell cultures convenient for use as an internal laboratory standard has been produced. Interferons from human foreskin, WI-38, and MA-160 cell cultures have been produced in serum-free media are suitable for study of their chemical and physical characteristics, and are convenient for use for immunization of lower animals.

Progress, Part II:

Sera obtained from rabbits and guinea pigs immunized with chick and mouse interferons were tested for anti-interferon activity. None was found in sera from animals immunized with chick interferon. Serum from guinea pigs immunized with mouse interferon has been shown to contain anti-interferon activity. In order to obtain isotopically labeled interferon for use as a test antigen, the induction of L-cell interferon by NDV (B_1 strain) was carried out in the presence of tritium labeled amino acids. Partial purification of the interferon was attempted by the use of gel chromatography. The yield of interferon from the labeling experiment was low and biological activity was not recovered from the column.

A study of the effect of attenuation of a virus on the sensitivity to and production of interferon was carried out with several strains of VEE and Western equine encephalomyelitis (WEE) viruses. The results show that at high multiplicities of infection (MOI) more interferon and less virus are produced by chick embryo cells (CEC). Furthermore when the virus inoculum is grown in CEC, more chick interferon and less virus are produced by CEC when compared to mouse brain inoculum at the same MOI. Chick interferon was demonstrated in the CEC grown inoculum and the results above are probably due to the "priming" phenomenon.[10] The Trinidad and TC-83 strains of VEE were found to induce approximately the same amount of interferon; the attenuated C1-15 strain of WEE induced

more interferon that the parent B628 strain. A large and small plaque variant were selected from the seed stock of Trinidad VEE virus. The virulent strains were found to be less sensitive to both chick and mouse interferon when compared with the TC-83 attenuated strain. These results suggest that interferon sensitivity may be one of the factors that should be selected for in the attenuation of a virus strain. The virulent and attenuated VEE virus strains may be useful in investigating the role of interferon as a specific host defense mechanism.

Summary, Part II:

Antisera which are active in neutralizing the biologic activity of mouse interferon were produced. The application of an immunological technique for the assay of interferon must await further developments in the labeling and purification of the interferons. A study of virulent and attenuated strains of VEE virus indicates that the interferon system is important in determining the yield of virus from tissue culture and suggests that interferon sensitivity may be one of the factors selected for in the attenuation of a virus strain.

Presentation:

Luscri, B. J. Several observations on the induction and bioassay of interferon from human cell cultures. Presented at Annual Meeting of American Society for Microbiology, Philadelphia, Pa. 23-28 April 1972.

Publications:

1. Luscri, B. J. Several observations on the induction and bioassay of interferon from human cell cultures. Abst. Annual Meeting ASM, 1972.

2. Jordan, G. W. 1972. Basis for the probit analysis of an interferon plaque reduction assay. J. Gen. Virol. 14:49-61.

3. Jordan, G. W. 1972. Quantitative aspects of interferon induced plaque reduction: Kinetics of interferon action. Virology. 48:425-432.

4. Jordan, G. W. 1972. Effect of interferon on the production of hemagglutinins and infectivity of GDVII virus. Arch. ges.Virusforsch. In press.

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1. Henry, C. J., and M. Slifkin. 1971. Inhibition of adenovirus type 2 by human and monkey interferon. Proc. Soc. Exp. Biol. Med. 137: 1432-1436.

2. Ho, M., Y. H. Tan, and J. A. Armstrong. 1972. Accentuation of production of human interferon by metabolic inhibitors. Proc. Soc. Exp. Biol. Med. 139:259-262.

3. Moehring, J. M., W. R. Stinebring, and D. J. Merchant. 1971. Survey of interferon production and sensitivity in human cell lines. Appl. Microbiol. 22:102-106.

4. Stewart, II, W. E., and R. Z. Lockart, Jr. 1970. Relative antiviral resistance induced in homologous and heterologous cells by cross-reacting interferons. J. Virol. 6:795-799.

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8. Siewers, C. M. F., C. E. John, and D. N. Medearis, Jr. 1970. Sensitivity of human cell strains to interferon. Proc. Soc. Exp. Biol. Med. 133:1178-1183.

9. U.S. Army Medical Research Institute of Infectious Diseases. 1 July 1969. Annual Progress Report, FY 1969, p. 219 to 221. Fort Detrick, Md.

10. Stewart, II, W. E., L. B. Gosser, and R. Z. Lockart, Jr. 1971. Priming: A nonantival function of interferon. J. Virol. 7:792-801.

ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1W662711A096 03:	Laboratory Identification of Biological Agents
Work Unit No. 096 03 403:	Separation, Purification and Concentration of Arbovirus Agents and Antigen-antibody Complexes for Military Medicine
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Virology
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Authors:	Neil H. Levitt, Captain, MSC (I) Carl E. Pedersen, Major, MSC (II, III)
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OL0856	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	03	403
b. [illegible]	62711A	1B662711A096		
c. [illegible]	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)
(U) Separation, purification and concentration of arbovirus agents and antigen-antibody complexes for military medicine

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 Clinical medicine; 004900 Defense; 010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
61 11	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER: NA
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 71	1.0	40
	CURRENT 72	1.0	100

19. RESPONSIBLE DOD ORGANIZATION
NAME: USA Medical Research Institute of Infectious Diseases
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21. GENERAL USE
Foreign intelligence considered

20. PERFORMING ORGANIZATION
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ASSOCIATE INVESTIGATORS
NAME: Pedersen, C. E.
NAME: DA

22. KEYWORDS (Precede each with Security Classification Code)
(U) Electrophoresis; (U) Immunology; (U) Military medicine; (U) Serology; (U) Antigen-antibody reactions

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Develop an in vitro diagnostic test for rapid detection of antibody to viruses causing militarily significant disease. Detect antigenic and biochemical variation among closely related viruses. This work unit is an essential element in a comprehensive program for medical defense against BW agents.

24 (U) Virus-antibody complexes are separated and detected by cellulose acetate electrophoresis and column chromatography.

25 (U) 71 07 - 72 06 - An identification procedure was developed to determine the strain grouping of unknown VEE field specimens using the microprecipitation test (MPT) to detect minor antigenic differences among the virus strains. Six field specimens, previously classified by subgroup by the kinetic hemagglutination-inhibition technique, were similarly identified by this laboratory using MPT. Methods are being studied to increase the sensitivity of the MPT using 125-I-labeled globulin. Clinical specimens from rhesus monkeys experimentally infected with rubella virus were examined for detection of specific antigen using the MPT.

Column chromatography of selected VEE viruses on calcium phosphate gel offered a simple and reproducible method for examination of biochemical characteristics and relatedness of strains within the VEE complex.

Isolation of VEE virus from mosquitoes captured in Louisiana during the Texas epizootic will be reported in Applied Microbiology soon to be published.

Publications: Appl. Microbiol. 22:143-144, 1971;

Terminated since FY 1973 funding will be under The Surgeon General, Army. New Project No. 3A062110A834; Accession No. DA 0C6418.

*Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE AND 1498-1 1 MAR 68 FOR ARMY USE ARE OBSOLETE

BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 03: Laboratory Identification of Biological Agents

Work Unit No. 096 03 403: Separation, Purification and Concentration of Arbovirus Agents and Antigen-antibody complexes for Military Medicine

Description:

Develop an *in vitro* diagnostic test for the rapid identification of viruses causing militarily significant disease. Detect antigenic variation among closely related viruses.

Progress, Part I:

The microprecipitation test (MPT) was employed to detect antigenic differences among strains of Venezuelan encephalomyelitis virus (VEE) representing each of the 8 groups characterized by Young and Johnson[1] using kinetic hemagglutination inhibition (KHI) technique. Tissue culture virus materials of these 8 strains of VEE were produced from the supernatant fluid harvests of infected duck embryo cell monolayers. These materials were concentrated by DEAE - cellulose chromatography and ultracentrifugation in order to prepare high titered antigens for use in the MPT. Table 1 shows PFU and hemagglutinin (HA) titers[2] of the antigens.

TABLE I. TITRATION OF VEE ANTIGENS

ANTIGEN		PFU/ml[a]	RECIPROCAL HA TITER
Name	Group		
Trinidad	Ia	10.9	3200
TC-83	unclassified	11.6	3200
Ica	1b	10.6	3200
P676	Ic	11.0	3200
3880	Id	10.1	3200
Mena II	Ie	10.7	800
Fe 3-7c	II	10.5	1600
Mucambo (MUC)	III	11.3	6400
Pixuna (PIX)	IV	10.1	400

a. Assayed on chick embryo cell monolayers.

TABLE 11. HEMAGGLUTINATION-INHIBITION TITERS OF RABBIT ANTISERA

ANTISERUM	RECIPROCAL HI TITER[a]
Trinidad	>5120
TC-83	5120
[illegible]	5120
[illegible]	5120
[illegible]	5120
Mena II	640
Fe 3-7c	2560
MUC	320
PIX	320

a. All sera tested for HI activity against 8 units of Trinidad VEE antigen.

Antisera for the MPT were produced in 2-2.5 kg New Zealand albino rabbits inoculated with formalin-inactivated suckling mouse brain preparations of the strains. Table II shows the hemagglutination-inhibition (HI) titers of the antisera. An antigen-antibody reaction pattern was established for each antigen using the MPT. Dilutions of (1:4 - 1:64) of each antigen were reacted in the MPT with dilutions of 1:10 - 1:160, respectively, of the different antisera. Such block testing revealed that each antigen possessed a unique pattern of reactivity differing from other antigen prototypes. Table III shows the scoring of the antigen-antisera reactions. From this tabulation a standard test pattern, as shown in Table IV was developed to identify the strain prototypes of unknown specimens.

Blind experiments were conducted using the 8 antigens coded, but not strain labeled. Correct identification of these antigens proved possible using the test standard. To prove the validity of the established standard for identifying strains of unknown field specimens, VEE virus strains obtained from Drs. Sherer and Shope were subjected to these identification procedures. The virus materials were grown in duck embryo cell monolayers and the supernatant harvest fluids concentrated by cellulose chromatography and ultracentrifugation to prepare antigens. Using the antisera to the 8 original strain prototypes, in dilutions selected for the standard pattern, the antigens were subjected to the MPT. The resulting values obtained in the standard test pattern were used to classify the antigens into VEE strain groups having similar antigenic reactivity. Six antigens have currently been examined with this identification procedure. This laboratory, was able to place 6 antigens into the identical strain groups Young and Johnson designated using the KHI technique.

Modification of the MPT utilizing ^{125}I labeled globulin is currently under investigation. Successful detection of small amounts of antigen in field or clinical specimens is not possible with the present MPT. Currently, antigen must have a titer of $\geq$10 logs of virus material before the antigen-antibody mixture will result in a visible precipitate in the MPT. Feasibility is being studied for using ^{125}I-globulin complexed directly with an antigen or complexed with an antigen-antibody reaction mixture in the MPT. Such successful modification of the MPT should allow the detection of small amounts of viral material as might be found in clinical or field specimens.

Currently experiments are being conducted to detect rubella virus in clinical specimens from rubella-infected rhesus monkeys. Tissue culture methods revealed virus in throat specimens; however, conventional MPT on replicate specimens failed to detect the antigen. Studies are continuing to use the MPT ^{125}I modification for detection of rubella antigen.

Summary, Part I:

An identification procedure was developed to determine the strain grouping of unknown VEE field specimens using the MPT to detect minor antigenic differences among the virus strains. Six field specimens, previously classified into subgroups by the KHI technique, have been similarly identified by this laboratory using the MPT. Methods are being studied to increase the sensitivity of the MPT using ^{125}I-labeled globulin. Clinical specimens from rhesus monkeys experimentally infected with rubella virus are being tested with MPT for detection of the antigen.

TABLE III. VEE ANTIGEN-ANTISERA REACTIVITY USING MPT

ANTISERUM		ANTIGEN Reciprocal of highest dilution showing + reaction[a/]								
Name	Dilution	Trin.	TC-83	Ica	P676	3880	Mena II	Fe 3-7c	MUC	PIX
Trinidad	1:10	64	32	32	32	4	16	8	32	4
	1:20	64	32	32	32	8	16	8	32	4
	1:40	64	32	32	32	8	8	8	32	-
	1:80	32	32	16	16	8	8	4	32	-
	1:160	32	32	16	8	4	-	-	-	-
TC-83	1:10	32	32	64	64	16	-	-	32	4
	1:20	32	32	32	32	16	-	-	32	-
	1:40	32	32	32	32	8	-	-	16	-
	1:80	8	32	8	-	-	-	-	-	-
	1:160	-	32	-	-	-	-	-	-	-
Ica	1:10	64	64	64	64	16	8	32	32	4
	1:20	64	64	32	64	16	4	32	8	-
	1:40	32	32	32	64	16	4	8	-	-
	1:80	32	32	32	32	8	-	-	-	-
	1:160	-	-	32	<8	4	-	-	-	-
P676	1:10	64	64	64	64	8	8	16	32	-
	1:20	64	32	64	64	8	8	8	32	-
	1:40	64	32	64	64	8	8	8	-	-
	1:80	64	32	64	64	-	-	8	-	-
	1:160	64	8	32	32	-	-	4	-	-

Name	Dilution	Trin.	TC-83	Ica	P676	3880	Mena II	Fe 3-7c	MUC	PIX
3880	1:10	32	16	32	16	16	8	4	16	-
	1:20	8	64	32	16	8	8	-	16	-
	1:40	8	32	32	16	8	4	-	8	-
	1:80	4	32	8	16	-	4	-	-	-
	1:160	-	-	4	-	-	-	-	-	-
Mena II	1:10	64	64	32	64	8	8	16	32	4
	1:20	32	64	32	64	4	8	8	16	-
	1:40	32	32	32	64	-	8	4	-	-
	1:80	32	16	-	16	-	4	-	-	-
	1:160	16	-	-	-	-	4	-	-	-
Fe 3-7c	1:10	4	8	16	32	-	8	8	-	-
	1:20	-	-	-	8	-	4	4	-	-
	1:40	-	-	-	-	-	-	4	-	-
	1:80	-	-	-	-	-	-	-	-	-
	1:160	-	-	-	-	-	-	-	-	-
MUC	1:10	32	32	32	16	8	16	-	64	8
	1:20	32	16	32	16	8	16	-	32	8
	1:40	8	-	16	-	4	8	-	32	-
	1:80	-	-	-	-	-	-	-	32	-
	1:160	-	-	-	-	-	-	-	-	-
PIX	1:10	32	32	64	16	8	8	4	32	8
	1:20	32	32	32	-	4	4	4	32	8
	1:40	8	4	4	-	-	-	-	32	8
	1:80	-	-	-	-	-	-	-	32	8
	1:160	-	-	-	-	-	-	-	24	-

a. - indicates < 1:4.

TABLE IV. STANDARD TEST PATTERN FOR VEE STRAIN IDENTIFICATION

ANTISERUM		ANTIGEN Reciprocal at highest dilution showing + reaction								
Name	Dilution	Trin.	TC-83	Ica	P676	3880	Mena II	Fe3-7c	MUC	PIX
Trinidad	1:40	64[a]	32	32	32	8	8	8	32	8
TC-83	1:20	32	32	32	32	16	2	4	32	-[a]
Ica	1:40	32	32	32	64	16	4	8	-	-
P676	1:40	64	32	64	64	8	8	8	-	-
3880	1:20	8	64	32	16	8	8	2	16	-
Mena II	1:20	32	32	32	64	4	8	8	16	-
Fe 3-7c	1:10	8	8	16	64	2	8	8	-	-
MUC	1:40	8	-	16	-	4	8	-	32	-
PIX	1:40	8	-	4	-	4	-	-	32	4

a. No reaction with undiluted antigen, when retested below 1:4.

TABLE V. PHOSPHATE MOLARITY OF PEAK VIRUS INFECTIVITY

ANTIGENIC GROUP[1]	VIRUS STRAIN	FRACTION NUMBER	PHOSPHATE MOLARITY
Ia	Trinidad	20	0.25
- -	TC-83	18	0.23
Ib	Ica	20	0.25
- -	9859	20	0.25
Ic	P-676	19	0.24
Id	3880	17	0.22
Ie	Mena II	18	0.23
II[a/]	Fe 3-7c	14 25	0.19 0.30
III	Mucambo	31	0.36
IV	Pixuna	27	0.32

a. Two peaks of virus infectivity have been observed.

TABLE VI VIRUS ISOLATIONS BY AREA

MOSQUITO SPECIES	POOL SIZE	VIRUS	COLLECTION REF. NO. AND DATE
Culiseta melanura	28	EEE	BR[1]-18-214/11-2 Aug
P. confinnis	100	VEE	AL[2]-26-222/17-10 Aug
Culex tarsalis	104	WEE	OD[3]-14-212/06-31 Jul
C. tarsalis	71	WEE	OD-14-219/08-7 Aug
C. tarsalis	30	WEE	OD-24-221/05 - 9 Aug
C. tarsalis	39	WEE	OD-25-221/08 - 9 Aug
C. tarsalis	100	WEE	OD-14-226/06 - 14 Aug
Aedes thelcter	18	WEE	OD-25-228/02 - 16 Aug
C. tarsalis	67	WEE	OD-25-228/09 - 16 Aug
Psorophora discolor	96	WEE	OD-25-228/14 - 16 Aug
C. tarsalis	15	WEE	OD-01-229/07 - 17 Aug
P. confinnis	106	VEE	OD-28-230/17 - 18 Aug
C. tarsalis	100	WEE	OD-24-235/01 - 23 Aug
C. tarsalis	100	WEE	OD-25-235/23 - 23 Aug
C. tarsalis	100	WEE	AM[4]-31-217/12 - 5 Aug
C. tarsalis	100	WEE	AM-31-217/13 - 5 Aug
Aedes nigromaculis	100	WEE	AM-32-217/03 - 5 Aug
C. tarsalis	23	WEE	AM-20-228/02 - 16 Aug
C. tarsalis	100	WEE	AM-05-231/01 - 19 Aug
C. tarsalis	100	WEE	AM-05-231/03 - 19 Aug
C. tarsalis	100	WEE	AM-05-231/04 - 19 Aug

TABLE VI. VIRUS ISOLATION BY AREA (Continued)

MOSQUITO SPECIES	POOL SIZE	VIRUS	COLLECTION REF. NO. AND DATE
C. tarsalis	104	WEE	AM-32-231/05 - 19 Aug
C. tarsalis	100	WEE	AM-17-235/03 - 23 Aug
C. tarsalis	100	WEE	AM-18-235/01 - 23 Aug
C. tarsalis	100	WEE	AM-18-235/03 - 23 Aug
C. tarsalis	16	WEE	AM-18-235/04 - 23 Aug
C. tarsalis	100	WEE	AM-21-235/12 - 23 Aug
C. tarsalis	100	WEE	AM-21-235/10 - 23 Aug
Culex Spp.	1	WEE	AM-01-237/15 - 25 Aug

1. Collecting team based at Baton Rouge, Louisiana.

2. Collecting team based at Alexandria, Louisiana.

3. Collecting team based at Odessa, Texas.

4. Collecting team based at Amarillo, Texas.

Progress, Part II:

In an attempt to differentiate virus strains within the VEE complex[1,3] we have used the brushite form of calcium phosphate, as described by Burness[4] for column chromatography. Adsorption to, and subsequent elution of virus populations from brushite, probably involves properties unique to each prototype virus, which may be a reflection of the charge on the viral membrane.

Column chromatography utilizing calcium phosphate provided reproducible chromatographic separation, good flow rates, and routine recovery of at least 90% of applied viruses. The results of chromatography with the TC-83 vaccine strain of VEE virus gave a pattern which was representative of the antigenic subgroup I VEE viruses examined. Both virus and HA activity eluted in a sharp peak which was associated with a minor peak of optical density. The phosphate molarity which corresponded with maximal infectivity of VEE viruses is shown in Table V. We found that antigenic subgroup I varieties elicited virus peaks in a very narrow range suggesting an intimate biochemical relationship.

Members of antigenic subgroups II, III and IV exhibited unique profiles. Examination of Fe 3-7c (subgroup II) exhibited 2 peaks of virus infectivity which suggested a differentiation of plaque size variants. The elution profiles of Mucambo, Pixuna and Mena II viruses indicated different surface properties among these strains. Mena II is a member of subgroup Ie, while Mucambo and Pixuna belong to subgroups III and IV respectively.

Summary, Part II:

Column chromatography of selected VEE viruses on calcium phosphate gel offered a simple and reproducible method for examination of biochemical characteristics and relatedness of strains within the VEE complex. Members of antigenic subgroup I demonstrated a series of elution profiles within a narrow range of 0.22 to 0.25 M phosphate buffer. Members of antigenic subgroups II, III, and IV differed substantially among themselves and viruses of antigenic subgroup I.

Progress, Part III:

Between 28 July and 26 August 1971, 13,193 pools of mosquitoes (501,992 mosquitoes comprising 52 species) were collected by U.S. Army Field Collection and Sorting Teams, Fifth U.S. Army Medical Laboratory, Fort Sam Houston, Texas. These pools were processed for the isolation of Group A arboviruses as recommended by the Center for Disease Control (Sudia, personal communication) by inoculation of primary duck embryo cell cultures followed by an agar-medium overlay to contain the toxicity of the mosquito pools. The supernatant fluid, obtained by freezing and thawing, from those cells producing plaques was inoculated into suckling mice and the brains of the mice which died were examined for viral antigen using monospecific sera with the complement fixing (CF) technique.

Table VI lists the isolations by area of collection. There were 26 isolations of western equine encephalitis (WEE) from pools collected in areas of West Texas known to be endemic for the virus. There was a single isolation of eastern equine encephalitis (EEE) from a pool of mosquitoes collected on 2 August near Madisonville, St. Tammany Parish, Louisiana.

Two strains of VEE were recovered: (1) an avirulent strain from a pool of 100 Psorophora confinnis collected in Evangeline Parish, Louisiana, on 10 August and (2) a virulent strain from a pool of 106 P. confinnis collected west of Del Rio, Valverde County, Texas, on 18 August. The avirulent strain has been extensively studied at the Center for Disease Control, Atlanta, Georgia, the Middle American Research Unit, Panama, as well as in this laboratory. It is type 1A virus; its in vivo virulence pattern indicates that it is the vaccine strain. Mechanical transmission did not seem to be important since all visibly engorged mosquitoes were excluded from pools; the titer of the pool (1.8×10^3 PFU/ml) indicated that this was a true infection with a subsequent extrinsic incubation period. Reisolations from the original pool were completed at the CDC as well as here at USAMRIID. Horses had been vaccinated in the area of Louisiana where this particular pool was collected during July and perhaps as last as 10 August. The P. confinnis mosquito has been the most highly infected species of those collected in the epidemic areas of Mexico and the U. S. (Sudia, personal communication); the single isolation during a project lasting 6 weeks indicates that the occurrence of the vaccine strain in mosquitoes while a true finding is extremely rare.

The initial pools were received on 3 August, the final pools on 20 September and the final report was forwarded to the coordinator, LTC Bruce Eldridge at Walter Reed Army Institute of Research on 12 October 1971.

Summary, Part III:

A total of 13,193 pools of mosquitoes collected in areas peripheral to the VEE epidemic which occurred in South Texas from June to August 1971, were examined for the presence of Group A arboviruses. A single isolation of EEE, 26 isolations of WEE, and 2 isolations of VEE were made. One of the VEE isolates was the vaccine strain.

Publications:

1. Levitt, N. H., K. R. Amsler and R. W. McKinney. 1971. Rapid detection of viral antibody by cellulose acetate electrophoresis. Appl. Microbiol. 22:143-144.

2. Pedersen, C. E. Jr., D. M. Robinson, and F. E. Cole, Jr. 1972. Isolations of the vaccine strain of Venezuelan equine encephalomyelitis virus from mosquitoes in Louisiana. Amer. J. Epidemiol. In press.

3. Pedersen, C. E. Jr., D. R. Slocum, and N. H. Levitt. 1972 Chromatography of Venezuelan equine encephalomyelitis virus strains on calcium phosphate. Appl. Microbiol. In press.

LITERATURE CITED

1. Young, N. A., and K. M. Johnson. 1969. Antigenic variants of Venezuelan equine encephalitis virus: Their geographic distribution and epidemiologic significance. Amer. J. Epidemiol. 89:286-307.

2. Clarke, D. H., and J. Casals. 1958. Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses. Amer. J. Trop. Med. Hyg. 7:561-573.

3. Young, N. A., and K. M. Johnson. 1969. Viruses of the Venezuelan equine encephalomyelitis complex. Infection and cross-challenge of rodents with VEE, Mucambo, and Pixuna viruses. Amer. J. Trop. Med. Hyg. 18:280-289.

4. Burness, A. T. H. 1967. Separation of plaque-type variants of encephalomyocarditis virus by chromatography on calcium phosphate. J. Virol. 1:308-316.

ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1W662711A096 03:	Laboratory Identification of Biological Agents
Work Unit No. 096 03 404:	Use of Antiglobulin for Early Detection of Arbovirus Antibody of Diseases of Military Importance
Reporting Installation:	U. S Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Virology
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Author:	Albert T. McManus, Captain, MSC
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OL0904	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. ENTER HISTORY	8A. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NL	X YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	03	404
b. CONTRIBUTING	62711A	1B662711A096		
c. CONTRIBUTING	CDOG 1212b(9)			

11. TITLE (Precede with security classification code): (U) Use of antiglobulin for early detection of arbovirus antibody of diseases of military importance

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical medicine; 004900 Defense; 010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
70 12	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION
b. NUMBER: NA
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR PRECEDING 71	1.0	8
FISCAL YEAR CURRENT 72	1.0	78

19. RESPONSIBLE DOD ORGANIZATION
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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME:
NAME: DA

21. GENERAL USE
Foreign intelligence considered

22. KEYWORDS (Each classification code): (U) Arboviruses; (U) Antigen-antibody reactions; (U) Serology; (U) Military medicine; (U) Encephalitis, equine (VEE)

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with security classification code.)

23 (U) Identify diseases by detection of early specific antibody production utilizing an arbovirus model system. This work unit is an essential element in a comprehensive program for medical defense against BW agents.

24 (U) Investigations will involve the use of antiglobulin neutralization in a plaque reduction test.

25 (U) 71 07 - 72 06 - A rhesus monkey Trinidad-VEE infectious system has been developed in coordination with the Animal Assessment Division of this Institute. An infectious dose of less than 500 PFU produced viremia within 48 hr and levels as high as one million PFU/ml. Antibody titers as high as 1:4096 were observed in 28-day convalescent serum. This system will be used to evaluate the proposed antiglobulin technique.

Publication: Appl. Microbiol. 23:654-655, 1972.

Terminated since FY 1973 funding will be under The Surgeon General, Army. New Project No. 3A062110A834; Accession No. DA OC6419.

* Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 68 AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 03: Laboratory Identification of Biological Agents

Work Unit No. 096 03 404: Use of Antiglobulin for Early Detection of Arbovirus Antibody of Diseases of Military Importance

Description:

Identify diseases by detection of early specific antibody production utilizing an arbovirus model system.

Progress:

Efforts to quantitate the viremia in humans infected with the strains of 17-D yellow fever virus have not been consistent. The 17-D strain has also been shown to be a poor model for plaque reduction serology due to the 4-6-day time requirement for the development of plaques.

Antiglobulin reagents to rhesus monkey serum became available during the 2nd quarter of this year. A cooperative studies with CPT Kastello of the Animal Assessment Division has been initiated. A pilot study showed that an infectious dose of $\leq$500 PFU (chick cell assay) of Trinidad strain Venezuelan equine encephalomyelitis (VEE) produces viremia within 48 hr. Titration of daily bleedings showed titers $\leq 10^6$ PFU/ml. Plaque reducing antibody titers $\leq$1:4096 have been assayed in 28-day convalescent serum by the plaque reduction method.

These results coupled with the ease and speed ($\sim$30 hr) of the Trinidad VEE plaque assay has directed major development efforts to this system. Evaluation of the antiglobulin system with the specimens which have been collected from the monkey project is expected in the near future.

Summary:

A rhesus monkey Trinidad-VEE infectious system has been developed in coordination with the Animal Assessment Division of this Institute. An infectious dose of $\sim$500 PFU produced viremia within 48 hr with titers $\leq 10^6$ PFU/ml. Antibody titers $\leq$1:4096 have been observed in 28-day convalescent serum. This system will be used to evaluate the proposed antiglobulin technique.

Publication:

1. McManus, A. T. and D. M. Robinson. 1972. Stability of live attenuated Venezuelan equine encephalitis vaccine. Appl. Microbiol. 23:654-655.

ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1W662711A096 03:	Laboratory Identification of Biological Agents
Work Unit No. 096 03 405:	Investigate Etiology of 1971 Hemorrhagic Fever Outbreak in Cochabamba, Bolivia
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Animal Assessment
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Authors:	Ralph W. Kuehne, B.S. Clarence J. Peters, Lt Commander, USPHS (NIAID) Richard O. Spertzel, Lt Colonel, VC
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OL0914	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISS'N INSTR'N	8B. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	03	405
b. [illegible]	62711A	1B662711A096		
c. [illegible]	CDOG 1212b(9)			

11. TITLE (Precede with security classification code)
(U) Investigate etiology of 1971 hemorrhagic fever outbreak in Cochabamba, Bolivia

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 Clinical medicine; 010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 06	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER: NA
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 71	0	0
FISCAL YEAR CURRENT 72	1.0	20

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21. GENERAL USE
Foreign intelligence considered

22. KEYWORDS (Precede EACH with security classification code)
(U) Hemorrhagic fever; (U) Etiology; (U) Virus disease; (U) Tacaribe complex

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS. (Furnish individual paragraphs identified by number. Precede text of each with security classification code.)

23 (U) Isolate and characterize the agent responsible for 6 cases of hemorrhagic fever which resulted in 5 deaths in Bolivia. This is a cooperative study with USA Middle America Research Unit and the National Institute of Allergy and Infectious Disease.

24 (U) A known, avirulent form of the Tacaribe virus complex will be examined in a manner identical with that planned for the virulent virus. This will include animal and tissue culture inoculation. Once an isolate is established it will be identified and characterized in terms of host range and pathogenicity. The isolate will be compared to Machupo virus which causes an endemic disease in the area.

25 (U) 71 07 - 72 06 - The causative agent of the 1971 Cochabamba, Bolivia, outbreak of hemorrhagic fever has been isolated and has been shown to be a member of the Tacaribe complex of the Arenovirus group. It appears to be a variant of Machupo virus, closely related serologically, but differing in its epidemiology, clinical disease in man, and histopathology and virulence for laboratory animals. Further studies are in progress to determine if other differences exist.

Terminated since FY 1973 funding will be under The Surgeon General, Army. New Project No. 3A062110A834; Accession No. DA 0C6420.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1 1 MAR 67 FOR ARMY USE ARE OBSOLETE

BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 03: Laboratory Identification of Biological Agents

Work Unit No. 096 03 405: Investigate Etiology of 1971 Hemorrhagic Fever Outbreak in Cochabamba, Bolivia

Description:

Isolate and characterize the agent responsible for 6 cases of hemorrhagic fever in Cochabamba, Bolivia.

Progress:

Details of the history and background of this outbreak of hemorrhagic fever were reported previously.[1]

As a preliminary to the isolation of the Cochabamba agent, a 4-week period was spent utilizing a prototype strain of Tacaribe virus (nonpathogenic for man) to test the procedures, teamwork, and equipment needed to work with the virulent virus. Tacaribe virus was inoculated by the intraperitoneal (IP) route into adult mice, adult hamsters and adult guinea pigs, and by the IP and intracerebral (IC) routes into suckling mice (SM) and suckling hamsters (SH). Characteristic deaths occurred in SM and SH. Brain suspension from SM was successfully passed to SH and VERO cell cultures. The original suspension was also inoculated into various cell cultures: VERO, WI-38, human embryo kidney, primary green monkey kidney, BHK-21, primary duck embryo, and primary chick embryo. Cytopathic effect was seen in the VERO system only. Leighton coverslips were prepared from all lines except primary monkey kidney and were stained by fluorescent antibody techniques. The complement-fixation (CF) test was shown to be functional using sera and antigens prepared at Middle America Research Unit (MARU).

Isolation of the Cochabamba agent was attempted from 5 tissue specimens: liver from Case No. 4; an unlabeled blood sample from either Case No. 3 or 4; and blood, spleen and throat swab from Case No. 6, the pathologist. These specimens were inoculated into a wide variety of animal hosts and tissue cell cultures. Machupo antisera were used in CF and indirect fluorescent (IFAT) antibody tests to attempt to demonstrate Tacaribe group antigen. SH brain material, obtained at MARU from Case No. 3 liver, and the unlabeled blood sample were passaged in SH. In both cases, the $SHBr_1$ passage from MARU was positive by CF test, as was the $SHBr_2$ passage made at USAMRIID. (The Br_1 pools were both bacterially contaminated, but this was not evident in the Br_2 pools.) The blood from Case No. 6 was positive in SH and SM, but no virus could be detected in the throat swab (some animals did show signs of illness, but CF antigen was not detectable).

Because of previous experience with Machupo virus, the spleen of Case No. 6 was chosen for major emphasis. The results are shown in Table I.

TABLE I. INOCULATION OF SPLEEN CASE NO. 6

HOST	DILUTION INOCULATED	RESULTS
Suckling hamster	10^{-1}	Death; CF brain antigen < 2.
	10^{-2}	Death; CF brain antigen 1:8; serum CF on survivors 1:32.
Suckling mouse	10^{-1}	Death; CF brain antigen < 2.
	10^{-2}	Death; serum CF survivors 1:2.
Adult hamster	10^{-1}	No illness; serum CF 1:64.
Adult mouse	10^{-1}	No illness; serum CF 1:4.
Adult guinea pig	10^{-1}	No illness; serum CF 1:32.
VERO tube	10^{-1}	No CPE, virus present on passage.
	10^{-2}	No CPE.
WI-38 tube	10^{-1}	CPE; virus present on passage.
	10^{-2}	CPE.
Primary monkey kidney cube	10^{-1}	No CPE.
	10^{-2}	No CPE.
Human embryo kidney cube	10^{-1}	No CPE.
	10^{-2}	No CPE.
HA-111 tube	10^{-1}	CPE.
	10^{-2}	CPE.
BHK-21 tube	10^{-1}	No CPE.
	10^{-2}	No CPE.
VERO Leighton tube	10^{-1}	Viral antigen on coverslip.
Human embryo kidney Leighton tube	10^{-1}	No viral antigen detected.
	10^{-2}	No viral antigen detected.

There are 4 notable features: (1) CF antigen titers in SM and SH brains are low. (2) SH and SM provide good animal hosts for isolation. (3) CF

the cell lines, only MA-111 (rabbit embryo kidney) and WI-38 (human embryonic lung) showed cytopathogenic effects. (4) VERO Leighton tubes proved to be adequate to detect virus, and harvest on day 6 showed abundant viral antigen. In anticipation of finding virus in the spleen of Case No. 6, extra SH were inoculated at 10^{-1}. Brains were harvested and used to prepare a large virus pool and an immunizing antigen. The pool was titrated in SM, SH, and tissue cell cultures. The titer was very low, about 10^{5} infective doses/ml. The antigen had a CF titer of < 1:2 and failed to immunize adult hamsters. Animals inoculated with 10^{-2} and harvested had a higher plaque titer, about 10^{8} PFU/ml, and a CF titer of 1:8. The reason for the low titers in general and the lower titer of 10^{-1} vs. 10^{-2} is not clear.

The 10^{-4} SMBr spleen material was used to prepare another lot of infected mouse material. Pools and antigens were prepared from brain, liver and kidney. The brain pool had a titer of 10^{6} PFU/ml and the brain antigen had a CF titer of 1:4, but both liver and kidney were < 1:2. The pool was used in a series of tests; the antigen was used to immunize adult hamsters and adult guinea pigs. The pool has been passed in SH brain 2 more times; harvests were made at varying times, in order to prepare a larger, higher titered pool and a better CF and immunizing antigen. We now have a large virus pool titering about 6×10^{6} PFU/ml and 5×10^{8} median lethal doses (LD_{50})/ml when inoculated into SH by a combination of the IP and IC routes. A large supply of antiserum has been prepared in adult hamsters.

Machupo virus seed from MARU was inoculated into the same host systems using another isolated Class III system for direct comparison, keeping the 2 viruses separated. It was passed IC in SH to prepare a pool and an antigen. We have produced a large pool titering 10^{6} PFU/ml and 10^{8} LD_{50}/ml for SH. Antiserum to Machupo has been prepared in adult hamsters and adult guinea pigs.

Although CF and IFAT antibody-staining indicated that our isolate was a member of the Tacaribe complex, a neucralization test was required to show type-specificity. Both viruses gave somewhat ragged titration results in SH; many sick animals were eaten by the mother, making exact end-point determinations impossible; therefore, plaque-reduction on VERO cell monolayers was attempted with varying degrees of success. Both viruses produced similar types of plaques in VERO cell cultures which developed at the same rate. However, approximately 80-90% experimental failure occurred due to degeneration of overlaid monolayers before complete development of virus plaques (5 days). Changes of media, pH, serum concentration, volumes and physiological age have not resulted in any consistent improvement. Other investigators have reported plaquing on MA-111 cell cultures,[5] but this, too, proved unsuccessful. Nevertheless, some successful tests were accomplished using VERO cells. Several constant-serum, varying-virus plaque-reduction tests showed type-specific reciprocal cross-neutralization between Machupo and Cochabamba viruses, even though homologous titers in general appeared greater than heterologous titers. A constant-virus, varying-serum dilution test was attempted using human serum. The cell sheet in the center of the VERO cell monolayers did not survive, but good plaques were seen around the periphery. Although quantitative interpretation was impossible, the results were nevertheless

considered significant. The test virus used was 10^2 PFU of Cochabamba SHRr$_2$. A 1:16 dilution of a Machupo antiserum pool, and a 1:64 dilution of serum from a known Machupo infection survivor, resulted in absence of plaque formation. (All higher dilutions showed plaques.) Normal human serum and serum from Case No. 5, the only survivor of the Cochabamba outbreak, showed plaques at the 1:4 dilution. It appears that the serum from this person had low neutralizing antibody, not only to Machupo virus, as determined earlier at MARU, but also to the causative agent of the Cochabamba outbreak. From these results, it is concluded that a very close serological relationship exists between the 2 viruses. The results of this test were recently confirmed by others at MARU.

Even though the Cochabamba isolate is related serologically to Machupo, other differences might exist which could define a distinct biological variant. Several laboratory differences have been observed, notably virulence and histopathology in laboratory animals. Small differences were noted in SH. SH inoculated with Machupo virus become ill on days 6-10; sick animals rarely recover, resulting in a clean titration. With Cochabamba virus, illness occurs on days 8-11; some hamsters recover, resulting in a more ragged titration. Machupo virus also seems to be somewhat more virulent for SH when the same number of PFU are inoculated.

With SM, inoculation with either virus does not always terminate in death of the animals. Many become ill, but recover, resulting in ill-defined endpoints. This observation was reported earlier by Johnson, et al.[4] with Machupo virus.

Greater differences in virulence have been noted in adult and suckling guinea pigs, Table II. When adult guinea pigs were first inoculated for antiserum production, 1 of 2 Machupo animals died while none of the Cochabamba-infected animals died. When adults were inoculated with a similar number of PFU of each virus, 3 of 4 Machupo and 0 of 4 Cochabamba animals died. Since investigators at MARU have been using the loss of virulence for adult guinea pigs as a marker for Machupo virus passage attenuation, it could be significant that a Machupo variant can exist which is not lethal for adult guinea pigs, but is still highly pathogenic for man. When suckling guinea pigs are inoculated by the IP and IC routes with each virus, significantly greater mortality occurs with Machupo virus than with Cochabamba.

Although a search of the literature failed to reveal any use of suckling guinea pigs with Machupo virus, they proved to be very sensitive animals and allowed the development of an in-vivo neutralization test which served as an alternative to the sporadic plaque neutralization test. A constant-serum varying-virus test was conducted using guinea pig serum and Machupo virus, Table III. The serum-virus mixtures were inoculated by the IC route and a log-neutralization index of 3.0 was attained with both Machupo and Cochabamba antisera.

SH were infected by IC route with the 3 strains, serially sacrificed at

TABLE II. GUINEA PIG MORTALITY OF MACHUPO AND COCHABAMBA VIRUSES

VIRUS (ROUTE)		DOSE	MORTALITY Dead/Total	%
ADULTS				
Machupo	(IP)	20 $SHLD_{50}$	1/2	50
Cocha	(IP)	60 $SHLD_{50}$	0/2	0
Machupo	(IP)	44,000 PFU	3/4	75
Cocha	(IP)	96,000 PFU	0/4	0
SUCKLINGS				
Machupo	(IP)	1000-3000 PFU	14/18	78
		1-3 PFU	6/18	33
Cocha	(IP)	6000-12000 PFU	4/14	29
		6-12 PFU	1/15	7
Machupo	(IC)	1000-3000 PFU	15/15	100
		1-3 PFU	15/19	79
Cocha	(IC)	6000-12000 PFU	3/8	38
		6-12 PFU	0/13	0

2-day intervals, and examined for comparative histopathology. Both groups developed lesions which indicated the presence of virus in the inoculum. Certain similarities and differences were observed: liver, lymphoid tissue, and central nervous system were the organ systems most affected by both virus isolates. Hepatic necrosis and fatty metamorphosis were observed earlier (days 4-7) with Cochabamba than with Machupo (days 7-11). A nonsuppurative encephalitis with vasculitis was commonly seen in the Cochabamba-infected animals (days 9-17). It was also present in 2 of 17 Machupo-infected hamsters. (It is interesting to note that 2 adult guinea pigs inoculated with Machupo virus developed a nonsuppurative encephalitis and vasculitis similar to the Cochabamba-infected SH.) Lymphoid depletion and necrosis were seen earlier (days 7-11) in the Machupo group than in the Cochabamba group (days 11-13). Despite these differences, it is possible that the 2 viruses are different strains of the same virus. The histopathological findings were somewhat inconclusive; further investigation is recommended.

Other properties of the Cochabamba isolate are currently being explored, including electron microscope structure and interferon sensitivity.

TABLE III. SUCKLING GUINEA PIG NEUTRALIZATION TEST

ANTISERUM	VIRUS DILUTION (Machupo)	DEAD/TOTAL	LD_{50}	LNI
Normal	10^{-1}	5/5		
	10^{-2}	2/3		
	10^{-3}	3/4	$10^{-4.0}$	
	10^{-4}	3/4		
	10^{-5}	0/3		
	10^{-6}	0/3		
Machupo	10^{-1}	2/4		
	10^{-2}	1/5		
	10^{-3}	0/3	$10^{-1.0}$	3.0
	10^{-4}	0/3		
	10^{-5}	0/4		
	10^{-6}	0/3		
Cochabamba	10^{-1}	2/4		
	10^{-2}	1/3		
	10^{-3}	0/3	$10^{-1.0}$	3.0
	10^{-4}	0/4		
	10^{-5}	0/4		
	10^{-6}	0/3		

Summary:

The causative agent of the 1971 Cochabamba, Bolivia, outbreak of hemorrhagic fever has been isolated and has been shown to be a member of the Tacaribe complex of the Arenovirus group. It appears to be a variant of Machupo virus, closely related serologically, but differing in its epidemiology, clinical disease in man, and histopathology and virulence for laboratory animals. Further studies are in progress to determine if other differences exist.

Publications:

Kuehne, R. W. Isolation and characterization of the Cochabamba virus. Presented at Annual Joint Meeting, Maryland - Washington Branches of the American Society for Microbiology, U. S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Md. 6 May 72.

Publications:

None.

LITERATURE CITED

1. Peters, C. J., and R. W. Kuehne. 1971. Hemorrhagic fever in Cochabamba. p. 129 to 136. In Commission on Epidemiological Survey Annual Report to the Armed Forces Epidemiological Board, FY 1971. Washington, D. C.

2. Wiebenga, N. H. 1965. Immunologic studies of Tacaribe, Junin and Machupo viruses. Amer. J. Trop. Med. Hyg. 14:802-808.

3. Rhim, J. S., B. Simizu, and N. H. Wiebenga. 1969. Multiplication of Machupo virus, the etiological agent of Bolivian hemorrhagic fever, in cell cultures. Proc. Soc. Exp. Biol. Med. 130:382-387.

4. Johnson, K. M., N. H. Wiebenga, R. B. Mackenzie, M. L. Kuns, N. M. Tauraso, A. Shelokov, P. A. Webb, G. Justines, and H. K. Beye. 1965. Virus isolations from human cases of hemorrhagic fever in Bolivia. Proc. Soc. Exp. Biol. Med. 118:113-118.

ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	**Medical Defense Aspects of Biological Agents (U)**
Task No. 1W662711A096 03:	**Laboratory Identification of Biological Agents**
Work Unit No. 096 03 800:	Immunological Studies with Microbial Toxins
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Divisions:	Bacteriology and Animal Assessment
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Authors:	Virginia G. McGann, Ph.D. Richard O. Spertzel, Lt. Colonel, VC Douglas W. Mason, Captain, VC Elizabeth O. Roberts, Ph.D.
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OL0876	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	8A. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. TERMINATION	U	U	NA	NL	YES / NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	03	800
b. CONTRIBUTING	62711A	1B662711A096		
c. CONTRIBUTING	CODG 1212b(9)			

11. TITLE (Precede with Security Classification Code)
(U) Immunological studies with microbial toxins of military medical importance

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 Clinical medicine; 004900 Defense; 016800 Toxicology; 010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
66 10	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER: NA
c. TYPE: d. AMOUNT
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 71	3.0	50
	CURRENT 72	3.0	80

19. RESPONSIBLE DOD ORGANIZATION
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21. GENERAL USE
Foreign intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Enterotoxin, staphylococcal; (U) Toxoid; (U) Immunology; (U) Antibodies; (U) Hypersensitivity; (U) Military medicine

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Investigate immunologic responses of a susceptible host after exposure to microbial toxin and after immunization with toxoid. This work unit is an essential element in a comprehensive program for medical defense against BW agents.

24 (U) Investigate serological activity of staphylococcal enterotoxins and their toxoids; evaluate the role of antibody in resistance to ingested toxin, and define the pathogenesis of acute response following reexposure to toxin.

25 (U) 71 07 - 72 06 - Humoral antibody per se does not participate in protection against alimentary intoxication with SEB but indicates a potential for rapid development of resistance. The number of IG exposures required to induce resistance to enterotoxemia is unpredictable but the time interval between exposures is not highly critical. Resistance develops before humoral antibody response can be detected and persists after antibody disappears. Intragastric administration of SEB, however, can be an effective means of priming humoral antibody-forming cells, as indicated by induction of Arthus-like reactions, development of systemic hypersensitivity and resistance to lethal effects of intravenous toxin, and by development of a secondary antibody response in antibody-negative monkeys following intravenous exposure. It is postulated that IG administration of SEB is even more effective as a stimulant to antibody-forming cells in the gastrointestinal tract for production of protective secretory antibody.

Publication: J. Infect. Dis. 124:206-213, 1971.

Terminated since FY 1973 funding will be under The Surgeon General, Army. New Project No. 3A062110A834; Accession No. DA OC6421.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498A 1 MAR 68 FOR ARMY USE ARE OBSOLETE

BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 03: Laboratory Identification of Biological Agents

Work Unit No. 096 03 800: Immunological Studies with Microbial Toxins

Description:

Investigate immunologic response of a susceptible host following intragastric exposure to microbial toxin.

Progress:

A study designed to investigate resistance of monkeys to intragastric (IG) exposure to staphylococcal enterotoxin B (SEB) was completed. Consideration was given to various factors that might modify development of resistance, humoral antibody exposure and cutaneous sensitivity, such as naturally-acquired antibody, SEB dosage and frequency of exposure.

Antibody-negative monkeys challenged at 2-week intervals with 1-10 IG median illness doses (ID_{50}) developed resistance prior to development of antibody. Following the 3rd challenge, only 3 of 12 monkeys had detectable humoral antibody but 10 resisted subsequent challenge. All monkeys were resistant after the 4th exposure, but only 1 of 6 in the low dose (1 ID_{50}) group and 3 of 6 in the high dose (10 ID_{50}) had antibody. Incidence of humoral response increased after additional exposures, but 2 monkeys in the low dose group remained refractory even after 7 challenges. Magnitude, as well as incidence, of hemagglutinin (HA) titers was dose-related; only 1 monkey developed precipitating antibodies. Although antibody titers generally disappeared within 3 mon after exposure, resistance to IG challenge remained unimpaired.

In groups of monkeys exposed at 4-mon intervals, a challenge dose of 10 ID_{50} was more effective than 1 or 100 ID_{50} doses for inducing development of resistance (Table I). Resistance developed more readily in groups with naturally-acquired antibody, but illness response of individual monkeys was not correlated with prechallenge antibody titer or with type of antibody, e.g. a monkey with an HA titer of 1:160 and precipitating antibody became ill after every challenge, while another with apparently the same antibody titers responded only to the 1st exposure. Similar patterns of response were observed with antibody-negative monkeys. Overall, approximately 30% of the monkeys consistently resisted challenge after 1 exposure, 10% after 2 and 14% after 3; essentially equal numbers of the remaining monkeys either never developed resistance or responded sporadically.

TABLE I. EFFECT OF SEQUENTIAL EXPOSURES AT 4-MON INTERVALS ON RESISTANCE OF ANTIBODY-NEGATIVE AND ANTIBODY-POSITIVE MONKEYS TO SEB ADMINISTERED BY GAVAGE.

CHALLENGE DOSE (ID_{50})	CHALLENGE NUMBER	EXPERIMENTAL MONKEYS				CONTROL MONKEYS	
		Antibody-Negative		Antibody-Positive			
		R/T[a]	MTO[b]	R/T	MTO	R/T	MTO
1	1	7/8	3.8	8/16	3.2	12/23	3.1±0.2
	2	4/6	3.0				
	3	3/6	4.0				
	4	2/5	2.9				
10	1	17/18	3.2	10/13	4.7	16/18	3.3±0.2
	2	13/16	2.7	5/13[c]	3.6		
	3	8/16[c]	2.6	2/13[c]	4.9		
	4	5/16[c]	2.7	4/13[c]	5.5		
100	1	9/9	2.8	10/10	3.7	8/8	2.5±0.2
	2	9/9	2.0	6/10[c]	3.1		
	3	5/9[c]	2.6	5/9[c]	3.8		
	4	6/8	4.2	4/9[c]	2.8		

a. R/T: Number ill/Number tested.

b. MTO: Mean time onset of illness (hr); difference between means not statistically significant.

c. Chi square analysis, with corresponding dose control, $P < 0.05$.

In general, with groups of monkeys that lacked naturally-acquired humoral antibody, incidence and magnitude of HA response were dose related and corresponded to findings observed following challenge at 2-week intervals (Table II). At each challenge dose, however, incidence of antibody response was significantly higher ($P < 0.005$) for monkeys that developed signs of enterotoxemia. Maximum HA titers appeared within 2 weeks postchallenge; thereafter titers decreased rapidly and were almost invariably negative within 4 mon. Precipitating antibodies appeared only in monkeys that had

naturally-acquired HA titers; maximum precipitin responses occurred after the first IG challenge and titers persisted at approximately the same levels throughout the study.

TABLE II. HEMAGGLUTININ RESPONSE OF ANTIBODY-NEGATIVE MONKEYS TO SEQUENTIAL CHALLENGE WITH SEB ADMINISTERED BY GAVAGE.

INTRAGASTRIC CHALLENGE		NUMBER OF MONKEYS	POSTCHALLENGE HEMAGGLUTININ RESPONSE					
			2 Weeks		6 Weeks		4 Months	
Dose (ID_{50})	Mon		Number Positive	GMT[a]	Number Positive	GMT	Number Positive	GMT
1	0	8	2	20	2	28	2[b]	28
	4	6	1	10	1	10	1	20
	8	6	1	20	0		0	
	12	5	0		0		ND[c]	
10	0	18	3	127	2	453	2[b]	453
	4	16	3	20	1	20	1	10
	8	16	6	36	3	20	0	
	12	16	7	22	5	15	ND	
100	0	9	2	20	1	20	0	
	4	9	4	24	2	20	0	
	8	9	7	66	1	80	1	80
	12	8	6	90	5	40	ND	

a. GMT: Reciprocal geometric mean titer of responders.

b. Monkeys not included in later challenges because of secondary type response.

c. ND: Not done, intravenous challenge administered at 6 weeks.

Monkeys subjected to sequential IG exposures developed hypersensitivity to parenterally administered SEB. Two mon after the last IG challenge, typical Arthus-like reactions were observed in 7 of 8 monkeys that were inoculated intracutaneously with 0.001 - 0.1 µg SEB. As in previous studies

of cutaneous sensitivity, the nonreactor had precipitating antibody far in excess of the intracutaneous challenge dose. No evidence of reagin-type or delayed hypersensitivity was observed. When administered 300 µg SEB/kg body weight intravenously (IV) 6-8 weeks after the last IG exposure, 20 of 31 monkeys demonstrated immediate acute signs of systemic hypersensitivity, i.e. emesis, respiratory distress and facial flush with 1 min, occasionally semicoma, and 3 monkeys died within 3-5 hr (Table III).

TABLE III. TOXIC AND HYPERSENSITIVE RESPONSES FOLLOWING INTRAVENOUS ADMINISTRATION OF ENTEROTOXIN B TO NONIMMUNE CONTROL MONKEYS AND TO MONKEYS WITH A HISTORY OF INTRAGASTRIC EXPOSURE.

INTRAVENOUS DOSE (ug/kg)	CHALLENGE DOSE	PRECHALLENGE ANTIBODY[a]	NUMBER OF MONKEYS	RESPONSE GROUP		
				Resistant	Hypersensitive	Typical Enterotoxemia
10	Control	0	10	2	0	8
	Previous IG experience	0	11	6	0	5
		<100[b]	2	0	1	1
		100	10	8	1	1
300	Control	0	10	2	0	8
	Previous IG experience	0	7	0	6	4(3)[c]
		<10[b]	10	2	7	2(1)
		10-90	12	4	7	1
		100	4	4	0	0

a. Estimate of % challenge dose inactivated by antibody in vivo based on in vitro combining activity of prechallenge serum; 0, no detectable antibody activity; 100, sufficient combining activity.

b. Sera had hemagglutinins but no measurable combining activity.

c. Parentheses indicate number in group that died 2-3 days after recovery from hypersensitivity reaction.

As in previous studies with parenteral sensitization,[1] monkeys with humoral antibody in excess, or at equivalence, for the challenge dose resisted IV challenge, while those with levels less than equivalence generally were hypersensitive. Unlike our previous findings, however, 6 of 7 monkeys that had no detectable antibodies developed immediate systemic reactions. Sensitized monkeys that had antibody and survived the immediate reaction were significantly better protected than nonimmune monkeys against toxic effects of parenteral SEB; resistance against lethality was high even in monkeys with no prechallenge precipitins. All monkeys that survived parenteral challenge had typical secondary antibody responses, with a 40% conversion rate to precipitating antibody within 2 weeks.

Until information becomes available regarding local immune responses to SEB in the gastrointestinal tract, it is impossible to evaluate the relative contribution of cellular and humoral factors in enteric resistance. It is obvious that vascular antibodies are not effective in protection against alimentary challenge, and although indicating the general immunoresponsive state of an individual, they do not reflect immunocompetence of secretory-antibody cells in the gastrointestinal tract. Alimentary exposure to SEB, however, is capable of priming humoral antibody-forming cells; this is indicated by the dose-related antibody response following IG challenge, the secondary antibody response to subsequent parenteral challenge, and the Arthus-like reaction to cutaneous inoculations. Demonstration of the latter responses by antibody-negative monkeys suggests extravascular localization of antibody, as well as the presence of significant numbers of primed cells. It is probable that local antigenic stimulation occurs earlier and is relatively greater for secretory immunocytes in the gastrointestinal tract. In consideration of the relatively short latent period between exposure and expression of enterotoxemia, effective protection probably would require the presence of fully competent, antibody-producing cells at or near sites of toxic action.

Summary:

Humoral antibody *per se* does not participate in protection against alimentary intoxication with SEB but indicates a potential for rapid development of resistance. The number of IG exposures required to induce resistance to enterotoxemia is unpredictable but the time interval between exposures is not highly critical. Resistance develops before humoral antibody response can be detected and persists after antibody disappears. Intragastric administration of SEB, however, can be an effective means of priming humoral antibody-forming cells, as indicated by induction of Arthus-like reactions, development of systemic hypersensitivity and resistance to lethal effects of intravenous toxin, and by development of a secondary antibody response in antibody-negative monkeys following intravenous exposure. It is postulated that IG administration of SEB is even more effective as a stimulant to antibody-forming cells in the gastrointestinal tract for production of protective secretory antibody.

Publication:

1. McGann, V. G., J. B. Rollins and D. W. Mason, 1971. Evaluation of resistance to staphylococcal enterotoxin B: Naturally acquired antibodies of man and monkey. J. Infect. Dis. 124:206-213.

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ANNUAL PROGRESS REPORT

Project No. 1W662711A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1W662711A096 03:	Laboratory Identification of Biological Agents
Work Unit No. 096 03 801:	Radioimmunological Assay of Physiologically Active Substances
Report Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland 21701
Division:	Pathology
Period Covered by Report:	1 July 1971 to 30 June 1972
Professional Author:	William S. Collins, II, Lt. Colonel, MSC
Reports Control Symbol:	RCS-MEDDH-288 (R1)
Security Classification:	UNCLASSIFIED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1 AGENCY ACCESSION	2 DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 010902	72 06 30	DD-DRE-R1498A

3 DATE PREV SUMRY	4 KIND OF SUMMARY	5 SUMMARY STAT	6 SUMR SECURITY	7 REGRADING	8A DISTR INSTR	8B SPECIFIC DATA CONTRACTOR ACCESS	9 LEVEL OF SUM
71 07 01	B. TERMINATION	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10 NO CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62711A	1W662711A096	03	801
b. [illegible]	62711A	1B662711A096		
c. [illegible]	C500 1212b(9)			

11 TITLE (Precede with Security Classification Code): (U) Radioimmunological assay of staphylococcal toxins and other exoproteins causing diseases of military importance

12 SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical medicine; 004900 Defense, 016800 Toxicology

13 START DATE	14 ESTIMATED COMPLETION DATE	15 FUNDING AGENCY	16 PERFORMANCE METHOD
70 07	CONT	DA	C. In-house

17 CONTRACT/GRANT
a. DATE/EFFECTIVE:
b. NUMBER: NA
c. TYPE:
d. AMOUNT:
e. KIND OF AWARD:
f. EXPIRATION:
g. ACCUM AMT:

18 RESOURCES ESTIMATE	A. PROFESSIONAL MAN YRS	B. FUNDS (In Thousands)
FISCAL YR PRECEDING	71	2.0	50
FISCAL YR CURRENT	72	2.0	61

19 RESPONSIBLE DOD ORGANIZATION
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ADDRESS: Fort Detrick, MD 21701

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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Collins, II, W. S.
TELEPHONE: 301 663-7211
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Metzger, J. F.
NAME: Johnson, A. D. DA

21 GENERAL USE: Foreign intelligence considered

22 KEYWORDS (Precede EACH with Security Classification Code):
(U) Toxins; (U) Radioimmunoassay; (U) Exoproteins; (U) Staphylococcus; (U) Military medicine

23 TECHNICAL OBJECTIVE, 24 APPROACH, 25 PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code):

23 (U) Develop a radioimmunoassay capable of detecting rapidly and quantitating small amounts of staphylococcal toxins and other exoproteins. This work unit is an essential element in a comprehensive program for medical defense against BW agents.

24 (U) Liquid- and solid-state techniques for radioimmunoassay are applied to develop a rapid test for the detection and quantitation of toxins.

25 (U) 71 07 - 72 06 - A solid phase radioimmunoassay system has been developed to assay staphylococcal enterotoxins A and B in body fluid, broths, food products and in purified form. The procedure for SEB assay has been published. Methods for assay of SEC and SED are still under investigation.

Preliminary work has begun on the production of antisera and assay methods for ACTH, bradykinin, prostaglandins and thyrotropic releasing hormone as a cooperative effort with the Physical Sciences Division. A usable antibody to ACTh has been produced in goats; preliminary results with the assay system are promising.

Publication: J. Immunol. 108:852-856, 1972.

Terminated since FY 1973 funding will be under the Surgeon General, Army. New Project No. 3A062113A[illegible]; Accession No. DA [illegible].

*Available from [illegible]

DD FORM 1498 [illegible]

BODY OF REPORT

Project No. 1W662711A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1W662711A096 03: Laboratory Identification of Biological Agents

Work Unit No. 096 03 801: Radioimmunological Assay of Physiologically Active Substances

Description:

Develop radioimmunological assay procedures for physiologically active substances.

Progress:

A sensitive, accurate and easy method has been developed to assay staphylococcal enterotoxins, types A and B (SEA and SEB). A report on the SEB portion was published in the Journal of Immunology. Further work is in progress on types C and D (SEC and SED).

The assay of other physiologically active substances is being investigated in cooperation with other divisions within the Institute. Assay systems are under development for ACTH, bradykinin, prostaglandins, and thyrotropic releasing hormone (TSH).

Production of anti-ACTH and -bradykinin was begun, in goats using a unique hapten system whereby the ACTH or bradykinin is chemically coupled to cellulose particles and used as an immunogenic agent. This system elicited precipitating antibody to ACTH after only 4 weeks after immunization.

Assay of antibody using a reverse inhibition competition system with 2 antibodies and 1 antigen in the system has been studied in some detail. It compares favorably with conventional hemagglutinin systems but has proved difficult to standardize.

Intensive investigation into the detection and assay of antibody is in progress since the inherent sensitivity of these procedures could lead to an important diagnostic tool.

Summary:

A solid phase radioimmunological assay method has been developed for the measure of SEA and SEB. Antisera to ACTH has been produced; and an assay system is being developed. Immunization to bradykinin is in progress.

Further work is in progress on SEC, SED and other physiologically active proteins.

Radioimmunoassay of antibody to specific antigens is under extensive study as this appears to be an extraordinarily sensitive system.

Publication:

Collins, II, W. S., J. F. Metzger, and A. D. Johnson. 1972. A rapid solid phase radioimmunoassay of staphylococcal B enterotoxin. J. Immunol. 109:752-756.

AUTHOR INDEX

APPENDIX A

TECHNOLOGY SUPPORT PLANS

(TSP)

TECHNOLOGY SUPPORT PLAN

TSP-01

AUTOMATED BIOCHEMICAL TECHNIQUES

Karen A. Bostian, B.S.

Objective:

Develop fast reliable methods to quantitate concentrations of various metabolites in biological fluids including blood, urine, and tissue extracts from both man and laboratory animals using the AutoAnalyzer (Technicon).

Progress and Summary:

New automated tests used this year have been:

1. Hexose: Automation of a manual method[1,2].

2. Tyrosine[3]: Application of a fluorometric analysis for tyrosine to serum stored frozen. Results were compared with those found with the TSM amino acid analyzer. Our tyrosine values were higher than those found by column analysis. We are probably detecting small peptides as well as the single amino acid. The two results seem to parallel each other, so this method may be useful for screening purposes.

3. Phenylalanine:[4] Approach and results were the same as for tyrosine.

4. α-Amino Nitrogen (Total amino acids):[5] This method showed results much more compatible with column analysis for total amino acids in serum than the ninhydrin method[6] used previously. Because this method is more pH sensitive, the ninhydrin method is still the best for measuring total amino acids in urine.

5. Glucose by o-Toluidine:[7] This method permitted making glucose determinations on whole serum rather than the Somogyi filtrates necessary for the glucose-oxidase method.[8] The disadvantages of this method are (1) strong acid reagent is necessary; (2) larger sample; and (3) stored samples clotted which often plugged the sample line. Results, however, were the same for both methods. The disadvantages of the o-toluidine method outweigh the inconvenience of preparing Somogyi filtrates for the glucose-oxidase method.

6. Alkaline phosphatase:[9] This method was set up to run serum samples from volunteer projects.

7. Bilirubin (Total & Direct):[10] This method was set up to run serum samples from volunteer projects. It has also been used for animal work.

8. Glycerol:[11] This method showed good results for plasma glycerol. The plasma glycerol is stabilized by addition of triethylamine when fresh; the samples will keep at -20C for at least 3 mon.

9. Automated immunoprecipitin system:[12] Standard curves were obtained for the proteins IgA, IgG, IgM, C_3, albumin, transferrin, α_1-antitrypsin, and haptoglobin. We are waiting for antisera for α_1-acid glycoprotein, β-lipoprotein, and α_2-macroglobulin. We are currently checking for stability of these proteins in stored serum, reproducibility of results, comparison of results from different sources of antiserum, and comparison of results from this method with standard immunoprecipitin techniques done by Dr. McGann's group in Bacteriology Division.

An estimate of the tests we have run during the past year are shown in Table I.

TABLE I. TESTS RUN DURING FY 1971

TEST	NO. SAMPLES	INVESTIGATOR
Cholesterol,[13] Triglycerides,[14] FFA[15], Phospholipids[16], Glucose[8]	1,000	R. H. Fiser
Inulin,[17] PAH,[18] Urea,[19] Creatinine,[20] Chlorides[21]	700	G. H. Bilbrey
Lowry Protein[22]	6,000	M. C. Powanda
Serum hexose[1],[2]	500	G. L. Cockerell
Lactate,[23] Pyruvate	200	J. B. Walford
Kjeldahl N,[24] Ammonia N[25] Creatinine,[20] Urea N[19] α-amino N[6]	100 rat urine	R. W. Wannemacher, Jr.
N (Kjeldahl)[24]	32 rat feces	R.W.Wannemacher, Jr.
Lowry Protein[22]	1,000	R.W.Wannemacher, Jr.
Kjeldahl N[24] and Lowry Protein[22]	84 vaccines	F. E. Cole, Jr.
Na,[26]Cl,[27] BUN,[19] P,[28] Creatinine,[20] Uric acid,[29] Alk. Phosphatase,[9] Bilirubin,[10] Cholesterol,[13] Triglycerides,[14] FFA,[15] Biuret Protein[31]	103 sera	C. S. White
Creatinine,[20] Protein[31],[32]	101 urines	C. S. White
Alkaline phosphatase,[9] Bilirubin,[10] Lactate,[23] Pyruvate,[23] Glycerol,[11] Cholesterol,[13] FFA,[15] Triglycerides,[14] Phospholipids,[16] Hexose[2]	126 sera	Med. Div. Protocol FY 72-2
Glucose,[8] FFA[15]	189 plasma	Med. Div. Protocol FY 72-2

Also we checked blood samples from 20 men for normal range as well as stability during storage of various metabolites. We found that samples kept at -20 C were stable for at least a month when run for cholesterol,[13] triglycerides,[14] glycerol,[11] SGOT,[30] CPK,[33] LDH,[34] glucose,[8] lactate,[23] and pyruvate.[23] Samples for SGPT[30] were stable only for 24 hours.

Using the Technicon Autoanalyzer basic modules including the digestor, fluorometer, and fluoronephelometer we maintain systems, reagents, and standards for running analyses for 33 metabolites and 11 specific proteins Also we try to set up, modify, or develop automated techniques as investigators require them. Our space and equipment permits a maximum of 6 analyses at one time.

Publications:

None.

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TECHNOLOGY SUPPORT PLAN

TSP-02

AUTOMATED AMINO ACID DETERMINATIONS

Richard E. Dinterman, B.S.

Objective:

Develop and use fast, reliable methods for quantitation of amino acids using the Technicon TSM Amino Acid Analyzer.

Progress and Summary:

Using an amino acid analyzer, 171 serial blood samples from the adenovirus project (Medical Division Protocol FY 71-2) and 168 samples from the yellow fever vaccine study (Medical Division Protocol FY 71-3) were analyzed and amino acid levels quantitated. The data from these 2 projects have been statistically analyzed for significant differences between control and infected subjects as well as differences between individual pre- and postinfection levels. Fifty-seven samples from an experiment to study the incorporation of various amino acids into free and bound pools of liver, serum, and muscle in the rat during infection were quantitated. The results are now being tested by the Computer Section to show any differences between normal and pneumococcus infected rats. In addition, amino acid measurement and quantitation of 144 serial urine samples taken from 2 sandfly fever volunteer studies (Medical Division Protocols FY 70-1 and 70-3) have been completed. These data also are being evaluated by the Computer Section.

A second Technicon TSM amino acid analyzer was placed in operation. Using modified columns which are smaller in diameter and slightly different buffers this system now provides for a more accurate measurement of amino acids then previously possible. Having also adapted the older TSM with this modification, it is now possible to obtain 11 complete physiologic amino acid chromatograms of $\geq$ 30 amino acids each.

In order to prevent the loss of heat-labile amino acids a larger coolant reservoir has been added to the analyzer systems. This cooler with its self-containing circulating system provides for a colder and more uniform temperature to the sample tray. However, with the more efficient air-circulating system in the new buildng our present overlay cooling system is not adequate. A work order has been placed for the manufacture of an aluminum sample tray with a cooling coil embedded through its center.

A Data Acquisition System has been purchased. A program is now being written by the Computer Section so that a computer-compatible punch tape will automatically be taken from the 2 analyzers, thus, eliminating the tedious and time consuming task of normal quantitation of chromatograms.

A new fast method by Eastman Kodak for testing amino acid concentrations has been evaluated. Because of poor resolution, this kit will not suffice as a fast screening tool.

APPENDIX B
VOLUNTEER STUDIES

PROTOCOL TITLE & NO. (No. Volunteers)	PURPOSE	COMMENTS & RESULTS
Infectivity of Human Plasma Presumed to Contain Sandfly Fever Virus. FY 72-1 (1)	To determine the infectivity of a unit of plasma presumed to contain sandfly fever virus, when administered intravenously to a single volunteer.	Volunteer developed typical sandfly fever.
Chemical Analysis of Blood and Urine Collected Under Standard Conditions. FY 72-2 (21)	To obtain additional data on diurnal periodicity of amino acids and determine periodicity of various lipid protein bound carbohydrates and trace metals obtained from venous blood of volunteers and establish normal values of growth hormone, insulin, free fatty acids, alanine and Cr following administration of oral or intravenous glucose.	See work unit 096 03 010 & 03 012 Collaborative study with Walter Reed Army Institute of Research.
Median Infective Titer of Sandfly Fever Virus in a Lot of Human Plasma. FY 72-3 (20)	To determine the infectivity of a single lot of human plasma known to contain sandfly fever virus (see FY 72-1) when administered intravenously in various doses to healthy volunteers.	The median illness dose (ID_{50}) of this plasma was approximately 0.0005 ml.
Associated Administration to Volunteers of Venezuelan equine encephalomyelitis vaccine, Live, Attenuated and Yellow Fever Vaccine, 17D Strain. FY 72-4 (32)	To compare single, simultaneous and closely spaced administration of YF and VEE vaccines on the basis of neutralizing and hemagglutination inhibition antibodies to both antigens in volunteers.	See work unit 096 02 008.

APPENDIX C

U.S. ARMY MEDICAL RESEARCH INSTITUTE OF INFECTIOUS DISEASES
GUEST LECTURE SERIES

DATE	GUEST LECTURER	TITLE OF PRESENTATION
30 Sep 71	Dr. Charles L. Wisseman, Jr. Professor and Head Department of Microbiology University of Maryland School of Medicine Baltimore, Maryland	Some Problems of Epidemic Typhus.
21 Oct 71	Colonel Harry C. Holloway, Jr., MC Director, Division of Neuropsychiatry Walter Reed Army Institute of Res Washington, D.C.	Drug Problems in Southeast Asia.
2 Dec 71	Dr. John B. Robbins Clinical Director of National Institute of Child Health and Development, NIH Bethesda, Maryland	Production of Hemophilus Influenza Vaccine.
27 Jan 72	Lt Colonel Carter L. Diggs, MC Deputy Director of Division of Communicable Disease and Immunology Walter Reed Army Institute of Res Washington, D.C.	Current Trends in Malaria Research.
17 Feb 72	Lt Colonel Paul K. Hildebrandt, VC Director, Division of Pathology Walter Reed Army Institute of Res and Major David L. Huxsoll, VC Chief, Department of Diagnostic Svcs Division of Veterinary Medicine Walter Reed Army Institute of Res Washington, D.C.	Tropical Canine Pancytopenia.
23 Mar 72	Dr Jay P. Sanford Professor of Internal Medicine University of Texas Southwestern Medical School at Dallas Dallas, Texas	Role of Immunity in Experimental Pyelonephritis.

DATE	GUEST LECTURER	TITLE OF PRESENTATION
20 Apr 72	Dr. Bennett L. Elisberg Chief, Department of Rickettsial Diseases Walter Reed Army Institute of Res Washington, D.C.	New World Tick Typhus - Fact or Fantasy.

APPENDIX D

U.S. ARMY MEDICAL RESEARCH INSTITUTE OF INFECTIOUS DISEASES PROFESSIONAL STAFF MEETINGS

DATE	LECTURER	TITLE OF PRESENTATION
10 Sep 71	Major Jean B. DuBuy, MC Physical Sciences Division	Endogenous Pyrogen.
	Dr. Robert S. Pekarek Physical Sciences Division	Endogenous Mediators of Trace Metal Changes during the Inflammatory Process.
	Major Frederick R. DeRubertis,MC Physical Sciences Division	Effect of Salmonella typhimurium Bacteremia on Host Thyroid Hormone Economy.
	Dr. Robert W. Wannemacher, Jr. Actg Chief, Physical Sciences Div	Endogenous Mediators of Amino Acid Transport.
15 Oct 71	Major Stanley H. Rabinowitz, MC Bacteriology Division	Host Defenses during Primary Venezuelan Equine Encephalomyelitis Virus Infection in Mice.
	Major Joseph Kaplan, MC Bacteriology Division	Electron Spin Resonance Studies of Cell Membrane Interactions.
	Mrs. Mary H. Wilkie Bacteriology Division	Analysis of Responses to Immunization.
	Captain Peter G. Canonico, MSC Bacteriology Division	Fractionation and Analysis of Killed Q Fever Vaccine.
19 Nov 71	Dr. Francis E. Cole, Jr. Virology Division	Current Status of Arbovirus Vaccine Program.
	Major David M. Robinson, VC Chief, Virology Division	Live Q Fever Vaccine.
	Dr. Richard H. Kenyon Virology Division	Development of an Improved Rocky Mountain Spotted Fever Vaccine.
	LT Daniel R. Woodman, MSC, USN U.S. Naval Unit, Fort Detrick	The Application of Indicators of Delayed Hypersensitivity in the Rapid Identification of Viruses.

DATE	LECTURER	TITLE OF PRESENTATION
21 Jan 72	Lt Colonel Peter J. Bartelloni, MC Chief, Medical Division	Clinical Evaluation of Eastern Equine Encephalitis Vaccine, Inactivated, Tissue Culture Origin, NDBR 104.
	Major William A. Christmas, MC Bacteriology Division	The Efficacy of a Pentavalent Vaccine in Animals.
	Captain Charles S. White, III, MC Medical Division	Clinical and Laboratory Studies in a Group of Selected Subjects Receiving Special Immunizations.
	Major William H. Adler, III, MC Pathology Division	Evaluation of Lymphocyte Function in a Group of Selected Subjects Receiving Special Immunizations.
25 Feb 72	Captain Philip C. Kosch, VC Animal Assessment Division	Dose-response Relationship of the Yellow Fever-infected Rhesus Monkey.
	Captain Thomas W. Davis, VC Animal Assessment Division	The physiological and Pathological Responses of Macaca mulatta to Staphylococcal Alpha and Delta Toxins.
	Mr. Ralph W. Kuehne Animal Assessment Division	Hemorrhagic Fever in Cochabamba--Virus Isolation and Identification.
	Captain Michael D. Kastello, VC Animal Assessment Division	Indications of Altered Immune Function in Hypercholesterolemic Monkeys.
	Lt Colonel Richard O. Spertzel, VC Chief, Animal Assessment Division	Venezuelan Equine Encephalomyelitis: Texas, 1971.
17 Mar 72	Dr. Leonard Spero Pathology Division	The Limited Digestion of Staphylococcal Enterotoxin B by Trypsin.
	Major John R. Warren, MC Pathology Division	Chemical Alteration of SEB.

DATE	LECTURER	TITLE OF PRESENTATION
17 Mar 72	Major William H. Adler, III, MC Pathology Division	Established Lymphoblast Cell Lines from Selected Individuals.
	Captain Thomas H. Hudson, MSC Pathology Division	The Ultrastructure of Soluble Protein Uptake by Liver Using Peroxidase as a Tracer.
	Captain Joe D. Burek, VC Pathology Division	Cytoplasmic Inclusions in Urinary Bladder Epithelium of Macaca mulatta. A Histochemical, Light and Electron Microscopic Study.
	Lt Colonel James L. Stookey, VC Pathology Division	Anatomy of a Case.
28 Apr 72	Mr. Daniel N. Harrison Microbiology Division	The Use of the Indirect Hemagglutination Inhibition Test for the Detection of Pasteurella pestis Fraction I.
	Colonel Dan C. Cavanaugh, MSC Department of Bacterial Diseases Walter Reed Army Institute of Res Washington, D.C.	Indirect Evidence of the Efficacy of Plague Vaccine USP in Preventing Bubonic Plague in Troops Exposed to Infection under Field Conditions in Republic of Vietnam.
	Captain James E. Williams, MSC Department of Bacterial Diseases Walter Reed Army Institute of Res Washington, D.C.	Maternal Antibody to Pasteurella pestis in New Born Rats.
	Colonel John D. Marshall, Jr., MSC Chief, Microbiology Division	Clinical and Serological Response to Multiple Plague Immunizations.
19 May 72	Captain Alan H. Rowberg, MC Administrative Division	Use of Data Processing Techniques for the Laboratory Diagnosis of Infection.

APPENDIX E

U. S. ARMY MEDICAL RESEARCH INSTITUTE OF INFECTIOUS DISEASES
FORMAL PRESENTATIONS AND BRIEFINGS

Date and Group or Individual	Individual(s) Participating	Subject
18-20 Jul 71 USPHS and Mexican Government VEE Conference, Brownsville, Texas	Colonel Dan Crozier, MC	Participant in conference on VEE.
19 Jul 71 Lieutenant General W. W. Vaughan Deputy Commanding General U. S. Army Materiel Command Washington, D. C.	Dr. William R. Beisel, M.D.	Briefing relative to transition of Fort Detrick program.
27-29 Jul 71 Conference at Center for Disease Control, Atlanta, Georgia	Colonel Dan Crozier, MC Major David M. Robinson, VC Major Carl E. Pedersen, MSC	Discussion of VEE Control Measures.
3 Aug 71 Colonel William S. Augerson, MC Military Assistant for Medicine and Life Sciences, Office of Director of Defense Research and Engineering, Washington, D. C.	Colonel Dan Crozier, MC	Briefing of USAMRIID research program and tour of unit facilities.
6 Aug 71 Colonel Reginald C. Thomas, MSC Medical Intelligence Office, Office of The Surgeon General, DA Washington, D. C.	Colonel Dan Crozier, MC	Orientation briefing of USAMRIID activities in relation to the Medical Intelligence Program.

Date and Group or Individual	Individual(s) Participating	Subject
9 Aug 71 Dr. Claude J. B. Bradish, Dr. Alastair Paterson Microbiological Research Establishment, Porton Downs, England	Colonel Dan Crozier, MC Dr. William R. Beisel, M.D. Colonel Joseph F. Metzger, MC Lt Colonel Peter J. Bartelloni, MC Lt Colonel Harry G. Dangerfield, MC Major David M. Robinson, VC Major Joseph Kaplan, MC	Briefing on USAMRIID research activities in areas of mutual interest; tour of Phase I of new medical facility.
16 Aug 71 Conference of the National Assembly of the Animal Health Association, Chicago, Illinois	Lt Colonel Richard O. Spertzel, VC	Impromptu briefing on VEE Epizootic.
22 Aug 71 Plague Symposium Colorado State University Fort Collins, Colorado	Colonel John D. Marshall, Jr., MSC	Participant in symposium on plague.
25-27 Aug 71 Wildlife Disease Association of America, Colorado State University Fort Collins, Colorado	Colonel John D. Marshall, Jr., MSC	Susceptibility of Rodents to Oral Plague Infection: A Mechanism for the Persistence of Plague in Interepidemic Periods. The Influence of Climate on the Seasonal Prevalence of Plague in the Republic of Vietnam.
30 Aug 71 Symposium on Pharmacology of Bacterial Toxins, Czechoslovak Academy of Sciences, Institute of Pharmacology, Albertov 4, Prague 2, Czechoslovakia	Dr. William R. Beisel, M.D.	Pathophysiology of Staphylococcal (Type B) Enterotoxin after the Administration to Monkey.

Date and Group or Individual	Individual(s) Participating	Subject
30 Aug-2 Sep 71 Western Hemisphere Nutrition Congress III, Bal Harbour, Florida	Dr. Robert W. Wannemacher, Jr.	Interrelationship between Nutrition and Infection with Regard to Changes in Plasma Amino Acids.
	Dr. Robert S. Pekarek	Metabolic Losses of Zinc and other Trace Elements during Acute Infection.
10 Sep 71 Foreign Animal Disease Training School, National Animal Disease Laboratory, Ames, Iowa	Lt Colonel Richard O. Spertzel, VC	Venezuelan Equine Encephalomyelitis
11 Sep 71 Horse Health Conference on VEE and Equine Infectious Anemia, Rutgers University New Brunswick, New Jersey	Lt Colonel Richard O. Spertzel, VC	VEE - The Clinical Picture and Production of a Vaccine for its Prevention.
12-17 Sep 71 American Chemical Society Meeting Washington, D. C.	Captain William J. Caspary, MSC	The In Vitro Reaction of 6-Hydroxylbenzo (a) Pyrene with DNA.
		Mechanism for the Covalent Linkage of Carcinogenic Polycyclic Hydrocarbons (HC) to DNA.
14-17 Sep 71 Workshop-Symposium on VEE Virus Pan American Health Organization Regional Office of World Health Organization, Washington, D. C.	Colonel Dan Crozier, MC Lt Colonel Richard O. Spertzel, VC Major David M. Robinson, VC	Participate as discussants in international symposium on VEE virus to review accumulated data and develop a program for reducing the impact of the disease on the health and economy of the continent.

Date and Group or Individual	Individual(s) Participating	Subject
15 Sep 71 Dr. Rober Wm. Brindlecombe Dr. Peter Holland Chemical Defence Establishment Porton Downs, England	Dr. Leonard Spero	Briefing on USAMRIID research activities of mutual interest.
20 Sep 71 Short Course, "Pathology of Laboratory Animals" Armed Forces Institute of Pathology Washington, D. C.	Lt Colonel James L. Stookey, VC	Systemic Mycotic Diseases.
23 Sep 71 Joint Annual Meeting of Commission on Epidemiological Survey and Commission on Immunization, Armed Forces Epidemiological Board, Washington, D. C.	Colonel Dan Crozier, MC	Introductory Remarks.
	Lt Colonel Richard O. Spertzel, VC	Overview of the 1971 Venezuelan Equine Encephalomyelitis Epizootic.
	Mrs. Mary H. Wilkie	Analysis of Sequential Humoral Antibody Responses.
	Major Stanley H. Rabinowitz, MC	Local Respiratory and Humoral Responses to Immunization.
	Major Joseph Kaplan, MC Captain William J. Caspary, MSC	Electron Spin Resonance Studies of Antigen-Cell Membrane Interaction.
	Captain Neil H. Levitt, MSC	Use of Microprecipitation and Electrophoresis in Diagnostic Virology.
	Major William H. Adler, III, MC	Mitogenic Effects of Enterotoxins in Lymphocytes.

Date and Group or Individual	Individual(s) Participating	Subject
24 Sep 71 Continuation of Joint Meeting of Commission on Epidemiological Survey and Commission on Immunization	Captain Peter G. Canonico, MSC	Lysosomal Responses during Infection.
	Dr. William R. Beisel, M.D.	Hypercholesterolemia and Altered Immunity.
	Dr. Robert S. Pekarek	Endogenous Mediators of Nonfebrile Host Responses.
	Dr. Richard H. Kenyon	Spotted Fever Vaccine.
	Major David M. Robinson, VC	Live Q Fever Vaccine.
	Colonel Joseph F. Metzger, MC	Staphylococcal Enterotoxoids.
27 Sep 71 Florida State Veterinary Medical Association Convention, Miami Beach, Florida	Lt Colonel Richard O. Spertzel, VC	Epizootic Control - Vaccination and Quarantine Procedures Pertaining to VEE.
28 Sep 71 Dr. J. B. Bateman Chief, Life Sciences Branch USA Research and Development Group (Europe), APO New York	Dr. William R. Beisel, M.D. Colonel Harry G. Dangerfield, MC Major David M. Robinson, VC	Briefing on USAMRIID research activities of mutual interest.
30 Sep 71 Meeting of Veterinary Biologics Div, U.S. Department of Agriculture and Veterinary Biologics Licensees, Hyattsville, Maryland	Lt Colonel Richard O. Spertzel, VC	Participant in meeting to discuss licensing requirements for VEE Virus Vaccine.

Date and Group or Individual	Individual(s) Participating	Subject
7 Oct 71 Colonels Lewis H. Huggins, MSC, Joseph A. Pastore, MSC, Milton C. Devolites, MSC; Lt Colonel Jack F. Heath, MSC; Majors Thomas H. Korte, MSC, James L. Peacock, MSC; all of Office of The Surgeon General, DA, Washington, D. C.	Colonel Dan Crozier, MC	Tour of USAMRIID facilities.
13 Oct 71 Grand Rounds, Veterans Administration Hospital, Baltimore, Maryland	Dr. William R. Beisel, M.D.	The VEE Story.
13 Oct 71 Colonel Marvin E. Nation, MSC, Lt Colonel Robert W. Twieto, MSC, U.S. Army Combat Development Command, Medical Service Agency, Fort Sam Houston, Texas; and Colonel Robert L. Krivulka, MSC, U.S. Army Medical Research and Development Command, Washington, D. C.	Colonel Dan Crozier, MC	Review of Department of the Army approved Qualitative Materiel Development Objective (QMDO) for Medical Defense against C and B Agents.
20 Oct 71 Frederick Rotary Club Luncheon Meeting, Frederick, Maryland	Dr. William R. Beisel, M.D.	Activities of USAMRIID.
20-22 Oct 71 Assistant Chief of Staff for Force Development CW/BR Program Symposium, U.S. Army Chemical Center and School Fort McClellan, Alabama	Colonel Dan Crozier, MC	Participant in CW/BR Program Symposium.

Date and Group or Individual	Individual(s) Participating	Subject
27 Oct 71 Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland	Captain Peter G. Canonico, MSC	The Cellular Biology of Staphylococcal Enterotoxin B.
28 Oct 71 Conference on the Design of Experiments in Army Research, Development and Testing, Walter Reed Army Institute of Research, Washington, D.C.	Major Robin T. Vollmer, MC	Experimental Design in Prospective Studies of Infection in Man.
29 Oct 71 Annual Meeting of U. S. Animal Health Association Oklahoma City, Oklahoma	Lt Colonel Richard O. Spertzel, VC	Overview of the 1971 Texas VEE Epizootic.
21-22 Oct 71 Meeting of Commission on Viral Infections, Armed Forces Epidemiological Board, Washington, D.C.	Lt Colonel Richard O. Spertzel, VC	Report on Status of VEE Vaccine Supply and Research.
5 Nov 71 Center for Disease Control Atlanta, Georgia	Colonel Dan Crozier, MC	Participant in conference on review of VEE.
10 Nov 71 Sigma Xi Meeting Colorado State University Fort Collins, Colorado	Colonel John D. Marshall, Jr., MSC	Plague.
11-12 Nov 71 Meeting of Commission on Rickettsial Diseases, Armed Forces Epidemiological Board, Washington, D. C.	Major David M. Robinson, VC Dr. Richard H. Kenyon	Live Q Fever Vaccine. Development of an Improved Rocky Mountain Spotted Fever Vaccine.

Date and Group or Individual	Individual(s) Participating	Subject
18-19 Nov 71 Annual Command Visit: Colonels Richard F. Barquist, MC, Donald W. Sample, MC, Dallas P. Wright, MSC; Lt Colonel Joseph R. Cataldo, MC; Major Robert A. Bates, MSC; all of USA Medical Research and Development Command, Washington, D. C.	Colonel Dan Crozier, MC, and Staff	Annual orientation briefing of overall mission and operation of USAMRIID.
2 Dec 71 Colonel David C. Cowling, Clinical Pathologist, Reserve Officer in Australian Army, Royal Melbourne Hospital, Melbourne, Australia	Colonel Joseph F. Metzger, MC	Briefing and discussions of research subjects of mutual interest.
15 Dec 71 Captain Gosta Logard, Instructor, ADC Defence School, Swedish Armed Services Kungsagen, Sweden	Colonel Dan Crozier, MC	Briefing on USAMRIID research activities of related interests.
20 Dec 71 Bureau of Biological Research Rutgers University New Brunswick, New Jersey	Captain Peter G. Canonico, MSC	Recent Advances on the Proteolytic Functions of Lysosomes.
21-22 Jan 72 Standardbred Short Course New York State College of Agriculture Cornell University, Ithaca, New York	Lt Colonel Richard O. Spertzel, VC	VEE.
25 Jan 72 Annual Meeting, Indiana State Veterinary Association Indianapolis, Indiana	Lt Colonel Richard O. Spertzel, VC	VEE.

Date and Group or Individual	Individual(s) Participating	Subject
26 Jan 72 Research Training Fellowship Group Walter Reed Army Institute of Res Washington, D.C.	Dr. William R. Beisel, M.D.	Host Metabolic Responses to Infectious Disease.
	Dr. Robert W. Wannemacher, Jr. Dr. Robert S. Pekarek CPT Michael C. Powanda, MSC CPT Gary L. Cockerell, VC	Interrelationship among Trace Metal Amino Acid and Protein Metabolism during Infection.
	Colonel Dan Crozier, MC	Vaccine Testing in Man.
	Lt Colonel Richard O. Spertzel, VC	VEE Vaccine.
	Major David M. Robinson, VC	Vaccine Development.
27 Jan 72 Southern Section, American Federation for Clinical Research New Orleans, Louisiana	Major Stanley H. Rabinowitz, MC	Host Defenses during Primary VEE Virus Infection in Mice.
27 Jan 72 Global Medicine Course Walter Reed Army Institute of Res Washington, D. C.	Colonel John D. Marshall, Jr., MSC	Plague.
28 Jan 72 Meeting of AD HOC Committee on Q Fever Vaccine, Commission on Rickettsial Diseases Baltimore, Maryland	Colonel Dan Crozier, MC Dr. William R. Beisel, M.D. Lt Colonel Peter J. Bartelloni, MC Major David M. Robinson, VC Captain Peter G. Canonico, MSC	Participants in AD HOC Committee on Q Fever Vaccine.
	Dr. Francis E. Cole, Jr.	Q Fever Phase I Vaccine Studies.
	Dr. Richard H. Kenyon	Rocky Mountain Spotted Fever Vaccine Studies.

Date and Group or Individual	Individual(s) Participating	Subject
7-8 Feb 72 72d Annual Conference of Veterinarians, University of Pennsylvania, Philadelphia, Pa.	Lt Colonel Richard O. Spertzel, VC	VEE in the United States.
14 Feb 72 Symposium on Military Veterinary Medicine, Walter Reed Army Institute of Research, Washington, D. C.	Lt Colonel Richard O. Spertzel, VC	Participant as instructor in principles of veterinary support of operations in future warfare; and presentation on VEE.
15 Feb 72 Dr. Walter Mertz, Chief, Vitamin and Mineral Nutrition Laboratory, U.S. Department of Agriculture, Beltsville, Md., and Dr. Richard J. Doisy, Department of Biochemistry, State University of N. Y. Upstate Medical Center, Syracuse, New York	Dr. Robert W. Wannemacher, Jr. Dr. Robert S. Pekarek Dr. William R. Beisel, M.D.	Orientation and demonstration on employment of the heated graphite atomizer for determination of Cr and Ni in Serum.
3 Mar 72 Life Sciences Research Program Army Research Office Arlington, Virginia	Colonel Dan Crozier, MC Captain Richard L. Coleman, MSC	Presentation of technical and budgetary review of FY 1973 USAMRIID program.
13 Mar 72 Veterinary Sciences Research Div Agriculture Research Service U.S. Department of Agriculture Beltsville, Maryland	Lt Colonel Richard O. Spertzel, VC	Participant in conference on research problem areas of VEE.
16 Mar 72 Dengue Task Force Meeting Walter Reed Army Medical Center Washington, D. C.	Lt Colonel Richard O. Spertzel, VC	Participant in task force meeting.

Date and Group or Individual	Individual(s) Participating	Subject
23 Mar 72 Commission on Rickettsial Diseases Meeting, Walter Reed Army Institute of Research, Washington, D. C.	Dr. Richard H. Kenyon	Rocky Mountain Spotted Fever Studies.
23 Mar 72 Dr. Jay P. Sanford Professor of Medicine University of Texas Southwestern Medical School of Dallas, Texas	Colonel Dan Crozier, MC Dr. William R. Beisel, M.D. Lt Colonel Peter J. Bartelloni, MC	Introductory briefing and tour of USAMRIID facilities.
	Dr. Robert S. Pekarek	Leucocyte Endogenous Mediators in Inflammation.
	Captain Charles S. White, III, MC	Clinical and Laboratory Follow-up Studies in Selected Immunized Subjects.
	Major William H. Adler, III, MC	Lymphoblast Culture Lines from Peripheral Blood Lymphocytes of Selected Individuals.
	Major Stanley R. Rabinowitz, MC	Host Defenses during Primary VEE Virus Infection in Mice.
	Major David M. Robinson, VC	Attenuated Q Fever Vaccine.
9-14 Apr 72 Annual Meeting of Federation of American Societies for Experimental Biology, Atlantic City, New Jersey	Dr. William R. Beisel, M.D.	Effects of Leukocytic Endogenous Mediator (LEM) on the Distribution in Tissues of Zinc and Iron.
	Captain Gary L. Cockerell, VC	Plasma Protein and Glycoprotein Changes in Inflammation Infection and/or Starvation.

Date and Group or Individual	Individual(s) Participating	Subject
9-14 Apr 72 Continuation of Annual Meeting of Federation of American Societies for Experimental Biology	Dr. Robert W. Wannemacher, Jr.	Several Factors Affecting Plasma Free Amino Acids Concentrations. Served as Chairman, Enzyme I Section, American Institute of Nutrition Program.
	Major Randall T. Curnow, MC	The Effect of Diplococcus pneumoniae (DP) Sepsis and Leukocytic Endogenous Mediator (LEM) on Liver Glycogen Synthetase (GS) and Glycogen Phosphorylase (GP) Activity in the Fasted Rat.
	Dr. Robert S. Pekarek	Direct Determination of Serum Chromium and Nickel by an Atomic Absorption Spectrophotometer with a Heated Graphite Furnace.
	Captain Michael C. Powanda, MSC	Nitrogen Metabolism during Sepsis in Rats.
23-28 Apr 72 Annual Meeting of the American Society for Microbiology, Philadelphia, Pennsylvania	Dr. Bruno J. Luscri	Several Observations on the Induction and Bioassay of Interferon Originating from Human Cell Cultures.
24 Apr 72 Lieutenant General Hal B. Jennings, Jr. The Surgeon General, DA, and Brigadier General Richard R. Taylor, MC Commanding General, US Army Medical Research and Development Command	Colonel Dan Crozier, MC	Tour of USAMRIID facilities.

Date and Group or Individual	Individual(s) Participating	Subject
25 Apr 72 Conference on VEE, Toronto, Canada	Lt Colonel Richard O. Spertzel, VC	VEE - Epidemiology and the Recent Spread Patterns of the Disease in South America, Central America, and the United States.
26 Apr 72 Rotary Club Members Frederick, Maryland	Colonel Dan Crozier, MC, and Staff	Tour of USAMRIID facilities.
29 Apr 72 Joint Meeting of American Federation for Clinical Research and American Society for Clinical Investigation, Atlantic City, New Jersey	Major Stanley R. Rabinowitz, MC	Host Defenses during Primary VEE Virus Infection in Mice.
4 May 72 Briefing for U.S. Public Health Service Officials, Edgewood Arsenal, Maryland	Colonel Dan Crozier, MC	Biological Warfare in Perspective.
6 May 72 Joint Meeting of Maryland and Washington, D.C. Branches of the American Society for Microbiology, Fort Detrick, Maryland	Dr. William R. Beisel, M.D.	Welcome and tour of laboratory facilities.
	Major Stanley R. Rabinowitz, MC	Host Defenses during Primary VEE Virus Infection in Mice.
	Mr. Ralph W. Kuehne	Isolation and Characterization of Cochabamba Virus.
	Colonel Dan Crozier, MC	Address to the evening session.

Date and Group or Individual	Individual(s) Participating	Subject
8-12 May 72 NATO Meeting of Editorial Committee, Medical Defense Aspects of NBC Operations, and Panel of Experts of The Technical Cooperation Program, Washington, D.C.	Colonel Dan Crozier, MC	Participant in meeting of the Editorial Committee on NATO Handbook; participant in TTCP Panel of Experts' Meeting.
11 May 72 NATO Panel of Experts for Medical Aspects of NBC Operations, Visit to USAMRIID, Fort Detrick, Maryland	Colonel Dan Crozier, MC	Welcome to USAMRIID and opening remarks.
	Dr. William R. Beisel, M.D.	Host Response to Infection.
	Major David M. Robinson, VC	Vaccine Studies.
	Major William H. Adler, III, MC	Immunology Studies.
	Dr. Robert S. Pekarek	Mediator Substance.
	Colonel Dan Crozier, MC	Building Orientation.
	Colonel Crozier Dr. Beisel Lt Colonel Richard O. Spertzel, VC Colonel Joseph F. Metzger, MC Colonel Harry G. Dangerfield, MC 1LT Jack W. Downing, MSC Mr. Roy W. Culler	Tour of building. Panel members were provided the opportunity of visiting with individual USAMRIID investigators in the afternoon. A brief outline of research projects provided visitors for choice of interest.

Date and Group or Individual	Individual(s) Participating	Subject
15-20 May 72 XLth General Session of the Office International des Epizooties Paris, France	Lt Colonel Richard O. Spertzel, VC	Participant in Round Table on VEE for purpose of establishing recommendations for VEE international zoo-sanitary code.
18-19 May 72 Meeting of the Armed Forces Epidemiological Board, Washington, D. C.	Colonel Dan Crozier, MC	Present report of the Commission on Epidemiological Survey to the Board.
25 May 72 The Society for Pediatric Meetings, Immunology Section Washington, D. C.	Major William H. Adler, III, MC	In vitro Studies on Role of Cell Mediated Immunity in Host Resistance to VEE Viral Infection in Mice.
25 May 72 Briefing for Dr. Chris J. D. Zarafonetis' Committee, Pentagon, Alexandria, Va.	Colonel Dan Crozier, MC	Briefing on the use of volunteers in research.
26 May 72 School of Aerospace Medicine Brooks Air Force Base, Texas	Colonel Dan Crozier, MC	Medical Defense Aspects of Biological Warfare.
30 May 72 Dr. Otto H. E. Westphal and Miss Ursula Haegele, Max-Planck-Institute for Immuno-biology, Freiburg, Germany	Colonel Dan Crozier, MC Dr. William R. Beisel, M.D. Lt Colonel Peter J. Bartelloni, MC Captain Charles S. White, III, MC	Tour of USAMRIID facilities. Vaccine Testing in man, including long term follow-up studies.

Date and Group or Individual	Individual(s) Participating	Subject
30 May 72 Continuation of seminar for Dr. Westphal and Miss Haegele	Major David M. Robinson, VC Dr. Francis E. Cole, Jr. Dr. Richard H. Kenyon	Experimental Vaccines under Development.
	Colonel Joseph F. Metzger, MC Lt Colonel William S. Collins, II Dr. Virginia G. McGann	Studies with Highly Purified Staphylococcal Enterotoxins.
	Major Stanley R. Rabinowitz, MC Major William H. Adler, III, MC	Studies on Cellular Immunity.
	Dr. Robert S. Pekarek Major Jean B. DuBuy, MC	Mediators of Nonfebrile Systemic Responses to Infection.
1-2 Jun 72 Biochemical Society/Nutrition Society Joint Colloquium Aberdeen, Scotland	Dr. Robert W. Wannemacher, Jr.	Ribosomal RNA Synthesis and Function as Influenced by Amino Acid Supply and Stress.
7-8 Jun 72 Conference on VEE, Kansas City, Kans. sponsored by Jensen-Salsbery Labs and American Association of Equine Practitioners	Lt Colonel Richard O. Spertzel, VC	1971 Outbreak of VEE in Texas: Spread and Control.
19 Jun 72 VEE Meeting, Montreal, Canada sponsored by Equine Practitioners Association of Quebec and Bluebonnets Raceway	Lt Colonel Richard O. Spertzel, VC	VEE - Epidemiology and the Recent Spread Patterns of the Disease in South America, Central America, and the United States.

Date and Group or Individual	Individual(s) Participating	Subject
20-23 Jun 72 1972 Army Science Conference, U. S. Military Academy West Point, New York	Lt Colonel Richard O. Spertzel, VC	Control of VEE Epizootic-Epidemic by Vaccine Developed at USAMRIID.
	Dr. Robert S. Pekarek	A Mediator for Triggering Non-Specific Host Defense Mechanisms.
18-22 Jun 72 Annual Meeting of the American Medical Association San Francisco, California	Colonel Dan Crozier, MC	Venezuelan Equine Encephalomyelitis (VEE) Vaccine; Its Use in Equines to Prevent an Epidemic in Man.
28-29 Jun 72 3d Annual Council of Army Veterinarians, Washington, D. C.	Lt Colonel Richard O. Spertzel, VC Lt Colonel James L. Stookey, VC	Participants in conference.

APPENDIX F

PUBLICATIONS OF U. S. ARMY MEDICAL RESEARCH INSTITUTE OF INFECTIOUS DISEASES

FISCAL YEAR 1972

1. Beisel, W. R. 1972. Food Poisoning, pp. 15-18. In Current Therapy 1972. H. F. Conn, ed., W. B. Saunders, Philadelphia.

2. Beisel, W. R., R. S. Pekarek, and R. W. Wannemacher, Jr. 1972. Effects of leukocytic endogenous mediator (LEM) on the distribution in tissues of zinc and iron. Fed. Proc. 31:667 (abstract).

3. Bellanti, J. A., R. I. Krasner, P. J. Bartelloni, M. C. Yang, and W. R. Beisel. 1972. Sandfly fever: Sequential changes in neutrophil biochemical and bactericidal functions. J. Immunol. 108:142-151.

4. Brown, J. A. W. L. West, T. A. Balourdas, W. M. Banks, and J. D. Marshall. 1971. Some possible mechanisms of action of a heat labile toxin from Pasteurella pseudotuberculosis. Cytobios 3:25-32.

5. Canonico, P. G., and M. J. Van Zwieten. 1971. Swelling of mitochondria from rabbit liver induced by staphylococcal enterotoxin B. J. Infect. Dis. 124:372-378.

6. Canonico, P. G., M. J. Van Zwieten, and W. A. Christmas. 1972. Purification of large quantities of Coxiella burneti rickettsia by density gradient zonal centrifugation. Appl. Microbiol. 23, In press.

7. Cavanaugh, D. C., and J. D. Marshall, Jr. 1972. The influence of climate on the seasonal prevalence of plague in the Republic of Vietnam. J. Wildlife Dis. 8:85-94.

8. Cavanaugh, D. C., H. E. Stark, J. D. Marshall, Jr., and J. H. Rust, Jr. 1972. A simple method for rearing fleas for insecticide testing in the field. J. Med. Entomol. 9:113-114.

9. Chapple, III, F. E., J. M. Crosbie, and B. E. Reisberg. 1971. Surgical technic for cross-circulation of rhesus monkeys. Lab. Anim. Sci. 21:610-612.

10. Cockerell, G. L. 1972. Plasma protein and glycoprotein changes in inflammation, infection, and/or starvation. Fed. Proc. 31:710 (abstract).

11. Cohen, B. I., W. J. Caspary, S. A. Lesko, and P.O.P. Ts'o. 1971. Mechanism for the covalent linkage of carcinogenic polycyclic hydrocarbons (HC) to DNA. Program, American Chemical Society, Washington, D. C., 12-17 September 1971 (abstract 131).

12. Cole, Jr., F. E. 1971. Inactivated Eastern equine encephalomyelitis vaccine propagated in rolling-bottle cultures of chick embryo cells. Appl. Microbiol. 22:842-845.

13. Cole, Jr., F. E., and R. W. McKinney. 1971. Cross-protection in hamsters immunized with group A arbovirus vaccines. Infec. Immun. 4:37-43.

14. Collins, II, W. S., J. F. Metzger, and A. D. Johnson. 1972. A rapid solid phase radioimmunoassay for staphylococcal B enterotoxin. J. Immunol. 108:852-856.

15. Curnow, R. T., and R. S. Pekarek. 1972. The effect of Diplococcus pneumoniae (DP) sepsis and leukocytic endogenous mediator (LEM) on liver glycogen synthetase (GS) and glycogen phosphorylase (GP) activity in the fasted rat. Fed. Proc. 31:684 (abstract).

16. DeRubertis, F. R., and K. A. Woeber. 1972. Accelerated host metabolism of L-thyroxine (T_4) during acute Salmonella typhimurium (ST) sepsis. Clin. Res. XX:424 (abstract).

17. DeRubertis, F. R., and K. A. Woeber. 1972. Evidence for enhanced cellular uptake and binding of thyroxine in vivo during acute infection with Diplococcus pneumoniae. J. Clin. Invest. 51:788-795.

18. DeRubertis, F. R., and K. A. Woeber. 1972. The effect of acute infection with Diplococcus pneumoniae on heptic mitochondrial alpha-glycerophosphate dehydrogenase activity. Endocrinology 90:1384-1387.

19. Dill, Jr., G. S., U. McElyea, Jr., and J. L. Stookey. 1972. Transitional cell carcinoma of the urinary bladder in a cat. J. Am. Vet. Med. Assoc. 160:743-745.

20. Fiser, R. H., J. C. Denniston, and W. R. Beisel. 1972 Gram negative septicemia versus endotoxicosis: Differential effects on lipid metabolism. Clin. Res. XX:233 (abstract).

21. Fiser, R. H., J. C. Denniston, and W. R. Beisel. 1972. Infection with Diplococcus pneumoniae and Salmonella typhimurium in monkeys: Changes in plasma lipids and lipoproteins. J. Infect. Dis. 125:54-60.

22. Fiser, R. H., J. C. Denniston, J. Kaplan, V. G. McGann, and W. R. Beisel. 1972. Hypercholesterolemia and altered immunity in rhesus monkeys. Clin. Res. XX:271 (abstract) and Fed. Proc. 31:727 (abstract).

23. Fiser, R. H., J. C. Denniston, M. D. Kastello, R. B. Rindsig, and W. R. Beisel. 1972. Cholesterogenesis during acute infection in chronically hypercholesterolemic rhesus monkeys. Proc. Soc. Exp. Biol. Med. 140:314-318.

24. Fiser, R. H., J. C. Denniston, R. B. Rindsig, and W. R. Beisel. 1971. Effects of acute infection on cholesterologenesis in the rhesus monkey. Proc. Soc. Exp. Biol. Med. 138:605-609.

25. Fiser, R. H., J. Kaplan, and J. C. Holder. 1972. Congenital syphilis mimicking the battered child syndrome - How does one tell them apart? Clin. Pediatrics, In press.

26. Fiser, R. H., J. B. Rollins, and W. R. Beisel. 1972. Decreased resistance against infectious canine hepatitis in dogs fed a high-fat ration. Amer. J. Vet. Res. 33:713-719.

27. Harrison, D. N., D. C. Cavanaugh, J. H. Rust, Jr., and J. D. Marshall, Jr. 1971. Characteristics of a bacteriophage-infected strain of Pasteurella pestis isolated from a human case of plague. Infec. Immun. 4:85-87.

28. Huxsoll, D. L., P. K. Hildebrandt, R. M. Nims, and J. S. Walker. Tropical Canine Pancytopenia, pp. 677-670. In Current Veterinary Therapy IV (R. W. Kirk, ed.,, W. B. Saunders, Philadelphia.

29. Jordan, G. W. 1972. Basis for the probit analysis of an interferon plaque reduction assay. J. Gen. Viol. 14:49-61.

30. Jordan, G. W. 1972. Quantitative aspects of interferon-induced plaque reduction: Kinetics of interferon action. Virology 48:425-432.

31. Jordan, G. W. 1972. Effect of interferon on the production of hemagglutinins and infectivity of GDVII virus. Arch. ges. Virusforsch., In press.

32. Kaplan, J. 1972. Staphylococcal enterotoxin B induced release of macrophage migration inhibition factor from normal lymphocytes. Cell. Immunol. 3:245-252.

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