### KINETIC OF ANTIBODIES DURING POST EXPOSURE RABIES VACCINATION WITH TISSUE CULTURE VACCINES

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The ELISA test has been used to follow the kinetic of antibodies during post exposure rabies vaccination in man.

Two tissue culture vaccines have been studied - one produced in human diploid cell strain (HDCS) the other in bovine embryo kidney cells (BEKC) primary explant. Both vaccines are inactivated and administered in 6 doses of 1.0 ml given on days 0, 3, 7, 14, 30 and 90.

Blood specimen were collected on day 0, 3, 7, 14, 30, 90 and 110. Sera were tested by ELISA test performed in microplates coated with purified rabies virus antigen with anti-Ig M, anti-Ig G and protein A, conjugated with peroxidase, and orthodianizidine as enzymatic substrate.

Results of level of Ig M, Ig G, and equivalents of neutralizing antibodies were given.

## STUDY OF HUMAN DIPLOID CELL RABIES VACCINE: PRE-AND POST-EXPOSURE TREATMENT OF RABIES IN THAILAND

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Rabies is a serious public health problem in Thailand, approximately 200-300 human deaths due to rabies have been reported annually from 1957 to 1979. The actual number of cases might be 2 to 3 times higher than the record. Measures to control animal rabies are not effective. In our study, 96% of human cases had been exposed to rabid dogs and about 3% of strayed dogs in Bangkok have been proved to be rabid. Since 1930, semple vaccines have been used for post-exposure treatment.

In 1977, a new type of rabies vaccine,

human diploid cell vaccine, inactivated type, (HDCV Institute Merieux) was granted a license for use in Thailand. So our objective was to study this vaccine in pre-exposure and post exposure treatment.

From 1978 to 1980, approximately 150 persons exposed to rabid animals in varying degrees: biten, licked and scratched were treated with HDCV on the schedule of six injections (day 0, 3, 7, 14, 30 and 90). In human contact cases, pre-exposure treatment using schedule of two injections on day 0 and 30 were given. Serum antibodies on day 0

and 45 were studied by mouse neutralization test compared to indirect fluorescent antibody test. All side effects have been recorded, 2.5% showed redness and edema, 0.5% had itching 1% and had low grade fever. All patients have been followed up by letters

and telephone, so far all 40 cases with definite bites of rabid animalsare still healthy in 1 year follow up.

Results of neutralizing antibody and fluorescent antibody were presented.

# ANTIGENIC DIFFERENCES AMONG VARIOUS RABIES VIRUS ISOLATES AS SHOWN BY MONOCLONAL ANTIBODIES

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Hybridomas have been used to produce monoclonal antibodies against antigenic determinants of rabies virus, by T.J. Wiktor and temporary co-workers (L.Schneider, P. Sureau) at the Wistar Institute, Philadelphia. These antibodies have been studied by T.J. Wiktor and A. Flamand. A set of anti-nucleocapsid and anti-glycoprotein monoclonal antibodies has been prepared and made available by T.J. Wiktor, to several laboratories around the

world, to undertake a cooperative study of the antigenic characterization of various isolates of rabies virus according to the species of animal and the country of origin.

Preliminary results obtained recently with isolates from wild and domestic animals and man, obtained from France, North Africa and Central Africa, using anti-nucleocapsid antibodies were reported. Work with antiglycoprotein antibodies is still in progress.

### SEROLOGICAL STUDIES ON POSSIBLE CAUSES OF INTRAUTERINE INFECTION IN THAI INFANTS

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Serological tests for toxoplasmosis, rubella, cytomegalovirus (CMV), Herpesvirus hominis (HVH) infections, syphilis (TORCHES) were carried out in 49 infants with showed signs of possible intrauterine infections and in 212 pairs of mothers and their newborn infants. The tests employed were ELISA for rubella and CMV infections, indirect haemaggluti-

nation for HVH infection and toxoplasmosis and RPR Macro-vue card test for syphilis. The immunoglobulin class of the antibody was also determined, and only infants with IgM antibody was considered indicative of intrauterine infection. It was found that 36.7% and 10.2% of infants with signs of intrauterine infections were positive for

rubella and CMV antibodies and the other 19% had mixed infections of rubella, CMV, Toxoplasma, syphilis and HVH. In contrast, only 6.1% of normal newborn infants had rubella antibody, 6% had HVH antibody and less than 1% had Toxoplasma antibody, and

none of them had CMV and Treponemaantibodies. Higher rate of seropositivities were found in their mother, the percentage seropositivities for rubella, HVH, CMV infections, syphilis and toxoplasmosis were 19%, 12%, 2% and 1% respectively.

## RECENT ADVANCES IN THE SERODIAGNOSIS OF PARASITIC INFECTIONS

#### F. AMBROISE-THOMAS

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The serology of parasitic diseases was recently improved by the new techniques now available, Some results obtained with two very different tests: Thin layer immunoassay and ELISA test are presented.

The thin layer immunoassay (Elwing' Nilsson and Ouchterlony, 1977) has a very simple principle. On a flat surface of polystyrene (Petri dish for example) some drops of antigenic solution are put and, after incubation and fixation of the antigens, the unknown sera, at different dilutions, are dropped. After incubation and washing, the polystyrene is exposed to water stream (above boiling water) for some minutes. Normally, polystyrene does not fix water and it is covered by very small condensation drops (negative results). On the circles where positive sera have reached, antibody antigen complexes made the polystyrene able to fix water and its surface is thereby covered with large condensation drops. This test is very quick to perform and it does not need sophisticated apparatus or materials. It is suitable for qualitative or semiquantitative screening. It has been utilized for several parasitic diseases and mainly for Echinococcosis with excellent results. Improvements on the conservation of Petri dishes pre-sensitized with antigens to have this material ready to use for long periods are being carried out.

The ELISA test is not so recent (Engvall and Perlmann, 1972). Its principle is well known and it offers advantages and disadvantages exactly opposite to the previous test; it is precise and very sensitive but it needs sophisticated machines for reading the results. With some technical modifications with the use of a new substrate, Orthotolidine it was applied to 12 different parasitic infections and the main results were:

- (i) The possibility to obtain actual quantitative results with only one dilution of sera by referring to a daily control curve.
- (ii) The utilization of metabolic antigens, excretory-secretory antigens by parasites cultivated or living only some days in vitro (Plasmodium falciparum, Toxoplasma gondii, Entamoeba histolytica, Onchocerca volvulus, Fasciola hepatica). Those antigens make the test more sensitive and with an increased specificity.
- (iii) The detection not of antibodies but of circulation antigens (or non antibody

saturated immune-complexes) with the possibility of a very early diagnosis and of post-therapeutic controls largely improved.

(iv) A quite easy study of antigenic properties of different proteic fractions extracted from a crude parasitic antigen. For such a purpose the GEDELISA test (gel Electro Diffusion-ELISA) is currently used which has a very elegant principle and gives really beautiful results for the study of parasitic antigens.

# FLUCTUATION IN THE RELATIVE PROPORTION OF DENGUE VIRUS SEROTYPES ISOLATED FROM PATIENTS WITH DENGUE HEMORRHAGIC FEVER (DHF) BETWEEN 1962-1980

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This report summarized the studies of isolation and identification of dengue viruses from DHF patients at the Children's Hospital, Bangkok, Thailand, between 1962-1980. Most of the earlier data (1962-1970) was extracted from the Annual Progress Reports of the Institute. Although many changes occurred in the techniques used to isolate and identify dengue viruses during the seventeen year period, technique changes could not account for variations in the serotypes dentified.

Dengue 2 virus was consistently isolated from the majority (> 50%) of DHF patients,

while isolation of other dengue virus types varied from year to year. Dengue type 1 isolations accounted for 10-30% of isolates from 1962-1975 but were not evident after 1975. On the other hand, Dengue type 4 represented 20-40% of the total beginning in 1976. Dengue type 3 was consistently detected in virus isolations normally accounting for about 10-20% of the total with the exception of 1963 when dengue 3 isolations represented more than 50% of the total. The disappearance of Dengue 1 in 1976 and appearance of Dengue 4 suggest that Dengue 4 had replaced Dengue 1 in causing 10-20% of DHF.

### STUDIES ON DENGUE VECTOR CONTROL IN YOGYAKARTA, INDONESIA

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The effectiveness of 3 methods: (a) public health education (PHE), (b) the application of 1% Abate sand granules and (c) combination of (a) and (b) were evaluated in four desas (small villages) in Yogyakarta, Central Java, Indonesia, in 1977 - 1978. These desas were designated as (i) P<sub>1</sub>, treated with PHE; (ii) P2, treated with 1% Abate larvicide; (iii) P<sub>3</sub>, treated with PHE and 1 % Abate larvicide and (iv) P<sub>4</sub> untreated (as the control area). They were similar in geography, demographical figures, socio-economical level, the presence of recently suspected Dengue Haemorrhagic fever cases and no vector control was accomplished by either local people nor health authorities.

First preliminary investigations were carried out in October 1977 during the dry season. These revealed a Breteau Index (BI) of 36, 36, 28 and 38 for *Aedes aegypti* and of 27, 41, 24 and 53 for *Ae. albopictus* in the villages P<sub>1</sub>, P<sub>2</sub>, P<sub>3</sub> and P<sub>4</sub>. The Biting/Landing Rate (BR) were 2.8, 2.4, 1.9 and 2.1 for *Ae. aegypti* and were 1.8, 2.9, 2.1 and 2.0 for *Ae. albopictus* in P<sub>1</sub>, P<sub>2</sub>, P<sub>3</sub> and P<sub>4</sub> respectively. First test.

Pre-test interviews showed that the local people had practically no knowledge on mosquito-borne diseases and their vectors and had no idea of control and preventive measures. They did not realize the danger that uncovered water in both natural and artificial containers could create good breeding places for *Aedes*.

Further preliminary entomological investigations were conducted in January 1978 during the rainy season. These included collections of larvae, adults, and eggs from ovitraps and bamboo cups set up 2 days before the survey. The results, namely BI, BR, Ovitrap Index (OI) and Bamboo Cup Index (BCI) were used as base line data for the control evaluation. Treatment were carried out two weeks after the last preliminary survey. The results were checked five times in four weeks intervals starting two weeks post-treatment.

Compared to the control village ( $P_4$ ) there was a significant decrease of Ae. aegypti and Ae. albopictus densities in the treated ones. For Ae. aegypti, a mean BI reduction of 48.8%, 82.6%, 78.2% and 18.1% and a mean BR reduction of 51.6%, 86.9%, 67.6% and 27.5% were found in the villages  $P_1$ ,  $P_2$ ,  $P_3$  and  $P_4$ , respectively. For Ae. albopictus a mean BI reduction of 59.3%, 63.8%, 52.8% and 32.2% and a mean BR reduction of 42.2%, 65.6%, 39.3% and 22.5% were recorded for the villages.

The OI and BCI which did not differentiate between the two species decreased by 66.7%, 82.4%, 79.3% and 30% and by 36.5% 45.5%, 39.6% and 24.3% in average respectively for the villages.

In conclusion, PHE could significantly reduced the *Aedes* population, and the use of Abate larvicide resulted in significant decrease of the mosquito densities with and

without PHE. From the post-test interviews on general knowledge on mosquito borne diseases and their prevention it was learned that the local people appeared to have been motivated by our activities even in the control village.

### EFFECT OF THE ANTIVIRAL DRUG RIBAVIRIN ON DENGUE VIRUS REPLICATION IN LLC-MK 2 CELLS

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The effect of ribavirin (virazole) on the growth of dengue type 1 virus was examined in confluent cell monolayers. Ribavirin, at concentrations ranging from 0.1 to 100 micrograms/ml, was added to growth media at various time intervals during the course of infection. Low concentrations of the drug (10 microgram/ml or less) delayed the production of infectious virus but had no effect on the ultimate virus titer at 7 days. Higher

concentrations (30-100 micrograms/ml) significantly suppressed the production of infectious particles. Suppression was more effective when the drug was added early in the growth curve, Ribavirin appeared to act by inhibiting viral RNA synthesis, as the 44S viral RNA was much reduced in SDS polycrylamide gels of labelled RNA extracted from drug-treated, infected cells. These in vitro results have led us to test ribavirin against in vivo flavivirus infections.

# POLIOANTIBODY SURVEY IN THAILAND AND POLIOANTIBODY RESPONSE AFTER ORAL OPV ADMINISTRATION, THAILAND, 1979

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In 1979, the Virus Research Institute, Department of Medical Sciences, Ministry of Public Health, Bangkok, Thailand, conducted an epidemiological survey of poliomyelitis in Chinat province in parallel with polioantibody survey in Bangkok Metropolis.

The result was used in surveillance of poliomyelitis.

In Chinat Province, 646 blood specimens were randomly collected from newborn children up to 14-year-old children in different

villages in October - December 1979. In Bangkok Metropolis 218 blood specimens were from 6-month-old children to 14-year-old in September 1979. Polioantibodies were tested by the microneutralization test, using Hep-2 cell culture.

According to outbreak of poliomyelitis in Chinat province; the OPV was administered to children of 0-4 age group. The prevaccinated and post vaccinated persons were tested for polioantibody and the sample from each lot of vaccines used in the operation were subjected to potency test.

The results showed that polioantibodies in all age groups of children under study from 6-month to 14-year-old revealed low percentage of all 3 types of polioviruses as compared with poliovirus type 3 which was the lowest. The pattern of polioantibodies among population in Chinat province was similar to population in Bangkok Metropolis. However, triple sero-negative polioantibodies in Chinat province was higher than in Bangkok Metropolis, which may bave been effected by OPV programme in Bangkok carried out previously.

Seven cases who received 1 dose OPV, all 7 children had antibody poliovirus type 2; 2 children to poliovirus type 1 and 3 children to poliovirus type 3; in 16 cases who received 2 dose OPV, 80% demonstrated polioantibodies for all 3 types; and 31 cases who received 3 dose OPV, 90% had polioantibodies for all 3 types. The OPV used in the operation met the standard requirement.

### SPECIFICITY OF URINE TESTS FOR ANTIMALARIALS

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Four urine tests for antimalarials; Haskin's, Eosin Colour, Wilson and Edeson's and Lignin tests were studied at the Hospital for Tropical Diseases in Bangkok. Daily urine sample for 20 days after antimalarial treatment in 41 children with malaria and daily urine sample for 10 days in 10 controls were examined.

Results of Haskin's, Eosin Colour and Wilson and Edeson's tests were: 75-100% of patients (7) given a single dose of 10 mg per kg of chloroquine showed strong reaction till day 4, 15% had such reaction till day 7; 15% - 20% of them had weak reaction till

day 20. All of 12 patients (100%) given quinine 10 mg base eight hourly for 5 days showed strong reaction during the 5 day treatment and lasted 3-4 days after the course of treatment, about 10% of them produced weak reaction till day 20. Almost all of 7 patients (100%) given quinine 10 mg base eight hourly for 14 days showed strong reaction throughout the 14 days of treatment and lasted one to two days after completion of the treatment, the latest of weak reaction in few cases were found 3 days after the course of treatment.

Results of Lignin test were: 15% of

patients (20) given a single dose of sulfadoxine 20 mg per kg plus pyrimethamine 1 mg per kg gave strong reaction at day 1 and 2, about 50% of them had weak reaction till day 4, only 5% had weak reaction till day 9. 70% of patients (7) given a single dose of sulfadoxine 30 mg per kg plus pyrimethamine 1.5 mg per kg gave strong reaction on day 1 and only 15% showed such reaction till day 4, 15% showed weak reaction till day 11.

In control group: 100 urine samples were examined, weak reactions were seen in 4 samples (4%); 3 by Haskin's and one by Eosin Colour tests.

## INCIDENCE OF MALARIA IN KHMER REFUGEES AT SRAKAO, PRACHINBURI PROVINCE, THAILAND

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A total of 686 Khmer refugees (222 from admission ward and 464 from out-patient clinic and camps) at Srakao, Prachinburi Province, were examined. Blood for malarial parasites by thick and thin blood smears were examined on 14 and 21 November 1979. The results showed no significant difference between the two groups. The total positive cases for malarial parasite were 334 (48.6%).

caused by *P. falciparum* 311 cases (45.3%), *P. vivax* 11 cases (1.6%) and mixed infection *P. falciparum* and *P. vivax* 12 cases (1.7%). No *P. malariae* was discovered. The incidence of liver and spleen enlargement showed no significance. The differential white blood count showed a high percentage of eosinophilia. The ratio of malarial parasite density between thick blood smear and thin blood smear was 1.4:1.

# SEROEPIDEMIOLOGY OF MALARIA IN ORANG ASLI CHILDREN IN KELANTAN, MALAYSIA

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A seroepidemiological study was made on Orang Asli (aborigines) children who live a semi nomadic life in the jungles of Ulukelantan, Malaysia. A total of 145 children below the age of 14 years were included in the study. Blood was collected on filter paper from finger pricks. Eluates from these samples were tested using IFA technique. Antigen for this study was prepared from washed schizonts harvested from continuous *in vitro* cultures maintained in the laboratory.

Plasmodium falciparum antibody prevalence rate was about 85% compared to 82% spleen and over 43% parasite rates. Both P. falciparum and P. vivax were present. The age specific pattern of antibody, spleen and parasite rates were that of a hyperendemic

community. There was a positive correlation between antibody and spleen rate up to 9 years. In older children the antibody rates increased while the spleen and parasite rates dropped. Although all slide positive cases were seropositive, there was no correlation between parasitaemia, spleen size and antibody titers. A few children with mixed infections demonstrated some degree of immunosuppression. The significance of the findings was discussed in relation to their socio-economic conditions.

## MALARIA ENDEMICITY IN THE VARIOUS PHYTOGEOGRAPHIC AND CLIMATIC AREAS OF AFRICA, SOUTH OF SAHARA

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Falciparum malaria is highly endemic in most of the Afrotropical Region and should be considered as a component of human ecology.

In Africa there is a succession of concentric climatic and vegetation belts from the rain forest to the deserts. Chorology and ecology of anopheline vectors are highly dependent on climatic and phytogeographic factors.

In predesertic areas malaria is more or less epidemic and humans do not acquire immunity.

In Sahelian Steppes of West Africa, transmission is concentrated during the short wet season. Malaria is unstable, generally mesoendemic.

In Sudan Savannas the transmission reaches its peak during the wet season but extends

during part of the dry season; malaria is stable, hyper or holoendemic. Children develop their immunity after several transmissions seasons.

In Rain Forest, or derived Savannas of equatorial areas, transmission occurs all along the year above the critical level. Children develop a strong immunity before they are four years old. Malaria is very stable. The parasitological index is very often below 50% even when the transmission is very high as it was observed in the Congo (more than one infected bite per child and per night). Breeding places of the main vector A. gambiae are very often man-made.

Malaria indexes are very low among pygmies living under the forest cover where anopheline vectors are generally very rare.

The classification of malaria endemicity

can hardly be applied to the African situations. Even when the parasitic and splenic indexes are below 50% they drop strongly in the children more than 10 years old. According to the indexes the malaria should be classified

as mesoendemic but the development of the premunition leading to a decrease of the indexes in adults are characteristic of holoendemy. Malaria classification has to be reviewed in a more pragmatic way.

# MALARIA AND MIGRATION IN SUGARCANE PLANTATIONS: A STUDY OF SEASONAL MOVES IN KANCHANABURI PROVINCE, THAILAND

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This study examines a process of seasonal migration occurring in Kanchanaburi Pro vince, Thailand. Special attention is given to the role migrant labour plays in replacing or supplementing family labour used on sugarcane plantations. The source of data is from a survey undertaken in early 1980. Analysis of the data reveals interesting insights. Approximately half of migrant workers are family movers, and a substantial number of these migrants come to work on sugarcane plantations seasonally for the past three years. The

decision to migrate is mainly economic in nature eventhough migrant workers do not appear to receive substantial benefits from their movement. The data further show that information concerning job opportunities on sugarcane plantations is obtained either directly through owners of plantations or indirectly through friends or relatives. Lastly, there is convincing evidence which suggests that migrant workers are able to adjust to the way of life on sugarcane plantations, but only a minority of them suffer from malaria attack in the area.

# STUDIES ON THE EPIDEMIOLOGY OF MALARIA IN SUGARCANE PLANTATIONS AT KANCHANABURI PROVINCE OF WEST THAILAND: A PRELIMINARY REPORT

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The studies on the epidemiology of malaria have been carried out since January 1980 at Nong Rhee Canton, Bor Ploi District, Kanchanaburi Province of West Thailand. The area is a relatively newly opened forested agricultural land with sugarcane plantations, and is considered as "a problem area" by the Malaria Division of the Ministry of Public Health. There are a considerable number of migrant labourers (thousands) moving in and out of the area twice a year, i.e. during sugarcane planting (June-July) and sugarcane harvesting time (January-March). Malaria has been found to be endemic, existing throughout the year. It has been postulated that the factors causing persistent and varying degrees of high rates of malaria in some villages of the canton include migration of the labour force, persistent and prevalent vector mosquitoes, more exophilic nature of local mosquito vectors, variable degrees of drug resistance of the parasites, different immune status of various groups of the people, community participation to the Malaria Control Programme, social and economic factors and behaviour of the people in connection with persistent high transmission of malaria in the area, and other unidentified factors. It is proposed that the studies on malaria in Nong Rhee Canton should be made especially on its epidemiology, and social and economic aspects in order to reveal the important factors contributing to the maintenance of the persistent transmission of malaria in the area and the impact of the social and economic components of malaria in 3 different groups of the villagers on the Malaria Control Programme of Thailand. Later on, alternate control measures of malaria in the area should be applied and operated for a period of time in order to obtain satisfactory results, then analysis and assessment of the results would be made finally.

In this investigation three groups of the people in Nong Rhee Canton were included, namely the household owners and relatives, local permanent labourers and migrant temporary labourers. The results of our studies in the dry season of the year during January-April 1980 revealed the low point prevalence of malaria among those 3 groups of the people, ranging from 0.8% to 2.7% and also a low prevalence in villages, i.e. 0-4.6%.

The malarial parasites were found to be more of *P. falciparum* (54.76%) than *P. vivax* (44.05%) and 1.2% of mixed infection.

Studies on local mosquitoes revealed the presence of the principal vectors, i.e. A. minimus every month throughout 5 months. A. maculatus was found occasionally and in small numbers.

# AN INTEGRATED VIEW OF ENTOMOLOGICAL AND PARASITOLOGICAL OBSERVATIONS ON FALCIPARUM MALARIA IN DJOUMOUNA, PEOPLE'S REPUBLIC OF THE CONGO

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A longitudinal malariological survey was carried out during 18 months in a village of the degraded forested area of the south-west of Brazzaville, Congo, dealing simultaneously with entomological and parasitological factors of the transmission.

It appeared that monthly *P. falciparum* malaria infection index of preschool children was relatively low and constant whatever the seasons slightly fluctuating around 31%, with a minimum of 23% and a maximum of 44%.

Regarding this situation the entomological inoculation rates due to A. gambiae s.s. were characterised by the classical 2 well marked peaks at the beginning and at the end of the rainy seasons (more than 2 infected bites per child/night). Even at their lowest they are 10 times higher (0.20) than the average actual parasitological incidence rate (0.021).

The analysis of what is called the "risk", which is the probability for children of receiving one, or more, infected bites each night, showed some important features with the unsurprising high risk correlated to the 2 peaks of anopheline transmission (R more than 0.80) and a lower risk during dry season (R = 0.20) but also with a quite half time decrease of this risk during the rainy season. Moreover, it was not possible to make any correlation between this entomological index and the evolution of plasmodial infection indicated by thin blood films.

In fact the explanation of the plasmodic index noticed was obtained when monthly

transition frequencies positive - negative blood examinations of children were analysed with the reversible catalytic model of Muench which allow the evaluation of the actual incidence  $(\hat{h})$  and recovery rate  $(\hat{r})$ .

Six points thus appeared clearly:

- (1) The recovery rate was usually higher than the incidence rate and this point explain why the plasmodic index observed were below 50%.
- (2) The average value of the recovery rate ( $\hat{r} = 0.04115$ ) means that parasites are thrown out of the peripheral blood in some 24.5 days *i.e.* 3 times faster than the 80 days usually admitted since MacDonald's work.
- (3) The average value of the incidence rate ( $\hat{h} = 0.02107$ ) means that from parasitological point of view a "new" infection appeared each 47.5 days,, or from an entomological point of view, just 7.7 inoculations were actually effective each year (while children could receive about 350-400 infected bites).
- (4) The average values of  $\hat{h}$  and  $\hat{r}$  indicated an equilibruim value of the parasite rate: L = h/h + r = 33.8 which is quite similar to the average plasmodic index effectively observed: O.P.I. = 30.8.
- (5) The expected plasmodic index thus calculated with h and r for each month fitted quite well with the observed prevalence and such a similitude definitively showed the permanent equili-

- brium which occurred between the parasite and its host all long the year.
- (6) The monthly correlative variations of h and f explained fairly well some special features of the observed prevalence such as: the normal increase at the beginning of the rain, at the time of the first anopheline peak; the unusual decrease during the rain and especially at the second anopheline peak and the unusual increase during the long dry season, when anopheline transmission seemed to be at its lowest.

Therefore we fully agree with Bekessy et al., (1976) considering that the estimations of  $\hat{h}$  and  $\hat{r}$  provide some interesting insights into the dynamics of infection with *P. falciparum* even in an area with a very high level of transmission but without seasonal parasitologic variations.

An ecological polyselective adaptative procedures was envisaged to explain how such a permanent dynamic equilibrium could have been attained between the vectors, the parasites and the hosts even before they are four years old in such a stable endemic malaria area.

### INITIAL RESPONSE TO SINGLE-DOSE OF CHLOROQUINE, SULFADOXINE-PYRIMETHAMINE AND PRIMAQUINE IN CHILDREN WITH VIVAX MALARIA

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The prospective study was carried out during July 1979 to August 1980 in 44 children with vivax malaria, one to twelve years of age, at the Hospital for Tropical Diseases in Bangkok. They were randomly assigned to one of 4 regimens. The drug dosage was adjusted to the body surface area based on an adult dose: Regimen CH, chloroquine 600 mg base; Regimen SP, Sulfadoxine 1,500 mg plus pyrimethamine 45 mg; Regimen SP-PR, Sulfadoxine 1,500 mg plus pyrimethamine 45 mg plus primaquine 45 mg base; Regimen PR, primaquine 45 mg base.

Regimen CH 10 cases: In all cases (100%) parasitaemia and fever were cleared within 7 days (average 46.8 hours and 37.2 hours respectively). Relapses were recorded in 5

cases on day 18, 24, 24, 24 and 44 (average 26.8 days).

Regimen SP 10 cases: In all cases, parasitaemia and fever were cleared within 7 days (average 68.6 hours and 45.33 hours respectively). Relapses were recorded in 5 cases on day 13, 16, 19, 21 and 26 (average 19 days).

Regimen SP-PR 10 cases: Parasitaemia and fever were cleared within 7 days in 9 cases (90%), both average 57.3 hours. Relapses were recorded in 6 cases on day 9, 10, 15, 19, 20 and 32 (average 17.5 days).

Regimen PR 14 cases: Parasitaemia was cleared within 7 days in 12 cases (85.7%) average 55.2 hours and fever was cleared within 7 days in 13 cases (92.8%) average

48 hours. Relapses were recorded in 5 cases on day 10,11,15,20 and 22 (average 15.6 days).

The results show that chloroquine is the

most effective drug for initial treatment of vivax malaria in children with rapid clearance of both parasitaemia and fever and delayed relapse.

# ACCIDENTAL HUMAN INFECTIONS BY *PLASMODIUM CYNOMOLGI BASTIANELLII*: A SEROLOGICAL AND CLINICAL STUDY OF TWO RECENT CASES

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Two cases of accidental human infection by *Plasmodium cynomolgi bastianellii* were recently observed in Paris and recalled us that man is highly susceptible of this simian parasite.

Both patients exhibited high fever, nausea, headache and various aches. In one case the *Plasmodium* was observed on blood smears (one per 20,000 red cells) and a successfull inoculation to Rhesus monkeys proved that it was due to *P.c. bastianellii*.

Sero-immunological studies using two tests, a fluorescent antibody test and a cellulose acetate immuno-electro-diffusion assay, were performed with 3 antigens; *P.c. bastianellii*, *P. falciparum* and *P. knowlesi*.

A rapid raise of specific antibodies occurred in both cases. However, using the homologous cynomolgi antigen, the antibody titer was much higher and remained detectable for a longer period, than using the heterologous antigens. This difference was obvious for the precipitating antibodies.

In the second case, blood smears were done too late, after initiation of treatment, and were therefore negative. Nevertheless the simian origin of this infection is highly suspected because this patient was exposed and bitten by mosquitoes infected by the same strain and his antibody response was strictly identical to the first one.

The high susceptibility of normal humans, the striking resemblance of the blood stages of *P. cynomolgi* and *P. vivax*, the low parasitaemia which has always been recorded in human cases, and the favourable evolution of this animal infection in man, lead us to suspect a possible existence of unknown human cases in regions where this infection naturally occurs in monkeys. In such case *P. cynomolgi bastianellii* would be a true zoonosis.

# MEFLOQUINE IN THE TREATMENT OF CHLOROQUINE RESISTANT FALCIPARUM MALARIA IN MALAYSIA

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Preliminary studies in human volunteers have shown that mefloquine is effective in the treatment of acute falciparum malaria.

A local study project was instituted to determine the efficacy of the drug in the treatment of falciparum malaria in a population group living in highly endemic areas in Malaysia. A particular note was also made to detect any side effects the drug may have in cases of G-6-PD deficiency.

### THE EFFECT OF PRIMAQUINE ON THE GAMETOCYTES AND SPOROGONY OF *PLASMODIUM FALCIPARUM* IN THAILAND

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The effect of a single dose of 15, 30 and 45 mg of primaquine base on the gametocytes and sporogony of *Plasmodium falciparum* in Thailand, possibly the chloroquine resistant strain was studied. Primaquine base was given to the naturally acquired falciparum malaria patients who had mature gametocytes. *Anopheles dirus* was used as the mosquito vector by blood feeding through an artificial membrane. It was found that a single dose of 15,30 and 45 mg of primaquine base cleared the gametocytes in an average 5.3,

4.5, 3.8 days respectively compared to 11.4 days in the control. The development of the gametocytes in the mosquito vector was nor accomplished. Infection in A. dirus after primaquine were obtained from the blood of 7 out of 30 patients in which the percentage of infectivity was very low compared to the day before treatment and no sporozoite formation was found. A single dose of 15, 30 and 45 mg of primaquine base when given to the patients with P. falciparum malaria were equally effective as gametocytocide and sporontocide in Thailand.

### SPECIES-SPECIFICITY OF MALARIAL LA, ANTIGENS

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Over the past 4 years we have been daily performing serological tests for human cases of malaria using an immuno-electro-diffusion assay (IED) with a crude soluble extract of *P. falciparum* ring forms. 20,000 tests were realized and compared with the standard fluorescent antibody test (FAT). The results enable us to conclude that among the thermolabile antigens described by Wilson and McGregor, the La<sub>1</sub> antigens induces species-specific humoral responses.

Antibodies against La<sub>1</sub> antigens were demonstrated in 100% sera from P. falciparum infected patients. They were not present in sera from individuals with a proved present infection by P. vivax, P. ovale or P. malariae, unless when they had been living for a long time in hyperendemic P. falciparum regions. In that case a previous infection by P. falciparum can be suspected.

Consequently this data suggest that the presence of antibodies against La<sub>1</sub> character-

izes *P. falciparum*. Thus the use of both serological tests gives some information about the infective species; When FAT is positive and IED also, it indicates a past infection by *P. falciparum*. When FAT is positive and IED negative it indicates that the infection is due to another species.

Using soluble extracts from other hemologous and heterologous species of malaria, we could not demonstrate any precipitin band specific for *P. vivax*, *P. ovale* and *P. malariae*.

While the diagnosis value of La<sub>1</sub> labile antigens is well established, their role in protective immunity is not proved. Antibodies against La<sub>1</sub> appear following a primary attack. Their level do not increase after repeated infections, and may remain for many years in the absence of reinfection. Proliferative responses of lymphocytes from sensitized individuals were inconsistently found in the presence of antigenic preparations containing La<sub>1</sub> antigens.

## CULTIVATION OF *PLASMODIUM FALCIPARUM* IN NORMAL AND HEMOGLOBIN E-CONTAINING RED BLOOD CELLS

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The high prevalence of hemoglobin E in endemic areas of malaria, together with the characteristics of hypochromic microcytic anaemia in carriers of this abnormal hemoglobin, have led to the suggestion that hemoglobin E may provide a protective advantage against malaria infection. Using an *in vitro* continuous cultivation technique, two iso-

lates of *Plasmodium falciparum* obtained locally and one isolate obtained originally from Vietnam were tested for their abilities to grow for 96 hours in normal (AA) and in hemoglobin E-contained red blood cells. Comparable results were obtained for AA,

heterozygous AE and homozygous EE cells under both reduced (17%) and hyperbaric (30%) oxygen tension. It appeared that hemoglobin E did not afford protection against *P. falciparum* malaria under the conditions tested.

### SYNCHRONIZATION AND PARTIAL PURIFICATION OF MATURE FORMS OF *PLASMODIUM FALCIPARUM* IN CULTURE BY SORBITOL LYSIS

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The effect of various sugars in the synchronization of the Malaysian Camp, strain of *Plasmodium falciparum* in *in vitro* culture, especially the sugar sorbitol, was studied. It was found that the mature parasites, i.e., the trophozoites and the schizonts, but not the immature ring forms, were lysed by exposure to an isotonic solution of sorbitol, glucose, or mannitol in distilled water. Exposure of the parasites to inositol, sucrose. and mannose solutions appeared to have no lytic effect. Multiple treatment of the *P*.

falciparum culture by sorbitol resulted in relatively high synchronous growth of the parasites. Free merozoites could be isolated from the culture supernatant at the time of reinvasion occurred. Moreover partial purification of the trophozoites and the schizonts from the synchronous culture of P. falciparum could be done by sorbitol lysis followed by differential centrifugation. This method provided a good source of the parasites for immunochemical studies of the stage-specific antigens.

### DISTRIBUTION OF CHLOROQUINE IN P. BERGHEI-INFECTED MOUSE ERYTHROCYTES

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The ability of *Plasmodium berghei*-infected mouse-erythrocytes to accumulate chloro-

quine is increased during the course of parasite maturation. Erythrocytes infected with

late stages of parasites accumulate up to 10 times more chloroquine than those infected with early stages. At low concentration of chloroquine (10<sup>-8</sup>M), differential fractionation of infected erythrocytes by saponin lysis or N<sub>2</sub>-decompression showed that a major proportion of the drug was found with the parasites, while minor proportions were found in the host cytosol and host membrane fractions. However, at higher concentration of chloroquine (10<sup>-6</sup>M and 10<sup>-3</sup> M), the proportion of drug in the host cytosol increased, while the host membrane-bound proportion

remained constant. Similar observation was found by using freeze thaw lysis. Unlike protease-treated cells which can accumulate comparable amounts of chloroquine, the drug in *P. berghei*-infected erythrocytes could not be easily washed out. Inhibitors of glycolysis or 2, 4-dinitrophenol can cause gradual leakage of the drug from intact infected erythrocytes. From this evidence it was therefore concluded that *P. berghei*-infected erythrocytes might accumulate chloroquine via an ATP-dependent active proton pump which was probably located on the membrane of the food vacuoles.

### ASYMPTOMATIC MALARIA INFECTIONS AND IMMUNITY IN MICE

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It has been found that a para-amino benzoic acid - deficient diet (PABA-DD) can totally suppress the infections of *Plasmo-dium vinckei petteri* in mice. Mice treated in this manner show strong resistance to parasite challenge. This implicates that asymptomatic infections may involve the immune responses leading to immunity.

In the present study, mice were subjected to infections with *P.v. petteri* and concurrently treated with PABA-DD for 56 days. Parasitaemias and the antibody titres were measured. The results were compared with those obtained from mice with the normal infection.

In the normal infections, parasitaemias rose rapidly until mice die or parasitaemias declined after reaching the peak. However,

these progressive parasitaemias did not hamper antibody production in the mice. Mice which survived from the infection, showed elevated IgM and IgG titres throughout life.

In contrast, the treated mice showed asymptomatic infections even after the treatment was terminated. Suprisingly important however, when it was found that the antibody production in these mice too was normal. Both the IgM and IgG titres remained high during or after the treatment period. Groups of these mice were later challenged with the homologous or the heterologous parasites. The results indicated that the immunity induced in this manner can confer protection to the homologous as well as to the heterologous parasites.

## COMPARISON OF COUNTERIMMUNOELECTROPHORESIS WITH IMMUNODIFFUSION TEST IN THE DIAGNOSIS OF AMOEBIASIS

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The counterimmunoelectrophoresis (CIEP) was compared with the immunodiffusion test (IDT) in regard to sensitivity and specificity and for its use as a diagnostic aid for amoebiasis in Jakarta. 182 sera were compared: Group I comprised of 23 cases of amoebic dysentery, group II of 104 cases of amoebic liver abscess and group III of 55 sera derived from healthy adults and non amoebiasis patients. The results showed that the CIEP was superior to the IDT as regard to sensitivity and specificity for amoebic disease since it was positive for all 23 sera from amoebic dysentery whilst the IDT was positive in only 15 of the group. It was furthermore positive for 103 sera (99 %) from the liver abscess group whilst the IDT was positive in only 57 sera (54.8%). In the control group both tests were negative for all sera (0%). The greater sensitivity of the CIEP was also shown by following the levels of antibody after metronidazole therapy of two individuals with liver abscess and of 8 individuals with colonic amoebiasis each month for 2-12 months. The CIEP was found consistently positive each month during the period of observation whilst the IDT showed irregular positive reactions during a shorter period. On comparison of the titers obtained by titration of 12 sera from liver abscess group by the two tests it was found that the IDT showed a better gradient of the antibody levels and giving two to four fold higher titers than the CIEP. In these studies it was also observed that some sera from patients were positive in the IDT and negative in the CIEP. This together with its use for titration of high level antibodies and its simplicity the IDT was found useful as an adjunct to the CIEP for the detection of antibodies in amoebiasis.

### SERIOUS ISOSPOROSIS BY ISOSPORA BELLI: A CASE REPORT TREATED BY FANSIDAR

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Coccidiosis infections are generally quite well tolerated, nevertheless in 9 cases per thousand the patients exhibited chronic diarrhoea, non absorption and debilitated general conditions. During 4 years such a patient received various therapies but without any improvement. Finally Fansidar was been administered on a weekly basis for 5 weeks, at a weekly dose of 3 tablets (25 mg of pyrimetha-

imine and 500 mg of sulfadozine per tablet). Symptomatologically there was tremendous

improvement within a week and the patient was cured at the end of the treatment.

### EFFECT OF MASS TREATMENT AND IMPROVEMENT OF ENVIRONMENTAL SANITATION ON THE ENDEMICITY OF HOOKWORM INFECTION

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Regarding the hypothesis set up from the results of the preliminary epidemiological survey, it showed that the control measures of hookworm infection in the community of Panasnikom District, Cholburi Province had the tendency to be able to control the endemicity down to the lower degree, if it would be possible to encourage the people to construct the sanitary latrines, up to 75% of the number of the households and to reduce the prevalence of the infection down below 9%.

The experiment had been done on the population of the two villages in the same area of preliminary survey. These two villages had no difference in the point prevalence and the intensity of infection. The samples were the population over 5 years of age which were proved as population at risk. One village was selected as experimental cohort with 232 complete follow up cases while the other village was a comparison cohort with 286 complete follow up cases.

During the dry season, around January, of the first and second years of study, the whole population of the experimental village were given a single dose of pyrantal pamoate, 10 mg per kg body weight, as mass treatment.

Encouragement of latrine construction had been carried out in a four year period of study and the percentage of the households constructed the latrine gradually increased from 17% to 52%.

Point prevalence rate in both cohorts had been studied by stool examination with Beaver standard smear method, zinc sulphate concentration method and modified culture method. The intensity of infection was measured via number of eggs per milligramme of faeces. The prevalence rates of the experimental cohort had been observed many times and they varied from 82.7% at the beginning of the study to 45.7% in the second observation, 7 days after the first mass treatment and it increased up to 65.1 % withing one year after that. After the mass treatment in second year of experiment the prevalence was reduced again, then the year after it was increased up to 68.9% by reinfection. The changing of point prevalence between the two follow up years was very little. In the comparison cohort, the point prevalence tended to increase every year. The mean egg count of the two cohorts observed at the same time of prevalence rates showed better pattern of endemic changes than the prevalence rates. In the second year of the experiment the

rainfall was very heavy thus it influenced on the endemicity of hookworm infection, the intensity of the both cohorts decreased. Then during the follow up year the amount of rainfall was rather low so the soil provided a poor condition for the reinfection; therefore, the increasing intensity of infection of both cohorts showed no significant difference.

Mean egg count which was examined once a year might have been considered to use in the statistical analysis for comparison of the dynamics of the disease within a period of year between the two cohorts. It could demonstrate the unit of the intensity reduction and the product of combination between the residue of the intensity reduction during the mass treatment or dry season and the reinfection during transmission period in the rainy season.

Mass treatment could reduce the level of endemicity and frequency of reinfection but it could not retain them at the low level if the environmental sanitation could not be improved to a satisfied quantity and quality.

### ANCYLOSTOMIASIS AND ITS PROBLEM IN INDONESIA

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A survey of intestinal parasites using Kato's method, among 246 children at the primary school in a rural area with low and moderate socio-economic levels were carried out. A modified Harada-Mori's method was used to culture 232 stool specimens.

Two hundred and thirty five children were examined hematologically e.g. haemoglobin, haematocrit, reticulocyte, serum iron, total iron binding capacity, saturated transferrin, and differential counting; anaemia was determined by criteria of W.H.O. and "rule of five", and for malaria parasites.

Among the parasites found, Ascaris lum-

bricoides and Trichuris trichiura are the highest in frequency (100%), followed by hookworm (92.68%).

Hematology finding revealed iron deficiency anaemia (63.17%) and  $B_{12}$  and folic acid deficiency anaemia (19.3%).

The stool cultured revealed *Necator americanus* (78%), *Ancylostoma duodenale* (12%) and none was found in 10%.

Considering all the facts, ancylostomiasis eradication should be carried out with improved environmental and personal hygiene, simultaneously with health education.

### TREATMENT OF TAENIASIS WITH PUAG-HAAD POWDER FROM ARTOCARPUS LAKOOCHA WOOD

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Puag-Haad is a local anthelmintic for tapeworms, traditionally used by the natives of the northern and northeastern parts of Thailand. It is prepared by simply boiling wood chips of *Artocarpus lakoocha*. Each patient was given 5 gm of the power on a fasting stomach in the morning, followed

by saline purgative and stools collected within 8 hours after purge were searched for the scolices of the tapeworms. Among the 32 cases treated, scolices of *Taenia saginata* were found in 24 cases and of *Taenia solium* in 2 cases. The common side effects were nausea and vomiting.

# THE EFFECT OF LIGHT AND TEMPERATURE ON THE RELEASE OF SCHISTOSOMA JAPONICUM CERCARIAE BY ONCOMELANIA HUPENSIS LINDOENSIS

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Shedding of S. japonicum cercariae by infected snails (O.h. lindoensis) was studied under two different temperature conditions over a period of 28 hours with three subsequent changes of the light conditions. Results of this studies showed that shedding of cercariae by the snails was influenced by both temperature and light intensity, but

temperature appeared to have a greater influence than light intensity. At 25° C, cercariae were released both in dark and high light intensity. At 30°C the number of cercariae released was significantly smaller as compared to 25°C. Likewise release only occurred under high light intensity. The importance of this aspect was discussed.

## THE SUSCEPTIBILITY OF TRICULA APERTA ON VARIOUS DOSAGES OF SCHISTOSOMA MEKONGI MIRACIDIA

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This experiment was designed to investigate the susceptibility of *Tricula aperta*, beta and gamma races, to the varying number of miracidia of *Schistosoma mekongi*.

Groups of 200 wild caught *T. aperta* (beta and gamma races) were exposed individually to 1, 2, 4, 8 and 16 miracidia of *S. mekongi* and were screened for cercarial shedding after 35 days of exposure. Screening was continued up to the 63 rd day and all negative snails were crushed and dissected. Snails found with the sporocyst were considered positive.

Laboratory test showed that the infection rates by 1, 2, 4, 8 and 16 miracidia in the beta race snails were 18.0, 29.5, 41.0, 58.5 and

61.5%, while in the gamma race snails were 7.0, 9.5, 9.5, 25.5 and 42.0%, respectively.

The mortality rates for both control groups, uninfected beta and gamma race snails were 0.5% but in the infected groups of the beta race snails were 2.5, 2.5, 2.0, 6.5 and 12%, while the gamma race snails were 3.5, 2.5, 2.5, 1.0 and 6%, respectively.

The experiment showed that the infection rates of *T. aperta* correlated well with the number of *S. mekongi* miracidia. At the same time, the mortality rate was also increased if the snails were exposed to more number of miracidia. The appropriate dose - infection rate of *T. aperta* for laboratory maintenance was discussed.

# COMPARISON OF CIRCUMOVAL PRECIPITIN TEST (COPT) FILTER PAPER METHOD AND FORMALIN - ETHER CONCENTRATION TECHNIQUE IN SCHISTOSOMIASIS FIELD SURVEY

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Schistosomiasis prevalence rate is higher among inhabitants of Barrio Buri than Barrio Pitogo as shown in both stool (formalin-ether and blood) COPT (filter paper) examination. The latter method of

examination appears to be more sensitive than the former in the detection of schistosomiasis cases because about 16% of cases are liable to be missed with stool (formalinether) concentration technique. There was not a single schistosomiasis case detected by stool (formalin-ether) concentration method that was not picked up by blood COPT (filter paper) method. Schistosomiasis is more prevalent in adults than in children and higher in males than in females in all age groups.

Among children, schistosomiasis is also more prevalent in males than in females with the youngest case found in a 5- year-old male. Males appear to acquire the infection earlier than females.

A blood COPT (filter paper) method is preferred than a stool (formalin-ether) concentration technique because it required less work and less time; it is not messy yet is very sensitive and highly specific. The method of obtaining blood from a finger prick (COPT filter paper) is more readily acceptable by subjects than the standard COPT method where blood specimen has to be obtained by veni-puncture.

Stool samples from 448 inhabitants of Barrios Pitogo and Buri of the Municipality

of Jaro, Leyte were examined and analyzed. There was a more or less equal overall prevalence rates for Ascaris, Trichuris and hookworm in the area. As expected, prevalence rates for Ascaris and Trichuris are slightly higher in children than in adults while rates for hookworm, schistosomiasis, paragonimiasis and taeniasis are higher in adults than in children. Except in few instances, males have higher parasitic prevalence rates than females. In addition to the aforementioned intestinal helminths, the area is likewise endemic for schistosomiasis, paragonimiasis and taeniasis at relatively high prevalence rates.

In conclusion it can be stated that circumoval precipitin test (filter paper) method is superior than formalin-ether concentration technique in the detection of schistosomiasis cases. Therefore this method is recommened for all schistosomiasis surveys, as well as for rural physicians in remote endemic areas for diagnosis of suspicious subclinical cases whereby specimens may be sent through the mail to a central laboratory doing circumoval precipitin test (COPT) for schistosomiasis.

# THE EFFECT OF PRAZIQUANTEL, LEVAMISOLE AND FANSIDAR AGAINST GNATHOSTOMIASIS IN HAMSTERS

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Gnathostomiasis is one of the major public health problems in Thailand and it has been estimated that about 900 people suffered each year. In the past decades various drugs had been used, none have been considered

effectuous in the treatment of gnathostomiasis. In this study various regimens of Praziquantel, Levamisole and Fansidar were tested in hamsters. None of them was effective against gnathostomiasis.

## ANGIOSTRONGYLUS CANTONENSIS: BIOGENIC AMINES IN THE LUNGS OF INFECTED RATS

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Sequential estimation of the levels of histamine, serotonin, norepinephrine and dopamine was done on rats parasitized by lungworm (Angiostrongylus cantonensis) between 30 and 75 days post infection. The highest level of histamine in the infected lungs was 52.10 µg/gm tissue, 13 times higher than the level found in control rats. The level of serotonin rose from the normal level of 6.41

µg/gm tissue to 10.27 µg/gm tissue after the worms had become lodged in the pulmonary artery for 15 days. There were no changes in norepinephrine or dopamine. Studies of host cell response to infection revealed that the rises in histamine and serotonin levels corresponded to a rise in the lung population of mast cells, suggesting that these cells produced the amines.

#### SERUM PROTEINASE INHIBITORS IN OPISTHORCHIASIS

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In mild and moderate *Opisthorchis viverrini* infection, it is difficult to detect clinical signs for diagnosis. Also routine clinical chemical reactions often failed to be detected in these patients. In 1974, Schelp *et al.*, reported the increase of serum ceruloplasmin-haemopexin band in infected people compared with non-infected people by using polyacrylamide gel electrophoresis. In this study the serum proteinase inhibitors, alpha<sub>2</sub>-macroglobulin, alpha<sub>1</sub>-antitrypsin and alpha<sub>1</sub>-antichymotrypsin which cannot be detected by the above method were investigated using rocket immuno-electrophoresis. Serum of 63 patients suffering from opisthorchiasis and 40 healthy

controls were determined for proteinase inhibitors. All these three proteinase inhibitors were increased significantly in infected group compared with controls. In infected group, serum alpha<sub>1</sub>- antitrypsin correlated with alpha<sub>1</sub>-antichymotrypsin, and serum glutamate oxaloacetate transaminase(SGOT). Serum alpha<sub>1</sub>-antichymotrypsin correlated not only with alpha<sub>1</sub>-antitrypsin and SGOT but also with SGPT (serum glutamate pyruvate transaminase). The increase of serum proteinase inhibitors together with other biochemical liver function tests may be useful as a guideline for the impairment of liver function and the host response to O. viverrini infection.

#### ALTERED COLLAGEN METABOLISM IN LIVER FLUKE DISEASE

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The histopathological studies in the liver infected by *Opisthorchis viverrini* has indicated liver fibrosis which can result in deterioration of liver functions. This finding has drawn our interests toward the effect of opisthorchiasis on hepatic collagen metabolism. Therefore, the biosynthesis of collagen, as determined by following the activity of enzyme prolyl

hydroxylase has been studied in hamsters infected with metacercarial cyst of *O. viverrini*. The result has shown an increase in prolyl hydroxylase activity as well as the collagen content in the infected liver when compared to uninfected control, and therefore suggested some abnormality in collagen metabolism in *O. viverrini* infected liver.

### VARIATIONS OF SERUM PROTEINASE INHIBITORS IN CLINICAL MALNUTRITION

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According to the classical theory, kwaskiorkor is caused by a lack of protein in the food and sufficient energy intake, whereas marasmus is the result of an insufficient protein and energy content of the diet. However, there are strong evidences that in addition to dietary intake other factors determine whether kwaskiorkor, marasmus or an intermediate form like marasmic-kwaskiorkor develops. There are reports from India that children on the same types of cereal based diets, some developed kwaskiorkor and others marasmus. Furthers marasmus and kwaskiorkor could be seen in the same children at different points of time. Based on these observations it had been postulated that marasmus is the end result of adaptation to protein-euergy malnutrition whereas kwaskiorkor represents a breakdown in the process of adaptation. One of the factors which may impose additional stress on the adaptation process is infection which is more common in kwaskiorkor than in marasmus. Among the responses of the organism to infection an increase of so called acute phase (AP) reactant proteins in human serum usually takes place. Some of the AP reactants proteins belong to the group of human serum proteinase inhibitors (PI), especially alpha 1- antitrypsin (AT) and alpha<sub>1</sub>- antichymotrypsin (Ach). An increase of PI in children suffering from marasmus and developing infection may be responsible, at least partially for the derangement of homeostasis in kwaskiorkor. It might be argued that production of PI in malnourished children may impair muscle protein breakdown. This results in inadequate mobilisation of endogenous amino acids which is necessary to maintain hepatic protein synthesis. In order to support this hypothesis AT and Ach had been studied together with albumin, prealbumin, and transferrin in 14 children with clinical PEM. All were suffering from infectious diseases mainly upper respiratory tract infection or dysentery. In seven children liver enlargement and edema were found. Since edema is the sine quanon of kwaskiorkor, these seven children were considered to have kwaskiorkor or marasmic-kwaskiorkor and the rest were considered to have marasmus. Nine healthy children were studied as controls. Plasma levels of Ach were higher in children with edema compared to either the control group or children without edema. AT levels showed no significant differences between the groups but tended to be higher in children with edema. The ratios of AT to albumin, prealbumin and transferrin were high in these children. In an additional study, where variations of PI in PEM children had been

observed for 50 days during treatment, attention had been focused to alphamacroglobulin (alpha<sub>2</sub>-M) a PI in human sera, but not belonging to the AP proteins. Alpha<sub>2</sub>-M had been found to be low in clinical PEM but it seems that the synthesis of this PI during treatment has a certain priority over the synthesis of other proteins like albumin when the level is very low before treatment. In case the early phase of recovery of the patient is complicated by infection, AT increased adding its inhibitory capacity to that of alpha<sub>2</sub>-M. Then the patient might be in a dangerous situation, where the balance of destruction of endogenous protein and the synthesis of proteins essential for maintaining homeostasis is disrupted by these PI despite treatment.

This hypothetical concept of PI action in PEM presumes that muscular proteinases are inhibited by serum PI, which could be proved for AT, and alpha<sub>2</sub>-M in vitro. However, further investigations are necessary for in vivo studies about the invasion of serum PI into the muscle cell or otherwise the relationship of serum PI to muscle PI in PEM associated with infection.

### ECCHINOCOCCAL CYST OF LUNG: THE FIRST INDIGENOUS CASE IN THAILAND

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A married woman aged 56 years was found, during a routine physical checkup, to have a well-defined mass in the anterior basal segment of the left lower lobe of lung (LLL). Tomographic study revealed well circumscribed non-calcified lesion. Obstruction of

bronchus supplying the anterior basal segment of LLL was noted by bronchography and bronchoscopy. Cytological study was negative for malignancy. Eosinophilia (560/mm³) was observed only in one of the two WBC examinations.

On October 30, 1975 thoracotomy was performed. Anterior basal segment of LLL was resected and found to contain a subpleural cyst which measured 2 × 2 cm. The

pathological findings were consistent with an ecchinococcal cyst.

The incidence, the epidemiology and the follow-up of this patient was discussed.

#### SNAKE BITE IN JAKARTA

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During 1978-1979, a total of 59 cases of snake bites were treated in our hospital. 79.6% of patients were below the age of 29 years, with a male - female ratio of 2:1. Bites frequently happened in the lower extremities (59.0%) and occurred often between 6 p.m. and midnight. The peak occurrence of snake bite was recorded in the month of July. Only 45.6% of the patients

could identify the species of snake. Most of the patients received medical treatment within 6 hours (84.6%). There were 22 cases of systemic poisoning, of which 4 were severe. All patients recovered and the fatality rate therefore was zero. A special case of bleeding tendency caused by a non-poisonous snake bite also recovered.

#### HOW DO THE FLIES DISTRIBUTE OVER INTO THAILAND

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Surveys of flies in the families Muscidae, Calliphoridae and Sarcophagidae in the different geographical parts of Thailand were made for zoogeographical study and their medical importance. From the studies it was found that most species seemed to distribute from China into the northern part and migrate throughout the eastern, central and western parts of Thailand. Most species collected in the western part were also similar to the species found in Burma where some of those species originated in India. General species of flies below the Isthmus of Kra were similar to the species found in Malaysia. This study based on the specimens which were collected by our teams only was discussed.

# THE SURVEY ON PARASITOIDS OF MEDICAL IMPORTANT FLIES IN THAILAND

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Synanthropic flies are admittedly important carriers of enteric infection. Resistance to insecticides by the species and chemical residues left in the environment have resulted in increased emphasis on biological control. Recent emphasis was placed on parasitoid as it was recognized that many species were involved in natural fly reduction.

The first survey of native parasitoid species attacking fly pupae was conducted at garbage piles of 31 provinces in Thailand from 1978-1979. The predominant fly species found breeding in study sites were *Musca domestica* and *Chrysomyia megacephala*. The parasitized pupae gave the emergence of 14 parasitoid species. *Spalangia endius* was the most widely distributed throughout the study sites accounted for 85.3%, followed by

S. nigroaenca with 44.1% of all study sites, Pachycrepoideus vindemmiae (23.5%), Dirhinuscrythroccrus (8.8%), S. cameroni, S. gemina and Trichopria sp. (5.9%) and 2.9% for each species of Brachymeria minuta, D. excavatus, Exoristorbia philippinensis, Psilus sp., Teleus spp, and unidentified Spalangia spp. Maximum parasitoid activity (55.3% parasitization) was found in Suphan-Buri whereas Bangkok at Phrakhanong section, Chumphon and Kalasin rendered no parasitization. Most study sites revealed rather low percentage parasitism.

As it was single sampled-survey, it can be expected that some parasitoid species were missed. Further collections perhaps in different seasons might reveal the presence of other species.

### STUDIES ON THE EPIDEMIOLOGY OF SUB-PERIODIC BRUGIA MALAYI IN MALAYSIA: ENTOMOLOGICAL ASPECTS

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The dynamics of the transmission of subperiodic *Brugia malayi* in a typical endemic area in North Malaysia was studied over a period of  $3\frac{1}{2}$  years. This is an area of intense zoonotic transmission from the adjoining forest into the oil palm and rubber estates. Infection rates in the 3 species of *Presbytis* i.e. obscura, melalophos and cristata varied from 60% to 85% with *Brugia malayi* during the period. The infection rates in the three groups of population varied from 4% at Pondok Tanjong to 6% at Foong Lee and

Pondoland estates. Despite 6 mass treatments with diethylcarbamazine, infections still persisted with the finding of new cases and mosquito infections with infective larvae. The primary vectors were *Mansonia bonneae* 

(34 infections), M. uniformis (11) and M. indiana(4) respectively in order of importance. Other entomological observations on flight range and distribution over the months were also discussed in some detail.

### THE CURRENT STATUS OF MALAYAN FILARIASIS IN THAILAND

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Malayan filariasis in Thailand at the present time is localized causing a public health problem only in small rural areas where some cases have been reported. The control programme of filariasis in Thailand during the last 19 years (1961 - 1979) covering the six endemic provincial areas of malayan filariasis with a total population of about

1.5 million (people at risk) has produced satisfactory results. The filariasis infection rates have decreased from 14.1% in Chumporn province in 1964 and 26.4% in Surat-Thani province in 1963 to 0.31% and 0.42% in those 2 provinces in 1979 respectively. The details of the results are as follows:-

Province	Filariasis Infection Rate (FIR) %		Current 1979	
	Previous data	in 1974-1976	FIR %	Microfilarial infection rate
1. Chumphon	14.1	0.30	0.31	0.014
	(1964)	(1974)		
2. Surat Thani	26.4	0.24	0.42	0.007
	(1963)	(1976)		
3. Nakhorn-Si	7.7	0.02	0.40	0.017
Thammarat	(1961)	(1976)		
4. Phatthalung	61.7	0.18	0.05	0
	(1962)	(1965)	(1978)	(1978)
5. Pattani	8.0	0.13	0.73	0.252
	(1971)	(1976)		
6. Narathiwat	11.1	0.89	0.24	0.187
	(1963)	(1976)		

The procedures of the filariasis control programme consisted of 4 major operations including (1) mass surveys to identify microfilarial carriers and cases with elephantiasis, (2) in the high endemic areas, application of mass treatment followed by re-treatment of the still positive microfilarial carriers with diethylcarbamazine, (3) in the low endemic areas, application of individual treatment with diethylcarbamazine followed by re-treatment if necessary, and (4) assessment of the

control measures by the study on the filarial infection in vector mosquitoes.

It has been noted that the DDT residual house spraying once or twice yearly through the National Malaria Eradication Programme in Thailand has contributed to the periodic decreases in the number of *Mansonia* mosquitoes particularly those comparatively marked indoor-biting species, which are the main vectors of malayan filariasis in Thailand.

## FILARIASIS IN DIFFERENT ECOTYPES IN WEST FLORES, INDONESIA

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Filariasis was studied in different ecological conditions in West Flores, Indonesia. The village of Jengkalang was situated near the coast; the village of Rabo was situated more inland in a rice field, while Mahima was located high up on a hill. In Jengkalang, among 139 people examined, 39% showed microfilaraemia. Mixed infections of Brugia timori and Wuchereria bancrofti were found, but more showed W. bancrofti infections, In Rabo, 108 villagers were examined, of which 28% showed B. timori microfilaraemia. In Mahima, a village situated 300 metres above sea level on a very steep hill, 20% out of the 239 people examined showed microfilariae of B. timori in their blood.

In general, filariasis occurs more in low

land areas, although present in hilly regions, the infection rates are usually lower. The microfilaria rate in Mahima is somewhat higher than expected which may be attributed to the movement of people. School children usually go to school in the lower villages while adults work in rice fields in the valley where filariasis is endemic. Nevertheless, people who never left the village also harbour microfilariae, which showed that transmission also occurs in the village itself.

The infection rates also showed differences. Jengkalang, although having the highest microfilaria rate, showed little clinical manifestations, while Rabo has the highest infection rate. Apparently W. bancrofti infection in that area does not show distinct clinical symptoms.

# DETERMINATION OF THE SUSCEPTIBILITIES OF LABORATORY BRED AEDES TOGOI AND ANOPHELES MOSQUITOES TO BRUGIA PAHANGI

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Seven species of mosquitoes were tested for susceptibilities to *Brugia pahangi*. They were allowed to feed on a cat with microfilarial densities which ranged from 10.2 to 13.4 mf per c.mm, and dissected 12 days after feeding. The infective rates of *An. sinensis*, *An. lesteri*, *Ae. togoi*, *An. campestris*, *An. maculatus*, *An. vagus* and *An. dirus* were 84.8 %, 79.5 %, 68.8 %, 46.9 % 21.5 %, 11.1 % and 0 % respectively.

In conjugation with a study of the levels of susceptibility, comparative degree of migration of *B. pahangi* were studied in *An. sinensis*, *Ae. togoi*, *An. maculatus* and *An. dirus*. These studies revealed that variations in the dynamics of filarial migration among vector species could depend in part on the degree of development of cibarial armatures in the fore-gut of mosquitoes. Microfilariae

were killed by the chewing action of the cibarial armatures and the proportion of ingested microfilariae that were killed was largely dependant on the presence and shape of these armatures. An. dirus has a well developed cibarial armature and killed 97.4% of ingested microfilariae. An. maculatus has a poorly developed cibarial armature and killed only 36.8% of the microfilariae. An. sinensis and Ae. togoi lack cibarial armatures. They killed only 0.8% and 2.7% of microfilariae.

In An. dirus the effects of the cibarial armatures were circumvented by injecting microfilariae into the thorax. These worms developed normally when compared with Ae. togoi and the body length and width of infective larvae obtained from these two species were not significantly different.

# STUDIES ON AQUATIC PLANTS AND GRASSES AS BREEDING HOSTS FOR MOSQUITOES

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Many aquatic plants and grasses act as breeding sites for *Mansonia* mosquitoes. Our studies revealed that there were 3 types of 11 species of aquatic plants and grasses in this area, including Type A: Floating

plants, i.e. (1) Pistia stratiotes, (2) Salvinia auriculata and (3) Eichhornia crassipes; Type B: Floating and semi-fixed plants, i.e. (4) Jussieae repens (Water pung-puay), (5) Hymenachne pseudointerrupta and (6)

Rattan grass (Ya wai); and Type C: Fixed plants, i.e. (7) Monochoria vaginalis, (8) Crinum thaianum (Water plubpleung), (9) Carex sp. (Ya-com-bang), (10) Scirpus grossus (Kok) and (11) Thelypteris sp. (Fern).

Searching Mansonia as well as Ficalbia mosquito larvae from aquatic plants and grasses in many shallow water-beds and swamps in Bangluke canton, the procedures of which were the same as Wharton. A hollow metal cylinder about 2 feet in diameter was pushed into the shallow swamp so that it lower end rested on the soil at the bottom of the swamp. The aquatic plants, grasses, etc. were pulled up and shaken vigorously in the water in a container; then the contents were bailed out and poured through the sieves. The materials collected by the sieves were washed in clean water which were then transferred into white enamel pans for observation of mosquito larvae.

As for the collection of floating water plants in the swamp, the manual method consisted of the collection of all water plants in an area of about 2 feet in diameter, for each collection. The collected aquatic plants were shaken vigorously in the water in a container, and then subsequent procedure was the same as described above.

The results revealed that of all 2,791 mosquito larvae collected, 1,526 were examined for their species. 44.7% were M. uniformis, 23.7% were Ficalbia hybrida, 11.9% were M. annulifera, 10.6% were M. indiana, 4.5% were M. crassipes and 2.6% were M. ochracea. There were also some larvae of M. bonneae, M. annulata, F. minima, F. chamberlaini, F. luzonensis and F. fusca.

As for the location of Mansonia mosquito species to the aquatic plants and grasses, it was found that the larvae of M. uniformis, M. indiana and M. annulifera were recovered from most plant species except Salvinia sp. where M. annulifera was predominant. M. annulata and M. bonneae were recovered from Crinum thaianum and Thelypteris and M. bonnea was also found from Jussieae repens and Rattan grass (Ya wai).

# STUDIES ON OVIPOSITION CHARACTERISTICS OF MANSONIOIDES MOSQUITOES WITH THE OBJECT OF FINDING A NEW METHOD FOR THEIR CHEMICAL CONTROL

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Mansonia (Mansonioides) mosquitoes, important vectors of Malayan filariasis, attach their eggs in clusters on floating leaves of aquatic plants. It is known that the gravid females prefer certain sites for oviposition and that egg-clusters are therefore found in aggregations. This behaviour could

offer a new method for their chemical control. We believe that a treatment of the aquatic vegetation, limited to areas of high attractiveness for ovipositing females, could be an effective method, especially if the breeding place is of controllable size. This method has one important advantage over the much

quoted alternative, the herbicidal destruction of the aquatic vegetation as it causes less damage to the ecosystem of the breeding place. This criterion should be taken into consideration, as *Mansonia* breeding places often serve as a source of daily food (fishes, crustaceans, vegetables etc.) for the rural people.

Studies are in progress to analyse the distribution pattern of *Mansonia* egg-clusters and to identify the factors which are responsible for the final choice of a certain host plant for oviposition. These studies shall clarify whether it is possible to predict places of high attractiveness for gravid *Mansonia* females. This would greatly simplify an economical application of insecticides.

Our studies revealed that the egg-cluster distribution follows a negative binomial expectation, a mathematical model for aggregations. This is found when variously sized sub-units of the breeding place are sampled,

the largest being an area of 100-200 m<sup>2</sup>, the smallest being the single plant. It seems that the factors which are responsible for the aggregations are characteristic for each size of sub-unit. They interfere in a certain hierarchical order with the flight of a *Mansonia* female in search of a suitable plant for oviposition. The highest category of factors leads to an egg-cluster aggregation in a certain large area (100-200 m<sup>2</sup>) of the breeding place, lower categories then lead to aggregations in progressively smaller sub-units of that area. The lowest category finally determines cluster aggregations on certain leaves of certain plants.

The nature of the factors is so far poorly understood and remains hypothetical. However it seems to be clear that the attractiveness of a certain plant species acts only as a factor within a relatively small area of 1-2 m<sup>2</sup>. We have therefore no guarantee that plants known to be attractive to *Mansonia*, e.g. *Pistia* or *Salvinia*, always harbour large numbers of egg-clusters.

## STUDIES ON THE COLONIZATION OF MANSONIA MOSQUITOES IN THAILAND

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Mansonia uniformis, M. indiana, M. annulifera and M. bonneae were successfully reared and bred in our insectaries. As for M. annulata, we could rear it up to the 2nd stage larvae, i.e. for about 10 days after hatching from egg, with the larva survival rate of 6.6%. After this period all larvae would die.

The blood meal and oviposition were easily carried out in all *Mansonia* species. In general *Mansonia* mosquitoes feed within 24-48 hours after emergence, and oviposition takes place 2 - 5 days after a blood meal. The oviposition is readily made on the under - surface of the leaf of *Pistia stratiotes*.

The larvae hatch from eggs within 3-6 days. The first stage larvae are reared in water containing dry and ground guinea pig dung infusion of 5 gm per litre (prepared on the day of its oviposition). This infusion is used as culture media in various dilution, but it has been found that \(\frac{1}{4}\) dilution gives the best result. The aquatic host plants which we usually use are Pistia stratiotes and Eichhornia crassipes. The percentages of survival of the larvae as observed on the 10th day after hatching were 73.5%, 72.6%,

69.1%, 13.5% and 6.6% in M. uniformis, M. indiana, M. annulifera, M. bonneae and M. annulata respectively while the percentages of adult emergence of these species except M. annulata were 26.9%, 18.8%, 12.7%, 14.1% respectively.

It has been observed that the survival rates of adult *Mansonia* mosquitoes in our insectaries was rather low. Further experiments to improve the techniques in rearing these *Mansonia* mosquitoes are still in progress.

### OBSERVATION ON TYPHOID ENCEPHALOPATHY IN CHILDREN

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During a two year period, 120 children constituting 1.2% of the 10,200 admissions in the Department of Child, Health, Cipto Mangunkusumo Hospital Jakarta were admitted for typhoid fever. All these cases were serologically and or bacteriologically positive.

Fourteen out of the 120 children (11.7%) presented a clinical picture of typhoid encephalopathy. Four cases were under 5 years of age, and there was a male preponderance viz. nine of them being boys.

The neurological symptoms and signs

in these 14 cases were: variable degrees of impairment of consciousness (comatose 3, semicomatose 6, irritable 3, disorientation2), involuntary movements (4 cases) decreased tendon reflexes (3 cases), increased tendon reflexes (4 cases), ankle clonus (5 cases) and paresis (4 cases).

Chloramphenicol and steroids were used in the treatment. Four out of the 14 patients died, and poor prognosis were observed in patients associated with convulsions and coma. Of the 10 patients who survived and could have a regular follow-up, 2 had paresis and 1 patient is mentally retarded.

#### MELIOIDOSIS AT SIRIRAJ HOSPITAL

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Melioidosis caused by *Pseudomonas pseudomallei* is a common infectious disease in tropical areas. The disease has protean manifestations and usually carries high morbidity and mortality. We encountered a total of 16 cases in two years period. Manifestations varied from fatal septicaemic form to chronic skin ulcer. The last 2 cases

merit some comment because of their presentation, localization and clinical course. These two middle - aged men presented with prolonged fever and the disease was localized in the liver. Since we do not have this form in Thailand the clinical course, management and review of all cases in Siriraj Hospital was discussed in detail.

### CHANGES OF LEUCOCYTES IN FALCIPARUM MALARIA

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The effects of falciparum malaria on the leucocyte profile and functions were studied in 120 patients admitted to the Bangkok Hospital for Tropical Diseases.

Blood samples were taken during acute illness before antimalarial therapy  $(D_0)$  and again on the 28th day or later as cured therapy  $(D_{28})$ . Leucocytes were enumerated and differentiated. The assessed neutrophil functions included the phagocytic and the microbicidal activities against *Escherichia coli* and *Candida albicans*, and the nitroblue tetrazolium (NBT) reduction activity.

It was found that on  $D_0$  there were leucopenia and increased percentage of band form of neutrophils while on  $D_{28}$  the total leucocyte count became normal but eosinophilia emerged. The phagocytic activity against both organisms were normal on  $D_0$  and  $D_{28}$ . The candidacidal activity was decreased on  $D_0$  but normal on  $C_{28}$  while the bactericidal activity against  $E.\ coli$  was decreased on  $D_0$  and  $D_{28}$ . The increases in the percentage of NBT positive cell, both unstimulated and endotoxin - stimulated, were higher on  $D_0$  than on  $D_{28}$ .

### TROPICAL PYOMYOSITIS

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Tropical pyomyositis is one of the not uncommon infectious diseases in tropics and subtropics. Staphylococcus aureus is the major causative agent, but the predisposing and precipitating factors are still controversial. The prevalence and clinical importance in Thailand is not well established, although sporadic cases have been reported from other hospitals.

This study reports two cases of tropical pyomyositis admitted to Phramongkutklao Hospital in 1979 and 1980. The first case was a 23-year old young male soldier, and another a 32 year-old female housekeeper. Both resided in Bangkok and had typical clinical

manifestations of tropical pyomyositis. The male patient had multiple abscesses, but the female a solitary one. None of them had history of traumatic injury prior to abscess formation.

The pus cultures yielded coagulase-positive *Staphylococcus aureus* in both cases, but the blood culture, performed in the female patient, was negative.

Both patients responded to the combined treatment with beta lactamase resistant penicillin and surgical drainage. Anaemia and pneumonia developed in the male patient which appeared to be complications of the disease.

## NON - FERMENTATIVE BACILLI ASSOCIATED WITH MAN : DETECTION AND IDENTIFICATION

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During the past ten years major advances have been made in the nutritional, chemical and genetic characterization and taxonomy of non-fermentative bacilli. These advances also represent a major contribution toward their correct identification and support the conclusion that many of these bacilli cannot

be correctly identified by means of the biochemical tests conventionally used in clinical bacteriology. More than 300 strains of non-fermentative gram-negative bacilli were examined to establish reliable procedures for their detection and identification in clinical laboratory. Growth on the surface but

neither growth nor acid in the butt of TSI agar was found to be a completely reliable means for detecting these bacilli. More than 98% of the strains were identified. Identifi-

cation was effected with a battery of 12 screening tests followed, with a minority of strains, by completion tests.

## MYCETOMA, A REVIEW OF 17 CASES SEEN AT THE INSTITUTE OF DERMATOLOGY, BANGKOK, THAILAND

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Although mycetoma is supposed to occur in Thailand with high frequency due to her geographic location; the exact statistics have not been reported. A review of 17 cases of mycetoma seen at the Institute of Dermatology diagnosed by clinical characteristics, cultures, histopathology and roentgenology was presented. Treatment was also discussed.

Eight cases (47.1%) of the patients were male between the age of 20-81 years, nine cases (52.9%) were female between the age of 27 - 62 years. The lesions appeared on the legs, ankles or foot in 14 cases (82.4%), one case (5.9%) on the index finger, one (5.9%) on the buttock, and one (5.9%) on the shoulders.

Culture identification showed (1) Actino-

mycotic mycetoma (64.7%); i.e. 5 cases of N. asteroides (29.4%), 3 cases of N. brasiliensis (17.6%), 2 cases of N. caviae (11.8%), 1 case of Strept. madurae (5.9%). (2) Eumycoltic mycetoma (35.3%); i.e. 3 cases of M. mycetomii (17.6%), 1 case of C. falciforme (5.9%), 1 case of A. boydii (5.9%), 1 case of Neotestudinia rosti. Systemic involvement was nil. Histopathology was of mixed cell granuloma, fungal grains lie in the abscess. Roentgenogram showed involvement of bone which occurred only in 41.2%.

In our series, treatment was not satisfactory Trimethoprim and sulfamethoxazole were given in nine cases. Good result occurred only in one case; eventhough *in vitro* response was sensitive.

### THE EFFECT OF METRONIDAZOLE ON TRICHOMONAS

# VAGINALIS BY USING IN VIVO AND IN VITRO METHODS

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This study was directed towards determining the effectiveness of metronidazole (Elyzol) upon *Trichomonas vaginalis*, both *in vivo* and *in vitro*, and the relationship between these two methods.

Total number of 67 patients with vaginal discharges were examined for *Trichomonas vaginalis*. Only 7 out of 8 positive patients were treated with metronidazole 250 mg twice daily for 7 days.

Daily examination of 5 patients that completed the schedule revealed that *Trichomonas vaginalis* was eliminated in 3 days after treatment. The in *vitro* test showed that the patient who recover from *Trichomonas vaginalis* on the second day after treatment had the minimum trichomonicidal concentra-

tion of  $1.5 - 3 \mu g/ml$  and the patient who recover from *Trichomonas vaginalis* in the third day after treatment had  $4 \mu g/ml$ . For hourly examination showed that the effect of the drug was influenced by 2 factors; the drug concentration and the exposure time, and there was no further drop of the minimum trichomonicidal concentration after 22 hours.

It was apparent that there was a correlation between the *in vivo* and *in vitro* effects of *Trichomonas vaginalis* taken from the patients who took longer time in being cured. These patients had higher minimum trichomonicidal concentration. However, because of the small sample size of this study, further studies are in progress to prove this relationship.

# THE ZOOGEOGRAPHY OF THE GENUS *LEPTOTROMBIDIUM*, INCLUDING THE VECTORS OF CHIGGER - BORNE RICKETTSIOSIS (SCRUB TYPHUS)

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Larval mites (chiggers) of the family Trombiculidae are vectors of chigger-borne rickettsiosis. All the known vectors are in the genus and subgenus *Leptotrombidium*. Although 7 species are proven vectors there are 163 known species in the subgenus. Approximately

65% are found in the Oriental region. The infection is known to occur in the Oriental, Australasian and Palaearctic Regions, but not in the New World, Europe and Africa. The subgenus, *Leptotrombidium* is present wherever the infection occurs. The common

hosts are shrews, voles, rats, mice, ground squirrels and birds. Species other than the vectors are infected in nature, and because they share similar habitats as the vectors, it is highly probable that these species also play a role in the transmission of chigger-borne rickettsiosis to man.

### PREVALENCE OF METHICILLIN - RESISTANT STAPHYLOCOCCI IN A UNIVERSITY HOSPITAL

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Staphylococcal diseases still causes high morbidity and mortality in Thailand. Since appropriate antimicrobial agents are methicillin and isoxazolyl penicillins, we therefore conducted susceptibility testing by agar dilution to determine prevalence of resistance. Of 101 isolates of *S. aureus* and 20 isolates of *S. epidermidis*, five (4.9%) *S. aureus* were

found to be resistant to methicillin and cephalothin. Others were susceptible with modes of MIC of  $3.1 - 6.2 \,\mu\text{g/ml}$ . This prevalence is not high, but increasing trend is anticipated due to indiscriminate use of antimicrobial agents by general practitioners and self-medication by patients.

### DEVELOPMENTAL HEALTH PLANNING AND UNICEF'S ROLE

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It is now almost universally accepted that improvements in health are more than a question of drugs, hospitals and health services. Thus "planning for health" has a much wider connotation than the conventionally used term "health planning" which is more or less confined to the task of the health sector. This is so because a multitude of factors are having effects on the status of health, for instance, nutrition, housing,

water and sanitation, education, employment and productivity etc. Thus planning for health should take into consideration of all these factors and forces which have a direct or indirect bearing on the health status of a population. This implies that the planning process should surpass the frontiers of medicine and the conventional health services sector and should be capable of embracing all other facets mentioned above.

The other dimension is the strong positive correlation that exists between health and general development. Health development should lead towards the overall development of a community or society and thus planning for health should form an integral component of the overall planning for development.

How can we put all these things together in practice? UNICEF (United Nations Children's Fund), in the past few years, has been using its "Basic Services Strategy" whereby health care is being developed along with other measures for expanding and improving facilities for education, nutrition, agricultural production, water supply, sanitation and various aspects of community development and social welfare.

The characteristic feature of this approach is to establish all the aforementioned basic services on an integrated basis so that they will be mutually supportive and reinforcing and the fact that communities will be involved in all stages of plan formulation and implementation.

