

SEATO MEDICAL RESEARCH STUDY ON MELIOIDOSIS

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Objective: This study is designed to determine the presence and distribution of Pseudomonas pseudomallei in Thailand and to evaluate its importance as the causative agent of the disease, melioidosis.

Description: Our previous efforts (see last Annual Report) have provided information regarding the general distribution of Ps. pseudomallei in soil and water samples obtained in various areas of Thailand and on the association of serological reactivity of Thai people with the presence of the organism in the external environment. Efforts to recognize active cases of infection in Thailand have been entirely unsuccessful, although isolated cases have previously been reported in the Thai medical literature. The conclusion which appears almost unavoidable is that Ps. pseudomallei is, in nature, a saprophytic organism which only rarely causes disease and then perhaps because of unusual routes of infection, abnormally low host resistance, or a combination of these factors. Nevertheless, when melioidosis does occur, it is a grave matter to the patient. Therefore some effort has been directed to examining experimental infection and the possibility of developing a more effective therapeutic regimen. Because of the departure of supporting personnel and the fact that little further can be gained by continuing the geographic and serological surveys, these activities have been deemphasized although the capability for isolation of Ps. pseudomallei and diagnosis, both bacteriological and serological, is being maintained. The laboratory still serves as a consultation laboratory for confirmation of isolates in Viet Nam and other areas and receives serum for serological study by the micro-hemagglutination test developed here. An extensive comparison of this serological test with that in use at WRAIR is in progress. The Thai Livestock Department has assumed some responsibility for further study of melioidosis, particularly in its veterinary aspects, using methodology and personnel developed and trained in this laboratory.

1 On loan from Thai Department of Health

2 Research and Education Division, Livestock Department, Ministry of Agriculture.

Progress:

1. Specimens received.

An additional 75 sputum samples, from supposedly non-tuberculous chronic and subacute pulmonary disease patients in Southern Provinces of Thailand (Pattalung, Songkhla, Pattani, Yala and Narathivas), where isolation rates from soil and water samples range from 25 to over 40%, have been examined for Ps. pseudomallei by hamster inoculation and cultural techniques. All were negative.

Thirty five specimens obtained from 31 swine at slaughter houses in the same region, and one cattle specimen, were also examined with negative results.

Nine natural water samples from Chandhaburi Province were negative for Ps. pseudomallei.

Eighteen cultures, representing 12 cases among U.S. servicemen in Viet Nam and one strain reportedly isolated from a rat, were confirmed as Ps. pseudomallei.

Results of antibiotic sensitivity tests of the isolates from Viet Nam, performed by the disc technique, were generally quite similar to those obtained with isolates from Thailand.

| <u>Sensitive</u> | <u>Resistant</u> |
|-----------------------------|-------------------------|
| Chloramphenicol, 30 μ g | Penicillin, 5 U |
| Neomycin, 30 μ g | Polymyxin B, 300 U. |
| Tetracycline, 30 μ g | Furadantin, 15 μ g |
| Kantrex, 30 μ g | Erythromycin, 5 μ g |
| Novobiocin, 10 μ g | Bacitracin, 10 U. |
| Ampicillin, 15 μ g | Methicillin, 5 μ g |
| Sulfathiazole, 1.0 mg | Colimycin, 5 μ g |
| | Streptomycin, 5 μ g |

One strain was found to be resistant to chloramphenicol and one to neomycin.

Virulence tests were performed by inoculating hamsters intraperitoneally with increasing dilutions of suspensions of three of the strains. The results indicated that the strains were highly virulent for hamsters with LD₅₀ values between 1 and 10 viable organisms.

Two hundred twenty-one sera from healthy American servicemen in Viet Nam were tested for melioidosis antibody by the sensitized erythrocyte micro-hemagglutination technique. None were reactive.

Sera from six confirmed cases were also tested. Of these, 4 showed either a rise in titer in paired specimens or a significant titer in a single (late) specimen.

Sera from 9 Special Forces soldiers in Viet Nam, who had been for prolonged periods in an area which yielded cases, were negative in the HA test.

2. Attempt to enhance susceptibility of a naturally resistant host.

In a discussion of the melioidosis problem with COL W.D. Tigertt, COL S. Vivona and LTC R.L. Taylor, the question arose as to whether some factor may be increasing susceptibility of servicemen in Viet Nam to melioidosis. A common denominator is the use of chloroquine. Accordingly, an attempt was

made to determine the effect of chloroquine on the resistance to melioidosis of a naturally resistant species, the rat. Initially, LD₅₀ assays were conducted in rats to determine the maximum sub-lethal dose of chloroquine and to ascertain their susceptibility to melioidosis. In these tests, it was found: 1) that rats would tolerate 2 subcutaneous doses at a 3 day interval of 1.0 ml of a 0.5% solution of chloroquine (Resochin-Bayer), higher doses resulted in some deaths, usually almost immediately; and 2) that the LD₅₀ for rats inoculated intraperitoneally was approximately 10⁸ organisms, the rats dying by 40 hours.

In the experiment to determine the effect of chloroquine, the rats were divided into 2 groups, one group receiving 0.5 ml of a 0.5% solution of chloroquine subcutaneously on day 0 and day 2, and both groups receiving graded doses of Ps. pseudomallei intraperitoneally. The results are summarized in Table I. Chloroquine apparently had a slight effect on enhancing susceptibility to the early (toxic?) deaths but no effect on susceptibility to delayed (infection type) deaths.

3. Attempt to enhance with chlorpromazine the effect of chloramphenicol.

In the same discussion (Par. 2, above), mention was made of the clinical failure of antibiotics which are effective in vitro. Col. Tigertt suggested that some attempt might be made to enhance the activity of antibiotics and recalled a report of an adjuvant effect of chlorpromazine on antibiotic action against Brucella. Accordingly, an attempt was made to determine whether chlorpromazine would enhance the effect of chloramphenicol in melioidosis infected hamsters.

The experiment was performed in 3 experimental groups of 20 hamsters, which were subdivided into sub-groups of 5 hamsters each, and a control group of 10 hamsters. The experimental and control groups each received an intraperitoneal inoculum of approximately 1 x 10² viable Ps. pseudomallei. On each of the next 3 days, the experimental groups received 2 doses of either chloramphenicol, chlorpromazine, or both. Appropriate control groups were inoculated with the drugs alone to observe any toxicity. Mortality was recorded twice daily. There were no deaths in the drug control groups. The results, summarized in Table II, did not indicate that chlorpromazine had any significant enhancing effect on chloramphenicol in this model although there was one long term survivor in the group treated with both drugs.

4. Effect of antiserum, alone and in combination with chloramphenicol, on experimental melioidosis in hamsters.

The disappointing results of the previous experiment, and especially the lack of absolute therapeutic effect of chloramphenicol, the drug of choice for this disease and one to which the infecting strain was sensitive in vitro, led us to test the effect of hyperimmune serum, alone and in combination with chloramphenicol, on the experimental infection of hamsters. The serum used was a pool of rabbit sera prepared against formalinized antigens of a variety of strains of Ps. pseudomallei and which had an HA titer of 1:20, 480. In this experiment, the dosage of chloramphenicol was increased 4-fold and therapy was initiated earlier following challenge. The results (Table III) were similarly disappointing. In the dosage used, the serum had little, if any, effect on the outcome of the experimental infection, and did not enhance the slight therapeutic effect (manifest as a delay in time of death) of chloramphenicol.

Summary:

1. We have, as yet, been unable to find active cases of melioidosis in Thailand despite the widespread presence of the causative organism in soil and water and the presence of antibody in people in endemic areas. Isolates of Pseudomonas pseudomallei from American servicemen in Viet Nam have been confirmed in this laboratory. They appear similar, in every way tested, to isolates from soil and water in

Thailand. Four of six proven cases demonstrated either a rise in antibody or a high titer in a single available specimen in the microhemagglutination test for melioidosis antibody developed here. In contrast, the sera of normal American servicemen in Viet Nam had no activity.

2. Chloroquine was found to have little effect on the natural resistance of the rat to melioidosis.

3. Chlorpromazine did not enhance the slight therapeutic effect of chloramphenicol on experimental melioidosis in hamsters, nor did hyper-immune pooled anti-Pseudomonas pseudomallei rabbit serum.

Table 1

Effect of Chloroquine on Susceptibility of Rats to Melioidosis

| Experimental Group | Inoculum* | Days following infection | | | | |
|--|-----------------------|--------------------------|-----|-----|-----|-----|
| | | 1 | 2 | 7 | 9 | 17 |
| <u>Ps. pseudomallei only</u> | 2.6 x 10 ⁸ | 6/8** | | | | 6/8 |
| | 2.6 x 10 ⁷ | 2/8 | | 2/8 | 3/8 | 3/8 |
| | 2.6 x 10 ⁶ | 3/8 | | | | 3/8 |
| <u>Ps. pseudomallei + Chloroquine***</u> | 2.6 x 10 ⁸ | 8/9 | | | | 8/9 |
| | 2.6 x 10 ⁷ | 6/9 | 7/9 | | | 7/9 |
| | 2.6 x 10 ⁶ | 1/9 | | | | 1/9 |
| | 2.6 x 10 ⁵ | 1/8 | | | | 1/8 |
| Chloroquine only*** | | 0/8 | | | | 0/8 |

* Viable cells in 0.1 ml intraperitoneally

** No. dead/Total

*** 0/5 ml of 0.5 % solution, subcutaneously, on initial day and 2 days later.

Table II

Effect of Chlorpromazine on Activity of Chloramphenicol in Infected Hamsters

| Experimental Group* | Cage | Days following infection | | | | | 11 |
|--|------------------------|--------------------------|-----|-----|-----|-----|----|
| | | 1 | 2 | 3 | 4 | | |
| <u>Ps. pseudomallei, only</u> | 1 | 1/5** | 5/5 | | | | |
| | 2 | 0/5 | 5/5 | | | | |
| | Cumulative % Mortality | 10 | 100 | | | | |
| <u>Ps. pseudomallei + Chloramphenicol***</u> | 1 | 0/5 | 3/5 | 5/5 | | | |
| | 2 | 0/5 | 4/5 | 5/5 | | | |
| | 3 | 0/5 | 5/5 | | | | |
| | 4 | 0/5 | 2/5 | 5/5 | | | |
| | Cumulative % Mortality | 0 | 70 | 100 | | | |
| <u>Ps. pseudomallei + Chlorpromazine****</u> | 1 | 0/5 | 5/5 | | | | |
| | 2 | 0/5 | 5/5 | | | | |
| | 3 | 0/5 | 5/5 | | | | |
| | 4 | 0/5 | 5/5 | | | | |
| | Cumulative % Mortality | 0 | 100 | | | | |
| <u>Ps. pseudomallei + both drugs*****</u> | 1 | 0/5 | 3/5 | 4/5 | 5/5 | | |
| | 2 | 0/5 | 2/5 | 4/5 | 4/5 | 4/5 | |
| | 3 | 0/5 | 5/5 | | | | |
| | 4 | 0/5 | 4/5 | 5/5 | | | |
| | Cumulative % Mortality | 0 | 70 | 90 | 95 | 95 | |

* Each group received 1×10^{12} viable Pseudomonas pseudomallei intraperitoneally on day 0.

** No. dead/Total

*** 5.0 mg, intramuscularly, at 0800 hours and 1600 hours on day 1, 2, and 3.

**** 0.1 mg as above.

***** Mixture of both drugs, as above.

Table III

Effect of Pooled Hyperimmune Ps. pseudomallei Antiserum and Chloramphenicol,
Alone and in Combination, in Infected Hamsters

| Experimental Group ¹ | Cage | Days following infection | | | |
|--|------------------------|--------------------------|-----|-----|-----|
| | | 2 | 3 | 4 | 6 |
| <u>Ps. pseudomallei</u> only | 1 | 5/5 ² | | | |
| | 2 | 5/5 | | | |
| | 3 | 5/5 | | | |
| | 4 | 4/5 | 5/5 | | |
| | Cumulative % Mortality | 95 | 100 | | |
| <u>Ps. pseudomallei</u> + serum ³ | 1 | 5/5 | | | |
| | 2 | 5/5 | | | |
| | 3 | 5/5 | | | |
| | 4 | 4/5 | 4/5 | 4/5 | 5/5 |
| | Cumulative % Mortality | 95 | 95 | 95 | 100 |
| <u>Ps. pseudomallei</u> + Chloram. ⁴ | 1 | 0/5 | 1/5 | 5/5 | |
| | 2 | 0/5 | 3/5 | 5/5 | |
| | 3 | 0/5 | 3/5 | 5/5 | |
| | 4 | 0/5 | 4/5 | 4/6 | 5/5 |
| | Cumulative % Mortality | 0 | 55 | 95 | 100 |
| <u>Ps. pseudomallei</u> + serum + chloramphenicol | 1 | 0/5 | 4/5 | 5/5 | |
| | 2 | 0/5 | 3/5 | 4/5 | 5/5 |
| | 3 | 0/5 | 4/5 | 4/5 | 5/5 |
| | 4 | 0/5 | 2/5 | 3/5 | 5/5 |
| | Cumulative % Mortality | 0 | 65 | 80 | 100 |

1 Each group received 9.5×10^1 viable Ps. pseudomallei, intraperitoneally on the morning of day 0.

2 No. dead/Total

3 0.2 ml per hamster, subcutaneously, 6 hours after challenge.

4 20 mg/hamster, intramuscular, 6 hours after challenge and twice daily thereafter.