NAMRU-2

SUMMARIES OF RESEARCH

1966

United States Naval
Medical Research Unit No. Two
Taipei, Taiwan

U. S. NAVAL MEDICAL RESEARCH UNIT NO. 2
BOX 14, APO SAN FRANCISCO 96263

SUMMARIES OF RESEARCH
1966

These summaries cover the research reported upon during 1966. Complete copies will be provided by mailing requests to the attention of the senior author in care of the department or division from which the report originated.

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SUMMARIES OF RESEARCH
1966

MR 005.09-0062
Report No. 1

Further Classification of TRIC Agents from Ocular Trachoma and Other Sources by the Mouse Toxicity Prevention Test (American Journal of Ophthalmology Supplement, April 1967)

E. R. Alexander, S. P. Wang and J. T. Grayston

From: Virology Division, Microbiology Department

Summary:

The mouse toxicity prevention test was used to examine antigenic relationships of 50 TRIC agent strains from various countries and various sites. These results are combined with previous reports from the laboratories to result in classification of 80 TRIC agents.

Sixty-two of 64 agents recovered from cases of ocular trachoma fall into 1 of 3 antigenically distinct types. Type A is characterized by SA-1 or G-17 strains, type B by TW-1 or TW-5 strains, and type C by TW-3 or ND-3 strains.

All of 16 strains recovered from sources other than ocular trachoma and 2 strains recovered from clinical trachoma fall into 3 distinct types: D (G-1 prototype), E (Bour prototype), and F (IC-Cal-3 prototype). These strains were recovered from inclusion conjunctivitis of infancy, from female cervix, from male urethra, and from 1 eye with punctate keratitis. They were recovered in London, San Francisco, Seattle and Taipei.

Five other strains received in this laboratory from 4 cases with atypical conjunctivitis in Adelaide and from the joint fluid in Reiter's syndrome in London do not fit into any of the 6 mouse prevention types and share other biologic properties which differentiate them from classical TRIC agents.

MR 005.09-0062
Report No. 2

Prevention of Anophylactic Death in the Mouse Toxicity Protection Test for TRIC Agents (American Journal of Ophthalmology Supplement, April 1967)

E. R. Alexander and J. W. Fresh

From: Virology Division, Microbiology Department

Summary:

The phenomena of prompt death and toxic death of immunized mice when challenged with intravenous TRIC agents may be distinguished clinically and pathologically. In both respects the prompt death phenomenon is similar to mouse anaphylaxis.

This prompt death phenomenon may be prevented by various drugs that adversely affect mouse anaphylaxis.

Aqueous epinephrine immediately before challenge is effective, as is chlorpromazine, and as are also promethazine and reserpine at increasing intervals. The latter 3 drugs have more marked side effects on experimental animals however.

Cortisone is effective in large doses.

The inbred strain of mice DEA/2 is refractory to prompt death, while it does exhibit toxic death.

On the basis of these findings we now routinely employ aqueous epinephrine (0.02 mgm I.P. per 15-gram mouse in less than 5 minutes before challenge) in the mouse toxicity prevention test.

MR 005.09-0062
Report No. 3

Trachoma Vaccine Studies in Monkeys (American Journal of Ophthalmology Supplement, April 1967)

S. P. Wang, J. T. Grayston and E. R. Alexander

From: Virology Division, Microbiology Department

Summary:

Experimental monkey eye TRIC agent infection can be prevented with vaccine. A series of immunization experiments were performed using 6 TRIC strains of 4 immunological types grown in egg yolk sac and made into vaccine by several methods. Protection against infection was found only with vaccines emulsified with oil adjuvant. Complete Freund's or mineral oil adjuvants were about equally effective. High potency vaccines (usually shown by particle counts of 10^9) were needed for protection. Highly purified vaccines prepared by sucrose gradient centrifugation or by genetron (fluorocarbon) extraction were protective. The addition of formalin to crude or purified vaccines had no noticeable effect on potency. In one experiment, vaccine was stored at 4°C for 1 year without loss of potency. Protection from monkey eye infection was highly specific among the 4 immunologic types as determined in mice by prevention of toxicity. The experimental infection was a vigorous test of vaccine potency. Good repetition of results was obtained when the challenge inoculum was about the minimal amount needed to infect the controls. The monkey eye infection is a good laboratory model to use for evaluating further improvements in trachoma vaccine.

MR 005.09-0062
Report No. 4

A Potency Test for Trachoma Vaccine Utilizing the Mouse Toxicity Prevention Test (American Journal of Ophthalmology Supplement, April 1967)

S. P. Wang and J. T. Grayston

From: Virology Division, Microbiology Department

Summary:

A quantitative modification of the mouse toxicity prevention test was described for potency assay of trachoma vaccine. The method of toxicity titration of toxin was revised. Closely spaced dilutions (3:4) of infected yolk sac suspension (ranging from 5 to 30%) were intravenously titrated in mice.

The 50% endpoint of lethality (LD_{50}) was obtained and the results of titrations were reproducible. The age and weight of mice were critical to reproducible titration.

Using this titration method, thermostability of toxin was investigated. Toxin was rapidly inactivated within 5 minutes at 56°C, but was stable for days at the refrigerator temperature of 2 - 5°C, and apparently stable for several months at -65°C. Although toxin was found to be labile, either live (DEAE-Sephadex purified) or killed (trypsin-genetron-sucrose gradient purified) organisms were effective immunizing antigens.

A quantitative measurement of protecting antigen was devised as follows: 2 doses of serial 10-fold dilutions of vaccine ranging from 10 to 0.001% concentration were injected intravenously into groups of 10 - 15 mice at weekly intervals. They were intravenously challenged 1 week after the last immunization with 1.5 LD_{50} of toxin. A graded pattern of protection was usually obtained by dilution of antigen and a 50% endpoint of effective dose (ED_{50}) of vaccine can be obtained. The results were reproducible in repeated testings of a given vaccine. Much better results were obtained with intravenous immunization than with intraperitoneal. Protection can be demonstrated without much loss 3 weeks after the intravenous immunization. In a parallel test, killed (genetron purified) and live (sucrose-gradient purified) preparations of vaccine derived from the same infected yolk sac materials were shown to be equally protective in the quantitative test.

This potency test has been used for evaluation of purification methods for vaccine. The results indicate that the immunizing antigen is closely associated with TRIC agent elementary body particles, and that treatment of the infected crude yolk sacs with 0.2% trypsin at 37°C for 15 minutes, ultrasonic disintegration at 18,000 - 20,000 cycles per second for 5 minutes, and repeated washings with SPG do not remove infectivity or protective antigens.

MR 005.09-0062
Report No. 5

Pannus with Experimental Trachoma and Inclusion Conjunctivitis Agent Infection of Taiwan Monkeys (American Journal of Ophthalmology Supplement, April 1967)

S. P. Wang and J. T. Grayston

From: Virology Division, Microbiology Department

Summary:

This is the first report of the reproduction in an animal model of eye disease similar to human trachoma. During a series of experiments studying the pathogenicity, prevention and treatment of monkey trachoma, 451 Taiwan monkeys (Macaca cyclopis) had 617 eye inoculations with TRIC agents which resulted in disease. Pannus developed during the course of 30 such infections in 26 monkeys. Trachomatous follicles in the tarsus, limbal follicles and Herbert's pits were all seen in some of these infections. In 2 prolonged infections with pannus, conjunctivae scars typical of trachoma progressed to cause distortion of the lids. Pannus occurred only in monkeys that had received TRIC agent vaccine (1 had received meningopneumonitis strain vaccine) and/or had had previous eye infection with TRIC agents. Pannus most frequently was associated with infection with the Bour atypical trachoma strain and the IC-Cal-3 inclusion conjunctivitis strain, but occurred only in

1 monkey of a large number inoculated with typical trachoma strains. It almost always occurred as part of a relatively severe disease and in animals with relatively high CF antibody titers. Monkeys with pannus in 1 eye usually developed pannus in the other if it was inoculated or if spontaneous cross infection occurred. It is concluded that pannus may result from infection with the inclusion conjunctivitis agent, that the host response to infection is most important in pannus formation and that hypersensitivity to homologous or heterologous TRIC agents is an important part of the host response in the pathogenesis of pannus formation.

MR 005.09-0062
Report No. 6

Immunization Against Trachoma

J. T. Grayston

From: Virology Division, Microbiology Department

Conclusions:

Under certain circumstances, it is possible to protect primates, including humans, from trachoma infection by immunization. Vaccines with higher potency and longer lasting effects which can be prepared more easily are needed. At the same time, it appears that methods must be found to reduce hypersensitivity effects of immunization. Discovery of a subfraction of the total organism containing the protective antigen offers the best hope for more effective immunization. It can be stated that a commercially produced effective vaccine, feasible for use in developing countries where trachoma is a serious problem, is not now available nor does it seem likely such a vaccine will be available in the very near future.

A great deal has been learned about the immunology of trachoma and the TRIC organisms since they were first isolated and grown in quantity fewer than 10 years ago. These intracellular organisms, with complex cell walls, cause diseases with interesting immunological and epidemiological characteristics. Further work directed toward prevention of trachoma is justified, both by the importance of this disease in the developing countries and by the broadly applicable immunological problems presented by this intermediate class or organisms.

MR 005.09-0063
Report No. 1

Infection of Pregnant Monkeys and Their Offspring with TRIC Agents
(American Journal of Ophthalmology Supplement, April 1967)

E. R. Alexander and W. T. Chiang

From: Virology Division, Microbiology Department

Summary:

An attempt was made to develop an experimental model in monkeys of the maternal-infant infection with TRIC agent in humans, producing follicular cervicitis in the mother and inclusion conjunctivitis in the infant.

In 20 attempts to infect monkey cervix, 4 monkeys have been infected as evidenced by recovery of TRIC agent one or more times from the cervix. All recovery has been in the first 3 weeks after inoculation.

Nine pregnant monkeys were inoculated in the cervix with TRIC agents, twice during the latter half of pregnancy, and once post-partum. None developed

significant cervicitis when compared with 4 placebo-inoculated controls. None of the 9 infant monkeys born to TRIC-inoculated mothers developed significant conjunctivitis, when compared to the 4 infants of placebo-inoculated mothers, and TRIC agents were not recovered from scrapings of the eyes, nor were inclusions seen on smear.

At about 7 months of age, all infant monkeys were challenged in the right eye with TRIC agents (Bour, IC-Cal-3 or GB-IOL-15 strains were used). All infants developed follicular conjunctivitis. Six of 9 infants born of TRIC agent-inoculated mothers developed severe follicular conjunctivitis with pannus. None of the 4 infants born of placebo-inoculated mothers developed severe follicular conjunctivitis.

Because pannus formation and follicular conjunctivitis of this severity is never seen in initial experimental trachoma infection of monkeys, it is assumed that 6 of 9 infant monkeys had inapparent infection with TRIC agent resulting in their sensitization to re-challenge. It is probable that this occurred in passage through an infected cervix.

MF 022.03.07-2013
Report No. 30

Isolation of Trachoma Inclusion Conjunctivitis Agents from Human Conjunctiva (Lancet 1:561-563, 1966)

D. O. Crompton, W. H. Howarth, D. Hardy, P. G. Surman and R. L. Woolridge

From: Virology Division, Microbiology Department

Abstract:

Four TRIC agents were isolated in embryonated eggs from eye cultures of a child from Alice Springs and 3 Adelaide adults. One patient presented with asthenopia, whilst the other 3 had mild symptoms associated with conjunctivitis. The cytoplasmic inclusion bodies seen in conjunctival epithelial cells of the 4 patients do not exhibit all the typical features of the HP body and have been designated "atypical inclusions". Microscopic examination of yolk sac smears revealed typical elementary bodies on serial egg passage. The yolk sacs produced a positive result to the PLT group specific fluorescent antibody test. Each strain carried the heat stable and purified elementary body group antigens. All 4 strains were pathogenic for mice and results of the mouse toxicity protection tests with I.M.V.S.-6 did not show a cross protection to the 2 Taiwan strains or the 2 previously studied South Australian strains (TW-1 group). The strain from 1 patient was not pathogenic for the Taiwan monkey eye. These properties suggest that the agents are closer to the psittacosis and lymphogranuloma-venereum agents than to the TRIC agents.

MF 022.03.07-2013
Report No. 33

Long-Term Follow-Up of the Initial (1959-60) Trachoma Vaccine Field Trials on Taiwan (American Journal of Ophthalmology Supplement, April 1967)

R. L. Woolridge, J. T. Grayston, I. H. Chang, C. Y. Yang and K. H. Cheng

From: Virology Division, Microbiology Department

Summary:

The initial trachoma vaccine field trial was begun on Taiwan in 1959-60 in pre-school aged children utilizing a partially purified vaccine. It showed statistically significant protection at a level of 60% at the end of 2 years of observation. However, beginning two and a half to three years after initiation

of the study, which was a year after the final booster vaccine inoculation, the protection afforded the vaccinated children began to disappear and at the end of 6 years of observation, there was no difference between the 2 groups, both of whom showed about 50% conversion to trachoma. This study while proving that trachoma could be prevented with vaccine pointed out that vaccines of greater potency or adjustments would be needed to prolong the protective effect. Field testing such vaccines will require long-term observation to assess the impact of the vaccine on the disease.

MF 022.03.07-2013
Report No. 34

Failure of Treatment of Trachoma with Antibiotic Eye Ointment and Oral Sulfa Drug Alone or in Combination with Trachoma Vaccine (American Journal of Ophthalmology Supplement, April 1967)

R. L. Woolridge, K. H. Cheng, I. H. Chang, C. Y. Yang, T. C. Hsu and J. T. Grayston

From: Virology Division, Microbiology Department

Summary:

A series of 3 field trials has been carried out designed to explore the usefulness of trachoma vaccine alone or in combination with drug therapy in Taiwan school children with active trachoma infection. Each of the studies was a failure because of the inability to cure trachoma with tetracycline eye ointment therapy alone or combined with increasing amounts of tri-sulfa drug orally. No reason was found for the failure of the standard drug treatment schedules to cure trachoma. It is clear that in these experiments where the treatment was inadequate, addition of trachoma vaccine showed no favorable effect on hastening the cure of the disease. Vaccine alone showed no effect on active trachoma. In 1 experiment, it was possible to show that there were fewer relapses or reinfections in children whose trachoma became inactive after vigorous therapy. This finding was not well established or confirmed because the extremely low cure rate associated with drug therapy in the 3 trials did not provide adequate numbers for evaluation.

MR 005.09-0011
Report No. 5

The Effect of Vancomycin on the Growth of Psittacosis-Trachoma Agents Cultivated in Eggs and Cell Culture (Applied Microbiology 00:00 1966)

H. M. Jenkin and S. C. Hung

From: Virology Division, Microbiology Department

Abstract:

Vancomycin is an antibiotic that is effective in preventing bacterial contamination when used in various studies of psittacosis and trachoma agents (PT). This antibiotic is not toxic to chick embryos at the 80/mg/egg level, or for HeLa 229 cells cultivated in a medium containing 3200 µg/ml vancomycin; it is toxic for LLC-MK2 cells at a concentration of 1600 µg/ml. Vancomycin does not affect the growth of a variety of PT agents when it is used at the concentration of 2 mg/egg or 800 µg/ml of cell culture medium, but does inhibit the growth of common bacterial air contaminants.

MR 005.09-0011
Report No. 6

Induction of Interferon by the Bour Strain of Trachoma in HeLa 229 Cells (American Journal of Ophthalmology Supplement, April 1967)

H. M. Jenkin and Y. K. Lu

From: Virology Division, Microbiology Department

Summary:

An inhibitor with many properties associated with interferon was induced in HeLa 229 cells by the Bour strain of trachoma, a member of the psittacosis-LGV-trachoma group of agents. Interferon induced by Bour was demonstrated by the inhibition of growth of poliovirus as well as the homologous trachoma strain. The yield of interferon was proportional to the inoculum dose. An inhibitor of growth of poliovirus was recovered from disrupted infected cells which has not been characterized and was not associated with the Bour agent. The significance of the inhibitor in relation to the problem of serial passage of trachoma agents in cell culture is discussed.

MR 005.09-0011
Report No. 7

Comparative Lipid Composition of Psittacosis and Trachoma Agents (American Journal of Ophthalmology Supplement, April 1967)

H. M. Jenkin

From: Virology Division, Microbiology Department

Summary:

The results suggest that it is possible to distinguish members of the psittacosis group of agents from host materials on the basis of their phospholipid and fatty acid components. Gas-liquid chromatography (GLC) of unhydrogenated methyl esters of fatty acids obtained from the phospholipid fraction of these agents were very similar. When hydrogenated methyl esters were examined, the fatty acids of 6BC and meningopneumonitis agents were different from each other and from host material. A large number of odd-numbered, unsaturated fatty acids seem to be present in these agents including a C17:1 compound which has been associated primarily with bacteria.

Two-dimensional thin-layer chromatography (TLC) was used to characterize the phospholipids of TW-3, 6BC and meningopneumonitis agents of the PLT group of agents. The phospholipid patterns of the 3 agents were quite similar. One unidentified phospholipid species was found in the meningopneumonitis agent but was a minor component or absent in the other agents. Phosphatidyl choline, phosphatidyl ethanolamine, sphingomyelin, phosphatidyl serine and phosphatidic acid were found and identified by their chromatographic behavior in the PLT.

MR 005.09-0053
Report No. 2

Isolation of Mycoplasma Organisms (PPLO) from the Human Eye: A Case Report (American Journal of Ophthalmology 62:1125-1127, 1966)

H. G. Arm, R. L. Woolridge, K. H. Cheng and I. H. Chang

From: Microbiology Department, Bacteriology Division

Abstract:

On 2 occasions pleuropneumonialike organisms were isolated from the conjunctiva of a 5-year old Chinese male in the absence of symptoms compatible with Reiter's syndrome. The significance of these findings is not known and cannot be evaluated until more satisfactory methods for isolation and maintenance of pleuropneumonialike organisms from the eye are developed.

MR 005.09-0053
Report No. 4

Observations Concerning Mycoplasma in Cervical Cultures from Pregnant and Nonpregnant Chinese Women (Journal of the Formosan Medical Association 65:541-546, 1966)

C. H. Lee, K. H. Cheng, H. G. Arm and R. L. Woolridge

From: Bacteriology Division, Microbiology Department

Summary:

The incidence of Mycoplasma organisms, commonly known as pleuropneumonia-like organisms or PPLO, in cervical canals of 85 women with normal cervices and 86 women with cervicitis and/or cervical erosion has been studied. The women were highly selected and were comparable in age, socio-economic level, and marital status. A higher incidence of "large colony" type PPLO was found in women with pathologic conditions of the cervix. The results of this study indicate that pregnancy inhibits Mycoplasma in the cervical canal. Normal defense mechanisms associated with pregnancy may be active against Mycoplasma in the genital tract.

MF 022.03.07-2014
L&R Report No. 023

Arthropod Tissue Culture: A Brief Outline of its Development and Descriptions of Several of its Applications

E. C. Suitor, Jr.

From: Virology Division, Microbiology Department

Abstract:

The development of arthropod tissue culture is outlined, with emphasis placed on lines of cells continuously propagated. Several results using this new laboratory tool at NAMRU-2, such as for arbovirus and microfilarial development, are presented. Examples of other applications and potentials are also listed.

MF 022.03.07-2014
Report No. 1

In Vitro Development of Microfilariae of Macacanema formosana in Mosquito Cell Cultures (Nature 211:868-870, 1966)

D. E. Wood and E. C. Suitor, Jr.

From: Virology Division, Microbiology Department

Abstract:

The in vitro development of microfilariae of Macacanema formosana was observed in the presence of mosquito cells in culture. Grace's Aedes aegypti cell line was used. Microfilariae developed through "sausage" form to second and possibly third stage larvae. Growth and development of the filarial larvae followed a pattern similar to that described for other microfilariae in insects.

MF 022.03.07-2014
Report No. 2

Growth of Japanese Encephalitis Virus in Grace's Continuous Line of Moth Cells (Virology 30:143-144, 1966)

E. C. Suitor, Jr.

From: Virology Division, Microbiology Department

Abstract:

The multiplication of Japanese encephalitis virus in Grace's continuously cultured line of Antheraea eucalypti cells is reported. When incubated at 28°C with the moth cells, in a culture medium consisting of 10% treated A. pernyi hemolymph in Grace's medium, the virus slowly multiplied. Over the course of 3-4 weeks, it reached titers approximately 10-100 times greater than that of the inoculum, rising 10-1000-fold after initial loss of infectivity in the first week.

MF 022.03.07-2014
Report No. 3

Establishment and Characterization of a Clone from Grace's In Vitro Cultured Mosquito (Aedes aegypti L.) Cells (Experimental Cell Research 44:572-578, 1966)

E. C. Suitor, Jr., L. L. Chang and H. H. Liu

From: Virology Division, Microbiology Department

Summary:

A spindle-shaped cell has been cloned from Grace's Aedes aegypti line. The clone varies from the parent in growth rate at different temperatures, and appears to be closely related in chromosome number to the 32 n member of the parent line. Whether there are other distinctive characteristics, such as viral susceptibility or insusceptibility, remains to be determined.

We believe this to be the first reported cloned line of continuously cultured insect cells. Samples of these cells are available to interested persons upon request.

MF 022.03.07-2005
Report No. 27

Cholera (International Pathology, 7:77-80, 1966)

J. W. Fresh

From: Pathology Department

Summary:

Although cholera occurred in western Europe and the United States in the 19th century, it has confined itself to the Middle East, India and Pakistan, and the Far East since then.

Antigenic classification of cholera vibrios has yielded 3 variants: (1) Inaba, (2) Ogawa and (3) Hokojima.

Some vibrios are hemolytic, and this can be demonstrated by adding a 72 hour broth culture to a 5% concentration of goat erythrocytes (Greig test), allowing it to stand 2 hours at 37°C or overnight in the refrigerator. The so-called El Tor vibrios are usually hemolytic while classical Vibrio cholerae is not.

The El Tor vibrios have been responsible for much of the serious disease in Asia since 1961. Most El Tor vibrios are Ogawa type and should be classified as such.

Pathologic studies by us and by others reveal no sloughing of the intestinal mucosa in cholera patients, rather, a diffuse mucosal inflammation and reduction in intestinal mucosal enzymes. There is no significant difference in pathologic changes produced by El Tor and by classical Vibrio cholerae infections.

MF 022.03.07-2005
IR Report No. 20

Quantitative Determination of Intestinal Ionic and Water Transfer During the Acute and Convalescent Phases of Asiatic Cholera (Proceedings of the 3rd World Congress of Gastroenterology, Tokyo, Japan, 1966)

L. S. C. Griffith, T. G. Mitchell, J. W. Fresh, J. W. Duckworth, W. E. Bullock, Jr., and R. A. Phillips

From: Physiology Department

Abstract:

A quantitative net loss of Na^+, Cl^- and water was measured during the acute phase study, while a net absorption occurred during convalescence. A significant correlation was demonstrated between the unidirectional L→P Na^+ and K^+ fluxes in both the acute and convalescent studies. A significant positive correlation between the net transfer of Na^+ and Cl^-, and between Na^+ and water was demonstrated during the acute and convalescent phase studies. The percent reduction (between 5-60%) in the unidirectional L→P flux during the acute phase, when compared to the convalescent value, reflected the severity of the patient's cholera.

MR 005.09-0040
Report No. 14

Studies on Levels of Penicillamine-Induced Cupriuresis in Heterozygotes of Wilson's Disease (Metabolism 00:00, 1966)

J. B. Tu and R. Q. Blackwell

From: Biochemistry Department

Summary:

Ten heterozygotes for Wilson's disease, all parents of Wilson's disease patients, were compared with 10 Wilson's disease patients and 26 normal subjects for their respective 24-hour urinary copper excretions before and after 1 gram doses of penicillamine. The mean urinary copper excretion by the heterozygotes was significantly higher than that of the normal subjects and significantly lower than that of the homozygotes for Wilson's disease. Differences were greater after penicillamine ingestion. Ranges for the normal subjects and the heterozygotes overlapped markedly thereby precluding any identification of heterozygotes. Heterozygotes and homozygotes for the disease had virtually separate ranges of urinary copper excretion after penicillamine ingestion.

These results suggest a slight tendency for tissue copper accumulation in the heterozygote. Twenty-four hour urinary copper excretion after penicillamine ingestion coupled with total plasma copper level was found to distinguish heterozygotes from those homozygous for Wilson's disease.

MR 005.20-0099
Report No. 15

The Identical Structural Anomalies of Hemoglobins $J_{Meinung}$ and J_{Korat} (Biochemical and Biophysical Research Communications 24:732-738, 1966)

R. Q. Blackwell and C. S. Liu

From: Biochemistry Department

Summary:

Hemoglobin $J_{Meinung}$ from a Hakkanese Chinese family in Taiwan and hemoglobin J_{Korat} from a Thai family have the same structural anomaly. In both hemoglobins the glycyl residue normally present at position 56 in the beta chain is replaced by aspartyl; therefore both can be designated as $\alpha_2 A \beta_2 56$ Asp

MR 005.09-0048
Report No. 1

Standards of Laboratory Animal Care on Taiwan (Journal of the Formosan Medical Association 00:00, 1967)

J. E. Del Favero and H. S. Chiang

From: Laboratory Animal Department

Summary:

A survey of the standards of laboratory animal care on Taiwan was performed in 14 institutions and departments utilizing experimental animals. The major deficiencies were noted in the areas of personnel, management and disease control. Guidelines for laboratory animal care and management are discussed. It appears that the major problems facing the scientific community are a general lack of knowledge of the fine points of laboratory animal care, and a lack of financial support. The formation of a national committee to foster the principles of laboratory animal care and management may provide the impetus necessary for general improvement of this discipline on Taiwan.

MR 005.20-0098
Report No. 19

Plasmodium japonicum, P. juxtanucleare and P. nucleophilum in the Far East (Journal of Protozoology 13:8-11, 1966)

R. D. Manwell

From: Parasitology Division, Medical Ecology Department

Abstract:

Plasmodium japonicum and P. juxtanucleare are two very similar species of avian malaria parasites. The former was discovered in domestic fowl in Japan, and the latter in the same host species in Brazil; it has since been found in chickens in Uruguay, Mexico, and Ceylon. The present study, based on a Ceylon strain of P. juxtanucleare and slides from the Bamboo Partridge (Bambusicola thoracica sonorivox) of Taiwan indicates that the latter host is a natural reservoir of P. juxtanucleare, since this species appears to differ in no significant way from P. japonicum. Infection is common in the Bamboo Partridge (5 of 26 birds showed it), but it has not so far been found in any other avian species although a total of 973 birds belonging to 17 orders, 45 families, 110 genera, and 183 species, have been examined from this area. Since the younger stages of Plasmodium nucleophilum look very much like similar stages of P. juxtanucleare, they are compared. The older stages however are usually easily distinguishable, and the former species is so far known only from non-gallinaceous birds whereas the latter appears to be limited to gallinaceous ones.

MR 005.20-0098
Report No. 35

New Pentastomida. Sambonia parapodum n. sp. from Varanus salvator and Armillifer agkistrodontis n. sp. from Agkistrodon acutus (Transactions of the American Microscopical Society 85:256-260, 1966)

J. T. Self and R. E. Kuntz

From: Parasitology Division, Medical Ecology Department

Abstract:

Sambonia parapodum n. sp. is described from Varanus salvator as the fourth member of the genus after Megadrepanoides, Self and Kuntz, 1957, with its 2 species, is relegated to synonymy with Sambonia based on Fain's (1961) redescription of the latter. Armillifer agkistrodontis n. sp. is described from Agkistrodon acutus.

MR 005.20-0098
Report No. 36

Occurrence of Entamoeba polecki in School Children in Taiwan (Journal of Parasitology 52:700, 1966)

D. E. Armstrong

From: Parasitology Division, Medical Ecology Department

Abstract:

This research note briefly describes the first reported occurrence of Entameoba polecki in Taiwan. The parasite was recovered from 2 of 234 Taiwanese elementary school children. Other protozoans recovered are listed in order of decreasing frequency.

MR 005.20-0098
Report No. 38

Desmogonius desmogonius Stephens, 1911 (Pronocephalidae: Digenea), a Redescription with Observations on Egg Filament Formation (Zeitschrift fur Parasitenkunde 25:506-509, 1965)

W. H. Coil and W. A. Reid

From: Parasitology Division Medical Ecology Department

Abstract:

The trematode, Desmogonius desmogonius Stephens, 1911 (Pronocephalidae: Digenea), is redescribed and observations are made on certain structures and the formation of the egg shell filaments.

MR 005.20-0098
Report No. 40

Plasmodium hegneri n. sp. from the European Teal Anas crecca crecca in Taiwan (Journal of Protozoology 13:437-440, 1966)

R. D. Manwell and R. E. Kuntz

From: Parasitology Division, Medical Ecology Department

Abstract:

Plasmodium hegneri n. sp. is described from the European teal duck, Anas c. crecca, from Taiwan. The blood stages, so far the only ones seen, are distinguished mainly by the elongate character of the gametocytes, which closely resemble Haemoproteus (though the pigment tends to be finer and less abundant), and the failure of both asexual and sexual forms to displace the nucleus or otherwise alter the host cell. Merozoites average 13.4 ± 2.2 (range 10-19) per segmenter. Trophozoites often adhere to the host cell nucleus, and may have a large vacuole and a remarkably long, slender pseudopodium. The species so far has been seen only in the European teal, although blood films from 194 species and over 1200 birds have been examined.

MR 005.20-0098
Report No. 41

New and Little-known Plagiorhynchid Acanthocephala from Taiwan and the Pescadores Islands (Journal of Parasitology 52:520-527, 1966)

G. D. Schmidt and R. E. Kuntz

From: Parasitology Division, Medical Ecology Department

Abstract:

The acanthocephalan subfamily Plagiorhynchinae is reviewed. Prosthorhynchus Kostylew, 1915 is synonymized with Plagiorhynchus Luhe, 1911. Plagiorhynchus subg. n. and Prosthorhynchus subg. n. are proposed within the genus Plagiorhynchus. A report is made on Acanthocephala of birds from Taiwan and Pescadores Islands including descriptions of Plagiorhynchus (Prosthorhynchus) taiwanensis sp. n., P. (Prosthorhynchus) golvani sp. n., P. (Prosthorhynchus) bullocki sp. n., P. (Prosthorhynchus) malayensis (Tubangui, 1935), comb. n., and P. (Plagiorhynchus) charadrii (Yamaguti, 1939) Van Cleave, 1951.

MR 005.20-0098
Report No. 39

Observations on Egg Shell Formation in Hydrophitrema gigantica Sandars, 1960 (Hemiuridae: Digenea) (Zeitschrift fur Parasitenkunde 25:510-517, 1965)

W. H. Coil

From: Parasitology Division, Medical Ecology Department

Abstract:

The author reports that in the case of Hydrophitrema gigantica the vitelline granules are used in the formation of the egg shell. The author also reports a new variation in the morphology of the system associated with the process of egg shell formation in digenetic trematodes. In H. gigantica the egg is formed in an elongate, granular ootype some distance from the Mehlis gland. Other material is added by the uterine gland which does not appear to be part of the egg shell itself.

The author postulates that in some trematodes a number of things may happen in the uterus which will alter the egg shell: 1) the operculum may be formed: 2) filaments may form at one or both ends; 3) the shell may be thickened by additional vitelline and/or uterine materials; 4) glycogen may be formed as the embryo matures.

MF 022.03.07-2013
Report No. 31

Field Trial of a Monovalent and Bivalent Trachoma Vaccine with Mineral Oil Adjuvant in Taiwan School Children (American Journal of Ophthalmology Supplement, April 1967)

R. L. Woolridge, J. T. Grayston, I. H. Chang, K. H. Cheng, C. Y. Yang, C. Neave

From: Virology Division, Microbiology Department

Abstract:

Two formalin treated purified trachoma vaccines (TW-3 monovalent and TW-1 + TW-3 bivalent) were tested in school aged children in central Taiwan. Both vaccines were given in mineral oil adjuvants to separate groups of children in 2 injections.

The cumulative conversion to trachoma of the children receiving bivalent vaccine followed for 2 to 3 years showed a low protection potency of the vaccine. This correlates well with the low particle count given the children.

Children receiving monovalent vaccine showed a statistically significant deleterious effect. Nearly two-and a half times as many children developed trachoma among those given vaccine as in the placebo group. The results are reminiscent of findings in cross protection studies in monkeys where immunization sufficient to prevent homologous infection resulted in more severe heterologous infection than was observed in controls.

MF 022.03.07-2013
Report No. 32

Natural History of Trachoma in Taiwan School Children (American Journal of Ophthalmology Supplement, April 1967)

R. L. Woolridge, J. T. Grayston, E. B. Perrin, C. Y. Yang, K. H. Cheng, I. H. Chang

From: Virology Division, Microbiology Department

Abstract:

First grade school children entering school in central Taiwan in 1960, 1961 and 1962 were examined periodically for the presence of trachoma infection. The 1960 group was followed through 1966 and the other 2 groups through 1964.

The series of eye diagnosis in individual children show many patterns other than simple conversion from normal to trachomatous eyes. Reversion from trachomatous to normal or inactive eyes was frequent. Periodic reinfection and reconversion to normal or inactive trachoma were also seen. The results give the impression that on Taiwan, trachoma is a disease where healing may occur without scarring and reinfection and relapse are common. The conclusions are supported by repeated examinations of children who were subjects in trachoma vaccine field trials.

These findings support the growing opinion that trachoma is a reinfectious disease in which the development of hypersensitivity to the organism contributes to the pathogenesis of the disease, particularly in the formation of pannus and scarring.

MR 005.09-0055
IR Report No. 18

Depression of the Delayed Type Allergic Response by Leprosy (Clinical Research 14:337, 1966 - Abstract)

W. E. Bullock

From: Clinical Investigation Department

Abstract:

The effect of leprosy on cellular hypersensitivity is poorly understood although it is agreed that lepromatous patients usually are anergic to skin test antigens of Mycobacterium leprae. Sulfone treated leprosy patients were divided into two age-equivalent samples; one with lepromatous (54 pts.) and one with tuberculoid (53 pts.) leprosy. Both samples were subdivided into "early Rx" (mean < 8 months) and "late Rx" (mean > 60 months) groups. Skin test responses in these four groups evoked by antigens of P. P. D., Candida albicans, Trichophyton, and picryl chloride were compared to a non-leprous control group (30 pts.). A statistically significant increase of negative response to all test antigens was present in lepromatous patients of the "early Rx" group as compared with controls. Negative responses to all antigens except Trichophyton were significantly increased in "early Rx" tuberculoid patients. "Late Rx" groups did not differ significantly from the control in response to protein antigens. Picryl chloride failed to evoke normal contact allergy in any treatment group. It is concluded that lepromatous or tuberculoid leprosy may cause generalized depression of the delayed type allergic response. Failure to induce normal primary sensitization to picryl chloride in "late Rx" patients suggests impairment of delayed allergic response well into the recovery phase of both leprosy types. Prolonged therapy of leprosy is associated with increased reactivity to protein antigens.

MR 005.20-0098
Report No. 44

Hepatocystis in Formosan Mammals with a Description of a New Species (Journal of Protozoology, 13:670-672, 1966)

R. D. Manwell and R. E. Kuntz

From: Parasitology Division, Medical Ecology Department

Abstract:

Hepatocystis has been found extremely common in a number of species of Formosan mammals, notably the large leaf-nosed bat (Hipposideros armiger terasensis), the Formosan macaque (Macaca cyclopis), and various subspecies of the red-bellied tree squirrel (Callosciurus erythraeus). Less often infected were the long-winged bat (Miniopterus schreibersii) and the Formosan giant flying squirrel (Petaurista grandis). The species occurring in H. a. terasensis is believed to be new, and is named Hepatocytis hipposideri. It causes considerable enlargement (but not other changes) of the host cell, is often amoeboid, and usually has numerous fine pigment granules.

MR 005.20-0098
Report No. 37

Sphaerechinorhynchus serpenticola Sp. N. (Acanthocephala:Sphaerechinorhynchinae), a Parasite of the Asian Cobra, Naja naja (Cantor), in Borneo (Malaysia) (Journal of Parasitology, 52:913-916, 1966)

G. D. Schmidt and R. E. Kuntz

From: Parasitology Division, Medical Ecology Department

Abstract:

Sphaerechinorhynchus serpenticola sp. n. is described from 17 specimens recovered from the mesenteries surrounding the large intestine of an Asian cobra, Naja naja, collected near Jesselton, North Borneo. It differs from S. rotundocapitus (Johnston, 1912), the only other species in this genus, in the number of cement glands, the number and size of proboscis hooks, lemniscus length, and shape of the posterior end of the female. The subfamily Sphaerechinorhynchinae is recognized, on biological and morphological grounds, as an aberrant group within the Plagiorhynchidae.

PAPERS READ BEFORE SCIENTIFIC SOCIETIES BY THE NAMRU-2 STAFF DURING CALENDAR YEAR 1966

Jenkin, H. M. Induction of an Interferon-like Effect in HeLa 229 Cells by a Trachoma Agent (Bour). Annual Meeting of the American Society of Microbiology, Los Angeles, California, 1-5 May 1966.

Bravi, E. M. Feeding Response of Two Closely Associated Vectors of Japanese Encephalitis Disease on Formosa. 50th Annual Meeting of the Pacific Branch of the Entomological Society of America, San Diego, California, 21-23 June 1966.

Blackwell, R. Q. (Co-authors B. N. Blackwell, J. T. H. Huang, L. C. Chien, T. Kathancharoen, A. Samaharn, C. Thephusdin and C. Borvornsin). Occurrence of Hemoglobin J-Korat in Northeastern Thailand. XI Congress of the International Society of Haematology, Syeney, Australia, 21-26 August, 1966.

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Woolridge, R. L. (Co-authors J. T. Grayston, E. B. Perrin, C. Y. Yang, K. H. Cheng and I. H. Chang). Natural History of Trachoma in Taiwan School Children. Symposium on the Trachoma and Inclusion Conjunctivitis, San Francisco, California, 25-31 August 1966.

Woolridge, R. L. (Co-authors J. T. Grayston, I. H. Chang, C. Y. Yang and K. H. Cheng). Long-term Follow-up of the Initial (1959-60) Trachoma Vaccine Field Trials on Taiwan. Symposium on the Trachoma and Inclusion Conjunctivitis, San Francisco, California, 25-31 August 1966.

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Alexander, E. R. (Co-authors S. P. Wang and J. T. Grayston). Further Classification of TRIC Agents from Ocular Trachoma and Other Sources by the Mouse Toxicity Prevention Test. Symposium on the Trachoma and Inclusion Conjunctivitis, San Francisco, California, 25-31 August 1966.

Alexander, E. R. (Co-author J. W. Fresh). Prevention of Anophylactic Death in the Mouse Toxicity Protection Test for TRIC Agents. Symposium on the Trachoma and Inclusion Conjunctivitis, San Francisco, California, 25-31 August 1966.

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Mitchell, T. G. (Co-authors J. W. Fresh, A. H. G. Love and R. A. Phillips. Sodium Transport Measurement in the Monkey Intestine. 11th Pacific Science Congress, Tokyo, Japan, 22 August - 10 September, 1966.

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Watten, R. H. (Co-authors J. B. Tu, R. Q. Blackwell). The Role of Copper Balance Determinations in the Investigation of Wilson's Disease. International Symposium on Wilson's Disease, Tokyo, Japan, 20-21 September 1966.

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Lee, Y. T. (Co-author E. C. Suitor, Jr.). Stability of Selected Arboviruses in Insect Hemolymph. 59th General Conference of the Formosan Medical Association, Taipei, Taiwan, 12-13 November 1966.

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Sun, W. K. C. Biting Midges (Diptera: Ceratopogonidae) from Kinmen (Quemoy). 59th General Conference of the Formosan Medical Association, Taipei, Taiwan, 12-13 November 1966.

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Fresh, J. W. The Pathology and Pathophysiology of Cholera. World Health Organization First Regional Seminar on Cholera Control, Manila, Philippines, 29 August - 9 September 1966.

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Clarke, E. J. Jr. Serological Survey for Chikungunya and Dengue Antibodies in the Inhabitants of a Southwest Taiwan Village: A Preliminary Report. Symposium on Recent Studies on Arthropod-Borne Diseases of Man and Animals on Taiwan, NAMRU-2, Taipei, Taiwan, 3-5 March 1966.

Suitor, E. C. Jr. Investigations on Arbovirus Growth in In Vitro Cultured Arthropod Cells. Symposium on Recent Studies on Arthropod-Borne Diseases of Man and Animals on Taiwan, NAMRU-2, Taipei, Taiwan, 3-5 March 1966.

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Jenkin, H. M. (Co-authors H. M. Yu and E. C. Suitor, Jr.) Cross Neutralization Studies of Various Arboviruses in Monkey Kidney Cells Using Plaque Inhibition and Plaque Reduction Tests. Symposium on Recent Studies on Arthropod-Borne Diseases of Man and Animals on Taiwan, NAMRU-2, Taipei, Taiwan, 3-5 March 1966.

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Chiang, W. T. Genital Infection with TRIC Agents in Taiwan. Trachoma Symposium, NAMRU-2, Taipei, Taiwan, 8 August 1966.

Alexander, E. R. Differentiation of TRIC Agents from Ocular and Non-Ocular Sources. Trachoma Symposium, NAMRU-2, Taipei, Taiwan, 8 August 1966.

Alexander, E. R. Anthropometric and Psychologic Studies in a Prospective Study of Cardiovascular Disease in Taiwan. Symposium on Cardiovascular Disease, NAMRU-2, Taipei, Taiwan, 18 November, 1965.

SYMPOSIA SPONSORED BY NAMRU-2 DURING CALENDAR YEAR 1966

Recent Studies on Arthropod-Borne Diseases of Man and Animals on Taiwan, NAMRU-2, Taipei, Taiwan, 3-5 March 1966.
Coordinated by Dr. E. C. Suitor, Jr., Arthropod Tissue Culture Laboratory, Microbiology Department.

Abstract:

The Symposium on Recent Studies on Arthropod-Borne Diseases of Man and Animals on Taiwan was divided into the following sections:

Viruses: Clinical, Epidemiological and Experimental
Bacteria and Rickettsiae
Parasites
Entomology and Vector Control

Presented were 27 papers covering a wide range of subjects related to arthropod-borne diseases by distinguished scientists and physicians from academic, military and research organizations locally and several papers by the NAMRU-2 staff. Each paper was followed by a discussion, and each section was headed by a discussion leader who maintained a high level of audience participation in the proceedings.

Conference on Clinical Research Problems in Leprosy, NAMRU-2, Taipei, Taiwan, 24 May 1966.
Coordinated by Dr. W. E. Bullock, Jr., Clinical Investigation Department.

Abstract:

A one-day Symposium on clinical problems in leprosy was held at NAMRU-2 on 24 May 1966. Participating were a group of scientists including Dr. Chapman H. Binford, Medical Director of Leonard Wood Memorial Hospital, Philippines; Dr. John H. Hanks, Director, Johns Hopkins Leonard Wood Memorial Laboratory; and several others who had recently attended a conference in Tokyo. Topics discussed were recent developments in tissue culture techniques for growth of fastidious microorganisms, the immuno-pathologic consequences of host-parasite antagonism in human leprosy, and the physiologic implications of the lipids in the micobacteria. Papers were also presented on the variegated clinical pattern of leprosy and its allergic reactions, recent epidemiologic contributions to the leprosy control program, and a keynote address by Dr. Binford entitled "Current Prospects for the Conquest of Leprosy." The meeting was attended by over 50 local physicians and scientists in the Taipei area.

Trachoma Symposium, NAMRU-2, Taipei, Taiwan, 8 August 1966.
Coordinated by Dr. E. Russell Alexander, Virus Immunology Division, Microbiology Department, NAMRU-2, and University of Washington.

Abstract:

The guest speaker at the one-day Symposium on Trachoma was Dr. Leslie H. Collier, Honorary Director, Trachoma Research Unit, Medical Research, Council, Lister Institute, London, England. The program included talks on immunogenicity of experimental trachoma vaccine in baboons by Dr. Collier, genital infection with TRIC agents in Taiwan, differentiation of TRIC agents from ocular and non-ocular sources, and a round-table discussion on trachoma and its prevention. The Symposium was attended by a number of local physicians and health experts.

Symposium on Cardiovascular Disease, NAMRU-2, Taipei, Taiwan, 18 November 1966. Coordinated by Dr. E. Russell Alexander, Virus Immunology Division, Microbiology Department, NAMRU-2, and University of Washington, Seattle.

Abstract:

Interest for this Symposium was generated by a collaborative project between the University of Washington and NAMRU-2. Six papers covering various aspects of cardiovascular disease, its detection and epidemiology were presented by University of Washington, NAMRU-2, and Chinese physicians. The keynote paper on "Circulatory Responses to Exercise and Heart Stress" was given by Dr. Robert A. Bruce, Department of Medicine, University of Washington, Seattle. The Symposium was attended by a large number of interested local physicians and the NAMRU-2 staff.