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COMMISSION ON
EPIDEMIOLOGICAL SURVEY
ARMED FORCES EPIDEMIOLOGICAL BOARD

SUMMARY OF THE ANNUAL REPORT OF THE COMMISSION
FISCAL YEAR 1965

FEBRUARY 1966
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ARMED FORCES EPIDEMIOLOGICAL BOARD
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THE DIRECTOR’S SUMMARY REPORT

The annual meeting of the Commission on Epidemiological Survey was held at the Walter Reed Army Institute of Research on 9 and 10 September 1965. Senior representatives of the Departments of Army, Navy, and Air Force, the U. S. Army Medical Unit and the U. S. Army Biological Laboratories attended the meeting. A number of ad hoc meetings were held during the year for discussions of specific problems. The Commission has been strengthened by the participation of Drs. Charles L. Wisseman, Sheldon E. Greisman and Richard B. Hornick as active members. Grateful appreciation was expressed to Captain Sidney A. Britten, USN, Executive Secretary of the Board and Miss Betty Gilbert, Administrative Assistant, for their help in administering the Commission’s affairs.

The first day’s meeting was devoted to hearing the reports which follow of the work completed or in progress by investigators of the U. S. Army Medical Unit and one of its contractors. Abstracts of the program follow. Details are given in the full report of the Commission.

An ad hoc group consisting of members of the Commissions of Rickettsial Diseases and Epidemiological Survey and staff members of the U. S. Army Medical Unit and Walter Reed Army Institute of Research has developed a program extending the studies of Q fever vaccine. Dr. Wisseman directs this Q Fever Ad Hoc Committee which is studying the antigenic and immunologic relationships of Phase I and II components of Coxiella burnetii. Phase I and II antigens, including viable strains, are being appraised as vaccines in volunteers under the University of Maryland contract.

The scope of the program of the Medical Unit and its contractors has been broadened. The fate of viruses within cells is being appraised by immunochemical techniques and the effect of ionizing radiation on susceptibility to Venezuelan equine encephalomyelitis (VEE) infection is under study. Metabolic studies have included appraisal of adrenal gland functions in human tularemia, sandfly fever and Q fever and thyroid function during pneumococcus infection of rats. Alterations of tissue and leukocyte alkaline phosphatase have been appraised in tularemia, sandfly fever and pneumococcus infections. Synthesis of protein and nucleic acid metabolism have been assessed in VEE infections in laboratory animals. There has been good progress in developing a VEE vaccine and an inactivated multivalent Arbovirus vaccine.

Studies of typhoid and tularemia vaccines in volunteers are nearing completion; and the physiologic changes of endotoxin have been extended.

The executive session on the second day was devoted to a general discussion of staphylococcal enterotoxin B and to administrative affairs of the Commission. The former is the subject of a special report to the Commission published separately by the U. S. Army Medical Unit.
In conducting the research described in this report the investigators adhered to the Principles of Laboratory Animal Care as established by the National Society of Medical Research.

THEODORE E. WOODWARD
Director
Commission on Epidemiological Survey
REPORT ON Q FEVER VACCINE STUDIES

Albert T. Dawkins, Jr., M.D., University of Maryland School of Medicine, Baltimore, Maryland

Q fever vaccine studies have been conducted under Contract DA-49-007-MD-751. It was desired to evaluate (1) the Phase I killed Henzerling strain vaccine given in a single dose to volunteers in terms of reactogenicity, immunogenicity, and efficacy in preventing aerogenically-induced disease and (2) the Phase II Nine Mile strain Coxiella burnetii with respect to its capacity to produce disease and its immunogenicity.

When volunteers were given 1 injection of formalin-inactivated Henzerling strain C. burnetii (rickettsial mass 30 μg) reactions at the sites of injection were mild. No vaccinees became ill while 5 of 6 controls developed disease when challenged by aerosol with 10^3.5 GIPID_{50} of the AD strain.

Volunteers given varying dilutions subcutaneously of Phase II living, apparently attenuated Nine Mile strain (BP-88) C. burnetii developed severe local reactions. The severity of local and febrile responses and the serological conversion rates were related directly to the dose of organisms injected. Eight volunteers "immunized" with various doses of the Phase II living attenuated Nine Mile strain organisms were challenged by the respiratory route with 10^3.5 GIPID_{50} of the AD strain. Three of 5 men who were seronegative prior to challenge became ill; none of 3 who were seropositive became ill. Postchallenge serological studies are incomplete.

Three volunteers were administered 10^3.5 and 4 were given 10^5.5 GIPID_{50} of the AD strain by the oral route. None of these men became ill. Postchallenge serological studies are in progress.

DEMONSTRATION OF VIRUSES IN LEUKOCYTES BY THE FLUORESCENT ANTIBODY TECHNIQUE

Donald S. MacNaill, Major, MC, and Robert F. Jaeger, B.S., U. S. Army Medical Unit, Fort Detrick, Maryland

By a rather simple and rapid method, the fluorescent antibody technique, it has been possible to demonstrate viremia due to Venezuelan equine encephalomyelitis in dogs. The test became positive on the 1st day after challenge; it was positive longer than viremia was detectable by other methods, and 3 days after antibodies were already detectable. It was positive on a day when neither viremia nor antibodies were detectable by other methods.

In an attempt to determine whether live virus was involved, the white cell suspension was diluted as much as 3 logs with rabbit serum buffer and was still lethal for mice. A similar study was performed in burros with comparable results.
Studies will be extended to specimens from humans receiving the attenuated live virus vaccine. Autofluorescence in leukocytes will still be a problem.

RECOVERY OF VIRAL PARTICLES FROM SERUM FOR ELECTRON MICROSCOPY
Anne Buzzell, Ph.D., U.S. Army Medical Unit, Fort Detrick, Maryland.

In its present stage of development the assay by electron microscopy for virus particles in serum is capable of detecting virus at concentrations of $10^6$ particles/ml in 1 ml of fluid by centrifuging onto a block of agar in conventional centrifuge cells. The procedure for transferring the virus to an electron microscope grid has been greatly simplified and now incorporates the step of negative staining which is of aid in identifying particle images. Final identification of the virus using specific antibodies with an electron-dense label such as ferritin, may also be simplified since ferritin should be visible in the thin uniform layer of negative stain. An improved centrifuge technique, still to be tested, may make possible detection of virus at concentrations as low as $10^2$-$10^3$ particles/ml in 0.1 ml of serum with about 1 hr required for the entire assay procedure.

EFFECTS OF IONIZING IRRADIATION ON THE IMMUNE RESPONSE TO VENEZUELAN EQUINE ENCEPHALOMYELITIS
Nelson R. Blemly, Lt Colonel, MC, U.S. Army Medical Unit, Fort Detrick, Maryland.

Attenuated Venezuelan equine encephalomyelitis virus produced a solid immunity in mice as demonstrated by resistance to challenge with the Trinidad strain of the virus 14 days after immunization. Mice infected with attenuated virus showed no ill effects and this state of well-being did not appear to be altered by the added stress of whole body irradiation. The resistance to challenge achieved was not affected by acute whole body irradiation before or after immunization or by repeated doses of irradiation before immunization when standard immunizing doses of attenuated virus were employed. When immunizing doses were reduced and the time between immunization and challenge shortened, an inhibitory effect of irradiation on the resistance to challenge resulted.

The exact cause of the irradiation effect is not fully understood, but it may be hypothesized that there is interference with replication of attenuated virus to a degree that infection and subsequent resistance to challenge are not established and/or that antibody production sites are damaged to such a degree that antibodies cannot be produced. Under this hypothesis, if adequate recovery of irradiated tissues has occurred before elimination of virus from the host system, resistance to challenge will become established.
Current and future studies consist in part in a determination of disappearance time of attenuated Venezuelan equine encephalomyelitis virus from the host system in irradiated and nonirradiated animals, and in examination of the protection exhibited by animals challenged 1-3 days after immunization to determine whether this protection is nonspecific, involving interferon or interference phenomena, or the result of production of significant amounts of antibody at this early date after immunization.

STUDIES ON THE PATHOGENESIS AND CONTROL OF TYPHOID FEVER
Richard B. Hornick, M.D., University of Maryland School of Medicine, Baltimore, Maryland.

Further investigations under Contract DA-49-007-MD-751 of the value of typhoid vaccines in preventing induced typhoid fever are reported. Use of challenge doses of 100,000 Quaiies strain Salmonella typhosa organisms, i.e., 1 $ID_{25}$, permitted demonstration of a significant level of vaccine effectiveness. Renewed interest in the role of Vi antigen led to immunization with Vi antibodies. Challenge with 1 $ID_{25}$ will be carried out during the coming year.

The laboratory strain, Ty2, was used as challenge material in 16 volunteers. When $10^7$ organisms were used 2 of 6 men became ill, while with $10^3$ organisms none of 5 became ill.

Based upon evidence in laboratory animals that endotoxin in proper dosage can enhance nonspecific resistance to infection an acetylated endotoxin was used as a vaccine in volunteers. Following challenge with typhoid or tularemia organisms there was no evidence that immune mechanisms had been enhanced by endotoxin vaccination.

A preliminary investigation into the possible role of colonic bacteroides species in protecting man against typhoid fever was begun.

Colymycin and Cephaloridine were tested in volunteers with induced typhoid fever; neither was as effective as chloramphenicol, the drug of choice, although they were effective in vitro.
ROLE OF ENDOTOXIN DURING TYPHOID FEVER AND TULAREMIA

Endotoxin studies were continued under Contract DA-49-007-MD-751. It was found that the sustained release of bacterial endotoxin into the circulation was not primarily responsible for the continuous febrile and toxic course of typhoid fever and tularemia in man. Rather, other mechanisms, presumably similar to those responsible for sustained fever and toxemia during infections induced by nonendotoxin containing microbes, must have been operative. It is emphasized, however, that while endotoxemia did not account for sustained illness, it could account for abrupt intensification of fever and toxemia at any time during the course of typhoid fever or tularemia. Thus, it was demonstrated that subjects with typhoid fever and tularemia exhibited remarkably hyperreactive febrile and toxic responses to a single intravenous injection of endotoxin. Release of a relatively small bolus of endotoxin into the circulation during illness (or upon institution of appropriate antibiotic therapy) could therefore produce an acute febrile and toxic spike, including shock, but such an event would be sharply circumscribed and would be superimposed upon the more basic mechanisms responsible for the characteristic sustained febrile and toxic state.

POSSIBLE POLYMER CONFIGURATIONS OF ANTHRAX TOXIN COMPONENTS
Anne Buzzell, Ph.D., U.S. Army Medical Unit, Fort Detrick, Maryland.

While studying anthrax ultrafiltrate in the ultracentrifuge, a large variety of components with and without biological activity were observed. Investigations were conducted on the possibility of molecular aggregation with persistence of lethal activity.

It is planned to test this postulate of lethal aggregates by additional experiments in order to gain better resolution of the components and to eliminate the need for theoretical corrections in the sedimentation constants occasioned by the presence of sucrose. In order to have enough fluid to test for lethal activity samples are pooled from all 3 tubes of the swinging bucket rotor. This means that for optimal resolution of components the 3 gradients must be identical. Substantial improvements in the technique of making gradients have been made. It is planned to dialyze the fractions before analyzing them in the ultracentrifuge and to determine diffusion constants as well as sedimentation constants so that the relationships among the aggregates can be specified in terms of molecular weights.
ADRENAL FUNCTION DURING INFECTIOUS ILLNESS
William R. Beisel, Lt Colonel, MC, U. S. Army Medical Unit, Fort Detrick, Maryland.

The studies reported permit an appreciation for the first time of the total, complex pattern of adrenal corticoid response to acute infection in man.

All functional portions of the adrenal cortex were involved in the responses observed.

These responses occurred with differences in timing, with the mineralocorticoid excretion rising after that of the glucocorticoid, and androgen, or pregnanetriol fractions. This observation is entirely compatible with the current concept that the 3 latter fractions respond primarily to ACTH stimulation, whereas aldosterone increases are stimulated primarily by a fall in circulating blood volume which activates the renin-angiotensin system. The timing of rise in aldosterone excretion could be correlated exactly with the observation, known for years, that during the height of, and following, acute infection there is also a marked fall in the urinary excretion of Cl\(^-\) and Na\(^+\).

The adrenal responses appear to be quite similar in each of the several illnesses studied (tularemia, sandfly fever, and Q fever) as well as in the artificially induced forms of fever.

The magnitude of adrenal response appears to be related to the severity of illness; this was true both for changes in plasma corticoids as well as the excretion of urinary metabolites.

With regard to plasma concentrations the pattern noted after infection or artificial fever was typical of Cushing's syndrome with loss of the normal diurnal fall in plasma corticoids.

Changes in the plasma protein binding of cortisol were not observed in any instance studied.

From the timing and magnitude of adrenal glucocorticoid response observed, it seemed impossible that changes in body nitrogen metabolism could be related solely to the alterations induced by adrenal cortical overactivity. Despite this conclusion based on our studies of short-duration illness, it is entirely possible that in long term, severe, untreated infectious disease, adrenocortical hyperfunction does contribute to negative nitrogen balance.

The increase in androgen secretion during infection failed to include those compounds with predominantly anabolic or fever-producing activity; instead, dihydroepiandrosterone of adrenal origin seemed to account in large measure for the increased excretion of 17-ketosteroids.
THYROID FUNCTION DURING GENERALIZED INFECTION
George E. Shambaugh, Major, MC, U. S. Army Medical Unit, Fort Detrick, Maryland.

The eight major alterations in thyroid physiology in infection can be explained by two key changes: an intrinsic defect in the thyroid gland and a fall in thyroxine (T4)-binding proteins.

Although the former has been well documented as a reaction to non-specific stress the fall in binding proteins in the rat has not been described previously.

Preliminary studies performed elsewhere showed a similar fall in T4-binding prealbumin in the chronically ill human and, when measured, in several instances of infection. Although T4-binding proteins in the rat behave in a similar manner pharmacologically to human T4-binding prealbumin, thyroidal uptake in man is increased during surgical stress while uptake in the rat decreases.

In spite of these species differences, a fall in T4-binding proteins in the rat, as in man, appears to be an important but hitherto unrecognized facet in the pattern of response to the specific stress of a generalized infection. Although some insight has been gained into changes in trophic hormones and binding proteins, there continues to be ignorance of alterations in the human thyroid gland itself. Until we can define these changes in the human, as has been done with the rat, the pattern of man's response to infection will remain incomplete.

INFLUENCE OF VIRAL INFECTION ON HOST PROTEIN AND RIBONUCLEIC ACID BIOSYNTHESIS
George Lust, Captain, MSC, U. S. Army Medical Unit, Fort Detrick, Maryland.

It was found in vivo that liver protein synthesis is depressed early in Venezuelan equine encephalomyelitis virus infection and subsequently increases above uninfected control levels. The same trend was found in liver microsomal protein synthesis experiments; the increase returning only to the control value. The effects in the brain were less pronounced.

In mouse fibroblasts, strain L, in tissue culture studies it was possible to see the effects again, but under more controlled conditions. By inhibiting host RNA synthesis with Actinomycin D, the appearance of virus-induced protein and virus RNA could be shown clearly. Specifically the appearance of a new RNA polymerase was demonstrated in the cytoplasm of the cell which was probably involved in the synthesis of virus RNA. This enzyme proved to be very difficult to work with, since it was apparently highly labile.
Presently, experiments are in progress to purify the enzyme further and to attempt to demonstrate the presence of the new polymerase in Venezuelan equine encephalomyelitis-infected mouse liver and brain.

**PROTEIN SYNTHESIS BY LIVER CELL COMPONENTS OF MICE INFECTED WITH DIPLOCCUS PNEUMONIAE**

David G. Van Ormer, First Lieutenant, MSC, U. S. Army Medical Unit, Fort Detrick, Maryland.

Protein biosynthesis occurs in at least 3 areas of the cell: the nucleus, mitochondria, and the endoplasm of the microsomal fraction. Increased protein synthesis had been noted in bacterial as well as viral infections in some of the viscera. The studies described showed that this increased activity occurred in both mitochondrial and microsomal liver-cell fractions of mice infected subcutaneously with 3-4 Diplococcus pneumoniae organisms. The label used was L-leucine-C\(^{14}\). Methods of fractionating are presented. Radioactivity of the tissue fractions was measured by liquid scintillation techniques. No direct explanation is available for the observed increases.

**ALTERATIONS IN TISSUE ALKALINE PHOSPHATASE ACTIVITY DURING ACUTE INFECTION**

George Lust, Captain, MSC, U. S. Army Medical Unit, Fort Detrick, Maryland.

Alkaline phosphatase is found in high concentrations in small intestine, bone and kidney and very low concentrations in brain and muscle. Its intracellular function is not understood.

Serial studies of leukocyte alkaline phosphatase carried out in volunteers during several infectious diseases revealed increased activity.

Diplococcus pneumoniae and Venezuelan equine encephalomyelitis virus infections were examined in mice. The former induced increases in alkaline phosphatase activity in serum and small intestine and a decrease in the liver whereas Venezuelan equine encephalomyelitis virus infection induced opposite changes.

While these studies still leave the specific intracellular function of alkaline phosphatase unanswered, they nevertheless provide additional information that alkaline phosphatase alterations do occur in tissues during acute bacterial and viral infections. The exact mechanism responsible for the changes must await additional investigations.
DETECTION OF PASTEURERRA PSEUDOTUBERCULOSIS ANTIBODIES BY MICROHEMAGGLUTINATION

John D. Marshall, Jr., Major, MSC, and Julius A. Currie, U. S. Army Medical Unit, Fort Detrick, Maryland.

The microhemagglutination procedure was applied to sera as a screening technique for antibodies to *Pasteurella pseudotuberculosis*. The microtiter technique was used throughout. A polyvalent antigen for the hemagglutination (HA) test has been prepared and used to screen 513 sera from humans and animals. Of sera from 47 individuals who had been in Madagascar during the plague epidemic of 1952, more than half were positive by the HA test for *Pasteurella pestis* antibodies and negative for *P. pseudotuberculosis*. Of 50 sera from patients in the Washington area with abdominal disease, 4 were positive for *P. pseudotuberculosis* and negative for *P. pestis*. Remaining sera tested were from rats experimentally infected with avirulent plague or *P. pseudotuberculosis*, and from various primates. Large numbers of sera could be screened in a relatively short time.

REVIEW OF ViroLOGY DIVISION PROGRAM

Robert W. McKinney, Major, MSC, U. S. Army Medical Unit, Fort Detrick, Maryland.

Progress on investigations on Venezuelan equine encephalomyelitis (VEE), Eastern equine encephalomyelitis (EEE), and Western equine encephalomyelitis (WEE) within the Virology Division are presented.

Storage of freeze-dried living VEE vaccine for 18 months resulted in no loss in titer. Procedures for serum neutralization tests for detection of antibodies following immunization were revised and continued under investigation.

Other tests for use with Group A arboviruses were studied. A metabolic inhibition test showed promise for use with animal sera, but has been disappointing when used with human sera. Further study is required.

Development of an inactivated multivalent Group A arbovirus vaccine was begun; VEE, WEE, EEE and Sindbis viruses are under consideration for this combination.

Preliminary studies were conducted on the use of immune serum for prophylaxis and treatment of VEE in guinea pigs.
Hartley strain guinea pigs were immunized with anthrax protective antigen (APA) or a combination of this antigen with live attenuated tularemia vaccine (LVS). Challenge of these guinea pigs with strain NH-6 Bacillus anthracis was carried out 30 days later. Serial sacrifices were made for 144 hr after challenge.

Sites of skin lesions in immunized and control animals were examined histologically. Numbers of organisms found in lesions were reduced in immunized animals when compared with controls; the APA-LVS vaccine combination appeared to reduce the numbers more than APA vaccine alone. The rate and intensity of neutrophilic and mononuclear cell mobilization to the challenge site were accelerated in the immunized animals; and were greatest in the APA-LVS immunized group. Neutrophilic and mononuclear phagocytosis of organisms was conspicuous in immunized animals but absent in controls. Eosinophilia at the inoculation site was notable in immunized animals, while it was absent or mild in controls.

It appears that the morphologic counterpart of the acquired increased resistance conferred on the guinea pig by immunization with APA has 4 recognizable components. These seem to have as their objective localization of the infection and consist of: (1) rapid mobilization of neutrophilic leukocytes to the site of challenge; (2) inhibition of replication of B. anthracis at the challenge site; (3) mobilization of mononuclear cells to the challenge site; and (4) phagocytosis of anthrax bacilli by neutrophilic leukocytes and mononuclear cells.

The enhanced protection against such challenge afforded guinea pigs by the combination of APA with LVS vaccine is reflected in acceleration and intensification of these 4 responses, with resultant increased efficiency in localization of the infectious process.


## Summary of the Annual Report of the Commission

### Abstracts, Annual Report to the Armed Forces Epidemiological Board, Fiscal Year 65

Abstracts of the progress reported in selected areas of research of the U. S. Army Medical Unit. A separate report covers research on staphylococcal enterotoxin B under Project No. 185330010164.
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