MATERIALS AND METHODS

I. Organisms.

Vibrio cholerae El Tor-Ogawa, Salmonella typhosa and Shigella flexneri type III. which had been isolated from patients admitted to Chiengmai Hospital, were employed in this study. The identification of strains of these organisms was confirmed by the Department of Bacteriology and Immunology, SEATO Medical Research, Bangkok. Before and during this study, the organisms were maintained on brain heart infusion agar* slants which were kept at 4°C and transferred monthly.

II. The Water Sample and Inoculation.

In this study water from various sources and locations was employed as shown in Figure 1 and Table I.

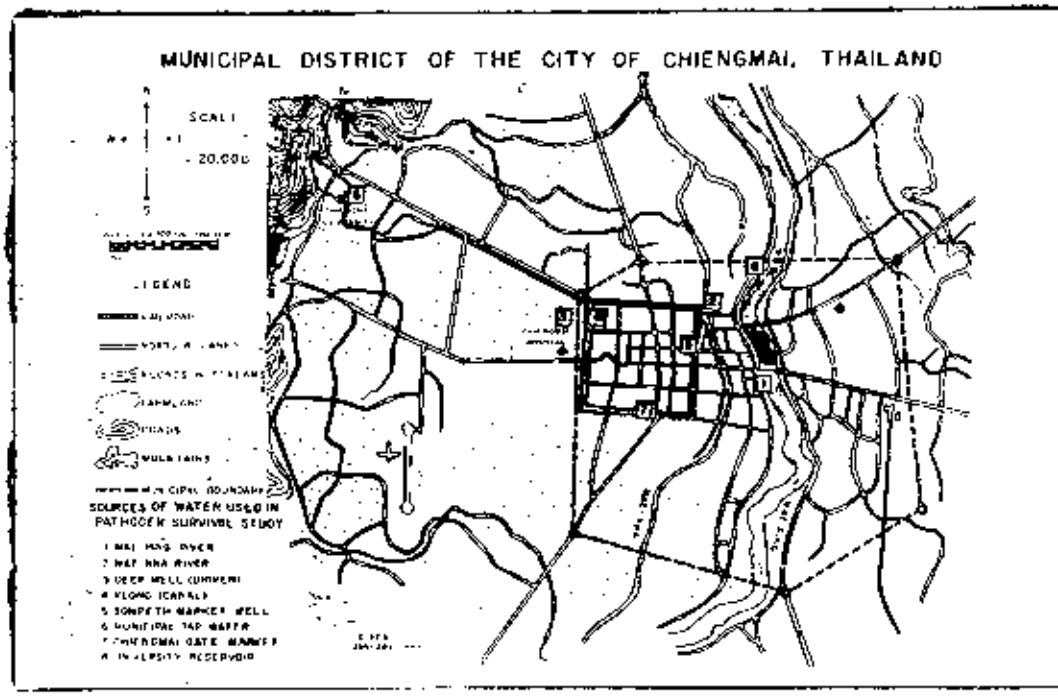

Figure I. Sites of Water Sources for Survival Studies.

*Difco Laboratories, Inc., Detroit 1, Michigan.

TABLE I.

Sources of Water Used in Survival Studies.

1. Mae Ping river (the major river draining the entire Ping valley and surrounding mountain, this river is the major source of water for Chiengmai).
2. Mae Kha river (A small river which flows through the city and serves as the major sewage run off. This river is considered particularly dangerous because it dries to a mere trickle in the hot season and floods surrounding areas in the rainy season).
3. Water from the deep, driven well at the Medical School (untreated but piped to all buildings on the campus).
4. The canal (klong) surrounding the old wall city (a shallow canal which is stagnant except in the rainy season).
5. Shallow dug well in Sompetch market.
6. Municipal tap water (this is obtained from the Mae Ping river, and it is precipitated and chlorinated. Treatment is well controled at the plant but the piping is subject to leakage and contamination in the vicinity of the consumer).
7. Shallow dug well at Chiengmai Gate market.
8. Chiengmai University reservoir which is a natural pond. (The water draining into this reservoir comes from the mountain nearby. Chlorinated water from this reservoir is piped to all areas of the campus).

The samples were collected at the depth of one to two feet below the water surface. Each sample was examined for iron and calcium content by the Clinical Chemistry Laboratory, Faculty of Medicine, Chiengmai University. Iron was determined by the modified method of Caraway (9) and calcium was determined by using the Flame Photometer Coleman Model 21. One hundred liter volumes of water from each source were impounded into each of three Shanghai jars (unglazed earthen vessels). In each jar the pH measurement was done by using Beckman's pocket pH meter model 180. The initial viable flora was determined by aerobic plate count method (48).

Each jar was inoculated to a final concentration of approximately 10^4 of each organism per milliliter using ten milliliters of 18 hours infusion broth cultures of Salmonella typhosa, Shigella flexneri type III and Vibrio cholerae El Tor-Ogawa. The jars were kept at room temperature and covered with aluminum lids (See Figure 2).

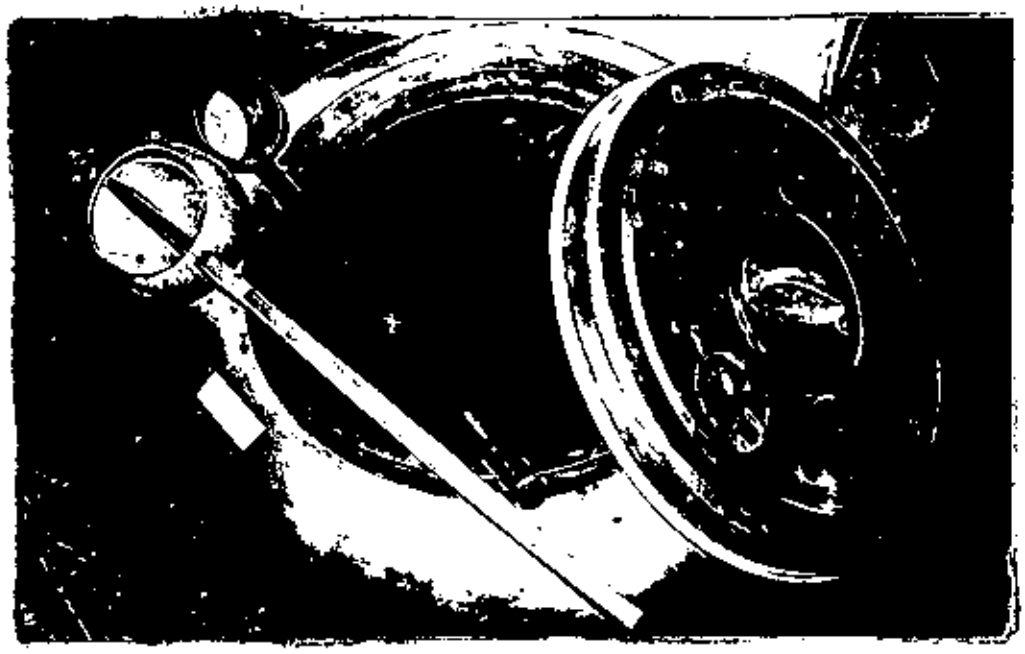

Figure 2. Water Jar and Ladles.

III. Examination of Water Jars for Viability of Enteropathogens.

Five hundred milliliter samples were collected from each jar periodically from zero hour to two weeks. A ladle was used to collect water from the jar throughout this experiment (Figure 2.). All water samples examined in this study were passed through millipore filters (the pore size of the filter membrane is 0.45 micron). One half of the millipore filter membrane was put into alkaline peptone broth (2.0 per cent peptone, 2.0 per cent Sodium chloride, pH 8.2 to 8.4) and the other half was placed in Selenite F broth.* The media were then incubated at 37°C for 18 to 24 hours. Subcultures were made on McConkey's agar* and Salmonella-Shigella agar* plates from Selenite F broth, and alkaline tellurite agar** plate from the alkaline peptone broth and incubated at 37°C for 24 hours.

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**Alkaline tellurite agar

Beef extract	3	gm.
Peptone	10	gm.
NaCl	25	gm.
agar	25	gm.
H_2O	1000	ml.

a. Mix the above ingredients in a large flask.
b. Take 11 ml. of 10% Na_2CO_3 in a screw-cap tube.
c. Take 50 ml. of 0.2% Sodium lauryl sulphate in a small flask.

Sterilize all three at 15 lbs. pressure for 15 minutes, take them out of the autoclave and now mix them together. Wait until the medium cools down to about 50°C and then add ....

d. 50 ml. of 20% sucrose which has previously been sterilized at 12 lbs. pressure for 15 minutes.
e. 1.0 ml. of 1% Potassium tellurite.

Suspected colonies of enteropathogens were picked and inoculated into Triple Sugar Iron (T.S.I.) agar* slants. After the slants were incubated at 37°C for 24 hours, those slants showing growth resembling enteropathogens were checked for biochemical reaction characteristics and tested with Difco Salmonella and Shigella "O" grouping serum and Burroughs-Wellcome anticholera serum by slide agglutination test.

The viability of Vibrio cholerae El Tor-Ogawa, Salmonella typhosa and Shigella flexneri type III in the water from Mae Nam Ping was also determined monthly from April 1965 to November 1965. These studies were conducted according to the procedures given above.

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