SUMMARIES OF RESEARCH
1 July - 31 December 1965

United States Naval Medical Research Unit No. Two
Taipei, Taiwan
These summaries cover the research reported upon during the last six months of 1965.

The work was supported in part by funds provided by the Bureau of Medicine and Surgery, Department of the Navy, Washington, D. C., and in part by funding under Public Law 480, Section 104(c).

R. H. WATTEN
CAPT MC USN
Commanding Officer

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THE CONTINUOUS PASSAGE OF AGENTS OF TRACHOMA IN CELL CULTURE. I. Characteristics of TW-3 and Bour strains of trachoma cultivated in serial passage in HeLa 229 cells
-- Jenkin, H. M.

Twelve strains and lines of mammalian cells were screened for their susceptibility to infection with the TW-3 and Bour strains of trachoma. The HeLa 229 cell line showed the highest susceptibility to infection but at no time were all of the cells infected nor was a cytopathic effect other than inclusion formation observed. Two other HeLa cell strains, S3 and calf, were resistant to infection. The TW-3 and Bour strains were passed continuously in the HeLa 229 cell line for 49 and 53 passages, respectively. Secondary cycling did not occur in this agent-cell system; therefore, it was necessary to break the cells and release the agent for serial propagation of the agents in cell culture.

Various cell culture passages of strains of trachoma cultivated in HeLa 229 cells remained pathogenic for monkeys and were nonpathogenic for mice. Problems of serial passage of agents of trachoma in cell culture were discussed.

(J. Infect. Dis. in press)

COMPARATIVE STUDIES OF THE OCULAR BACTERIAL FLORA OF CHINESE CHILDREN WITH CLINICAL TRIC AGENT INFECTIONS
-- Arm, H. G., and Woolridge, R. L.

The eyes of 473 Chinese school children, ranging in age from 9 to 11 years, have been studied bacteriologically. Six hundred and three isolations representing 19 species of 12 genera were made. Approximately one-half of the cultures yielding organisms were pure cultures. The distribution of bacterial species by clinical category indicated that several species present in normal children's eyes may become associated with infection as opportunists, if environmental conditions permit. Cultures of the eyes of 417 children with various clinical symptoms failed to detect pleuropneumonialike organisms by the methods employed.

(Med. J. Austral. in press)
TRIC AGENT INFECTIONS ON TAIWAN: Epidemiology, prevention and chemotherapy studies
-- Woolridge, R. L., and Grayston, J. T.

Trachoma treatment experiments in Taiwan school children showed that vaccine alone had little effect in curing trachomatous children. When trachoma vaccine was combined with tetracycline eye ointment and oral sulfonamides the cure rate was not significantly greater than the cure without the trachoma vaccine. The poor curative effect of vigorous combined tetracycline eye ointment and oral sulfamethoxydiazine with trachoma vaccine prevented an evaluation of the ability of simultaneously administered trachoma vaccine to prevent trachoma relapse or reinfection.

(Med. J. Austral. in press)

ZOOPHILIC SCHISTOSOMIASIS ON TAIWAN: Reestablishment of Schistosoma japonicum subsequent to drastic alteration of habitats by flood
-- Kuntz, R. E.

Drastic alterations in the habitats of Schistosoma japonicum in the enzootic area of Taiwan practically eliminated the parasite in the focus studied. Observations over a period of several years allowed a comparison of the status of host-parasite relationships before and after interruption of the parasite cycle by natural causes. Although excessive flooding reduced the snail (Oncomelania formosana) population and the parasite infection in definitive hosts (lower vertebrates) to near nil, Oncomelania populations returned to near normal after approximately 1 year, followed by appearance of infection in rodents. An unusual compensation in biological productivity seemed to allow for rapid reestablishment of the schistosome cycle after abnormal circumstances had placed the parasite-snail-definitive host associations in jeopardy.

(J. Parasit., 51:747, October 1965)

INTESTINAL PARASITES IN PEOPLES OF TAIWAN: A cursory survey of Hua-fan-she Village (Sun Moon Lake, Nan-tou): A new locality for endemic clonorchiasis
-- Kuntz, R. E.

(continued on next page)
A cursory survey of Tsou aborigines in central Taiwan has shown a sparseness of the protozoans commonly found in man, and the pathogen Entamoeba histolytica was not detected. Rates of infection for the common helminths of man fell within the ranges of expectation and were commensurate with helminth infection potentials for the area. Strongyloides occurred in 3% of persons examined, representing a record high rate for the island. Clonorchis was detected in 39% of people sampled in an area not previously recognized as endemic for the Chinese liver fluke.

(Fromosan Science, in press)

MF022, 03. 07-2003. 32
INTESTINAL PARASITES OF PEOPLES OF TAIWAN: Intestinal parasites of aborigines (Yami) of Lan Yu (Orchid Island)
-- Kuntz, R. E., and Lawless, D. K.

A survey of the intestinal protozoans and helminths, based upon an examination of a single fecal specimen from each of 325 aborigines (Yami) on Lan Yu revealed the presence of 9 species of protozoa and the eggs of 10 species of helminths. The list of protozoans, including Dientamoeba fragilis and Entamoeba hartmanii is longer than given for other surveys in this general geographic area. In contrast to many investigations, E. coli occurred in fewer persons than the pathogenic ameba E. histolytica. Balatidium appeared in all age groups reflecting the sanitary conditions under which these people live.

Ascaris was common in all age groups of people examined but Trichuris occurred in fewer persons than expected based upon other surveys in this area. Several aborigines were infected with Enterobius and Strongyloides, and rates of infection for hookworm were commensurate with existing epidemiological conditions. Diphyllobothrium and Taenia were recorded, the presence of the former suggesting possible consumption of amphibians and/or reptiles and the latter probably is present as a result of consumption of raw or partially cooked pork.

(J. Formosa Med. Ass. in press)

MF022, 03. 07-2003. 33
NOTES ON SOME MALLOPHAGA FROM FORMOSAN Mammals
-- Emerson, K. C.

(continued on next page)
This is a report of Mallophaga (biting lice) recovered from mammals native to Formosa. Five species of Mallophaga are reported:

Felicola jucii from Paguma larvata taivana (the masked civet);
Felicola rohani from Herpestes urva (the crab-eating mongoose);
Tricholipeurus indicus from Muntiacus reevesi micrurus (the Formosan Reeve's muntjac);
Bovicola thompsoni from Capricornis swinhoei (the Formosan serow); Trichodectes kuntzi from Melogale moschata subauranticaca (the orange-tinted tree civet).
Trichodectes kuntzi is described as a new species.

MR005.09-0057.2
OBSERVATIONS ON THE BIONOMICS OF THE BITING MIDGE CULICOIDES ARAKAWAE (ARAKAWA) (DIPTERA: CERATOPOGONIDAE) IN TAIWAN
-- Sun, W. K. C.

Culicoides arakawae (Arakawa) is widely distributed in Taiwan and its adjoining islands: the Pescadores, Small Liu Chiu Island and Orchid Island, most abundantly at the plains from 200 m. elevation down to the sea coast. The yearly pattern of C. arakawae collected shows two eminences in spring and fall alternating with two depressions. The spring eminence is the dominant one. The nocturnal activity, sex ratio of the midge, and the fed/unfed index of the females are also reported.
(Tunghai University Biological Bulletin 26, p. 1, February 1966)

MR005.09-0046.1
EXPERIMENTAL INFECTION OF A MONGOOSE WITH ANGIOSTRONGYLUS CANTONENSIS (CHEN)
-- Wood, D. E.

A "crab eating" mongoose, Herpestes urva, was fed 1000 Angiostrongylus cantonensis (Chen) larvae in the laboratory. The larvae were obtained from laboratory infected snails (Australorbis glabratus). Thirty-three days after the feeding, first stage larvae appeared in the stool of the mongoose. On autopsy 75 days after infection, 5 adult A. cantonensis were recovered from the heart and 100 or more dead worms were found in the pulmonary arteries. Large hardened masses in the lungs contained viable eggs and first stage larvae of A. cantonensis.
(Research Note in J. Parasit. 51:941, December 1965)
LIVER DISEASE ON TAIWAN
-- Chu, P.C., Sun, S.C., Liang, W.H., and Fresh, J.W.

Review of 762 liver biopsies from two Chinese military hospitals on Taiwan over a five year period revealed the major distribution of clinically manifested liver diseases to be as follows: hepatitis, presumably viral, 42%; cirrhosis 19%; and primary carcinoma 9.0%.

The incidence of subclinical liver disease found in three surveys of seemingly healthy people (two on 1,266 Chinese servicemen and one on 1,012 adult female Chinese military dependents) based on 37 liver needle biopsies was as follows: hepatitis, presumably viral, (16) 43%; nonspecific reactive hepatitis (12) 32%; and fatty metamorphosis (2) 5%. The only instance of postnecrotic cirrhosis was in the acute icteric group of the first survey. The overall incidence of anicteric (viral) hepatitis, based on survey methods and liver needle biopsies, was 0.9%.

Nonspecific reactive hepatitis was present in about 0.5% of seemingly healthy people, usually representing either an early or a convalescent stage of acute hepatitis.

The high incidence of hepatitis, presumably viral, and resulting cirrhosis in the Chinese people may be related to the frequent occurrence of primary liver carcinomas. When biopsies were adequate and included non-neoplastic tissue, 65% of the liver carcinomas discussed here had associated posthepatitic or postnecrotic cirrhosis.


ANICTERIC VIRAL HEPATITIS: A clinicopathologic follow-up study in Taiwan
-- Cooper, W.C., Gershon, R.K., Sun, S.C., and Fresh, J.W.

A long-term study of acute anicteric hepatitis in young Taiwanese and Chinese military men is described. Patients were obtained by screening 3529 randomly selected asymptomatic men with the serum glutamic pyruvic transaminase test.

The evidence in this study indicated that anicteric hepatitis not only is frequent but often becomes chronic and may progress to significant liver damage, both functional and structural. The large number of cases of cirrhosis of the liver without apparent antecedent cause in Taiwan, and probably elsewhere, may be explained by the insidious progression of anicteric hepatitis. The existence of this great pool of anicteric hepatitis in varying states of chronicity generates a number of important problems whose ramifications extend into all branches of medicine, and especially into the fields of epidemiology, preventive medicine and public health.

STUDY OF SERUM BILIRUBIN LEVELS IN RELATION TO WEIGHT LOSS IN CHINESE PREMATURE INFANTS
-- Lu, T. C., and Blackwell, R. Q.

Daily serum bilirubin concentrations and body weights were determined in 15 Chinese premature infants with birth weights less than 2,000 grams. The mean total serum bilirubin levels for the first 7 days of life respectively were 5.4, 8.9, 11.7, 13.1, 13.5, 12.9, and 12.2 mg per 100 ml. The mean accumulative percentage weight losses in the infants from the second through the seventh day were 3.0, 6.7, 7.2, 7.7, 7.8, and 7.6 per cent respectively. From these values and from the correlation in individual cases between peak serum bilirubin levels and the maximum accumulative percentage of body weight loss it was concluded that a definite relationship exists between the two conditions.

Although the present results require additional clinical verification they lend further weight to previous work suggesting the advisability of early feeding of the premature infants, particularly those below 2,000 grams birth weight. The results also appear to indicate the importance of adequate fluid intake in jaundiced infants.

(Submitted to Amer. J. Dis. Child.)

INCREASED INCIDENCE OF SEVERE HYPERBILIRUBINEMIA AMONG NEWBORN CHINESE INFANTS
-- Lu, T. C., Wei, H. Y., and Blackwell, R. Q.

Twenty-six G-6-P D deficient cases, 3%, were found among 878 Chinese newborn males. Serum bilirubin levels were significantly higher in the G-6-P D deficient infants than in premature and full-term Chinese neonates studied previously. Ten of the 26 subjects had peak bilirubin levels above 20 mg per 100 ml. These results suggest that a substantial proportion of the male Chinese neonates which develop severe neonatal jaundice come from the relatively small proportion with G-6-P D deficiency.

(Pediatrics, in press)
HEMOGLOBIN J<sub>Korat</sub> IN THAIS
Blackwell, R. C., et al

Hemoglobin J<sub>Korat</sub>, a "fast" hemoglobin with an anomaly in its beta chain different from the anomalies previously reported, was the major hemoglobin component in the blood of 9 subjects among 1923 Thais from northeastern Thailand. After hemoglobin E, J<sub>Korat</sub> is the second most frequent of the anomalous hemoglobins among Thais.
(Science, 150:1614, December 1965)

CYTOGENETIC STUDY OF CASES OF SEX ANOMALY AND GENITAL AMBIGUITY
Huang, S. W.

Cytogenetic studies were done on 7 individuals with sex anomalies and genital ambiguity. These cases include: 2 with Turner's syndrome (45/XO), 1 with triple-X syndrome (47/XXX), 2 with female pseudohermaphroditism (46/XY) and 1 with multiple congenital anomalies and ambiguous sex (46/XX).

Rendering a prognosis and successful treatment of intersex problems often depends on the early and accurate diagnosis of genetic sex, which is best accomplished by means of the cytogenetic method. The report of diagnosis of many sex anomalies should be done carefully only after both buccal smear and karyotyping are studied.

SPECIFIC AND NONSPECIFIC PASSIVE IMMUNITY IN INFANT RABBIT CHOLERA
Gillmore, J. D., Versage, P. M., and Phillips, R. A.

The passive protective effect of several specific and nonspecific hyperimmune antisera was studied in suckling rabbits using Dutta's intra-intestinal method of challenge. The challenge, an egg and rabbit passaged NIH 35A-3 (Inaba) strain, was administered in numbers of from 60 to 840 colony forming units/100 gm, which produced a profuse diarrhea leading to acidosis and death in 18-26 hours.
(continued on following page)
A single 0.5 ml intraperitoneal injection of antiserum obtained from mature rabbits immunized IV with the live homologous strain was found to protect 84.0% of the suckling rabbits when administered 48 to 72 hours before challenge. Hyperimmune antisera prepared in like manner with an egg and rabbit passaged Vibrio cholerae, Ogawa strain gave only 13% protection as compared with 27.6% protection obtained with antiserum from rabbits immunized IV with a commercial typhoid-paratyphoid vaccine and 20% protection with antisera from animals immunized with a vibrio-free mucinase preparation. No protection was afforded by antiserum from tetanus toxoid immunized animals while 9.4% and 3.4% of suckling rabbits survived receiving normal rabbit serum or saline, respectively, as controls.

(J. Infect. Dis. in press)

MR005.09-0041.1
NASOPHARYNGEAL CARCINOMA AND ENVIRONMENTAL CARCINOGENS -- Fresh, J. W., Sun, S. C., and Rampsch, J. W.

Nasopharyngeal biopsies were obtained from suspected cancer cases. The 83 patients with malignancies selected for study had lived at their present address at least 15 years.

All of the cancers were differentiated or undifferentiated epidermoid carcinomas. More carcinomas were found in men than women in a ratio of 3 to 1. Seventy-Eight of the patients were born on Taiwan. Of 8 patients with family histories of malignancies, 2 had family histories of nasopharyngeal carcinomas. Forty-nine patients were smokers of long duration (36% of the men and 17% of the women). About half of the patients' relatives had chronic pharyngitis.

Elevated levels of arsenic were found in the hair and fingernails of 81% of the patients. Significant quantities of proven carcinogenic agents, arsenic, nickel and chromium, were found in the environmental dust, ashes, water and fuel of the patients' homes. The presence of these carcinogens may be possible predisposing factors for the production of carcinomas, especially nasopharyngeal carcinoma, in Chinese.

(Series Cancerologica, in press)

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The following reports are referred to only by title. Complete copies of any of these reports will be provided on request.

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MR005. 09-0040.13  Erythrocyte acid phosphomonoesterase activity in G-6-P D deficient Chinese neonates
-- Lu, T. C., Wei, H. Y., Blackwell, R. Q.
(Submitted to Nature)

NAMRU-2-029(CS)  Digeneic trematodes of fishes from North Borneo (Malaysia)
-- Fischthal, J. H., Kuntz, R. E.

NAMRU-2-030(CS)  Six digenetic trematodes of mammals from North Borneo (Malaysia)
-- Fischthal, J. H., Kuntz, R. E.

NAMRU-2-031(CS)  Digeneic trematodes of amphibians and reptiles from North Borneo (Malaysia)
-- Fischthal, J. H., Kuntz, R. E.

NAMRU-2-033(CS)  The significance of the number of vibrios seen in rice water stools
-- G. S. Huber
(Public Health Service Publication No. 1328, p. 41, 1965)

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Paper presented by NAMRU-2 staff members at scientific society meetings during the last six months of 1965.

The 25th Annual General and Scientific Meeting of the Ophthalmological Society of Australia (A.M.A.), 20-24 September 1965, Adelaide, Australia


Woolridge, R. L., and Grayston, J. T. Tric agent infections on Taiwan: Epidemiology, prevention and chemotherapy studies.

The 9th International Conference on the Biochemistry of Lipids, 5-10 September 1965, Noordwijk, Netherlands.

Jenkin, H. M. A comparison of the phospholipids found in agents of psittacosis and trachoma.

The 23rd International Congress of Physiological Sciences, 1-9 September 1965, Tokyo, Japan

Neptune, E. M. Jr. Respiration of homogenates of mucosal cells from rabbit and guinea pig ileum.


The International Congress of Pediatrics, 7-14 November 1965, Tokyo, Japan

Tu, J. B., Blackwell, R. C., and Hou, T. Y. Diagnosis and treatment of Wilson's disease in the asymptomatic stage.

Establishment and characterization of a clone from Grace's in vitro cultured mosquito (Aedes aegypti L.) cells.

First description of the complete life cycle of the biting midge, Forcipomyia (Lasiohelea) t'aiwana (Shiraki) (Diptera: Ceratopogonidae) reared in the laboratory.

Observations on the bionomics of the midge Culicoides arakawae (Arakawa) (Diptera: Ceratopogonidae) found bearing viruses in Taiwan.

Basic research developments with agents of trachoma and psittacosis.

Behcet's syndrome with superior vena cava thrombosis: presentation of a case report and immunologic studies.