

MELIROIDOSIS

A Report of Clinical Cases and of a
Survey for Antibodies among Non Indigenous Men Living
in an Endemic Area.

Thesis submitted to the University of Edinburgh
for the Degree of M.D.

by

R.N.T. Thin M.B.Ch.B. (Edin), M.R.C.P. (Edin).



ACKNOWLEDGMENTS

I am most grateful to all those at Terendak Camp, Malaysia, who co-operated so enthusiastically to make this work possible. I particularly thank Lieutenant Colonel G. Rapmund, USA MC, Officer Commanding the United States Army Medical Research Unit at the Institute for Medical Research, Kuala Lumpur, Malaysia, and the two veterinarians, Captain J.M. Strauss USA VC and Captain M. Groves USA VC, for all their help and encouragement. I also thank Miss E. Gan, the serologist, who kindly carried out all the serological tests. Mr A.H. Gould B.Sc., F.I.A., Principal Scientific Officer, Ministry of Defence (Army Department), A.M.D. Stats., kindly did the statistical analyses.

	<u>Page</u>
 CHAPTER III EPIDEMIOLOGY	
Introduction	49
Method	50
Results	50
Discussion	
Habitat of the Organism	50
Portals of Entry	51
 CHAPTER IV THE SEROLOGICAL SURVEY	
Introduction	56
Methods	56
Results	57
Raised Titres	59
Positive Titres	60
Age and Time Spent in Endemic Areas	63
Clinical Findings Among Men with Raised Titres	63
Summary of Results	64
Discussion	
The Haemagglutination Test	66
Incidence of Positive Titres	66
Exposure to Surface Water in Men with Positive Titres	68
Positive Titres in New Zealanders and Inpatients	69
Clinical Features	69
 CONCLUSIONS	 71
 BIBLIOGRAPHY	 72
 Appendix I	
Biographical Note on Lt Col Alfred Whitmore	78
Appendix II	
The name <u>Pseudomonas pseudomallei</u>	79
Appendix III	
Diagnoses of Inpatients	80
Appendix IV	
Diagnoses of Outpatients	81
Appendix V	
The Haemagglutination Test	82

INTRODUCTION

Melioidosis is a rare infection due to a Gram negative bacillus, Pseudomonas pseudomallei. The main endemic areas are Burma, Malaysia, Viet Nam and Indonesia but the disease has also been found in the northern part of Australia and Thailand, while a few cases have been reported from the United States of America and elsewhere. Most of the patients have been diagnosed in the Far East, but a few Europeans and Americans have only developed symptoms after returning to the Western Hemisphere from endemic areas (Grant and Barwell 1943, Maegraith and Leithead 1964 and Patterson, Darling and Blumenthal 1967).

The author treated seven patients suffering from melioidosis while stationed in Malaysia and Singapore. Three of them died and it seemed probable that in such patients early diagnosis was essential so that vigorous treatment could be started as soon as possible. Review of the literature showed there was a wide spectrum of clinical forms of melioidosis, and serological surveys, which mainly concerned Asians, showed that a small proportion of the subjects had raised titres of melioidosis antibodies (Nigg 1963 and Strauss et al 1968). In asymptomatic individuals these titres may indicate previous melioidosis. The author carried out a serological survey to see if non-indigenous soldiers in Malaysia had raised antibody titres and men with raised titres were examined to try to detect features of previous melioidosis.

Epidemiological studies by Ellison and his colleagues (1968) showed that Ps pseudomallei was widely distributed in the surface water of Malaysia. As they had not sampled the surface water in the localities where the author's patients had developed melioidosis, he collected a number of surface water samples for bacteriological examination.

The history of melioidosis will be reviewed, the seven patients will be described, and the epidemiological and serological studies will be presented. This work was carried out while the author held the post of Specialist in Medicine, first at the Military Hospital at Terendak Camp in Malaysia, and later at the British Military Hospital in Singapore.

CHAPTER IHISTORICAL AND GEOGRAPHICAL REVIEW

	<u>Page</u>
Original Description	3
Early Work and Discovery in Malaysia, Viet Nam and Indonesia	4
Melioidosis since 1959	
South East Asia	5
Australia	5
America	6
Melioidosis in Other Countries	7

CHAPTER I

HISTORICAL AND GEOGRAPHICAL REVIEW

ORIGINAL DESCRIPTION

Melioidosis was first recognised in 1911 by Whitmore when carrying out a post-mortem examination on the body of a Burman in Rangoon. He observed lesions identical with those of glanders, but found that the organism isolated from them, although Gram negative and morphologically resembling the glanders bacillus, differed in that it was motile and grew more luxuriantly on culture media. In 1912 Whitmore and Krishnaswamy published an account of 38 similar patients, and in the same year Whitmore presented a paper to the Bacteriological Section of the 80th Annual General Meeting of the British Medical Association describing the characteristics of the organism which he had confirmed while working in the Bacteriology Department of Guy's Hospital in London.

In 1913 Whitmore published a more complete account of the 38 cases. All were male paupers, most were morphine addicts, and in every case the diagnosis was only established after death, many of them having been found dead on the streets. The clinical features of those observed in hospital were fever, prostration, cough, signs of pneumonia or pleurisy, and diarrhoea. Whitmore described the pathological lesions noting that they were caseous nodules, usually present in the lungs but also affecting the liver and spleen. The organism was pathogenic for guinea pigs, producing the Straus Reaction (Straus 1889) in male animals and death within a few days. Whitmore described an experiment where feeding guinea pigs with pure cultures of the organism produced death. Many of the morphine addicts had abscesses at their injection sites but he never isolated Ps pseudomallei from these abscesses, nor from the regional lymph nodes. Whitmore concluded that morphine addiction predisposed patients to melioidosis by its general effects and, having isolated the bacillus from urine, he suggested that the disease was spread by infected urine and probably by sputum among those with unhygienic habits and living in overcrowded accommodation.

EARLY WORK AND DISCOVERY IN MALAYSIA, VIET NAM AND INDONESIA

In 1913 Fletcher (1919) observed a severe epizootic septicaemia among animals in the laboratories of the Institute for Medical Research at Kuala Lumpur in Malaysia. The causative organism was isolated and subsequently found to be Ps pseudomallei. Stanton reported human cases of melioidosis from Kuala Lumpur in 1917 and during the next twelve years Stanton and Fletcher (1932) discovered 39 Asian patients in Kuala Lumpur of whom only two survived. In addition to the clinical features reported by Whitmore they described vesicles and pustules, enlargement of the liver and spleen, parotitis and wild delirium in one patient. They observed that lesions occurred in almost every organ in the body.

Stanton and Fletcher thought that melioidosis occurred as a natural infection in rats, rabbits and guinea pigs, but it appears that most of the examples they quote were animals in captivity. They ^{saw} single cases in a dog, a cat and a horse, and they observed the disease could be produced experimentally in monkeys but not in pigs and horses. These workers observed that the Mallein Test, so useful in diagnosing glanders in horses, was positive in one man suffering from chronic melioidosis. They found agglutinins to Ps pseudomallei in this man's serum. In 1921 Stanton and Fletcher suggested the name "Melioidosis" since Greek physicians used the name "Melis" for a variety of conditions resembling glanders.

In 1925 Pons and Advier (1927) diagnosed melioidosis in a woman from a village near Saigon in South Viet Nam, and many more reports from Viet Nam have followed including those of Fournier and Chambon in 1958 and Sheehy, Deller and Weber in 1967. The first European found to have melioidosis was a tea broker in Ceylon who died after a month's illness characterised by pleurisy and diarrhoea (Denny and Nicholls 1927). In 1929 Mesnard, Joyeux and Gaulene reported another European patient who died from the disease in Tonkin in North Viet Nam. Gambier (1930), writing from Phnom Penh in Cambodia, described a Russian who, on arriving from Bangkok, was suffering from a febrile illness which proved to be melioidosis and was rapidly fatal. This case has been cited as evidence of melioidosis in Cambodia, but this patient most probably acquired his infection in Thailand.

In 1931 Gilmour described a case in Singapore: the initial diagnosis was small-pox because of the dense pustular rash, but the character and distribution were atypical and bacteriological investigation showed that the correct diagnosis was melioidosis. De Moor, Soekarmen and Walle reported the first case from Indonesia in 1932 and other reports have followed such as those of Bezemer (1935) and Sudibyo (1938).

MELIOIDOSIS SINCE 1939

South East Asia

During World War II melioidosis occurred in Allied soldiers serving in South East Asia. Grant and Barwell (1943) and Mayer and Finlayson (1944) reported chronic forms which developed after the patients had left the Far East. Harries, Lewis, Waring and Dowling (1948) described melioidosis in five West African soldiers stationed near Rangoon in Burma. Cox and Arbogast (1945) also reported an American serviceman who contracted the disease in Burma. Paton, Peck and Van de Schaaf (1947) and Peck and Zwanenberg (1947) reported cases from Bangkok. There are also descriptions of melioidosis among Japanese soldiers who had invaded South East Asia (Hayakawa 1945 and Sakihara 1952). Mirick, Zimmerman, Maner and Humphrey reported two patients from Guam in 1946, and Ziskind, Pizolatto and Buff (1954) described a patient who presented after his return to the United States of America following service in the Philippine Islands, Guam and Okinawa.

Australia

During the last 19 years melioidosis has appeared in northern Australia. The first case was seen in a sheep in 1949. Since then epizootics have developed in sheep, goats and pigs as described by Cottew in 1955. The disease has occurred in Malaysia in pigs, goats (Omar and horses (Davie and Wells 1952) but the main movement of animals is from Australia to Malaysia et al) (Davie and Wells, and Stanton and Fletcher 1952), so it appears unlikely that melioidosis was imported from Malaysia by animals. Rimington described six human cases from the same part of northern Australia in 1962. This sudden appearance and explosive spread among animals suggests recent importation, probably by Australian servicemen returning from endemic areas after World War II.

America

All the cases mentioned above occurred in or near the main centre of infection in South East Asia, or the patients visited this region. However, there are a few reports from the United States of America, Panama and Ecuador concerning patients who never left the American continent, and who had no known contact with imported melioidosis. McDowell and Varney described the first of these patients in 1947. He was a 31 year old man who had spent two years in Panama 17 years before, but had lived in the United States of America for the rest of his life; he had had many illnesses before developing persistent ulcers and sinuses from which Ps pseudomallei was cultured. In 1948 Beamer, Varney, Brown, McDowell and Eck reported the case of a 25 year old woman who had not left the United States, and in 1954 Beamer and his colleagues described the bacteriological features of the strains of Ps pseudomallei isolated from these two patients. In 1951 Garry and Koch described a third patient who had never left the United States and there are two reports of patients contracting melioidosis in Panama (Joy, Scalettar and Sodee 1960, and Rubin cited by Biegeleison, Mosquera and Cherry 1964). Biegeleison and his colleagues described a case from Ecuador concerning a man who died following a toe injury which became infected.

As these are the only well documented reports of the disease arising in the Western Hemisphere they merit careful examination. According to Biegeleison and his colleagues, the organism isolated by Garry and Koch was later shown in another laboratory to be a *Pseudomonas* of a different species, but they give no details. Garry and Koch discussed the atypical features of their strain, noted that Blanc, Delage and Martin (1943) had found a similar strain, and concluded that the organism which they had isolated was Ps pseudomallei. Biegeleison and his colleagues were also critical of the bacteriological findings of Beamer and his colleagues, but they are more detailed than those of many earlier accepted reports and appear satisfactory according to the account given by Haynes and Burdholder in Bergey's Manual of Determinative Bacteriology (1957). On the other hand Biegeleison and his colleagues accept two cases from Panama for which no bacteriological details have yet been published (Joy et al 1960 and Rubin 1964). They described their own careful bacteriological studies and stated that the identification of Ps pseudomallei was confirmed by Dr Clara Nigg who has studied this organism in some detail.

(Nigg et al 1956 and Nigg 1963). It seems reasonable to accept that these were all cases of melioidosis but their origin is not clear.

Melioidosis in Other Countries

Sutmoller, Kraneveld and Van de Schaaf (1957) reported an epizootic of melioidosis among sheep, goats and pigs on Aruba Island in the Dutch West Indies, but they did not comment on the source of the infection. Although melioidosis may be present naturally in the islands, the nature of this epizootic suggests recent importation, perhaps by settlers from Indonesia. Aruba Island is near the Panama Canal through which many travellers pass on their way from South East Asia.

In 1953 Ives and Thompson reported a patient who apparently developed melioidosis in India. He was a British mining engineer who worked for 22 years in Central India and had only travelled between India and the United Kingdom. He died after a febrile illness lasting nine months and Ps pseudomallei was isolated at necropsy. The patient described by Mægraith and Leithhead (1964) probably acquired his infection at Chittagong which is in East Pakistan near the Burmese border.

In 1961 Professor Ertug in Ankara described a patient from southern Turkey who developed a febrile illness which was diagnosed as melioidosis and successfully treated. Apart from this all the cases have occurred in warm climates. One area which has not been mentioned so far is Africa. In 1931 Duke reported melioidosis as the probable cause of the death of a native of Uganda (Duke cited by Stanton and Fletcher 1932) and the absence of further reports suggests, but does not confirm, that melioidosis is not present there.

CHAPTER IITHE CASES

	<u>Page</u>
Introduction	9
The Patients	
Patient Number 1	9
Patient Number 2	13
Patient Number 3	16
Patient Number 4	21
Patient Number 5	26
Patient Number 6	29
Patient Number 7	34
Pathology	38
Bacteriology	
Morphology	38
Cultural Characteristics	38
Biochemical Properties	39
Pathogenicity	39
Antibiotic Sensitivity	39
Discussion	
Incidence	41
Predisposing Factors	41
Diagnosis	42
Treatment	44
Mortality	47

CHAPTER II

THE CASES

INTRODUCTION

Seven patients will be described. The first six were admitted to the Military Hospital at Terendak Camp and the seventh was admitted to the British Military Hospital at Singapore.

Terendak Camp is situated near Malacca on the west coast of the Malayan peninsula. British, Australian, New Zealand, Gurkha and Malaysian soldiers and their families were stationed there. The Terendak Military Hospital was a small modern general hospital. While the author was there he had a close link with the United States Army Medical Research Unit in the Institute for Medical Research at Kuala Lumpur, Malaysia; this unit kindly carried out all the serological tests for the author, and confirmed the presence of Ps pseudomallei in the positive cultures.

The British Military Hospital at Singapore is a general hospital situated on Singapore Island.

THE PATIENTS

Patient Number 1

A 39 year old white British sergeant was admitted to hospital with a history of increasing thirst and polyuria over the preceding ten weeks. Eleven days before admission he had developed a painful swelling of the right knee. He had served in Malaysia for three years and was a storeman for most of this time. He frequently drank beer to excess, and his father suffered from diabetes mellitus.

On examination he was obese, confused and dehydrated. His temperature was 99.0° Fahrenheit. The right pupil was smaller than the left but they both reacted briskly to light. There was a sparse pustular rash, the right knee was acutely inflamed and there were several old and recent scars on his legs. His liver extended one inch below the right costal margin; it was smooth, firm and non-tender. The spleen was not palpable. Crepitations were present over the lower zones of both lungs and his blood pressure was 110/64 millimetres of mercury.

Investigations The haemoglobin was 14.6 grammes per 100 millilitres, the total white count was 5,400 cells per cubic millimetre with 76 per cent neutrophils and the erythrocyte sedimentation rate was 70 millimetres in the first hour (Westergren). No malaria parasites were present in numerous films of his peripheral blood. On admission his urine contained sugar and acetone and his blood sugar was 276 milligrammes per 100 millilitres, thereafter varying between 100 and 300 milligrammes per 100 millilitres. The serum glutamic pyruvic transaminase was 100 Smith Frankel units per millilitre. Serial portable chest radiographs showed an ill-defined rounded opacity in the right mid-zone (Figure 1). Ps pseudomallei was grown in pure culture from the blood, sputum, skin pustules and from synovial fluid aspirated from the right knee. The urine remained sterile, the stools did not contain any pathogens, and nose and throat swabs showed various organisms. The cerebrospinal fluid was normal. The electrocardiogram showed sinus rhythm, a PR interval of 0.24 seconds, depressed ST segments and inverted T waves in all leads. The serum electrolytes were within normal limits throughout. The Widal Reaction, Weil Felix, Brucella Agglutination, Paul Bunnell, Leptospirosis Haemolytic and Toxoplasma Dye Tests, and the Venereal Diseases Research Laboratory slide test were all negative. The melioidosis haemagglutination titre was 1:640, the agglutination titre rose from 1:160 to 1:320 and the complement fixation titre was 1:20. These are all positive titres.

Progress After the initial investigations had been carried out diabetic ketosis and septicaemia were diagnosed and treatment was started with intravenous fluids, water soluble insulin, penicillin and streptomycin. Within a few hours he became hypotensive requiring intravenous hydrocortisone to restore his blood pressure. Thereafter his pressure fell whenever corticosteroid supplements were temporarily withdrawn. He was a little better on the day after admission when melioidosis was considered to be a possible cause of his septicaemia, so chloramphenicol in a daily oral dose of 2 grammes was started and, once the results of the sensitivity tests were available, he was also given chlortetracycline in the same dose. His diabetes was readily controlled with water soluble insulin but his general condition slowly worsened. He developed a coarse irregular tremor, plucking at the bedclothes, profuse watery diarrhoea, and he died four weeks after admission. He received a total of 29.75 grammes of chloramphenicol and 21.0 grammes of chlortetracycline.

Post-mortem findings Many caseous nodules and abscesses were found in the lungs and the right middle lobe contained an abscess 4.5 centimetres in diameter. The liver was enlarged, contained a few small nodules and histology showed marked fatty degeneration. The kidneys contained many tiny nodules just visible macroscopically, and an abscess had largely destroyed the prostate. The spleen was enlarged. The pancreas was macroscopically normal and histological sections showed marked interstitial fibrosis but no abnormality of the Islets of Langerhans. Ps pseudomallei was isolated in pure culture from the heart blood and abscesses in all the affected organs.

Summary

This patient had a history of thirst, polyuria and a painful knee. He was found to have diabetic ketosis and septicaemia. The diagnosis of melioidosis was established by isolating Ps pseudomallei and he developed positive haemagglutination, agglutination and complement fixing titres. He died four weeks after admission to hospital despite treatment with chloramphenicol and chlortetracycline. Necropsy showed multiple abscesses and fatty degeneration of the liver.

Patient Number 1

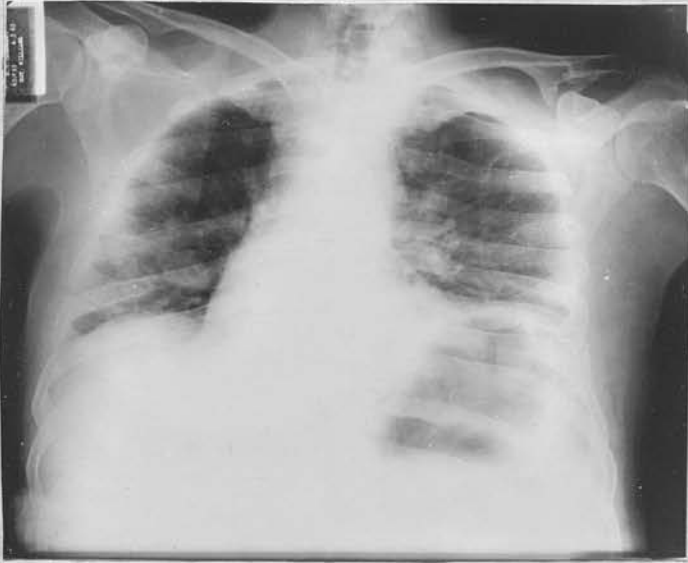


Figure 1.

This is a portable radiograph showing a rounded opacity in the right lower zone.

Patient Number 2

A 32 year old white New Zealand sergeant was admitted to hospital with one month's history of malaise, nausea, vomiting, diarrhoea and weight loss of two stones. During the five days before admission his condition had worsened with fever, shivering, sweating, thirst and painful swelling of both knees preventing weight bearing.

Three months before admission he had been treated elsewhere for a carbuncle but swabs had not been taken for culture. He had been in Malaysia for a total of three years. He worked as a cook in an infantry battalion and was a heavy beer drinker.

On examination he was obese, pale and breathless at rest. His temperature was 103.0° Fahrenheit. He had a left ptosis and acute inflammation of both knees. A few pustules were present over the trunk and limbs, and there were several small scars on his knees. Breath sounds were diminished over the bases of both lungs but no adventitious sounds were present. The liver extended one inch below the right costal margin; it was smooth, firm and non-tender. His blood pressure was 120/90 millimetres of mercury.

Investigations The haemoglobin was 13.1 grammes per 100 millilitres, the total white count was 9,200 cells per cubic millimetre with 73 per cent neutrophils and the erythrocyte sedimentation rate was 115 millimetres in the first hour. No malaria parasites were found in numerous films of his peripheral blood. His serum bilirubin was 3.1 milligrammes per 100 millilitres but other tests of liver function were normal. The serum electrolytes remained within normal limits. A portable chest radiograph showed a raised right hemidiaphragm without obvious pulmonary collapse or consolidation (Figure 2). The electrocardiogram was normal. Ps pseudomallei was isolated in pure culture from the blood and from a pustule overlying the right knee. Urine and synovial fluid aspirated from the left knee on admission were sterile, and no pathogens were isolated from his stools. The cerebrospinal fluid was normal. The melioidosis haemagglutination titre was 1:640, the agglutination titre 1:320 and the complement fixation titre 1:20.

Progress The initial diagnosis was septicaemia, possibly due to typhoid fever as there was an outbreak in Malacca at the time. Treatment was started with oral chloramphenicol and ampicillin, 0.5 grammes of each every 6 hours. The following day he was worse with dysphagia, profuse watery diarrhoea, an increase in the number of pustules, a coarse irregular tremor and plucking at the bed-clothes. As the clinical picture resembled the previous patient, melioidosis was considered to be a possible cause of the septicaemia so treatment was changed to 1 gramme of chloramphenicol and 0.5 grammes of chlortetracycline 6 hourly by intramuscular injection. On the third day he only passed 320 millilitres of urine and his blood urea rose to 242 milligrammes per 100 millilitres. He died suddenly on the fourth day after admission. He was given a total of 23.5 grammes of chloramphenicol and 10.5 grammes of chlortetracycline.

Post-mortem Findings Abscesses were scattered throughout both lungs and an inflammatory vegetation was attached to the endocardium. The liver was enlarged and showed the histological changes of cirrhosis. The enlarged spleen contained one abscess. A diffuse abscess was present in the subcutaneous tissue of the scalp and another overlay the frontal cortex of the brain. Ps pseudomallei was isolated in pure culture from the heart blood, the intracardiac vegetation and from abscesses in all the affected organs.

Summary

This patient had a history of malaise, weight loss and painful knees. He was febrile, had a sparse pustular rash and acute inflammation of both knees. The diagnosis of melioidosis was established by isolating Ps pseudomallei, and he had positive haemagglutination, agglutination and complement fixation titres. He died four days after admission to hospital despite treatment with chloramphenicol and chlortetracycline. Necropsy revealed multiple abscesses and hepatic cirrhosis.

Patient Number 2.



Figure 2.

This is a portable radiograph showing only a raised right hemidiaphragm.

Patient Number 3

A 26 year old Maori infantry soldier was admitted to hospital with a four day history of malaise, headache, lassitude, tightness in the chest and watery brown diarrhoea without blood or mucous. He had been in Malaysia for five weeks.

On examination his temperature was 106.6° Fahrenheit. He had a few pustules on the trunk and arms and signs of consolidation over the middle and lower zones of the right lung. There were numerous old and recent scars on both legs. His blood pressure was 120/70 millimetres of mercury.

Investigations The haemoglobin was 12.2 grammes per 100 millilitres, the total white count was 16,000 cells per cubic millimetre with 88 per cent neutrophils and the erythrocyte sedimentation rate was 101 millimetres in the first hour. No malaria parasites were found in numerous films of his peripheral blood. Serial chest radiographs showed cavitated segmental consolidation in the right middle lobe and in the apical segment of the right upper lobe which gradually healed (Figures 3 to 5). Stool cultures did not show any pathogens and urine and blood cultures were sterile. Serial sputum and skin swabs produced growths of various organisms. His Heaf test was positive, Grade 2. The routine serological tests listed for Patient number one (page 10) were all negative. The melioidosis haemagglutination titre rose from 1:10 to 1:320, the agglutination titre rose from 1:10 to 1:160 and the complement fixation titre rose from 1:5 to 1:20. These are all rises to diagnostic levels.

Progress A few hours after admission his condition worsened with profuse watery diarrhoea, a cough productive of mucopurulent sputum, right sided pleuritic pain, confusion, coarse tremors and plucking at the bedclothes. The initial diagnosis was acute pneumonia perhaps due to Klebsiella pneumoniae. He was given 0.5 grammes of tetracycline orally every 6 hours with slow improvement after the first few days. A month after admission his melioidosis haemagglutination titre was found to be positive but as he was improving chloramphenicol was not added to his regime. Although he required a long period of convalescence he was entirely well when last seen six months after admission and he was known to be well 21 months later. He received a total of 100.5 grammes of tetracycline.

Summary

This patient had a four day history of malaise, tightness in the chest and diarrhoea. On examination there was evidence of right sided pneumonia which was confirmed radiographically. The diagnosis of melioidosis was established by a rise in the titre of all three serological tests. Slow but satisfactory improvement followed treatment with oral tetracycline.

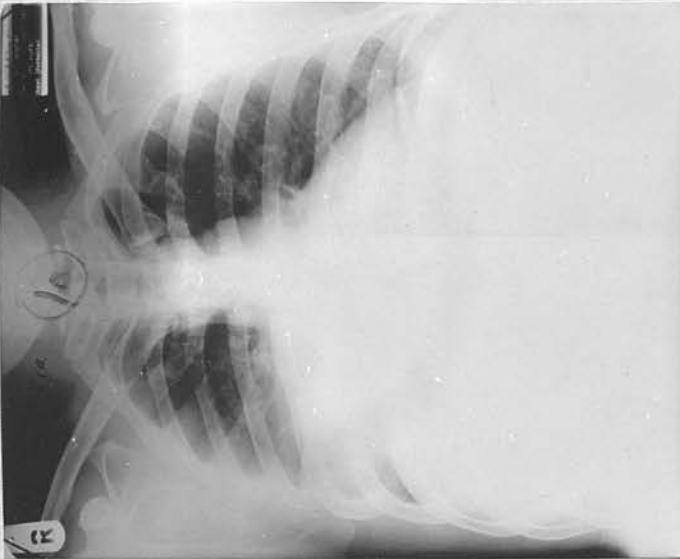


Figure 3a.

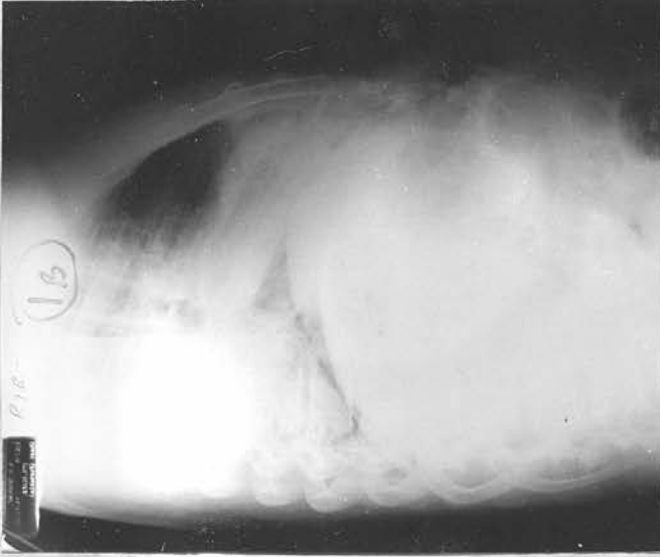


Figure 3b.

Antero-posterior and right lateral radiographs taken on admission showing segmental consolidation of the right middle lobe and of the apical segment of the right lower lobe.

Patient Number 3.

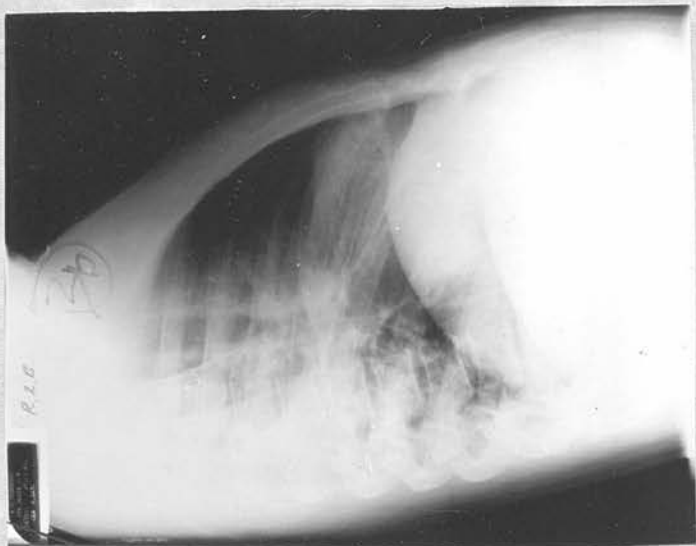


Figure 4b



Figure 4a

Postero-anterior and right lateral films taken two weeks after admission showing improvement. Cavitation is visible in the right middle lobe.

Patient Number 3.

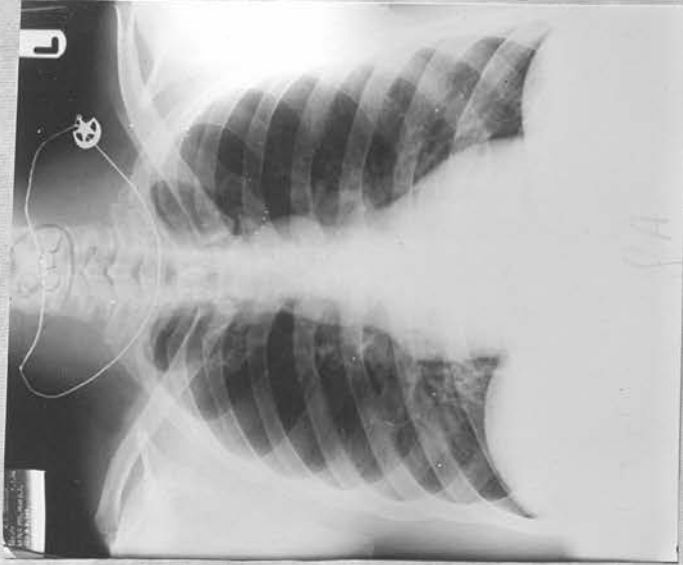


Figure 5a.

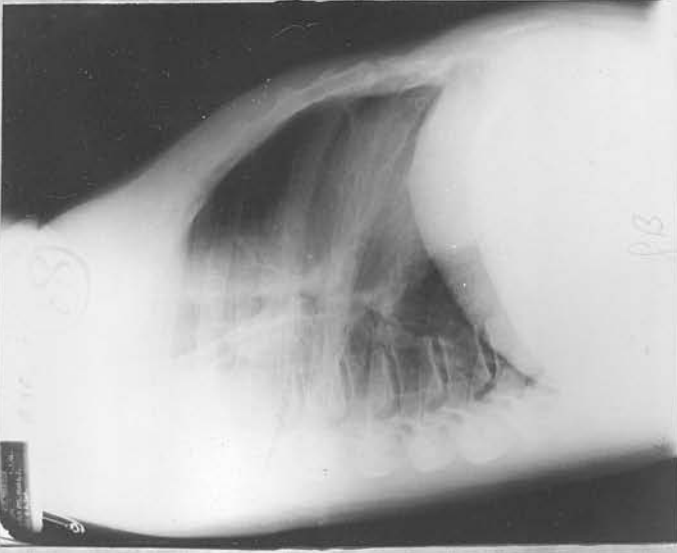


Figure 5b

Postero-anterior and right lateral films taken 10 weeks after admission showing residual fibrosis.

Patient Number 4

A 32 year old white British infantry sergeant was admitted to hospital with one day's history of fever, headache and malaise. He had been in Malaysia for seven months. He was a keen footballer who had received many minor injuries, and one month before admission he had received an extensive abrasion which had become infected but swabs had not been taken for culture.

On examination his temperature was 100.2° Fahrenheit, there were several scars on his legs but no other abnormality was detected. His blood pressure was 114/68 millimetres of mercury.

Investigations The haemoglobin was 14.8 grammes per 100 millilitres, the total white count was 9,200 cells per cubic millimetre with a normal differential count and his erythrocyte sedimentation rate was 25 millimetres in the first hour. No malaria parasites were found in numerous films of his peripheral blood. The only abnormal liver function test was a raised beta-globulin on electrophoresis of his serum proteins. Ps pseudomallei was isolated in pure culture from his sputum. His urine was sterile and no pathogens were isolated from his stools. His Heaf test was positive, Grade 3. His chest radiograph showed a rounded cavitated opacity in the left upper zone 1.5 centimetres in diameter (Figure 6) which enlarged before resolving (Figures 7 to 10). The melioidosis haem-agglutination titre rose from 1:40 to 1:640 and the agglutination titre rose to 1:160 but no complement fixing antibodies were detected.

Progress The clinical diagnosis was pyrexia of unknown origin but this was changed to lung abscess when the abnormality on his chest radiograph was found. A course of cloxacillin produced no improvement so pulmonary tuberculosis was tentatively diagnosed and triple chemotherapy prescribed. Despite this he deteriorated with a non-productive cough, enlargement of his liver and spleen, elevation of his erythrocyte sedimentation rate to 79 millimetres in the first hour and the lesion on his chest radiograph enlarged. Two months after admission he developed haemoptysis and a few days later Ps pseudomallei was isolated from his sputum. His treatment was at once changed to chloramphenicol, 3 grammes daily by intramuscular injection, and chlortetracycline, 3 grammes daily by mouth. He improved

satisfactorily and when last seen four months after his discharge from hospital he was entirely well. A report was received 18 months later that he remained well. His total antibiotic treatment was 69 grammes of chloramphenicol and 93 grammes of chlortetracycline.

Summary

This patient had a 24 hour history of malaise and fever, and the principal finding was a cavitated opacity on his chest radiograph. The diagnosis of melioidosis was established by isolating Ps pseudomallei from his sputum and by the development of positive titres of melioidosis haemagglutinins and agglutinins. He responded satisfactorily to chloramphenicol and chlortetracycline.

Patient Number 4



Figure 6.

This radiograph shows the cavitated opacity in the left upper zone. (This film was taken on the day after this patient's first admission to confirm the abnormality; this is why it has the number 2. It shows the cavity better than the first film.)

Patient Number 4.

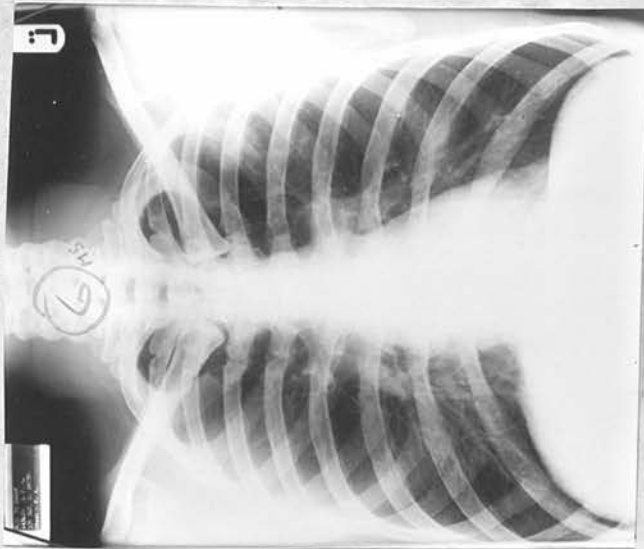


Figure 7.

Figure 7: This shows enlargement of the lesion.



Figure 8.

Figure 8: A tomogram confirming the cavity.

Both films were taken 6 weeks after the beginning of the patient's illness.

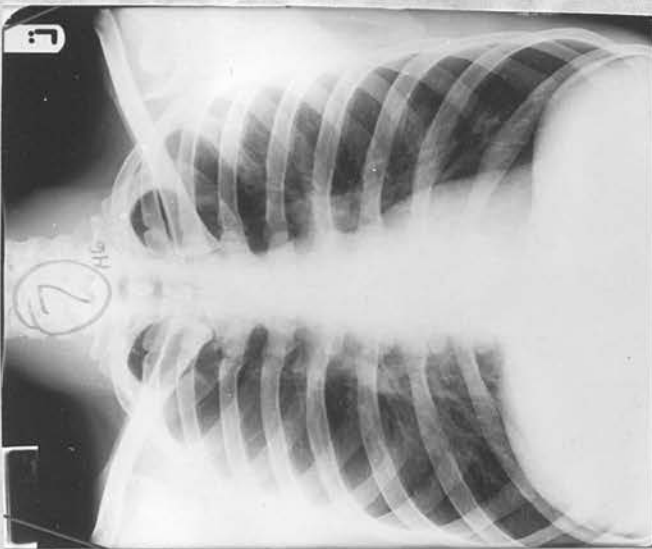


Figure 9.

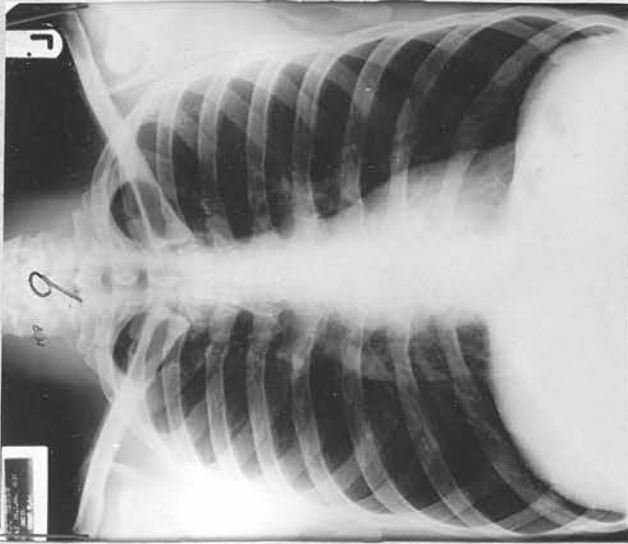


Figure 10.

Figure 9 : This radiograph was taken 1 week after Figures 7 and 8, and shows diffuse streaky shadowing in the left upper zone.

Figure 10: This was taken 2 months after Figure 9. It shows considerable resolution.

Patient Number 5

A 31 year old Maori infantry soldier was admitted to hospital with a two day history of malaise, anorexia, shivering and sweating. His stools had been loose but had not contained any blood or mucous. He had been in Malaysia for nine months and he had been drinking very heavily during the month before admission.

On examination his temperature was 104.2° Fahrenheit. There were several recent abrasions on his legs and signs of consolidation over the middle and lower zones of the left lung. The liver extended one inch below the right costal margin; it was smooth and soft but not tender. His blood pressure was 110/64 millimetres of mercury.

Investigations The haemoglobin was 14.8 grammes per 100 millilitres, the total white count was 20,000 cells per cubic millimetre with 86 per cent neutrophils and the erythrocyte sedimentation rate was 96 millimetres in the first hour. No malaria parasites were found in films of his peripheral blood. The liver function tests were normal apart from a rise in the serum glutamic pyruvic transaminase to 56 Smith Frankel units per millilitre. Serum electrolytes were within normal limits but the blood urea rose terminally to 83 milligrammes per 100 millilitres. The chest radiograph initially showed consolidation in the left lower zone (Figure 11) and later in both lower zones (Figure 12). Ps pseudomallei was isolated in pure culture from the blood and sputum. His urine and swabs from pustules were sterile. Stool culture did not show any pathogens. The melioidosis haemagglutination, agglutination and complement fixation tests, and the routine serological tests listed for patient number one (page 10) were all negative.

Progress On the evening of admission he developed a cough productive of tenacious mucoid sputum containing a few streaks of blood. Intramuscular penicillin was started. After 24 hours a blood culture contained an organism with the appearance of Ps pseudomallei. Treatment was at once changed to intravenous chloramphenicol and chlortetracycline, 6 grammes of each every 24 hours. Despite this he worsened with signs of consolidation over the right lung, dyspnoea, cyanosis, profuse watery diarrhoea, a sparse

pustular rash and mental confusion. He was given oxygen, physiotherapy and, later, bronchoscopy and tracheostomy were carried out, but he died on the fifth day after admission. During the last 86 hours of his illness he received 21 grammes of chloramphenicol and the same dose of chlortetracycline, both intravenously.

Post-mortem Findings Both lungs showed widespread bronchopneumonia and multiple abscesses. There was histological evidence of hepatitis and myocarditis, and the spleen showed septicaemic changes. Ps pseudomallei was isolated in pure culture from heart blood and from all abscesses sampled.

Summary

This patient had a two day history of malaise and fever. On examination he had signs of left sided pneumonia which was confirmed radiographically. Ps pseudomallei was isolated from his blood and sputum but he did not have any homologous serum antibodies. He died five days after admission despite treatment with intravenous chloramphenicol and chlortetracycline, and vigorous supportive measures. Necropsy showed widespread bronchopneumonia and multiple lung abscesses.

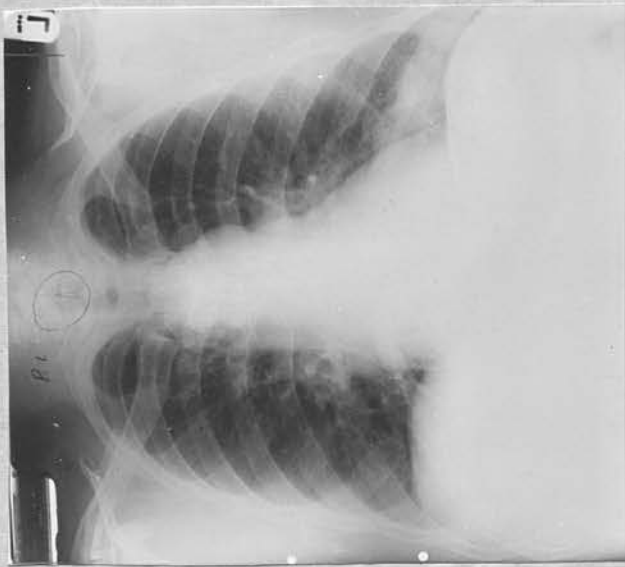


Figure 11.

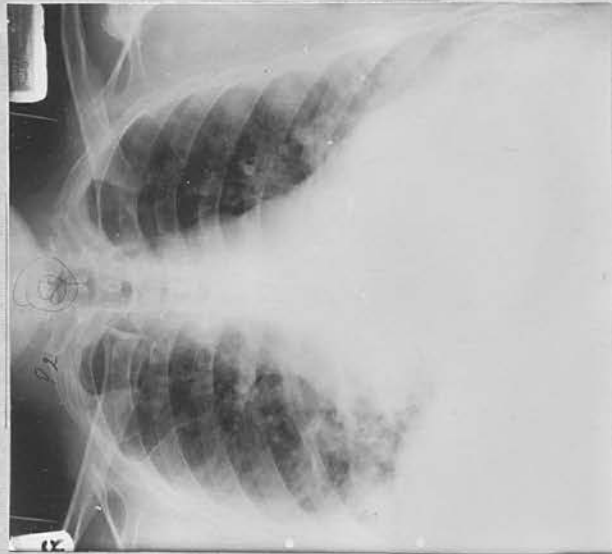


Figure 12.

Figure 11: An antero-posterior radiograph taken on admission showing consolidation in the left lower zone.

Figure 12: This radiograph was taken 3 days later and shows bilateral basal consolidation.

Patient Number 6

A 36 year old white British housewife was admitted to hospital with a two month history of malaise, lethargy, headache, weight loss and recurrent sore throats associated with fever. She did not have a cough. Three weeks before admission she had noticed aching pains in her wrists, elbows, knees and ankles. She had been in Malaysia for two years and was a keen gardener.

On examination her temperature was 100.4° Fahrenheit. There was pain on passive movement of all the affected joints which were warm and slightly tender. She had a few small scars on her hands and one on her right knee, but there were no other abnormalities. Her blood pressure was 104/72 millimetres of mercury.

Investigations The haemoglobin was 10.5 grammes per 100 millilitres, the total white count was 7,300 cells per cubic millimetre with 84 per cent neutrophils and the erythrocyte sedimentation rate was 78 millimetres in the first hour. No malaria parasites were found in the peripheral blood films. The liver function tests were normal apart from a raised alpha 2 globulin on electrophoresis of the serum proteins. Serial chest radiographs showed a cavitated opacity in the left upper zone which worsened before clearing (Figures 13 to 18). Repeated blood cultures, urine cultures, gastric washings and laryngeal swabs were negative on culture. Her Heaf test was positive, grade 3. Serum was tested on three occasions for rheumatoid factor. In the second specimen the Latex test was weakly positive and the Rose Waaler test was positive, but the other samples were negative. The specimens sent for the anti-streptolysin "O" titre were all reported to be unsuitable for testing. No antinuclear factor was present, nor were lupus erythematosus cells found, the serum uric acid was normal and the Gonococcal Complement Fixation Test was negative. The routine serological procedures listed for Patient number one (page 10) were all negative throughout. The melioidosis haemagglutination titre rose from 1:40 to 1:160 and the agglutination titre rose from 1:10 to 1:160 but no complement fixing antibodies were detected.

Progress While the initial investigations were being carried out she was given methicillin, but she remained febrile and developed a dry cough. The chest radiograph showed slightly increased consolidation after two weeks so pulmonary tuberculosis was suspected and triple chemotherapy prescribed. This did not produce any clinical improvement and the appearance of the chest radiograph did not change. After another two weeks 0.5 grammes of chlortetracycline every six hours were added to her regime because melioidosis was considered to be a possible cause of her illness. She slowly began to improve. Six weeks after admission her melioidosis haemagglutination titre rose to a diagnostic level; as she was improving chlortetracycline was continued alone. She made an uneventful recovery and one year after her discharge a report was received that she remained well. She received a total of 66 grammes of chlortetracycline.

Summary

This woman had two months' history of malaise, weight loss and vague joint pains. On examination she was febrile and her chest radiograph showed a cavitated opacity in the left upper zone. Melioidosis was suspected when she failed to respond to methicillin followed by anti-tuberculous chemotherapy, and confirmed by diagnostic rises in the haemagglutination and agglutination titres. She responded slowly but satisfactorily to oral chlortetracycline.

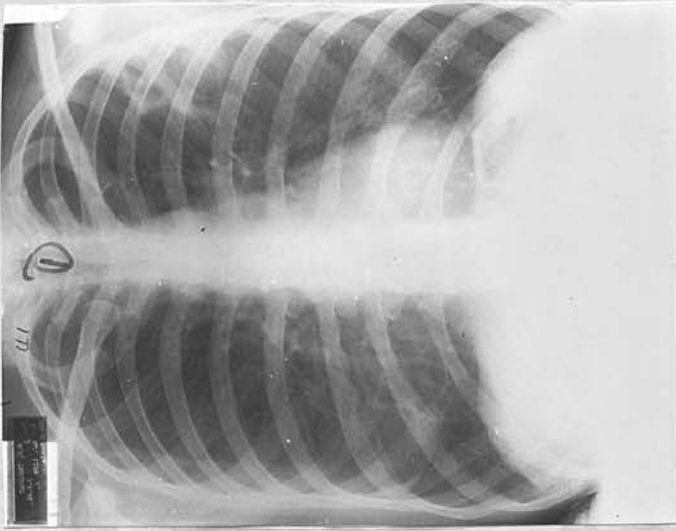


Figure 13.

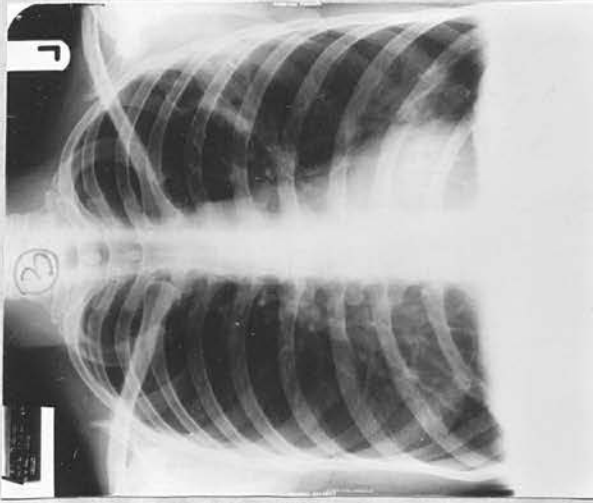


Figure 14.

Figure 13: This radiograph shows a cavitated lesion in the left upper zone.

Figure 14: Two weeks later there is an increase in the consolidation.

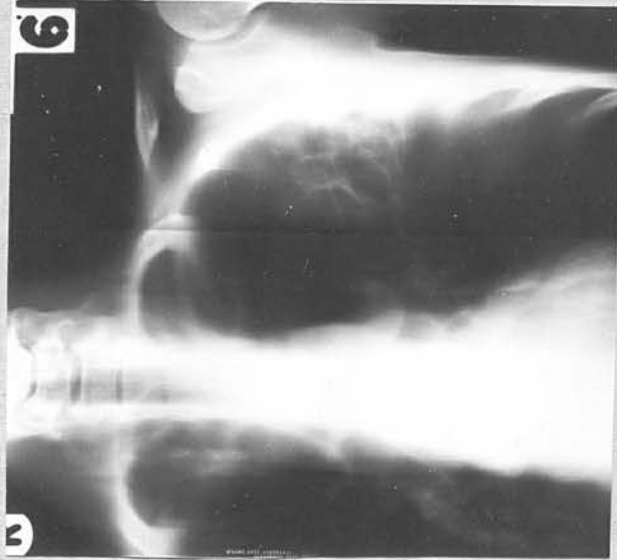


Figure 15.

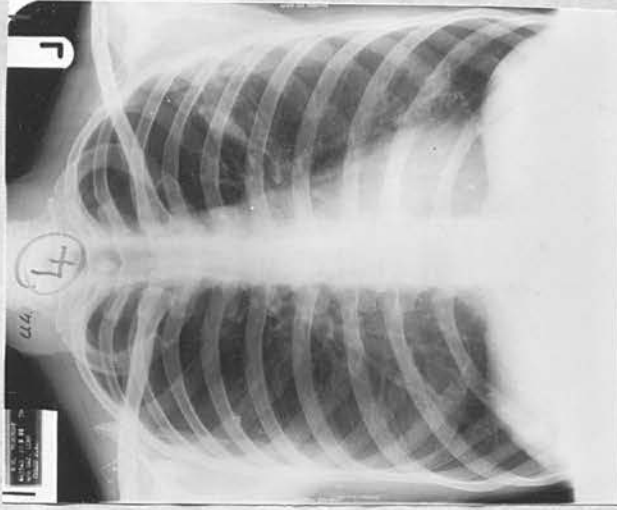


Figure 16.

Figure 15: This is a tomogram taken 1 week after the radiograph shown in Figure 14 confirming the cavitation and showing that the lesion is more extensive than the postero-anterior films indicate.

Figure 16: This shows slight improvement 10 days after the patient started tetracycline.

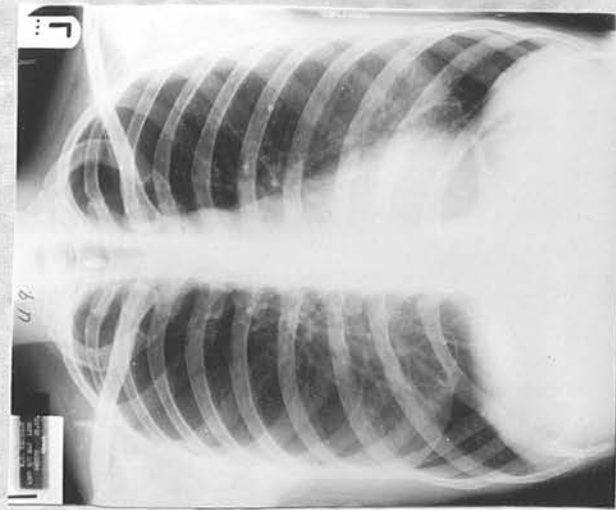


Figure 17

Figure 17: Ten weeks later there is considerable resolution.

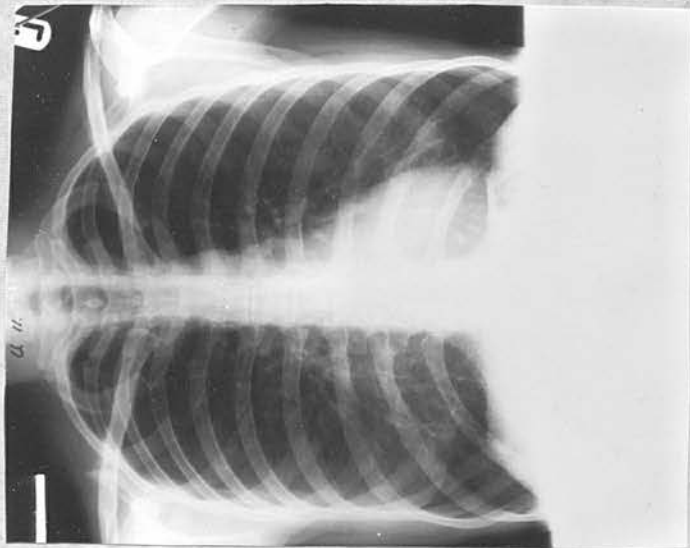


Figure 18

Figure 18: Four months after admission only minimal fibrosis remains in the left upper zone.

Patient Number 7

A 47 year old white British housewife was admitted to hospital with a two week history of malaise, headache and fever but no cough. She had been in Malaysia and Singapore for a total of two years and nine months.

On examination her temperature was 103.2° Fahrenheit and her spleen was palpable but no other abnormality was found.

Investigations The haemoglobin was 12.8 grammes per 100 millilitres, the total white count was 10,200 cells per cubic millimetre with 86 per cent neutrophils and the erythrocyte sedimentation rate was 65 millimetres in the first hour. No malaria parasites were found in her peripheral blood films. Her chest radiograph showed an ill-defined irregular cavitated opacity in the left middle zone (Figure 19) which worsened before it gradually cleared (Figures 20 to 22). Her Heaf Test was negative.

Ps pseudomallei was not cultured from the single specimen of sputum she coughed up, nor was it isolated from laryngeal swabs, blood or urine. The melioidosis haemagglutination titre rose from 1:40 to 1:2560, the agglutination titre rose to 1:320 and the complement fixation titre rose to 1:40. The routine serological tests listed for Patient number one (page 10) were all negative.

Progress The initial diagnosis was aspiration pneumonia and she was given 250 milligrammes of chlortetracycline every six hours by mouth, but her condition gradually worsened with persisting fever, dry cough and enlargement of her spleen. As soon as the diagnosis of melioidosis was suspected from her rising haemagglutination titre, the dose of chlortetracycline was increased to 1 gramme every six hours by mouth and she was also given 1 gramme of chloramphenicol every eight hours by intramuscular injection. Clinical and radiographic response was satisfactory but five weeks after starting chloramphenicol she developed pancytopenia with a haemoglobin of 7.0 grammes per 100 millilitres, a total white count of 2,400 cells per cubic millimetre and a platelet count of 100,000 cells per cubic millimetre. Chloramphenicol was stopped. She made a satisfactory recovery from this incident and by the time of her discharge she was well, her blood picture was normal and her chest radiograph was clear. She received a total of 112 grammes of chloramphenicol and 107 grammes of chlortetracycline.

Summary

This woman had a two week history of malaise and fever. Her spleen was palpable and her chest radiograph showed an ill-defined opacity in the left middle zone. The diagnosis of melioidosis was established by a rise in the titres of all three serological tests. She responded satisfactorily to chloramphenicol and chlortetracycline although her recovery was complicated by a transient episode of pancytopenia attributed to chloramphenicol.

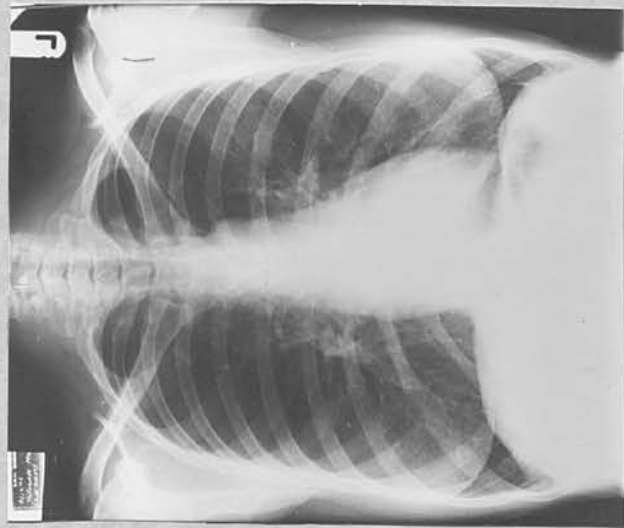


Figure 19.

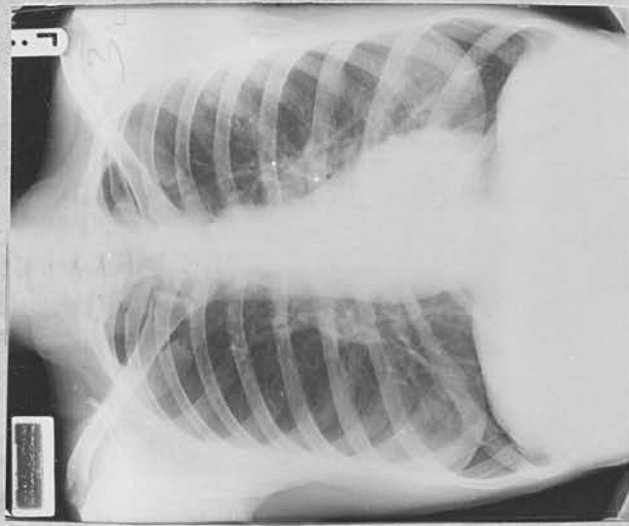


Figure 20.

Figure 19: This radiograph shows an ill defined cavitated shadow in the left middle zone.

Figure 20: This was taken two weeks after Figure 19 and shows increased shadowing in the left middle zone.

Patient Number 7.

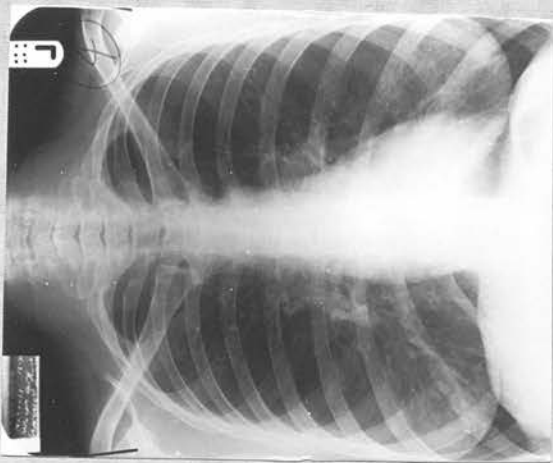


Figure 21.

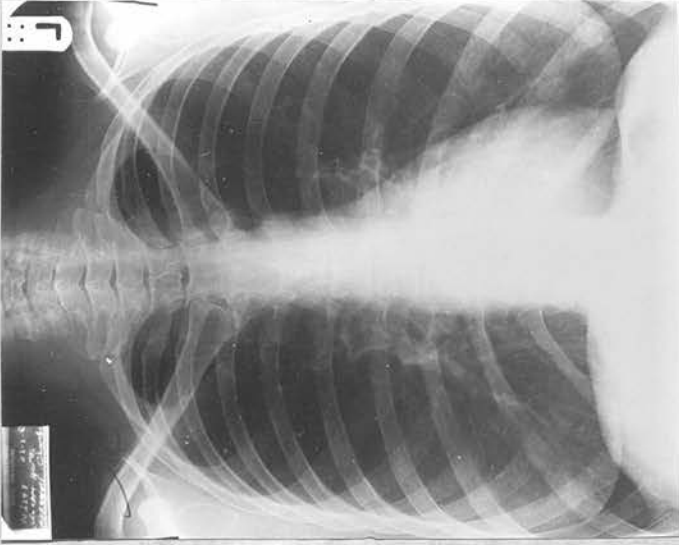


Figure 22.

Figure 21: This was taken 1 week after stopping chemotherapy and 8 weeks after

Figure 20. Considerable resolution has occurred.

Figure 22: Two months after Figure 21, there is only slight residual fibrosis.

PATHOLOGY

These case and necropsy reports showed that lesions occurred most frequently in the lungs and, in descending order of frequency, in the liver, spleen and kidneys, but almost every organ could be affected. The lesions started as microscopic foci which developed into inflammatory nodules and multiple nodules coalesced to form abscesses.

The lesions were characteristic. They started as collections of neutrophils surrounded by zones of congestion. When larger they had centres of necrotic, pink staining, caseous material containing nuclear debris, surrounded by a narrow zone of more proliferative activity with lymphocytes and histiocytes, and a thin developing capsule of fibrous tissue. At the periphery intensely congested dilated capillaries formed a distinctive collar sharply demarcating the lesion from the surrounding tissue. Gram negative bacilli were not demonstrated.

BACTERIOLOGY

All the strains were identical.

Morphology

The organism was a short Gram negative rod with rounded ends showing no characteristic arrangement. Bipolar staining was well demonstrated in Leishman stained films. It was actively motile at 37° and 24° Centigrade.

Cultural Characteristics

No growth was observed on blood agar after 24 hours incubation at 37° Centigrade, but after 48 hours smooth, round, low convex, translucent, greyish white colonies one or two millimetres in diameter appeared. Further incubation produced large rough wrinkled colonies and slight haemolysis.

Small pink "poached egg" colonies appeared on MacConkey Agar after 48 hours incubation.

Nutrient agar incorporating 3 per cent glycerol and 1:200,000 crystal violet (Miller et al 1948) proved to be the most useful medium for the study of this organism. Colonies, deeply tinted by the dye, were

visible after 24 hours incubation at 37° Centigrade. They gradually enlarged to a maximum of seven millimetres after 14 days incubation when they had a dry wrinkled beaten-aluminium appearance.

Twenty four hours incubation at 37° Centigrade in nutrient broth produced an even turbidity and an early surface pellicle. A heavy viscous deposit appeared after a few days.

Biochemical Properties

There was a faint acid reaction without gas formation after 24 hours incubation with glucose, sucrose, maltose and mannite; no further change occurred after 21 days. Adonitol, dulcitol and lactose gave no reaction after 30 days incubation at 31° Centigrade. These reactions were studied using peptone water and Andrade's indicator. It is known that weak and unreliable sugar reactions occur in liquid media when the organism oxidises sugar, and this was assumed here so the oxidation fermentation test was not carried out (Hugh and Leifson 1953 and Cowan and Steel 1965).

Catalase was produced, the oxidase test was positive, gelatin was liquified and growth occurred in Potassium Cyanide medium. Neither indole nor hydrogen sulphide were produced and urease activity was absent.

Pathogenicity

Female guinea pigs died on the fifth day after intra-peritoneal inoculation of 0.05 millilitres of 1:10 and 1:100 dilutions of overnight broth cultures. At necropsy nodules were present in the lungs and transverse colon. The organism was isolated from the nodules and from heart blood. Male animals could not be obtained to demonstrate the Straus Reaction (Straus 1889).

Antibiotic Sensitivity

All strains were markedly sensitive to chloramphenicol, moderately sensitive to the tetracyclines, particularly to chlortetracycline, and slightly sensitive to novobiocin, sulphatriad, ampicillin and neomycin. They were resistant to benzylpenicillin, methicillin, cloxacillin, streptomycin, erythromycin, cephaloridine, fucidin, colomycin, kanamycin

and polymyxin B. These findings were identical in necropsy isolates. Sensitivity was tested using "Multodisks" (Messrs. Oxoid Ltd.) but the technique used was not sufficiently reproducible to allow the minimum inhibitory concentrations to be estimated.

Where possible sensitivity tests were carried out daily during treatment. No evidence of drug resistance developed. Simple tests showed no evidence of synergism or antagonism between chloramphenicol and the tetracyclines, but sophisticated techniques such as those described by Garrod and Waterworth (1962) were not available.

DISCUSSION

Incidence

These patients presented over the course of two and a half years in a comparatively small population and no other cases of melioidosis were diagnosed in West Malaysia during the same period. Whitmore's report of 38 cases discovered in Rangoon in one year appears to be the highest documented incidence, though Cooper (1967) indicates a high incidence among American soldiers in South Viet Nam. Stanton and Fletcher (1932) reported 39 patients discovered in and around Kuala Lumpur over twelve years and this is probably a higher incidence than the author's. There have only been occasional reports from Malaysia since then, recent ones being those of Khaira, Young and Hart in 1959, Montgomery in 1963 and Baird and Meers in 1965. Most early reports concerned Asian patients but recently many of the published accounts have been of non-indigenous patients suggesting that cases among Asians are being missed. The sporadic pattern of the reports and the change in the type of patient suggest that melioidosis is only diagnosed where there is not only awareness of its existence but also adequate laboratory facilities for isolation and identification of the causative organism. Stanton and Fletcher (1932) estimated from their necropsy findings that melioidosis was responsible for 200 deaths in the Federated Malay States every year, but it is impossible to estimate the incidence of melioidosis at the present time. It is probably more common than is realised.

Predisposing Factors

There was evidence of pre-existing disease in the first two fatal cases and possible in the third, Patient 5. Patients 2 and 4 had local cutaneous infections before the onset of melioidosis, and the single positive Rose Waaler Test in Patient 6 may have been due to early rheumatoid arthritis but is of doubtful significance. Thus only patients 3 and 7 were definitely well before they developed melioidosis.

Predisposing factors have been mentioned by a number of writers. The general effect of morphine addiction has already been noted, the patient described by McDowell and Varney (1947) had a long history of

serious infections and five of the six patients Rimington described in 1962 had severe underlying disease. Montgomery (1963) suggested that diabetics are particularly liable to melioidosis, but diabetics are susceptible to all bacterial infections. Kalazar, pertussis and syphilis have been mentioned as predisposing factors, but there are no reports of tuberculosis predisposing patients to melioidosis. Although Fournier and Chambon (1958) considered that predisposing factors were important there do not appear to be any conditions particularly associated with melioidosis. Ps pseudomallei appears to be a widespread usually avirulent organism which rarely becomes established in the body unless resistance is lowered by some other condition.

Diagnosis

Although a review of the literature shows that melioidosis produces very variable clinical features even in the acute form, the clinical pictures of the author's acutely ill patients were remarkably alike. There was evidence of septicaemia, pneumonia, profuse watery diarrhoea, confusion, coarse tremors and plucking at the bedclothes. The sub-acute cases had various clinical features but were all febrile and they all had pulmonary lesions. Several classifications of melioidosis have been suggested and the sub-division into acute, sub-acute and chronic forms put forward by Alain, St Etienne and Reynes in 1949 is the most practical. Patients 1, 2 and 5 had acute melioidosis and the remainder had sub-acute melioidosis.

Simple laboratory investigations helped little towards diagnosis. Two patients had a marked neutrophil leucocytosis and three had a relative neutrophilia, the highest neutrophil response being in one of the fatal cases and the lowest in one of the moderately severe cases. Other accounts show equally variable findings. Raised erythrocyte sedimentation rates were, however, prominent in these patients during active infection varying from 65 to 115 millimetres in the first hour. High erythrocyte sedimentation rates have not been emphasised by other authors, but the very high values found in Patients 2 and 5 on admission were helpful in making an early diagnosis. However high erythrocyte sedimentation rates are found in many conditions.

Like other workers the author concluded that the most satisfactory method of diagnosing melioidosis is to isolate Ps pseudomallei and demonstrate rising homologous antibody titres, but if isolation is not possible it is necessary to rely on antibody studies alone. The bacillus was isolated from the blood in all the acute cases and the presence of bipolar rods in blood cultures from these patients after 24 hours incubation was the first bacteriological indication of the diagnosis. Other workers have also found blood culture to be a valuable investigation (Whitmore 1913 and Fournier and Chambon 1958). Cultures should be taken from all febrile patients suspected to be suffering from melioidosis, since patients who have positive blood cultures are likely to be just those in whom early diagnosis is vitally important to save their lives.

Ps pseudomallei was also isolated from sputum, pustules and synovial fluid from the acutely ill patients. Nutrient agar containing glycerol and crystal violet proved to be particularly useful for isolating this organism and colonies were visible earlier on this medium than on any other. Although Miller and his colleagues first described the medium in 1948, it does not appear to be widely used. Fournier and Chambon (1958) and Cooper (1967) do not mention it although it was used by Sheehy, Deller and Weber (1967) and some other American writers. It was used routinely in the U.S. Army Medical Research Unit at the Institute for Medical Research in Kuala Lumpur and in the laboratory at the Terendak Military Hospital.

Ps pseudomallei was isolated from only one of the sub-acute cases, the remainder being diagnosed by rises in their antibody titres. Neither the agglutination nor the complement fixation tests differentiate between Ps pseudomallei and Ps mallei, but the haemagglutination test is specific for Ps pseudomallei and more sensitive than the other two (Fournier and Chambon 1958). Several workers including Darby and Mendez (1960) have described patients diagnosed by serological methods alone. However Patterson, Darling and Blumenthal (1967) point out that serological studies should not be relied on to diagnose acute cases, for they may die without developing antibodies. Patient number five was one such patient. It therefore appears that the place for serological studies is in the diagnosis of the less acute forms of melioidosis.

Although none of the patients presented with pulmonary symptoms they all had pulmonary abnormalities on their chest radiographs and these were the principal findings in the sub-acute cases. The abnormalities varied but four patients had cavitated lesions; in two patients these lesions were tentatively diagnosed as pulmonary tuberculosis and this condition was also considered in the other two. Cooper (1967) mentions that pulmonary infection appears to be the most common presentation, and Fournier and Chambon (1958) and Spotnitz, Rudnitzky and Rambaud (1967) indicate that pulmonary cavitation and confusion with pulmonary tuberculosis are common. Fournier and Chambon also mention that pulmonary lesions are often bilateral; this was true for the author's acute cases, but none of his sub-acute cases had bilateral radiographic changes. It appears that melioidosis should be considered in the differential diagnosis of all patients with pulmonary abnormalities on their chest radiographs.

Profuse watery diarrhoea was prominent in the acutely ill patients and the ensuing electrolyte disturbance was probably partly responsible for the fatalities. Whitmore in 1913 and Stanton and Fletcher in 1932 mentioned diarrhoea as a feature of melioidosis but since then other writers have rarely commented upon it. Though many of the acute diarrhoeal illnesses occurring in endemic areas are relatively benign, melioidosis may be fatal. It seems prudent therefore, to consider melioidosis in the differential diagnosis of all such patients presenting in endemic areas.

Treatment

Until the discovery of antibiotics, supportive measures and surgical drainage of abscesses formed the only treatment for melioidosis. Now, though vigorous supportive measures and drainage of accessible abscesses may still be required, chemotherapy is the most important part of treatment.

The three patients suffering from acute melioidosis were treated with chloramphenicol and chlortetracycline. Patient 1 died following conventional doses of both drugs so Patient 2 was given larger doses. After Patient 2's death it was decided to treat any further acute cases more intensively and Patient 3 was given 6 grammes of each drug intravenously every 24 hours, but he also died.

The remaining four patients had sub-acute melioidosis. Two patients, numbers 4 and 7, were diagnosed before definitive chemotherapy was started, and responded satisfactorily to intramuscular chloramphenicol and oral chlortetracycline in doses of 3 or 4 grammes daily. Patient 7's response was interesting: before the diagnosis was established she was slowly deteriorating while receiving chlortetracycline alone but improved rapidly as soon as chloramphenicol was added to her treatment. The other two patients, numbers 3 and 6, were given tetracyclines alone and only improved slowly.

All writers agree that chloramphenicol is the drug of choice in the treatment of melioidosis, and Fournier and Chambon (1958) recommend 3 or 4 grammes daily for several weeks, implying that this is adequate even for septicaemic cases. However this dose was insufficient for two of the author's septicaemic patients. According to recent American accounts from South Viet Nam (Cooper 1967 and Sheehy, Deller and Weber 1967) the best chemotherapy for acute cases is a combination of chloramphenicol, kanamycin and novobiocin, all given intravenously in massive doses. Laboratory studies of the action of this combination on Ps pseudomallei have not yet been published, but are due to appear shortly (Weber, Stalkamp and Douglas cited by Sheehy, Deller and Weber 1967). Kanamycin was not considered by the author for all the strains isolated from his patients were resistant to this drug in vitro and at that time he was not aware that the Americans were using it. Furthermore, Maegraith and Leithhead (1964) mention that their patient was unable to tolerate 1 gramme of kanamycin daily for more than five days, so at the moment it is difficult to determine the place of the large doses (4 grammes per 24 hours) recommended by American writers. There was only very slight sensitivity to novobiocin in vitro, and as there are contraindications to prescribing a bacteriocidal agent with bacteriostatic drugs such as chloramphenicol and tetracyclines (Jawetz and Gunnison 1952), novobiocin was not given.

Khaira, Young and Hart (1959), writing from Kuala Lumpur, successfully treated three patients suffering from sub-acute melioidosis with chloramphenicol and tetracyclines prescribed in moderate daily doses in short courses of one or two weeks. The strains of Ps pseudomallei isolated from the author's patients showed greater in vitro sensitivity to chloramphenicol and chlortetracycline than to any other antibiotics

and several workers have made similar observations. In addition, in the only published accounts of the in vitro action of antibiotic combinations on Ps pseudomallei the authors (Chambon, Lajudie and Fournier 1954 and Fournier and Chambon 1958) found that this combination is more often synergistic than any other. However Chambon (1955a) observed that this combination may induce resistance to chloramphenicol in the laboratory but the author has been unable to trace any other report confirming this work. Fournier and Chambon mention that several French authors found resistance to chloramphenicol developing in patients treated with this drug alone but it appears that they were giving small doses in long courses. All these reports came from Viet Nam and there have been no reports of drug resistance in Malaysia or elsewhere, so it was decided to prescribe chloramphenicol and chlortetracycline together. No resistance developed in the author's patients and the combination was satisfactory in Patients 4 and 7 who improved more rapidly than the patients treated with tetracyclines alone.

Patient number seven developed pancytopenia after a month's treatment with chloramphenicol. Blood dyscrasias are a well known complication of chloramphenicol therapy occurring in 1:10,000 to 1:100,000 patients receiving the drug according to Dunlop and Murdoch (1960). In view of the poor prognosis of severe melioidosis the author has no doubt that chloramphenicol should be prescribed as soon as the diagnosis is reasonably certain. In acute cases and in sub-acute cases not responding to conventional therapy, it appears that dosage should be higher than that given to Patient number five, and ten or twelve grammes per 24 hours are probably required for such patients as indicated by American writers. The drug should probably be given intravenously at first, the course lasting at least four weeks. In acute cases it seems advisable to add other drugs, the choice depending on sensitivity tests and previous experience in the locality. Chlortetracycline appeared to be the choice in Malaysia but kanamycin and novobiocin may be better elsewhere. However, until the Americans publish full details of their experience in Viet Nam it is difficult to determine the most satisfactory treatment for acute melioidosis. In sub-acute melioidosis 3 or 4 grammes of chloramphenicol and the same dose of chlortetracycline will probably be sufficient. In mild cases chlortetracycline alone may be adequate as

shown by Spotnitz, Rudnitzky and Rambaud (1967) who successfully treated nine patients suffering from pulmonary melioidosis with tetracyclines alone.

Mortality

The mortality among the author's patients was 3 out of 7 or 43 per cent, and the three patients who died were suffering from acute melioidosis. Before the introduction of chemotherapy the mortality rate was 95 to 100 per cent for all cases (Whitmore 1913 and Stanton and Fletcher 1932) but since the discovery of antibiotics the mortality has fallen. It was 31 per cent in the French Expeditionary Force to Viet Nam in the early 1950's and 23 per cent among patients in the care of the U.S. Army Medical Services in South Viet Nam in early 1967. However these rates are not entirely comparable for the proportion of acute cases is not known, and as recently as 1967 Sheehy, Deller and Weber indicated that the mortality in acute fulminating cases may still exceed 90 per cent. Although the overall prognosis is very much better than it was, much remains to be done to reduce the mortality.

CHAPTER IIIEPIDEMIOLOGY

	Page
Introduction	49
Method	50
Discussion	
Habitat of the Organism	50
Portals of Entry	51

CHAPTER III

EPIDEMIOLOGY

INTRODUCTION

Whitmore originally suggested that melioidosis was spread by infected excreta contaminating food and water, and the form of the epizootic in 1913 in Kuala Lumpur suggested to Fletcher (1919) that the infection was due to soiling of foodstuffs by rat excreta. These suppositions appear to have led to a widespread belief that the disease is spread by rodents, especially rats, but review of the literature shows no evidence to support this view. In fact there is considerable evidence to the contrary. Stanton and Fletcher (1932) observed that melioidosis was rare among the large number of rats examined at Kuala Lumpur. Necropsy examination of 20,000 rats at the Pasteur Institute of Saigon did not show any of the characteristic lesions and blood culture of several thousand of them were negative (Delbove and Reynes 1942). Harries and his colleagues (1948) did not find melioidosis in 500 rats examined at Rangoon. Strauss and his associates (1968) carried out a careful study of 421 rats trapped on Carey Island off the west coast of Malaysia, an island where melioidosis is endemic, and their only positive finding was one rat with a haemagglutination titre of 1:80.

On the other hand there is an increasing body of evidence that Ps pseudomallei is present in surface water. Vaucel (1937) infected guinea pigs by immersing them in lakes and rice paddies in Viet Nam after scarifying their flanks and legs. Chambon (1955b) found seven strains of Ps pseudomallei in 150 specimens of water, earth and mud collected from lakes, streams and rice paddies around Saigon. Laws and Hall (1964) discovered Ps pseudomallei in water specimens collected at Townsville in northern Australia, and Ellison and his colleagues (1968) found the organism in many parts of Malaysia, but they did not study Terendak Camp or Singapore Island. The author therefore collected samples in these latter areas.

METHOD

Samples were collected in sterile containers and sent to the U.S. Army Medical Research Unit at the Institute for Medical Research in Kuala Lumpur where they were examined. Two millilitre doses of water were injected intraperitoneally into weanling hamsters. Heart blood of all hamsters dying between the first and sixth days after injection was inoculated on to three per cent glycerol agar containing 1:200,000 crystal violet. Colonies that appeared to be Ps pseudomallei were examined by the methods previously outlined. This technique is described by Strauss and his colleagues (1968) who used it in their study of Carey Island. It was also used by the same group (Ellison et al 1968) in their study of the surface water of Malaysia.

RESULTS

Thirty one specimens were collected at Terendak Camp from playing fields, the golf course, drains, streams and private gardens. Twenty of them contained Ps pseudomallei. At Singapore 121 samples were collected from playing fields, a golf course, drains, streams, private gardens and the roadside. Ps pseudomallei was isolated from eight specimens. The results are tabulated below.

	<u>No.of Samples</u>	<u>No.Positive</u>	<u>Percentage</u>
Terendak	31	20	64.5
Singapore	121	8	6.6

DISCUSSION

Habitat of the Organism

The collection of water samples was not systematic, but the positive results show that Ps pseudomallei is present in the surface water and can be readily isolated, especially at Terendak. This is the first report of Ps pseudomallei in the surface water of Singapore. This technique appears to be a sensitive method for detecting the bacillus in surface water.

During this study the author observed that Ps pseudomallei was more frequently isolated when the water was fresh than when it had been lying for some time. Many of the positive isolates were from specimens collected shortly after rain had started to fall. Similarly Strauss and his colleagues (1968) observed that the organism was isolated from drains on Carey Island more often after rain than during dry periods. Rain either improves the conditions for multiplication of the bacillus or washes it out of the soil into the surface water. The latter suggestion is less likely since the organism has only rarely been isolated from soil. The type of soil may be relevant but hydrogen ion concentration and exposure to the sun seem to be unimportant. The disappearance of the bacillus from water which has been lying is curious since it survives for months in specimens kept in the laboratory at room temperature (Strauss et al 1968).

French workers observed that melioidosis frequently occurred during the rainy season (Fournier and Chambon 1958). Patient number seven developed her infection towards the end of an exceptionally wet season in Singapore. Despite widespread flooding in Malaysia and Singapore at this time no other cases of melioidosis occurred in Singapore, and none were found in Kuala Lumpur, Malacca or Kota Bahru (personal communications from Snelling, Eapen and Cheah Tjong Siang in 1967). It may be that although a little rain is associated with an increase in the number of Ps pseudo-mallei in the surface water, massive flooding washes them away in the same manner as *Leptospirae*.

Portals of Entry

It appears to be more likely that melioidosis is acquired from surface water than from rats. Although Ps pseudomallei may enter the human body by ingestion or by inhalation, Fournier and Chambon (1958), who had wide experience of melioidosis in Viet Nam, consider that entry is usually through lacerations and abrasions. They point out that there are numerous accounts of men developing melioidosis after accidents in which open injuries were contaminated in padi fields or drains. If this is so it is perhaps surprising that melioidosis is apparently so rare among Asian women and children.



There is one possibility that has not been mentioned by other authors. Leeches carry Ps pseudomallei (Strauss personal communication 1966) so the organism may be introduced directly into the leech bites suffered by all who go into the jungle in Malaysia. All the author's male patients had been in the jungle.

Although all the author's patients with melioidosis had recent injuries exposed to surface water, so had some of the other patients admitted to hospital under his care. It therefore appears that insufficient evidence has so far been found to determine the usual portal of entry of Ps pseudomallei.



Figure 23.

A typical garden at Terendak. Ps pseudomallei was isolated from these puddles.



Figure 24.

Ps pseudomallei was isolated from surface water on this pitch.

CHAPTER IVTHE SEROLOGICAL SURVEY

	Page
Introduction	56
Methods	56
Results	57
Raised Titres	58
Positive Titres	59
Age and Time Spent in Endemic Areas	63
Clinical Findings Among Men with Raised Titres	63
Summary of Results	64
Discussion	
The Haemagglutination Test	66
Incidence of Positive Titres	66
Exposure to Surface Water in Men with Positive Titres	68
Positive Titres in New Zealanders and Inpatients	69
Clinical Features	69

CHAPTER IV

THE SEROLOGICAL SURVEY

INTRODUCTION

Serological surveys, mainly among native populations, have shown that a small proportion of people living in endemic areas have positive titres of melioidosis antibodies in their blood (Nigg 1963 and Strauss et al 1968). When there is no evidence of active disease positive titres may indicate previous melioidosis, usually mild and self-limiting.

The author carried out a serological survey among non-indigenous soldiers at Terendak Camp to see if any of them had raised antibody titres. Men with raised titres were examined to try to detect features of previous melioidosis.

METHODS

During a 30 week period two groups of soldiers were studied. The first consisted of all soldiers admitted to the main medical ward of the hospital (the Inpatient Group). The second comprised all the Outpatients attending the Venereal Diseases Clinic with a fresh infection plus healthy soldiers (together they form the Other Subject Group). Both groups included British, Australians and New Zealanders. The healthy soldiers consisted of the hospital staff, who were all British, and New Zealanders selected from the New Zealand Infantry Battalion by using their regimental numbers and random sampling numbers (Hill 1966).

A sample of venous blood was taken from each subject; the serum was separated and sent to the U.S. Army Medical Research Unit in the Institute for Medical Research at Kuala Lumpur where the melioidosis haemagglutination test was carried out as described in Appendix V. Acute and convalescent specimens of blood were taken from inpatients with pyrexias of unknown origin, pneumonias of obscure aetiology and non-specific enteritis. Shortly afterwards simple individual questionnaires were sent to the units of the men concerned requesting details of their work, age and time spent in endemic areas during previous and current tours. The men were also asked if they played rugby football, association football or hockey. A pilot study showed that this was a more accurate method of obtaining the information than questioning the men when the

blood was taken, for it enabled the units to check the answers.

All patients were examined on admission to hospital. They suffered from a wide variety of conditions and their diagnoses are detailed in Appendices III (Inpatients) and IV (Outpatients). Three patients who had melioidosis have already been described (Patients 4, 5 and 6 in Chapter II) and will not be considered further here. Usually the results of the haemagglutination tests arrived after the patients had been discharged from hospital, so soldiers with raised titres were reviewed as outpatients at a follow-up clinic where they were examined, blood was taken for another haemagglutination titre and a routine blood count, and they all had a chest radiograph. Their personal medical documents were scrutinised to confirm details of previous illnesses.

Later, for comparison, the documents of another group of 350 British infantrymen were examined after their return to the United Kingdom following a two and a half year tour at Terendak.

With the help of an experienced British officer each man was placed in one of four grades of exposure to surface water according to his outdoor work. These grades have been numbered I to IV, Grade I being maximum exposure, Grades II and III being intermediate and Grade IV being minimum exposure. Subjects in Grade I included infantrymen, field gunners, combat engineers, field signallers and field medical orderlies. Drivers, tank crew and wireless operators were in Grade II. Mechanics and other technicians were in Grade III, while storemen, clerks and other personnel who rarely left their offices were in Grade IV.

RESULTS

A total of 1,287 specimens of blood were collected from 905 subjects. There were 339 inpatients and 556 Other Subjects. The British predominated with 548 men, and there were 176 Australians and 181 New Zealanders (see Table 1 overleaf).

TABLE 1

NATIONAL DISTRIBUTION

	<u>Inpatients</u>	<u>Other Subjects</u>	<u>Total</u>
British	204	344	548
Australians	88	88	176
New Zealanders	47	134	181
<u>Totals</u>	339	566	905

Raised Titres

Raised titres have been divided into doubtful titres of 1:10 and 1:20, and positive titres of 1:40 and higher. A total of 47 men had raised titres (5.2 per cent of the total studied): 18 were positive (2.0 per cent) and 29 doubtful (3.2 per cent).

Inpatients had 18 doubtful and 10 positive titres and Other Subjects 11 doubtful and 8 positive titres (see Table 2 below). The incidence of raised titres among Inpatients (8.3 per cent) was significantly higher ($p < 0.01$) than the incidence among Other Subjects (3.3 per cent).

TABLE 2

DISTRIBUTION OF SUBJECTS WITH RAISED TITRES

	<u>Inpatients</u>				<u>Other Subjects</u>			
	<u>N</u>	<u>D</u>	<u>P</u>	<u>R</u>	<u>N</u>	<u>D</u>	<u>P</u>	<u>R</u>
British	189	13	2	15 (7.4%)	336	5	3	8 (2.3%)
Australians	84	2	2	4 (4.5%)	87	0	1	1 (1.1%)
New Zealanders	38	3	6	9 (19.1%)	124	6	4	10 (7.5%)
<u>Totals</u>	311	18	10	28 (8.3%)	547	11	8	19 (3.3%)

N Negative Titres
 D Doubtful Titres (1:10 and 1:20)
 P Positive Titres (1:40 and higher)
 R Raised Titres (Doubtful plus Positive Titres)

New Zealanders had a higher incidence of raised titres than British or Australians. It is convenient to compare New Zealanders with British plus Australians added together to form one group. Among Inpatients 19.1 per cent of New Zealanders had raised titres, but only 6.5 per cent of British plus Australians had raised titres. Among Other Subjects 7.5 per

cent of New Zealanders had raised titres, but only 2.1 per cent of British and Australians had raised titres (see Table 3 below).

TABLE 3 COMPARISON OF NEW ZEALANDERS WITH BRITISH AND AUSTRALIANS

Titres	<u>Inpatients</u>		<u>Other Subjects</u>	
	<u>Negative</u>	<u>Raised</u>	<u>Negative</u>	<u>Raised</u>
New Zealanders	38	9 19.1%	124	10 7.5%
B & A	273	19 6.5%	423	9 2.1%

B & A British and Australians

When games players and non-players were compared, players had 7.3 per cent of raised titres overall and non-players had 4.1 per cent (see Table 4 below). The highest incidence was 23 per cent in New Zealand players (Section 1a). The only sub-group in which this finding was reversed was British and Australian Inpatients: players had 6.0 per cent raised titres and non-players 7.1 per cent (Section 1b).

TABLE 4 RAISED TITRES
COMPARISON OF PLAYERS AND NON PLAYERS

	<u>PLAYERS</u>	<u>NON PLAYERS</u>
<u>(1) INPATIENTS</u>		
a. N.Z.		
Raised Titres	6 23%	2 15%
Negative Titres	20	11
b. B. & A.		
Raised Titres	6 6.0%	12 7.1%
Negative Titres	95	157
<u>(2) OTHER SUBJECTS</u>		
a. N.Z.		
Raised Titres	8 9.4%	1 2.3%
Negative Titres	77	43
b. B. & A.		
Raised Titres	4 3.0%	5 1.9%
Negative Titres	127	255
<u>TOTAL</u>		
Raised Titres	24 7.3%	20 4.1%
Negative Titres	319	466

N.Z. = New Zealanders

B. & A. = British & Australian

When the men were distributed into the four grades of exposure to surface water at work, more subjects in Grade I had raised titres than in any other grade. Seven point five per cent of all subjects in Grade I had raised titres, 5.3 per cent in Grade II, 1.4 per cent in Grade III and 3.9 per cent in Grade IV (see Table 5 below). The highest incidence in Grade I was 27.0 per cent among New Zealand Inpatients (Section 1a).

TABLE 5 EXPOSURE TO SURFACE WATER AT WORK BY GRADES

	I		II		III		IV	
<u>(1) INPATIENTS</u>								
a.N.Z.								
Raised Titres	7	27.0%	1	12.5%	0		0	
Negative Titres	19		7		5		0	
b.B. & A.								
Raised Titres	5	6.0%	7	8.4%	1	1.6%	5	12.2%
Negative Titres	78		76		62		36	
<u>(2) OTHER SUBJECTS</u>								
a.N.Z.								
Raised Titres	8	8.5%	1	5.0%	0		0	
Negative Titres	86		19		15		0	
b.B. & A.								
Raised Titres	4	3.2%	4	3.0%	1	1.9%	0	
Negative Titres	114		129		52		87	
<u>TOTAL</u>								
Raised Titres	24	7.5%	13	5.3%	2	1.4%	5	3.9%
Negative Titres	297		231		134		123	

Positive Titres

The relative proportions of men with positive titres were similar to those of men with raised titres in the various sub-groups (see Table 6 overleaf).

Three per cent of Inpatients had positive titres, but only 1.4 per cent of Other Subjects (see Section 1). Five point five per cent of New Zealanders had positive titres compared with 1.2 per cent of British and Australians (Section 2).

When players and non-players were compared, the incidence of positive titres was higher in players (Section 3), being highest in New Zealanders who had 7.2 per cent (Section 3c).

When the men were distributed into the four grades of exposure to surface water at work the percentages of positive titres were higher in Grade I than in the other grades (Section 4). The highest incidence of positive titres in Grade I was 7.5 per cent in New Zealanders, (Section 4c).

TABLE 6POSITIVE TITRES

SECTION (1)

COMPARISON OF INPATIENTS AND OTHER SUBJECTS

	<u>INPATIENTS</u>		<u>OTHER SUBJECTS</u>	
Positives	10	3.0%	8	1.4%
Remainders	329		558	

SECTION (2)

COMPARISON OF NEW ZEALANDERS AND BRITISH + AUSTRALIANS

	<u>NEW ZEALANDERS</u>		<u>BRITISH + AUSTRALIANS</u>	
Positives	10	5.5%	8	1.2%
Remainders	171		653	

SECTION (3)

COMPARISON OF GAMES PLAYERS AND NON PLAYERS

		<u>PLAYERS</u>		<u>NON PLAYERS</u>	
(a) INPATIENTS	Positives	5	4.3%	5	2.7%
	Remainders	112		177	
(b) OTHER SUBJECTS	Positives	7	3.2%	1	0.3%
	Remainders	209		303	
(c) NEW ZEALANDERS	Positives	8	7.2%	2	3.5%
	Remainders	103		55	
(d) B & A	Positives	4	1.7%	4	0.9%
	Remainders	228		425	

SECTION (4)

EXPOSURE TO SURFACE WATER BY GRADES

	I	II	III	IV	
(a) INPATIENTS	Positives	6 5.5%	4 4.4%	0	0
	Remainders	103	87	68	41
(b) OTHER SUBJECTS	Positives	6 2.8%	1 0.65%	1 1.5%	0
	Remainders	206	152	67	87
(c) NEW ZEALANDERS	Positives	9 7.5%	1 3.6%	0	0
	Remainders	111	27	20	0
(d) B & A	Positives	3 1.5%	4 1.8%	1 0.65%	0
	Remainders	196	212	115	128

B & A = British and Australians



Figure 25.

Typical heavy (Grade I) exposure.

Age and Time Spent in Endemic Areas

The age distribution and mean time spent in endemic areas was similar in men with raised and negative titres.

Most of the soldiers in the camp were young men. Seventy eight point one per cent of subjects with negative titres were between 20 and 29 years old and the ages of those with doubtful and positive titres did not differ significantly from this.

The mean time spent in endemic areas was 20 months for those with negative titres, 19½ months for those with doubtful titres and 20½ months for those with positive titres.

Clinical Findings Among Men with Raised Titres

When the men with raised titres were examined at the follow-up clinic no abnormal physical signs were found. However 14 of the 18 men with positive titres (77.8 per cent) had a history of short term fever since arriving in Malaysia, compared with 20.7 per cent among those with doubtful titres and 18.8 per cent among the 350 British infantrymen whose documents were examined after their return to the United Kingdom from Terendak. The groups are compared in Table 7 below and the high incidence in men with positive titres is significant ($p < 0.01$). One man with a positive titre had no personal medical documents and as his history could not be confirmed elsewhere he has been omitted.

TABLE 7

CLINICAL FINDINGS

	No Fever	Fever	Percentage
No. with Positive Titres	3	14	77.8
No. with Doubtful Titres	23	6	20.7
Other British Infantrymen	284	66	18.8

No significant differences were found when the incidence of other illnesses and accidents were compared.

During the short term fevers the men had complained of headache and malaise, and on examination they had been pyrexial but no abnormal clinical signs had been found. Nine of the men with positive titres had had neutrophilias and seven had had raised erythrocyte sedimentation ^{rates} while acutely ill. Seven had had chest radiographs taken while they were febrile but all had been normal. Bacteriological investigations had not shown any pathogens and bacterial and viral serological studies had been negative. All had recovered satisfactorily.

At the follow-up clinic the melioidosis haemagglutination tests were repeated. Among men with positive titres, 6 titres remained the same, 10 fell, 1 rose and then fell and 1 rose (see Table 8^{overleaf} for details). Haemoglobin estimations, white cell counts and erythrocyte sedimentation rates were all normal, and they all had normal chest radiographs.

Summary of Results

Forty seven men (5.2 per cent of the total) had raised titres: 18 were positive (2.0 per cent) and 29 doubtful (3.2 per cent). Raised titres were more common among games players than non-players and among outdoor workers than office workers. Inpatients had a higher incidence of raised titres than Other Subjects, and New Zealanders a higher incidence than the British and Australians. Men with positive titres had a significantly higher incidence of short term fever since arriving in Malaysia than men with doubtful titres or a control group of British infantrymen.

TABLE 8

DETAILS OF SUBJECTS WITH POSITIVE TITRES

<u>NATIONALITY</u>	<u>DIAGNOSIS</u>	<u>PAST HISTORY</u>	<u>GRADE</u>	<u>GAMES</u>	<u>TITRES</u>
<u>INPATIENTS</u>					
British	Duodenal Ulcer	P.U.O.	II	No	1:40, 1:40
"	Acute Infective Hepatitis	None	I	No	1:20, 1:40, 1:20
Australian	Chronic Urethritis	P.U.O.	II	Yes	1:80, 1:80
"	P.U.O.	P.U.O.	II	No	1:40, 1:20
New Zealander	Aspiration Pneumonia	P.U.O.	I	No	1:40, 1:20
"	Chronic Otitis Media	P.U.O.	I	Yes	1:160, 1:160
"	P.U.O.	P.U.O.	I	Yes	1:160, 1:160, 1:40
"	P.U.O.	P.U.O.	I	Yes	1:80, 1:80, 1:40
"	Aspiration Pneumonia	P.U.O.	I	No	1:80, 1:80
"	Acute Tonsillitis	P.U.O.	II	Yes	1:40, 1:40
<u>OUTPATIENTS</u>					
British	Urethritis	P.U.O.	I	Yes	1:40, 1:10
"	Urethritis	Acute Enteritis	III	Yes	1:40, 1:40
"	Urethritis	Not known	II	Yes	1:40, 1:20
Australian	Urethritis	P.U.O.	I	No	1:40, 1:20
<u>HEALTHY SUBJECTS</u>					
New Zealanders		P.U.O.	I	Yes	1:80, 1:40, 1:20
"		Laceration of Leg	I	Yes	1:10, 1:280, 1:280
"		P.U.O.	I	Yes	1:160, 1:20, 1:20
"		P.U.O.	I	Yes	1:80, 1:20

P.U.O. = Pyrexia of Unknown Origin

The second titre was 3 to 6 weeks after the first

The third titre was 6 to 12 months after the second.

DISCUSSION

The Haemagglutination Test

Dr. A.D. Alexander developed the haemagglutination test used in the examination of these sera at the Walter Reed Institute for Research in Washington DC, and several thousand sera have been examined there and at the U.S. Army Medical Research Unit in the Institute for Medical Research at Kuala Lumpur (Raymond personal communication 1966). Melioidosis is not endemic in the United States of America, but titres of 1:10 and 1:20 were found in sera from 700 United States residents, though none of them had higher titres. Titres of 1:40 and more develop in patients suffering from active melioidosis. Therefore titres of 1:10 and 1:20 are considered to be of doubtful significance and higher titres are regarded as positive.

Doubtful titres may represent minor exposure to Pg pseudomallei or ingestion or inhalation of the bacillus, for ingested or inhaled antigens produce less antibody response than antigens introduced parenterally. They are unlikely to represent cross reactions with other antigenically related agents for careful tests have not shown any such reactions. One possible cause is the anamnestic response, but this is also unlikely for doubtful titres were found in some men very soon after their first arrival in Malaysia.

It is not known if these or any of the other detectable antibodies are associated with immunity.

Incidence of Positive Titres

Brygoo (1953) discovered 2 per cent of positive titres of more than 1:80 using the agglutination test to examine sera from 465 Europeans and Asians living in Saigon. The incidence was lower in Europeans than in Asians. Cook (1962) discovered one positive complement fixation titre among sera from 619 subjects living in a region of northern Australia where melioidosis is endemic. Most of his subjects were meat or stock handlers, so it is interesting that the positive titre was from a canefield worker. Dr. Clara Nigg (1963) found 8.3 per cent of positive titres among 337 sera from Thai males examined with a complement fixation test, and using a haemagglutination test based on the method of Chen and Meyer (1954) she found 29.1 per cent of positive titres among 405 sera from Thais. Strauss and his colleagues (1968) discovered positive titres among 1.8 to 15.7 per cent of 1,606 sera from a mixed population of Malaysians; they employed

the haemagglutination test used for the author's sera. The lowest incidence was among Forest Aborigines, subjects from plantations were intermediate and the incidence was highest among people from rice growing areas. This correlates with a low isolation of Ps pseudomallei from jungle streams, intermediate rate from plantations and high isolation rate from rice growing areas found by the same group of workers (Ellison et al 1968).

The author's subjects were selected in that many were patients. However, all the military units were represented and the numbers in the various nationalities and occupations were proportional to the numbers in the population the hospital served. The age distribution of men with positive and negative titres was the same, so it appears that age was not an important factor in the appearance of melioidosis haemagglutinins in this population. The mean time spent in endemic areas was also similar in men with positive and negative titres so it is concluded that mean time spent in endemic areas was also unimportant. This is in keeping with the different times spent in Malaysia by the patients with clinical melioidosis.

The incidence of positive titres among the author's subjects was the same as the overall incidence in Brygoe's series and higher than the incidence he found among Europeans. It was similar to the incidence among Forest Aborigines studied by Strauss and his colleagues. The soldiers were exposed for a relatively short time, but Terendak Camp appeared to be heavily contaminated with Ps pseudomallei. This may explain why in such a short time the soldiers had the same incidence as the Aborigines. The higher incidence among the other Asians studied by Strauss and his colleagues is presumably due to life-long exposure in heavily contaminated areas. The lower incidences reported by Brygoe and Cook may be because they used less sensitive tests or their subjects may have been less exposed to Ps pseudomallei.

The author's sera were examined for leptospiral antibodies using the leptospirosis haemolytic test (Cox et al 1957). Only eight had positive titres (0.88 per cent). Hart (personal communication 1968), who studied 1000 New Zealand infantrymen observed that a similar proportion developed positive leptospirosis antibody titres after a three year tour in Malaysia. These figures indicate that mild self-limiting melioidosis is slightly more common than mild self-limiting leptospirosis, so supporting the suggestion that melioidosis may prove to be more common than is at present realised.

Exposure to Surface Water in Men with Positive Titres

One man who initially had no melioidosis antibodies reported again when his titre had risen to the high level of 1:1280; during the intervening period he had received a laceration while playing rugby football at Terendak. Strauss (personal communication 1966) observed an equally high titre in a serum sample taken from a Malay woman living in a district where Ps pseudomallei was present in the surface water. She had a history of a recent laceration to her foot. It seemed likely that similar injuries occurred among other footballers and in men engaged in rough outdoor work such as infantrymen. This prompted the questions about the men's games' playing habits with particular reference to rugby football, association football and hockey. The results showed more positive titres among games players and outdoor workers.

Most New Zealand players played rugby football but only half the British and Australian players were rugby footballers, the rest playing association football and hockey. Fewer injuries are sustained during association football and hockey and they are less liable to contamination with surface water than rugby injuries. Since New Zealand players had a higher incidence of positive titres than British and Australian players, the findings suggest injuries exposed to surface water are associated with positive titres of melioidosis antibodies. The presence of Ps pseudomallei in surface water suggests that the bacillus may be inoculated directly into such injuries and may lead to melioidosis.

Fournier and Chambon (1958) based their suggestion for the organisms' entry through superficial injuries on their observation that melioidosis often followed the contamination of injuries by surface water. All the author's patients with clinical melioidosis had scars from recent superficial injuries which had been exposed to surface water in Malaysia, and the two Maoris, Patients 3 and 5, had received injuries playing rugby football at Terendak. The histories of Patients 4 and 7, in particular, support this suggestion for Ps pseudomallei's portal of entry. Patient 4 who had suffered an extensive infected abrasion shortly before admission to hospital, had received this playing association football at Terendak. Two weeks before the onset of Patient 7's illness she had cut her feet and had then walked barefoot in a region where Ps pseudomallei was present in the surface water.

Positive Titres in New Zealanders and Inpatients

The high incidence of positive titres among New Zealanders may have been due to their heavy exposure to surface water since many were rugby footballers and infantrymen.

The reasons for the high incidence of positive titres among Inpatients are not clear. They had a slightly higher percentage of players and more were heavily exposed at work than the Other Subjects, but the differences are less marked than among the New Zealanders. Although seven had acute infective illnesses (see Table 8) these did not appear to be associated with melioidosis.

Clinical Features

Some details of the short term fevers were taken from the men's personal medical documents. Acceptance of the diagnoses primarily depended on the temperature records, and discussion with the doctors concerned showed that they had personally confirmed the raised temperatures, but wherever possible other documents were consulted for confirmation.

Short term fever, or pyrexia of unknown origin, is a common diagnosis for mild febrile illness among Europeans living in hot climates. The patients complain of the non-specific symptoms of fever and clinical signs are usually absent. Simple laboratory investigations are unhelpful and sophisticated tests are sometimes difficult to carry out because of lack of nearby facilities. Most patients recover before the second specimen of blood for serological studies should be taken, and may be unwilling to attend again after a relatively trivial illness, so no definite diagnosis may be reached. However, Deller and Russell (1967), writing from South Viet Nam, demonstrated that many such fevers can be diagnosed when vigorously investigated. It is interesting to note that in their series of 110 patients they discovered 2 cases of melioidosis and only 1 of leptospirosis.

The high incidence of previous short term fever in the men with positive titres suggests that these episodes might have been mild melioidosis. Review of the seven patients with clinical melioidosis shows that five of them had varying periods of vague febrile illness before admission to hospital. These early symptoms resembled those of the short term fevers, so vague febrile illness appears to be a feature of mild

melioidosis and an early manifestation of severe melioidosis. Short term fever is usually a benign self-limiting condition, but melioidosis may be fatal. Therefore, investigation of all patients with a fever of unknown origin developing while they are in an endemic area, or after they have visited any of these areas, should include bacteriological and serological studies for Ps pseudomallei. In view of the long latent period which may elapse after patients have left endemic areas and the increasing volume of world travel, any doctor may be consulted by a patient suffering from melioidosis, so all doctors should be aware of this disease and its dangers.

CONCLUSIONS

1. Debilitated individuals appear to be more susceptible to melioidosis than healthy people.
2. The clinical features are variable and although acute cases may show a characteristic clinical picture, the diagnosis can only be established by bacteriological studies.
3. Treatment must be vigorous, particularly in acute cases, and even with such treatment the mortality in acute cases remains high.
4. Pseudomonas pseudomallei, the causative organism, is present in the surface water in Malaysia and Singapore.
5. A small proportion of non-indigenous men at a military camp in Malaysia had positive titres of melioidosis antibodies.
6. Positive titres were more common among men engaged in vigorous outdoor activities than among other men.
7. Infection may occur when open injuries are contaminated with surface water containing Ps pseudomallei.
8. Fever appears to be an important feature of mild melioidosis and an early sign of severe melioidosis.

BIBLIOGRAPHY

- Alain, M., St-Etienne, J. and Reynes, V. 1949, La Melioidose: Considerations etiologiques, Cliniques et Pathogeniques à propos 28 Cas. *Med. trop.* 2, 119.
- Baird, J.P. and Meers, P.D. 1965, Melioidosis-successful treatment. *Trans. R. Soc.Trop.Med.Hyg.* 59, 359.
- Beamer, P.R., Varney, P.L., Brown, W.G., McDowell, F. and Eck, B., 1948, Melioidosis: Report of a second case from the Western Hemisphere. *Amer.J.Path.* 24, 717.
- Beamer, P.R., Varney, P.L., Brown, W.G. and McDowell, F., 1954, Studies on Malleomyces pseudomallei isolated from Melioidosis originating in the Western Hemisphere. *Amer.J.Clin.Path.* 24, 1231.
- Bezemer, F., 1935, Melioidosis in Celebes. *Genesk. T.Ned-Ind.* 75, 1577.
- Biegeleison, J.Z., Mosquera, R. and Cherry, W.B., 1964, A Case of Human Melioidosis: Clinical, Epidemiological and Laboratory Findings. *Amer.J.trop.Med.Hyg.* 13, 89.
- Blanc, G., Delage, B. and Martin, L.A., 1943, Etude comparative de caracteres biochimiques et serologiques du bacille de Whitmore et du bacille pyocyanique. *Ann.Inst.Past.* 69, 65.
- Brygoo, E.R., 1953, Contribution à l'étude des agglutinins naturelles pour le bacille de Whitmore. Les agglutinins dans quelques cas de melioidosis. *Bull.Soc.Path. exot.* 46, 347.
- Chambon, L.a, 1955, Résistance du bacille de Whitmore acquise in vitro et in vivo à l'égard du chloramphenicol, de l'aureomycine et de la terramycine. *Ann.Inst.Past.* 88, 315.
- Chambon, L.b, 1955, Isolation du B. Whitmorei à partir du milieu extérieur. *Ann.Inst.Past.* 82, 229.
- Chambon, L. a. De lajudie, P. and Fournier, J. 1954, Etude de la sensibilité du bacille de Whitmore aux antibiotiques in vitro et chez les malades atteints de melioidose. *Bull.Soc.Path. exot.* 47, 139.
- Chen, T.H. and Meyer, K.F., 1954, Studies on Immunisation against Plague. VII. A. Haemagglutination Test with the Protein Fraction of Pasteurella Pestis: A Serologic Comparison of Virulent and Avirulent strains with observations on the Structure of the Bacterial Cells and its Relationship to Infection and Immunity. *J. Immunol.* 72, 282.

- Cook, I., 1962, Survey for Antibodies to Melioidosis in Man and Native Animals. *Med.J.Aust.* 2, 627.
- Cooper, E.B., 1967, Melioidosis. *J.Amer.med.Ass.* 200, 452.
- Cottew, G.S., 1955, Melioidosis. *Aust.vet.J.* 31, 155.
- Cowan, S.T. and Steel, K.J. 1965, Manual for the Identification of Medical Bacteria. 23, London, Cambridge University Press.
- Cox, C.D. and Arbogast, J.L. 1945, Melioidosis. *Amer.J.Clin.Path.* 15, 567.
- Cox, C.D., Alexander, A.D. and Murphy, L.C. 1957, Evaluation of Haemolytic Test in Serodiagnosis of Human Leptospirosis. *J.Infect.Dis.* 101, 210.
- Darby, J.P. and Mendez, E., 1960, Chronic Melioidosis: Serologic Diagnosis and Antibiotic Treatment. *U.S. armed Forces med.J.* 11, 1203.
- Davie, J. and Wells, C.W., 1952, Equine Melioidosis in Malaya. *Brit.vet.J.* 108, 161.
- Delbove, P. and Reynes, V., 1942, Acquisitions recentes sur quelques maladies infectieuses. I. volume, Saigon. Portail ed. Cited by Fournier and Chambon 1958.
- Deller, J.J. and Russell, P.K. 1967, An Analysis of Fevers of Unknown Origin in American Soldiers in Viet Nam. *Ann.Intern.med.* 66, 1129.
- De Moor, C.E., Soekarnen, and Walle, N.V.D., 1932, Melioidosis in Java. *Meded. Dienst d. Volksgezondheid in Neder-Ind.* 21, 206.
- Denny, C.R. and Nicholls, L. 1927, Melioidosis in a European. *Ceylon.J. Sci.D.* 1, 27.
- Dunlop, D.M. and Murdoch, J.McC., 1960, The Dangers of Antibiotic Treatment. *Brit.med.Bull.* 16, 67.
- Ellison, D.W., Strauss, J.M., Baker, H.J., Marcarelli, J.L. and Alexander, A.D., 1968. Distribution of *Pseudomonas pseudomallei* in Soil and Surface Waters of Malaysia. In Press.
- Ertug, G., 1961, Report of the First Case of Pulmonary Melioidosis from Western Countries. *Dis.Chest.* 40, 693.
- Fletcher, W., 1919, Annual Report of the Institute of Medical Research at Kuala Lumpur.
- Fournier, J. and Chambon, L., 1958, La Melioidose. Maladie d'Actualité et le bacille de Whitmore. Paris, Editions Medicales Flammarion.

- Gambier, A., 1930, Un cas de melioidose observé à Phnompenh. Bull.Soc. Path.exot. 23, 436.
- Garrod, L.P. and Waterworth, P.M., 1962, Methods of testing combined antibiotic bacteriocidal action and the significance of the results. J.Clin.Path. 15, 328.
- Garry, M.W. and Koch, M.L., 1951, Chronic Melioidosis: Bacteriological and Clinical Correlations in Diagnosis. J.Lab.Clin.Med. 38, 374.
- Gilmour, C.C.B., 1931, A Case of Melioidosis in Singapore. Malayan.med.J. 6, 12.
- Grant, A. and Barwell, C. 1943, Chronic Melioidosis. A Case diagnosed in England. Lancet i, 199.
- Harries, E., Lewis, A.A.G., Waring, J.W.B. and Dowling, E.J., 1948, Melioidosis treated with Sulphonamides and Penicillin. Lancet i, 363.
- Hayakawa, K., 1945, Personal Communication and Demonstration (Serum and Vaccine Institute, Singapore). Quoted in Studies from the Institute for Medical Research, Federation of Malaya, Jubilee Volume Number 25 "The Institute for Medical Research 1900-1950" by various authors. Printed at the Government Press, Kuala Lumpur in 1951.
- Haynes, W.C. and Burkholder, W.H., 1957, The Genus Pseudomonas, in Bergey's Manual of Determinative Bacteriology. 7th Edition. 87. London, Bailliere, Tindall and Cox Ltd.
- Hill, A.B., 1966, Principles of Medical Statistics. 8th Edition. 21. London, The Lancet Ltd.
- Hugh, R. and Leifson, E., 1953, The Taxonomic Significance of Fermentative versus Oxidative Metabolism of Carbohydrates by various Gram Negative Bacteria. J.Bact. 66, 24.
- Ileri, S.Z., 1965, The Indirect Haemagglutination Test in the diagnosis of Melioidosis in Goats. Brit.vet.J. 121, 164.
- Ives, J.C. and Thomson, T.J., 1953, Chronic Melioidosis: The first Report of a Case Infected in Central India. Glasgow.med.J. 34, 61.

- Jawetz, E. and Gunnison, J.B., 1952, Studies on Antibiotic Synergism and Antagonism: a scheme of Combined Antibiotic Action. *Antibiot. and Chemother.* 2, 243.
- Joy, R.J.T., Scalettar, R. and Sodee, D.B., 1960, Optic and Peripheral Neuritis. *J.Amer.med.Ass.* 173, 1731.
- Khaira, B.S., Young, W.B. and Hart, P. de V. 1959, Melioidosis. *Brit.med. J.* i, 949.
- Laws, L. and Hall, W.T.K., 1964, Melioidosis in Animals in North Queensland. IV. Epidemiology. *Aust.vet.J.* 40, 309.
- Maegraith, B.G. and Leithead, C.S., 1964, Melioidosis: A Case Report. *Lancet.* i, 862.
- Mayer, J.H. and Finlayson, M.H., 1944, Chronic Melioidosis: A Case showing Bone and Pulmonary Lesions. *J.roy.Army med.Cps.* 82, 4.
- McDowell, F. and Varney, P.L., 1947, Melioidosis: Report of First Case from Western Hemisphere. *J.Amer.med.Ass.* 134, 361.
- Mesnard, J., Joyeux, B. and Gaulene, 1929, Un cas de Melioidose au Tonkin. *Bull.Soc.Med.Chir. Indochine.* 16, 32.
- Miller, W.R., Pannell, L., Cravitz, L., Tamer, W.A., and Ingalls, M.S., 1948, Studies on certain Biological Characteristics of M.Mallei and M. pseudomallei. (1) Morphology, Cultivation, Viability and Isolation from contaminated Specimens. *J.Bact.* 55, 115.
- Mirick, G.S., Zimmerman, H.M., Maner, G.D., and Humphrey, A.A., 1946, Melioidosis on Guam. *J.Amer.med.Ass.* 130, 1063.
- Montgomery, R., 1963, Melioidosis: Report on a Fatal Case in a British Soldier. *J.roy.Army med.Cps.* 109, 223.
- Nigg, C., 1963, Serological Studies on Sub Clinical Melioidosis. *J.Immunol.* 91, 18.
- Nigg, C., Ruch, J., Scott, E. and Noble, K. 1956, Enhancement of Virulence of M pseudomallei. *J. Bact.* 71 530
- Omar, A.R., Cheah Kok Kheong, and Mahendranathan, T., 1962, Observations on Porcine Melioidosis in Malaya. *Brit.vet.J.* 118, 421.
- Paton, J.P.J., Peck, C.R., and Van de Schaaf, A., 1947, Report on a Case of Melioidosis from Siam. *Brit.med.J.* i, 336.
- Patterson, M.C., Darling, C.L. and Blumenthal, J.B., 1967, Acute Melioidosis in a Soldier home from South Viet Nam. *J.Amer.med.Ass.* 200, 447.

- Peck, C.R. and Zwammenburg, T., 1947, A Case of Melioidosis presenting as an Abscess in the Neck. *Brit.med.J.* 1, 337.
- Pons, R., and Advier, M., 1927, Melioidosis in Cochin China. *J.Hyg.* 26, 28.
- Rimington, R.A., 1962, Melioidosis in North Queensland, *Med.J.Aust.* 49, 50.
- Sakihara, R., 1952, A Case of Melioidosis in a Japanese Soldier in Malaya. *Jap.J.med.Sci.Biol.* 5, 425.
- Sheehy, T.W., Deller, J.J. and Weber, D.R., 1967, Melioidosis. *Ann.intern. med.* 67, 897.
- Spotnitz, M., Rudnitzky, J., and Rambaud, J.J., 1967, Melioidosis Pneumonitis. *J.Amer.med.Ass.* 202, 950.
- Stanton, A.T., 1917, Studies of the Institute for Medical Research, Kuala Lumpur No. 14.
- Stanton, A.T. and Fletcher, W., 1921, Melioidosis, a new disease of the Tropics. *Trans.4th Congress Far Eastern Ass. trop. Med.* 2, 196.
- Stanton, A.T. and Fletcher, W., 1932, Studies from the Institute for Medical Research, Federated Malay States. No. 21 London. John Bale, Sons and Davidson.
- Straus, I., 1889, Sur un moyen Diagnostic rapide de la morve. *Arch.Med. exp.* 1, 460.
- Strauss, J.M., Alexander, A.D., Rapmund, G., Gan, E. and Dorsey, A.E., 1968, Ps pseudomallei antibodies in Human Beings in Malaysia, an Endemic Area of Melioidosis. In Press.
- Strauss, J.M., Ellison, D.W., Jason, S., Marcarelli, J.L. and Rapmund, G., 1968, Melioidosis in Malaysia. An Intensive Study of Carey Island, Selangor, for Ps pseudomallei. In Press.
- Sudibyo, R.M.S., 1938, Two Cases of Cutaneous Melioidosis. *Genesk.T. Ned-Ind.* 78, 1424.
- Sutmoller, P., Kraneveld, F.C. and Van de Schaaf, A., 1957, Melioidosis (Pseudomalleus) in Sheep, Goats, and Pigs on Aruba (Netherlands Antilles). *J.Amer.vet.med.Ass.* 130, 415.
- Vaucel, M., 1937, Presence probable du B.Whitmore dans l'eau de mare au Tonkin. *Bull.Soc.Path.exot.* 30, 10.

- Whitmore, A., 1912, On the Bacteriology of an Infective Disease occurring in Rangoon. *Brit.med.J.* ii, 1306.
- Whitmore, A., 1913, An Account of a Glanders-like Disease occurring in Rangoon. *J.Hyg.* 13, 1.
- Whitmore, A. and Krishnaswamy, C.S., 1912, An Account of the Discovery of a hitherto undescribed Infective Disease occurring among the Population of Rangoon. *Indian. med. Gaz.* 47, 262.
- Ziskind, J., Pizzolatto, P. and Buff, E.E., 1954, Characteristics of a Strain of Malleomyces pseudomallei from Chronic Melioidosis. *Amer.J.Clin.Path.* 24, 1241.

The original descriptions of melioidosis were published by Whitmore in 1912 and 1913.

Alfred Whitmore was born in 1876 at Botcherley in Cumberland where his father was clergyman. He received his early education at St Bees School before going up to Cambridge University. After graduating B.A. he went to St. Mary's Hospital for his clinical training. He obtained the B.Chir. in 1900, the M.B. in 1903 and proceeded to the degree of M.D. in 1913. He was commissioned into the Indian Medical Service in 1903 and spent most of the next twenty years in Rangoon where he served as pathologist and surgeon. He played an important part in the early development of the Burma Medical School. After retiring from the Indian Medical Service in 1927 he worked in the Pathology Department of the University of Cambridge until he died in 1946. As far as the author can discover Whitmore did not publish any papers apart from those on melioidosis.

Unfortunately no record of his M.D. Thesis remains but the style of his second paper, published the same year as he received his doctorate, suggests that it was founded on a thesis.

The author is indebted to the Registry of the University of Cambridge and the Clerk to Gonville and Caius College for much of this information. Some details were taken from Dr. Whitmore's obituary in the British Medical Journal (1946, ii, 68).

APPENDIX IIThe name *Pseudomonas pseudomallei*

Since Whitmore suggested the name *Bacillus pseudomallei* for the causative organism in 1913, many other names have been suggested such as *Bacillus Whitmori*, *Loefflerella whitmori*, *Loefflerella pseudomallei*, *Pfeiferella whitmori* and *Malleomyces pseudomallei*. The last two have been widely used but have been rejected, the first by the Judicial Commission of the International Committee on Bacterial Nomenclature (Report 1958) and the second in the Report of the International Bulletin of Bacterial Nomenclature (1959). I have adopted the term *Pseudomonas pseudomallei* because it is used by Haynes and Burkholder in Bergey's Manual of Determinative Bacteriology (1957).

APPENDIX IIIDiagnoses of In-Patients

Pyrexia of unknown origin	36
Scrub Typhus	9
Leptospirosis	5
Malignant Malaria	10
Benign Tertian Malaria	6
Bronchopneumonia	11
Acute Tonsillitis	14
Other Acute Upper Respiratory Infections	8
Peptic Ulcer (Duodenal 15, Gastric 1.)	16
Acute Infective Hepatitis	21
Mononucleosis	12
Acute Enteritis	16
Bacillary Dysentery	5
Amoebic Dysentery	4
Acute Meningo-encephalitis	7
Helminthiasis	5
Patients stung by Jellyfish	22
Pulmonary Tuberculosis	1
Bronchitis and Asthma	8
Psychiatric Disorders	7
Skin Conditions	23
Venereal Infections	7
Melioidosis	3
No Disease found	10
Miscellaneous Conditions	73
Total	339

The terminology is adapted from the recommendations of "The Nomenclature of Disease" eighth Edition, published by H.M.S.O. 1960.

APPENDIX IVDiagnoses of Out-Patients

Early Syphilis	4
Chancroid of Penis	32
Urethral Gonorrhoea	233
Non-gonococcal Urethritis	77
Lymphogranuloma Venerum	3
No disease found	46
Total	395

APPENDIX VThe Haemagglutination Test

This haemagglutination test is a modification of the method described by Ileri in 1965 using sensitised sheep erythrocytes.

Antigen Preparation

A culture of a smooth strain of Ps pseudomallei was inoculated into a broth medium with a pH of 7.0 to 7.2, and incubated for two weeks at 37° Centigrade. The culture was then autoclaved at a pressure of 15 pounds per square inch for 15 minutes. After centrifuging for one hour at 1600g the supernatant fluid was removed, preserved with 0.5 per cent phenol and stored at 4° Centigrade.

Preparation of Sensitised Sheep Cells

Sheep blood was mixed with an equal volume of Alsever's Solution and stored at 4° Centigrade for two to four weeks. On the day of the test the preserved cells were centrifuged at 1000g for five minutes and washed three times in physiological saline. They were centrifuged again and physiological saline was added to the cells to give a ten per cent suspension. The erythrocytes were sensitised by adding ten volumes of a predetermined dilution of antigen to one volume of the cell suspension. The mixture was placed in a water bath at 37° Centigrade for one hour. It was then centrifuged for five minutes at 1000g. The supernatant fluid was removed; and the sensitised cells were washed in physiological saline and centrifuged again. The supernatant fluid was removed, and the cells were re-suspended in sufficient physiological saline to give a one per cent suspension.

The appropriate sensitising dose of antigen was determined by titrating the antigen against a standard anti-pseudomallei rabbit serum with a haemagglutination titre of approximately 1:40,960. The optimum sensitising dilutions of antigen varied from 1:50 to 1:100.

The Test

The sera for examination were inactivated by warming them in a water bath at 56° Centigrade for 30 minutes. Washed non-sensitised sheep erythrocytes were added to remove non-specific antibodies that might agglutinate sheep cells; the mixture was incubated at 37° Centigrade and then centrifuged to remove the cells. The sera were then serially diluted

two-fold with physiological saline starting with a dilution of 1:10. To 0.4 millilitre of each dilution 0.1 millilitre of the one per cent suspension of sensitised sheep erythrocytes was added. The tubes were incubated at room temperature and shaken intermittently. Readings were made after two hours and after 18 hours; they were usually the same. Normal serum, antigen, diluent and antiserum controls were included with each test. Results were graded as follows: 4 plus for complete granular agglutination, 3 plus for a smooth mat with folded edges on the bottom of the tube, 2 plus for a smooth mat with irregular edges on the bottom of the tube, 1 plus for a narrow ring of red around the edge of a smooth mat, and negative for a discrete red button in the centre on the bottom of the tube. The titre was the highest dilution of the serum giving a reading of 2 plus or greater.

Results

Preliminary evaluation studies were made on serial serum samples from bacteriologically proven cases of melioidosis, 500 sera from normal human beings, and rabbit sera for 14 strains of Ps pseudomallei, 3 of Ps mallei, one each of Ps aeruginosa, Pasteurella Tularensis, Pasteurella Pestis, Listeria monocytogenes, Brucella abortus, Salmonella dublin, Corella species and Leptospira species. The results showed excellent specificity and sensitivity (unpublished data, Walter Reed Army Institute of Research, Washington D.C., U.S.A., cited by Strauss et al 1968).