APPENDIX AA

Report on the Occurrence of Respiratory Anthrax and Haemorrhagic Anthrax Meningitis following the Intrusion of U.S. Military Planes over Northeast China

(ISCC/5)

INTRODUCTION

Following the invasions by American airplanes which disseminated insects and other objects (houseflies, anthomyiid flies, wolf spiders, ptinid beetles and feathers) carrying anthrax bacilli, there occurred suddenly and successively in some invaded areas of Northeast China a hitherto rare disease—anthrax infection through the respiratory route. The following report concerns the dissemination of insects and other objects carrying anthrax bacilli by American airplanes, the sudden and successive occurrence of anthrax infection through the respiratory route in Northeast China, and finally, discussion and conclusion.

CHAPTER I

Insects and Other Objects Carrying Anthrax Bacilli Dropped by American Airplanes

(A) *Feathers*

(1) At 11 a.m. March 11, 1952 when more than four hundred inhabitants of Pei-ching Village, Chang Shan (i.e. the 5th) District of Antung Hsien were holding a meeting, village chief Wu Ching-ming and a farmer Chiang Wen-ch'ang first saw three American planes flying from northwest to southeast. Soon afterwards they observed a greyish object dropped from one of the planes, slowly falling toward southeast. Men and women of the whole village were immediately mobilized to search for that object. A large amount of feathers was found at the Lan-shih-shan region, south east of the village. Two days prior to this, the villagers had been engaged in catching insects in the same region for two days without seeing any feathers. The feathers collected were examined by bacteriologists Hsin Chün, Ching Kuan-hua and Chao Cheng-lin and were found to carry anthrax bacilli (Document AA-1).

(2) At 12:52 March 12, 1952, eight American planes invaded K'uan-tien Hsien. One of the planes dropped a cylindrical object—a bacteria bomb. The remnants of this bacteria bomb were found by a school boy, Li Ssu-chien of K'uan-tien Middle School in the afternoon of March 21 in the maize field of Lou-ho-t'ao outside the east gate of K'uan-tien city. Feathers were found in the vicinity of the bomb fragments. (Please refer to App. V.) Bacteriological examinations of the feathers carried out by bacteriologists, Hsieh Shao-wen (Samuel Zia) and Chang Nai-chu, revealed anthrax bacilli.

(B) *Anthomyiid flies and wolf spiders*

Anthomyiid flies and wolf spiders were also found in the vicinity of the remnants of the bacteria bomb in the maize field at Lou-ho-t'ao, outside the east gate of K'uan-tien City. Specimens of the flies and spiders were collected by entomologists, Prof. Liu Ch'ung-lo and Dr. Ma Shih-chün, and were identified by Dr. Chen Sicien H., Director of the Laboratory of Entomology, Academia Sinica, Assistant Professor Lu Pao-ling, Peking College of Agriculture, and Professor Wang Feng-chen of Tientsin Army Medical College.

The flies and spiders were examined by Dr. Hsin Chün, bacteriologist, and were found to carry anthrax bacilli. (It may be also mentioned that anthrax bacilli were also isolated by bacteriologists Hsieh Shao-wen and Chang Nai-chu from anthomyiid flies and wolf spiders disseminated at Tsingtao by American planes.)

(C) *Houseflies*

At 10 p.m. March 14, 1952 American airplanes invaded Ssuping area. In the afternoon of March 17 Wang You-ts'ai, an inhabitant of San-ho Village, First District, Ssuping City noted large numbers of flies at San-tao-lin-tze outside the village. He called together Ch'iu Jung-sheng and Chao Sheng-tien to burn up as many of the flies as they could with hemp stalk. They reported the incident to their group leader Ts'ao Mei-chiu, who mobilized 6 more members to search out and exterminate a great many more. The chief Chao of the Hsiao-hung-tsui-tze police station happened to pass by the spot at that moment and witnessed the above facts. Next day (March 18) at 3 p.m. Liu Chi-an and Hsü Chung-lin, sanitary inspectors of the city, went to the spot. They searched and found large numbers of flies which were duly destroyed by some 40-50 persons mobilized by the District Government.

The flies were identified by the entomologists Prof. Ch'in Yao-ting and Dr. Feng Lan-pin to be houseflies *Musca vicina*. From such flies

anthrax bacilli were isolated by the Epidemic Prevention Station of Ssuping city. This was confirmed by bacteriologists Hsin Chün and Cheng Keng. (Documents AA-2 & AA-3)

(D) *Ptinid beetles*

In the evening of March 20, 1952, Lu Li-tsun, an inhabitant of Pei-chiao-ch'ang Village, Liu-erh-pu District of Liaoyang Hsien heard the noise of airplanes flying over the village. His sister-in-law also heard the noise; she went out of the house to look for, but could not see any plane. According to the Air Observer Corps, two American planes invaded Liaoyang area at 6 p.m. of that day and again at 6:30 p.m. on March 27. At the time when Lu Li-tsun heard the noise of airplanes, Jen Wan-ku, a militiaman of Pei-chiao-ch'ang Village was on his way to the 4th group of inhabitants on patrol duty. He saw about 160 meters away on the southeast a red object of the size of a thermos bottle dropping from the air above the houses of Chang Chia-feng, Wang Wen-ch'ang and Huang Yü-ch'eng. The object exploded when it was about 3-4 meters above the roof of the houses producing a feeble noise and an offensive smell. At the same time Wang Yung-ch'ang, an inhabitant of Ah-lao-ch'iao also saw the red object from a distance of about 700 meters away on the southwest. Wang Hua-ming, a member of Wang Wen-ch'ang's family saw, through the window, the red object falling in front of their gate when he was sitting on his kang (brick bed). He rushed out of his room but the red object had already disappeared. He went back to his room again and lighted the lamp and saw numerous insects on the outer surface of the window pane. On careful inspection numerous insects were found on the ground and on the outer side of the walls of the above mentioned three houses. Action was then taken to catch and burn these insects. Similar insects were found on other houses in the village. Next day the District Government received reports on the discovery of similar insects in neighboring villages and towns. Up to March 28th, these insects were found in 36 villages and towns including the town of Liu-erh-pu. An-shan city which is not far from Liu-erh-pu was also found to have such insects. The area in which these insects were found covered 30 kilometers from east to west and 20 kilometers from north to south. According to the District Government, such "red object" as described above was also seen to have fallen in various places and similar insects were found also in the fields. The weather then was still very cold. The earth froze at night, melting only in the day time. The insects were identified by entomologists Liu Ch'ung-lo and Lu Pao-lin, as *Ptinus fur*. Bacteriological examination by Drs. Hsieh Shao-wen (Samuel Zia) and Chang Nai-chu, proved that these insects carried anthrax bacilli. (Documents AA-4 & AA-5).

(E) *Entomological Identification*

(1) *Housefly* (*Musca vicina* Macquart)

The fly, specimen No. 13033, discovered in San Ho Village of Ssuping on March 17, 1952, has been identified as housefly, *Musca vicina* Macquart, by entomologists Ch'in Yao-ting, Professor of National Medical College, Shenyang, and Feng Lan-pin, Lecturer of the same College.

This species of housefly belongs to Family *Muscidae,* Order *Diptera.* The characteristics of this species are: Four black longitudinal bands on the mesonotum. In the male, width of front about one-fourth to one-third that of the compound eye, the abdomen light orange in color with a central black stripe dorsally. In the female, width of front slightly narrower than the compound eye, and abdominal tergite orange in color, with grayish yellow tomentum. The identification is based on Li and Feng (3), Tokunaga (1) and a series of publications by Patton (11, 12).

This species of housefly is widely distributed. Outside of Asia, it is the commonest species in Hawaii (2). It has been recorded in China (4, 13). Its habits resemble those of the common housefly (*Musca domestica* Linn.), breeding in such media as horse dung and excreta, garbage and decayed organic materials. There may be many generations a year, varying with the climate of the locality. The adult flies invade houses. (see Document AA-2)

(2) *Ptinid beetle* (*Ptinus fur* Linn.):

The beetle, specimen No. 362-1 (2007), discovered in Pei-chiao-ch'ang Village of Liaoyang on March 20, 1952, has been identified as ptinid beetle, *Ptinus fur* Linn., by Prof. Liu Ch'ung-lo of the Department of Entomology of Peking College of Agriculture, and by Assistant Professor Lu Pao-lin of the same institution. This species of beetle belongs to Family *Ptinidae,* Order *Coleoptera.* Its characteristics are: A pair of moderately yellowish brown subtomentose cushions on the pronotum. Punctures on elytra arranged parallelly in serial rows, also fine brownish setae on the surface. A white hairy marking near the base and apex of each elytron (see Document AA-4).

The identification is based on Reitter (20) and Hinton (18).

Ptinid beetle has a wide distribution. According to Hsin (15) and Kuan (16), it has been recorded in China.

(3) *Anthomyiid fly and wolf spider*

Details are given in the Report on the Calcareous Bacteriological Bomb Dropped by U.S. Military Plane at K'uan-tien. (App. V).

(F) *Bacteriological Examinations*

(1) *Methods*

The methods for the isolation of anthrax bacilli are briefly given here. Details are obtainable in the Documents at the end of this Appendix.

1. For isolation of bacteria from insects: The insect is first washed in sterile normal saline and cultures are then made of the washing fluid. The insect is then ground up in a sterile mortar with sterile saline. The suspension of the ground insect is cultured and inoculated into white mice. The media used are plain agar plate, blood agar plate, S.S. agar plate and cooked meat broth.

2. For examination of feathers: The feather is washed in sterile normal saline. This saline is then centrifuged at 3000 revolutions per minute for 20 minutes. The sediment is used for culture and animal inoculation as mentioned above.

3. For the examination of human post-mortern material: The material is divided into 2 parts, 1 part is used for direct culture and the other part for animal inoculation.

When organisms are first isolated from any material, further steps are taken for identification. *Bacillus anthracis* is preliminarily diagnosed if the morphology and staining quality of the organisms, appearance of colonies, manner of growth in the broth, the presence or absence of motility and pathogenicity for white mice are identical with those of that organism. Further steps include the test on other laboratory animals for pathogenicity, biochemical characteristics and immunological studies. When all the findings are identical with those of the anthrax bacillus, the isolated organism is finally identified as *Bacillus anthracis.*

(2) *Diagnostic Criteria*

1. Preliminary identification:

a) The colonies are greyish and non-hemolytic slightly elevated with curled-hair appearance at the periphery; their surfaces are rough.

b) Morphology. Gram-positive large bacilli with square ends, non-motile an dlined up in long chains. There are centrally placed spores.

c) Growth in broth, flocculated sediment. No turbidity. No pellicle.

d) Pathogenic to white mice and from the viscera of the dead mice Gram positive, encapsulated large bacilli with square ends are found.

2. Confirmatory tests:

a) Pathogenic for guinea pigs, encapsulated, large Gram positive bacilli with square ends are found in the viscera of the dead animal.

TABLE I. RESULTS OF BACTERIOLOGICAL EXAMINATION

Specimens	Serial No.		355	48004B	48003	17006	362-1	13033
	Name		Feathers	Wolf Spiders	Anthomyiid flies	Feathers	Ptinid beetles	House flies
	Districts		K'uan Tien	K'uan Tien	K'uan Tien	Antung Peiching village	Liaoyang	Ssuping
	Date received		April 14	March 24	March 24	March 15	April 14	March 30
General characteristics	Colonies on agar plate		Grayish white in color, not transparent. Edge uneven and curled hair like.					
	Morphology		Gram positive large bacilli with square ends and in chain form, spore formation at centre.					
	Growth in meat broth		Flocculent precipitation					
	Motility		—	—	—	—	—	—
Biochemical Properties	Milk Coagulation Test		+	+	+	+	+	+
	H_2S		—	—	—	—	—	—
	Indol		—	—	—	—	—	—
	Hemolysis	Goat's red blood cells	—	—	—	—	—	—
		Rabbit's red blood cells	—	—	—	—	—	—
		Guinea pig's red blood cells	—	—	—	—	—	—
	Lactose		—	—	—	—	—	—
	Glucose		+	+	+	+	+	+
	Maltose		+	+	+	+	+	+
	Mannitol		—	—	—	—	—	—
	Sucrose		+	+	+	+	+	+
	Inositol		—	—	—	—	—	—
	Xylose		—	—	—	—	—	—
	Arabinose		—	—	—	—	—	—
	Dulcitol		—	—	—	—	—	—
	Salicin		—	—	—	—	—	—
Animal Inoculation	White Mice	Death of animal	Died	Died	Died	Died	Died	Died
	Guinea pigs	Death of animal	Died	Died	Died	Died	Died	Died
		Gelatinous change at site of injection	+	+	+	+	+	+
		Enlargement of spleen	+	+	+	+	+	+
		Congestion and enlargement of liver	+	+	+	+	+	+
		Congestion of lungs	+	+	+	+	+	+
		Cultures of heart blood	+	+	+	+	+	+
		Smear from internal organs	+	+	+	+	+	+
Precipitation Test			+	+	+	+	+	+

TABLE II. RESULTS OF BACTERIOLOGICAL EXAMINATIONS

Autopsy Cases	Case No.		I	II	IIIa	IV
	Name		Chü Chan-yun	Wang Tze-pin	Wei-Liu-shih	Tien Cheng-ho
	District		Manching	Shenyang	An-shan	An-tung
	Occupations		Railway worker	Pedicab driver	House wife	Farmer
	Date of onset of disease		March 19	March 20	April 11	April 16
	Date of death		March 22	March 25	April 14	April 18
	Pathological diagnosis		Anthrax of respiratory system	Anthrax of respiratory system and Anthrax Meningitis	Anthrax of respiratory system and Anthrax Meningitis	Anthrax of respiratory system and Anthrax Meningitis
General Characteristics	Colonies on agar plate		Grayish white colonies. Not transparent. Edge uneven and curled hair like.			
	Morphology		Gram positive large bacilli with square ends and in chain form. Spore formation at center			
	Growth in meat broth		Flocculent precipitation			
	Motility		Non motile			
Biochemical Properties	Milk Coagulation test		+	+	+	+
	H_2S		—	—	—	—
	Indol		—	—	—	—
	Hemolysis	Goat's red blood cells	—	—	—	—
		Rabbit's red blood cells	—	—	—	—
		Guinea pig's red blood cells	—	—	—	—
	Lactose		—	—	—	—
	Glucose		+	+	+	+
	Maltose		+	+	+	+
	Mannitol		—	—	—	—
	Sucrose		+	+	+	+
	Inositol		—	—	—	—
	Xylose		—	—	—	—
	Arabinose		—	—	—	—
	Dulcitol		—	—	—	—
	Salicin		—	—	—	—
Animal Inoculation	White Mice	Death of animal	Died	Died	Died	Died
	Guinea pigs	Death of animal	Died	Died	Died	Died
		Gelatinous change at site of injection	+	+	+	+
		Enlargement of spleen	+	+	+	+
		Congestion and enlargement of liver	+	+	+	+
		Congestion of lung	+	+	+	+
		Culture of heart blood	+	+	+	+
		Smear from internal organs	+	+	+	+
	Precipitation Test		+	+	+	+

b) There is fermentation of glucose, sucrose and maltose. Acid is produced without gas. Milk is coagulated.

c) No hemolysis on the red blood cells of goat, rabbit and guinea pig.

d) The liver or spleen of animals died from anthrax is ground up with saline and then boiled and used for precipitation with the filtrate as an antigen, a precipitation test (Ascoli test) is made with anti-anthrax diagnostic serum. The result is positive.

(3) *Results of Examination*

The characteristics of strains of organisms isolated are given in Tables I and II.

CHAPTER II

Sudden and Successive Occurrence of Anthrax Infection through the Respiratory Route

Case 1.

Chü Chan-yun, male, 55 years old, a railway foreman at the Man Ching Station and railwaymen Li Tso-hsiang and Liu Chung-ko on March 16, 1952 took part in catching and killing flies at a place one and one half kilometers to the north of the Man Ching Station. (In the night of March 14 American airplanes invaded Ssuping area including Man Ching Station.)

On March 19, Chü Chan-yun became ill with fever, headache and aching in the limbs. On March 21 he was admitted to the Railway Hospital when he also developed cough, nausea, vomiting and insomnia, mental confusion and rigidity of neck.

Examination of sputum revealed the presence of Gram positive encapsulated large bacilli. White blood cell count 28,000. Death occurred at 2:45 p.m. March 22, 1952.

Autopsy was done by Dr. Sung Teh-yu of the Epidemic Prevention Station of Ssuping. There were congestion, oedema and small hemorrhages in both lungs. Increased consistency was felt in the upper lobe of both lungs. Hilum lymph glands were enlarged. Bilateral pleural effusion and pericardial effusion were present. Ulcerative hemorrhagic spots were seen on the mucosa of small intestine. The tip of the appendix showed hemorrhagic inflammatory process. The mesenteric lymph glands were however not enlarged. Bacteriological examination was done in the Epidemic Prevention Station of Liao-hsi Province. Anthrax bacilli were isolated from the lung, liver and spleen. The organisms were re-examined by bacteriologists Hsin Chun and Cheng Keng and the original diagnosis

of *Bacillus Anthracis* was confirmed. The pathological diagnosis of respiratory anthrax was confirmed by pathologists Professors Wu Tsai-tung and Li Pei-lin.

(The clinical symptoms indicate that the meninges were involved but the skull was not opened for examination at autopsy because of the refusal of the family.) (Document AA-7 and Document AA-10)

Case 2.

Wang Tze-pin, male of 47 years was a tricycle-rickshaw driver in Shenyang. He became ill on March 20 with general malaise. Next day he was confined to bed. On April 22 aching in legs, general weakness, upper abdominal discomfort, nausea and headache were felt. He was slightly better in the morning of April 23 but got worse by the afternoon. Until 6 a.m. April 24 he was still conscious and could move about. However since then he sank progressively into coma with restlessness. Neck was rigid. Kernig's sign was positive. Death occurred at 9 a.m. April 25. Autopsy was done by Prof. Chu Feng-ch'un and assistant Wang Hung-lieh of the National Medical College with the diagnoses of hemorrhagic anthrax meningitis, anthrax bronchopneumonia of right lower lobe, peribronchitis, interlobular cellulitis, suppurative hemorrhagic anthrax lymphadenitis of hilum glands, pulmonary oedema, pleural effusion, pericardial effusion and multiple punctate necrosis and ulceration of intestinal mucosa due to anthrax infection. (No enlargement of mesenteric lymph glands.) The pathological diagnosis was confirmed by Professors Wu Tsai-tung and Li Pei-lin. Anthrax bacilli were isolated from the brain tissue, heart blood and spleen by bacteriologists Chu Chi-ming and Liu Shih-ming and confirmed by bacteriologists Hsin Chun and Cheng Keng. (Document AA-9 and Document AA-10)

Case 3a.

Wei Liu-shih, female of 32 years, was an inhabitant of Anshan City. She was repeatedly engaged in catching and killing insects (*Ptinus fur*) dropped by American airplanes. In the evening of April 11 she felt general malaise. Next day headache, chilliness and fever were noticed. Condition became worse on April 13 and she was confined to bed. Cough, chest pain, shortness of breath and vomiting developed. On April 14 mentality became confused with delirium, restlessness and dyspnea. She had attacks of convulsion. The neck was markedly rigid. Kernig's sign was positive. Dry and moist rales were heard all over the lungs. Death occurred at 11:50 p.m. April 14. Post mortem examination was done by Drs. Chiang Ying-kai and Kuo Cheng-teh, assistants in the department of Pathology of the National Medical College. The pathological diagnoses

were: Hemorrhagic anthrax meningitis, necrotic anthrax pneumonia of left lower lobe, hemorrhagic anthrax lymphadenitis of the hilum, oedema of lungs, bilateral pleural effusion, pericardial effusion and acute splenic tumor. The pathological diagnoses were confirmed by Professors Wu Tsai-tung and Li Pei-lin. Anthrax bacilli were isolated from the brain tissue by bacteriologists, Chu Chi-ming and Liu Shih-ming and confirmed by bacteriologists Hsin Chun and Cheng Keng (Document AA-8 and Document AA-10)

Case 3b

Wang Shu-chih, female of 23 years, was a primary school teacher at Liu-erh-pu of Liao-yang Hsien. She was healthy in the past, and was very actively engaged in catching and killing insects dropped by American airplanes. On April 6 she felt dryness of throat with hoarseness of voice. Some headache and general joint aching was also felt. She was however working as usual until 9 a.m. April 8, when she went to toilet and collapsed there. When picked up by her colleagues from the toilet she was unconscious. Cyanosis was noted and the light reflex of pupils was lost. Death occurred at 10:30 a.m. on the same day. Autopsy was done by Drs. Chao Wen-tou and Wang Hung-lieh of the National Medical College. There were diffuse subarachnoid hemorrhages in the brain and spinal cord. Lungs showed congestion and oedema with bronchopneumonia in the right upper lobe. Cerebral arteries and endocardium were normal. On microscopic examination of sections, Gram-positive bacilli with square ends morphologically identical with anthrax bacilli in the bronchopneumonic lesions, hepatic sinuses, capillaries of the brain, and large vessels of the meninges were seen. Culture was however not done because the autopsy was made at Liu-erh-pu where facilities for bacteriological examination were not available. The pathological material was examined by pathologists Wu Tsai-tung and Li Pei-lin and the diagnoses of acute hemorrhagic anthrax meningitis, anthrax bronchopneumonia of right upper lobe and pulmonary oedema were made. (Document AA-10)

Case 4

Tien Cheng-ho, male, 44 years old, was a farmer of Eastern Shuang Shan Village, Chang Shan district of Antung Hsien. The village is 1¾ kilometers away from Pei Ching Village to the northeast. On April 14, farmer Tien and his son discovered the feathers disseminated by American airplanes and took part in their collection and disposal. He fell ill on April 16 with sudden onset of chills, fever, generalized joint pain and mild headache. Condition became worse the next day. He vomited twice. After daybreak on April 18 he became unconscious with both hands tightly

clenched as in spasm. Temperature was 38.7°C. Death occurred at noon of the same day.

Autopsy was done by Dr. Sung Wei-yi, superintendant of the Liaotung Provincial Hospital. Diffuse hemorrhages were found in the leptomeninges. Internal organs showed marked post-mortem changes. Right lung weighed 850 gms. and left lung 700 gms. The cut surface was purple-black in color with hemorrhages. The hilum glands were as big as the thumb. Direct smears made from the brain, lungs, spleen and kidneys showed numerous Gram positive bacilli with square ends. From cultures and animal inoculations of heart blood, brain, lung, spleen and kidney anthrax bacilli were isolated. The diagnosis of hemorrhagic anthrax meningitis was confirmed by pathologists Professors Wu Tsai-tung and Li Pei-lin. The primary infection was in the lung. The organism, isolated from the heart blood, was confirmed by bacteriologists Drs. Hsin Chun and Cheng Keng to be *Bacillus Anthracis* (Document AA-6 and Document AA-10).

CHAPTER III

Discussion

(A) *Entomological Consideration*

I. *Housefly*:

In North China, under normal conditions, this species of house fly passes winter mainly as pupae, and the adults appear comparatively late in the year. Meng and Winfield (4, 5, 7, 9, 10) have studied this species of flies. According to their report (6), this fly appears in Tsinan, Shantung, in May. They have collected all the flies in a single house throughout a year. Although upon analysis of the 1831 flies collected, this species was found to be 91.77% of the total population, yet not a single specimen of this fly appeared in the period from January to April.

The regional temperature in Northeast China is lower than that in Tsinan. At Ssuping, the average temperature in March, 1952 was 1.4° C below zero. The appearance of this species of housefly in Ssuping should be later than in Tsinan. However, large numbers of these flies were discovered in the field at Ssuping on March 17th, 1952. This is definitely abnormal.

II. *Ptinid beetle*:

This species of beetle is a pest of stored products. According to the reports of Hsin (15), Li (14), Patton (19) and Cotton (17), under natural conditions, the beetle frequents warehouses, granaries,

flour mills and other factories and storage houses for animal and plant products. It damages stored grains, flours, furs, leathers, etc. It is also a well-known museum pest, destroying especially dried specimens of animals and plants.

This species has one to three generations a year, varying with the temperature. In China, it usually passes the winter as larva, but occasionally a few adults may also survive the cold. It takes three and a half months to complete a generation. The adults are active in the night and retire into hiding during daytime; they also have the habit of feigning death.

When the circumstances under which ptinid beetles were discovered at Liaoyang are compared with their behavior under natural conditions, the following four points are noteworthy:

1. Under natural conditions, ptinid beetles should be found at places such as warehouses, especially those for storing grains, flours, furs and leathers, or any other place for storing such materials. However, at Liaoyang and the other localities these beetles were found not around store-houses, but outside ordinary houses. They were found not only on the ground and walls outside the houses, but also in the fields. This is definitely not a natural phenomenon.

2. All the publications mentioned above have pointed out that ptinid beetles are nocturnal. However, at Liaoyang, these beetles were discovered in daytime at places where they do not normally appear. This is at variance with the normal habits of these beetles.

3. On May 26th, 1952, Ma Shih-chun, Assistant Research Member of the Laboratory of Entomology, Academia Sinica, and Lu Pao-ling, assistant Professor of the Peking College of Agriculture went to Pei Chiao Chang Village. The purpose of their visit was to investigate from entomological point of view whether at the time of the discovery of these beetles was there any possibility of appearance in large numbers of ptinid beetles under natural conditions at that place. They found that neither was there any storehouse for grains, furs, leathers, or other substances, nor was there stored in the houses of the people any furs, leathers, or large amounts of grains or other materials on which ptinid beetles might develop. They have come to the conclusion that it is impossible for large numbers of ptinid beetles to appear at that place under natural conditions.

4. After a large number of ptinid beetles had been discovered in Pei Chiao Chang Village of Liaoyang on March 20, 1952, Feng Lan-pin, Lecturer of National Medical College and Assistant Li Shao-hua investigated further whether in the neighboring areas of Liao-

yang were ptinid beetles appearing naturally. They searched in the vicinity of Shenyang and Liaoyang such places as granaries where these beetles might appear, but they were unable to find any. This shows that this species of beetle had not appeared at that time under natural conditions. Thus it is concluded that the appearance of large numbers of ptinid beetles at Liaoyang is unusual and moreover, that these beetles could not have migrated or transferred from the neighboring areas.

It is very evident from the four points mentioned above that at that time no ptinid beetles had appeared at places where they could naturally occur, while on the contrary they suddenly occurred in abundance at places such as Pei-chiao-chang Village of Liaoyang where natural conditions did not permit their appearance. Therefore, from the entomological point of view, the occurrence of ptinid beetles at Liaoyang and Anshan is entirely unusual.

Finally, it is observed that the unusual occurrence of the house flies and ptinid beetles, as mentioned above, was preceded by the intrusion of U.S. military planes into those places.

(B) *Bacteriological Consideration.*

As mentioned in the literature (21, 23) different strains of anthrax bacilli may show differences in certain biochemical reactions. This is amply confirmed by personal observations of Prof. Cheng Keng in this country, especially in the speed of sucrose and salicin fermentations. But the biological and biochemical features of the 10 strains of anthrax bacilli isolated from feathers, insects and human autopsy material are entirely identical. This speaks for the common origin of all the organisms isolated from *Ptinus fur,* housefly, feathers and the clinical cases of anthrax infection.

In 1894 Heim (25) reported the finding of pathogenic anthrax spores on the body surface and in the excreta of *Ptinus.* Cao (22) experimented with the ova of housefly (*Musca domestica*). The surface of the ova was sterilized. They were then hatched in the flesh of an animal died of anthrax. Anthrax bacilli could be found in the larvae, and, in the adults, for at least 9 days after hatching. As far back as 1869 Raimbert had experimental proof that the fly could carry anthrax bacilli (27). Nuttall (26) mentioned Bollinger as having caught flies from a cow died of anthrax and isolated anthrax bacillus from the digestive tract of the flies. These flies were inoculated into 2 rabbits which later died of anthrax. In 1912 Graham—Smith (24) reported the feeding of

larvae of housefly with foods carrying anthrax bacilli. The organism could be isolated from most of the adult flies.

The above works indicate that the *Ptinus fur* and housefly could be used to carry anthrax bacilli.

Some control observations have been made in our laboratory (See Appendices E and F). In June 1952, 3106 flies of various kinds were collected from Shen-yang including *Fannia scalaris, Muscina stabulans, Lucilia sericata, Sarcophaga* sp., *Calliphora* sp. etc. From such a large number of flies no anthrax bacilli were isolated. At the same time 13 specimens of feathers from Shen-yang and 3 specimens from Kuantien were examined. No anthrax bacilli were found in these local specimens of feathers. No control studies have been made for *Ptinus fur* since it was impossible to find a local specimen. The control observations would further indicate that the isolation of anthrax bacilli from insects, (*Ptinus fur* from Liaoyang, housefly from Ssuping, anthomyiid fly from Kuantien) wolf spiders (from Kuantien) and feathers (from Kuantien and Pei-ching Village of Antung) is not merely an accident.

That *B. anthracis* could be used as a bacteriological weapon is well known. Rosebury and Kabat (28) put it on the top of the list of organisms for air-borne infection because: "The anthrax bacillus is one of the most thoroughly studied bacteria. Its properties make it an obvious possibility as an agent in warfare." "*B. anthracis* is surpassed by few micro-organisms in infectivity for animals, and by none in host range." Zelle *et al* (29-32) in Camp Detrick carried out extensive studies on *B. anthracis* as a bacteriological weapon. They reported on the selection of strains, nutrition studies and the use of chemicals to enhance the invasiveness of the organism. Some variants were obtained especially suitable for infection through the respiratory route. The virulence of the organism could be artificially increased for the purpose of bacterial warfare.

From the facts mentioned above it is not difficult to unravel the plot of American government of disseminating *B. anthracis* in order to wage bacteriological warfare in Northeast China.

(C) *Pathological Consideration*

In the 5 cases of anthrax infection the etiological diagnosis was established either by culture or by the demonstration of the organisms in pathological sections. Clinically there were manifestations of respiratory infection in every case. Case 1 had congestion and oedema of both lungs with increase of consistency in upper lobes, enlargement of hilum glands and bilateral pleural effusion etc., lesions en-

tirely similar to those of case 2 and case 3a. Case 3b had anthrax bacillus bronchopneumonia though the bronchial lymph glands were not enlarged. In case 4 the lungs were evidently increased in weight and the bronchial lymph glands were enlarged. All 5 cases had lesions in the respiratory system. There was no cutaneous lesions in any one of these. Although hemorrhagic ulcers were found in intestines in case 1 and case 2 but the mesenteric glands were not enlarged so the intestinal lesions could not be primary in nature. They were probably produced after the swallowing of bacteria in the sputum or secondary to septicaemia (34) shortly before death. So we conclude that all the 5 cases were anthrax infection through the respiratory route (36). 4 of the 5 cases had proven lesions of hemorrhagic anthrax meningitis. The extensive diffuse subarachnoid hemorrhage and the lack of striking cellular reaction were characteristic of anthrax meningitis (33—40). In case 1 the brain was not examined at post-mortem but clinically the patient also had headache, vomiting, coma and nuchal rigidity, pointing to the presence of meningitis. The isolation of anthrax bacilli from heart blood, liver, spleen, lung, brain and other internal organs proves the presence of septicaemia. The death was evidently due to a combination of hemorrhagic meningitis and septicaemia.

Anthrax is primarily a disease of herbivorous animals. The susceptibility of man to *B. anthracis* is comparatively low. So anthrax infection is rare in human beings (33, 37, 39, 41). The mode of infection in the human disease is principally contact with diseased or dead animals or their hides and excreta harboring anthrax bacilli. The usual form of anthrax in man is cutaneous infection. Infection through the respiratory route is extremely rare (35). Under natural conditions respiratory infection of anthrax is also related to animal hides and wool, so it is called wool sorters disease. In the 5 cases mentioned in this report no history of contact with such things could be elicited.

Anthrax meningitis is a very rare disease. Lin and Chen (38) saw 2 cases from 1937 to 1946. They searched the world's literature from 1927 to 1940 and came across only 11 papers, mostly of single case reports. Most of the cases in the literature started as cutaneous infection and the meninges were secondarily infected. Since 1940 such reports became even scantier in the literature. In 1947 Shanahan *et al* (42) reported 1 case, a rug worker who had anthrax meningitis following a malignant pustule of the upper lip.

In China post-mortem and clinical statistics from medical colleges and their teaching hospitals likewise indicate the rarity of

anthrax meningitis. The Shanghai Medical College had 1178 autopsies from 1928 to 1952. The China Union Medical College performed 3942 autopsies from 1916 to 1952. In the National Medical College at Shenyang 1093 post-mortem examinations were done from 1940 to 1952. Not a single case of anthrax meningitis was found among the total member of 6213 autopsies in the above mentioned three institutions. Professor Wu Tsai-tung saw only 1 case of anthrax meningitis in 21 years of his career as a pathologist and the patient he saw had definite contact with hides before the onset of the disease. Therefore there is no doubt that this disease is very rare in this country and abroad.

In Man Ching, Shenyang, Liaoyang, Anshan and Eastern Shuang-shan Village, Chang-shan district of Antung Hsien anthrax of skin was rare in the past. Anthrax infection through the respiratory route and anthrax meningitis had never been observed. In the teaching hospitals of National Medical College, Shenyang from 1949 to June 1952 only 1 case of skin anthrax was seen among 1,400,000 out-patients. It means that anthrax is very rare in Shen-yang area. In the Liu-erh-pu district including the town of Liu-erh-pu and 35 villages, with a total population of 61,372 persons in 14,029 families no case of anthrax has ever been discovered in the past 10 years. In Anshan City, from Jan. 1950 to March 1951, among the 760,212 patients of all the public and private hospitals and clinics, not a single case of anthrax was observed.

Anthrax was rare not only in man but also in animals. In Shenyang only 6 cases of animal anthrax were recorded in the past 5 years but has not been seen in the past 3 years. According to the records of the Veterinary Hospital and the personal records of the veterinarians at Liu-erh-pu anthrax has not occurred among 4646 domestic animals under their care in the whole district in the past 10 years. A similar result was obtained on investigation into the animal diseases in Anshan. In the recent years, there was also no anthrax among domestic animals in Man Ching station and Eastern Shuang Shan village. This is not just an accidental occurrence, it is the result of widespread use of prophylactic vaccinations.

Conclusion

(1) Anthrax was a rare disease in man and animals in Man-Ching, Liaoyang, Anshan, Shen-yang and Antung Hsien in the past. But from March 19 to April 16, 1952, within a short period of less than one month, cases of anthrax infection through the respiratory route occurred in succession. This is an extremely unusual phenomenon.

(2) *B. anthracis* was isolated from insects and other objects disseminated by American airplanes. The same organism was obtained from brain and other internal organs of persons dead from anthrax infection. The biological and biochemical properties of *B. anthracis* from insects and pathological materials were entirely identical.

(3) All the 5 cases of human anthrax infection had manifestations and lesions of the respiratory system indicating that the infection was introduced through the respiratory route and produced fatal results through septicaemia and hemorrhagic meningitis.

(4) Summing up the facts mentioned above, we conclude that the human anthrax infection was produced by the bacteriological warfare of the American government.

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Entomology (*Ptinus fur*)	14-20
Bacteriology	21-32
Pathology	33-42

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DOCUMENT AA-1

REPORT ON BACTERIOLOGICAL EXAMINATION OF FEATHERS

1. Specimen No.: 17006.
2. Source of specimen: sent from Peiching Village, Antung City.
3. Date received: March 15th, 1952.
4. Kind of specimen: Feathers.
5. Procedures: The feather was put in a mortar, washed and ground with sterile physiologic saline. The washing fluid was injected intraperitoneally into 2 mice, each receiving 0.5 ml., and was cultured on plain agar plate and blood agar plate for bacterial isolation. Smears were also made for microscopic examination.

(1) Under microscope, no bacteria were seen on the smears.

(2) The cultures made from the above-mentioned washing fluid on agar plate and blood agar plate resulted in growth of grayish white, irregularly edged and rough-surfaced colonies. Stained smears revealed large-sized bacilli with square ends, lined up in chains. There were centrally placed spores in the bacteria. Gram positive. No motility. Pure cultures were made from this kind of colony.

(3) 0.5 ml. of the above-mentioned washing fluid was inoculated into the peritoneal cavity of two mice. One of the mice died after 24 hours and the other died 36 hours afterwards. The spleen smear was stained and examined under microscope. There were found encapsulated large Gram positive bacilli with square ends. Cultures yielded the same kind of bacteria.

(4) Examination of the pure cultures.

I. Morphology:

(1) Cultured on plain agar plate and meat broth, the bacteria are large bacilli with square ends, lined up in chains, with centrally placed spores. No motility. Gram positive.

(2) Direct smears made from heart blood, liver and spleen of the dead mice and guinea pigs inoculated with this bacteria showed Gram positive encapsulated large bacilli.

II. Cultural characteristics:

(1) Plain broth: Growth with flocculent sediments, broth not turbid.

(2) Plain agar plate: Grayish white colcred opaque colonies with irregular edges and rough surfaces. Under hand lens the periphery of the colony was curly-hair in appearance.

(3) Gelatin: Stab culture grew like an inverted fir tree. Very slow liquefaction of the gelatin.

(4) Blood agar: No hemolysis.

(5) Milk: Coagulated.

III. Biochemical characteristics: It fermented glucose, maltose and sucrose without gas formation, but did not ferment lactose, mannitol, dulcitol, inositol, xylose, arabinose and salicin. Hydrogen sulfide negative. Indol reaction negative.

IV. Hemolysis test: 2% suspensions of red blood cells in normal saline were prepared respectively with blood from rabbit, guinea pig and goat. Bacteria suspension (1 loopful in 1 ml.) was mixed with the red cell suspension and kept in an incubator for 2 hours and control test was done with *B. subtilis*. The results are shown in the following table:

Bacterial suspension / 2% R.B.C. suspension	Suspension of bacteria isolated	*B. subtilis* (control)
Rabbit	No hemolysis	Hemolysis
Guinea pig	No hemolysis	Hemolysis
Goat	No hemolysis	Hemolysis

V. Pathogenicity test:

(1) Mouse: Died in one day after subcutaneous injection of ¼ loopful of the bacteria, and in three days after injection of 1/100 loopful. Smears of liver, spleen and heart blood showed the presence of encapsulated Gram positive large bacilli with square ends.

(2) Guinea pig: Died in two days after subcutaneous injection of 1/4 loopful of the bacteria. Autopsy findings: gelatinous exudate at the site of injection. Spenic enlargement. Smears of liver, spleen and heart blood showed the presence of Gram positive encapsulated large bacilli with

square ends. General and biochemical characteristics of the bacilli isolated from these organs were identical with those isolated directly from the feathers.

VI. Immunological characteristics:

Ascoli test. The liver and spleen of mice and guinea pigs died after inoculation were ground separately, and their respective suspensions were made. These suspensions were immediately boiled for 30 minutes in water bath and filtered through filter paper. Precipitation reactions were carried out with these filtrates and diagnostic anti-anthrax serum. Control matreals included the liver and spleen of healthy mice and guinea pigs and also normal rabbit serum. The results are as follows:

Antigen / Serum	Boiled Antigen		Boiled filtrates of visceral organs of healthy animals		Saline
	Mice	Guinea pigs	Mice	Guinea pigs	
Diagnostic anti-anthrax serum	(+)	(+)	(—)	(—)	(—)
Normal rabbit serum	(—)	(—)	(—)	(—)	(—)

Conclusion: *Bacillus anthracis* isolated from the specimen of feathers.

Examined by:

Ching Kuan-hua, M. B.

Assistant Prof. of Bacteriology, National Medical College, Shenyang.

Chao Cheng-lin, M. B.

Assistant Prof. of Micrology, Harbin Medical College.

Reexamined and Reported by:

Hsin Chün, M. D.

Chief Technical Expert of Northeast Epidemic Prevention Institute.

Date of Report: April 25, 1952.

DOCUMENT AA-2

REPORT ON ENTOMOLOGICAL IDENTIFICATION OF HOUSEFLIES

Serial No. of specimen: 13,033	Original No. of specimen:	Date received: March 22, 1952

Circumstances of discovery:

At 10 p.m., March 14, 1952, American military planes invaded the area of Ssuping municipality. On March 17, inhabitants of San Ho Village, Ssuping municipality found large quantities of flies at San Tao Lin Tze, outside the village.

Results of identification:

Scientific name: *Musca vicina* Macquart **(Diptera, Muscidae)**

Common name: Housefly.

Comments: Houseflies usually inhabit the vicinity of houses and animal enclosures. In the Northeast they do not appear outdoors until May. Their sudden discovery in the cold weather of March in open fields must be attributed to the activities of American aircraft.

Remarks:

Identified by:

Ch'in Yao-ting, Professor of Biology, National Medical College, Shenyang.

Feng Lan-pin, M.B., Lecturer, Department of Medicine, National Medical College, Shenyang.

Date: March 24, 1952

DOCUMENT AA-3

REPORT ON BACTERIOLOGICAL EXAMINATION OF HOUSEFLIES

Date of receiving specimen: March 18, 1952, at 5 p.m.
Kinds of specimen: Houseflies.
Source of specimen: Ssu-ping City.
Procedure and Results:

20 flies, crushed with a pair of sterile forceps were used for the following bacteriological tests.

1. Direct smear: Large Gram-positive spore-bearing bacilli with square ends were seen.

2. Culture on agar plate: After incubation for 18 hours, there was a growth of grayish white colonies with rough surfaces and irregular edges. When examined with a hand-lens, the edge was in the form of curled hair appearance. These colonies were taken for further examinations as follows:

A. Direct smear examination showed Gram positive bacilli with square ends and arranged in long chains. Hanging drop examination showed no motility.

B. Culture in meat broth: After incubation for 18 hours, there was flocculent sedimentation at the bottom of the tubes. The supernatant liquid was clear. Microscopic examination of the direct smear revealed also Gram positive bacilli in long chains. Hanging drop examination showed no motility.

C. Culture in peptone water revealed the same findings as in broth. No indol formation.

D. Intraperitoneal inoculation with the suspension of the pure culture into 3 white mice and 3 guinea pigs 0.2 ml. of the bacteria suspension was used for each mouse and 0.5 ml. for each guinea pig. They died one after another from 26 to 42 hours. On autopsy, smears made from the spleen revealed the same kind of large Gram-positive bacilli.

E. Cultures of the heart blood of these dead animals yielded pure growth of the same bacteria.

Preliminary Diagnosis: *Bacillus anthracis* isolated from the houseflies.

Examined by Chang Yü-chia
Ssu-ping Health Station

Date of report: March 23, 1952.

REPORT ON BACTERIOLOGICAL IDENTIFICATION

1. No. of specimen: 13033
2. Source of specimen: A strain of bacteria isolated from the house-flies at Ssu-ping, sent by Anti-epidemic Station of Ssu-ping.
3. Date Received: March 30, 1952.
4. Procedures: The following examinations were done after receiving the specimen:

I. Morphology:

Gram-positive large bacilli with square ends and central spore formation. Lined in the form of a chain. Hanging drop examination revealed no motility of the organism.

II. Cultural Characteristics:

1) Plain agar plate: The colonies appeared rough and opaque with uneven edges and curled-hair appearance.

2) Meat broth: Growth with flocculent precipitation. The supernatant fluid clear. No pellicle formation.

3) Blood agar plate: The appearance of colonies was the same as on the plain agar plate. No hemolysis.

4) Gelatin media: Stab culture showed inverted ür tree-like growth in 24 hours, while liquefaction took place gradually several days later.

5) Milk media: Coagulated

III. Biochemical Characteristics: A pure culture of this strain was used for the following biochemical examinations. The results were:

Indol reaction	(—)	Mannitol	(—)
Lactose	(—)	Xylose	(—)
Glucose	(+)	Inositol	(—)
Maltose	(+)	Arabinose	(—)
Sucrose	(+)	Salicin	(—)
Dulcitol	(—)	Hydrogen sulfide	(—)

IV. Hemolytic Reaction:

Three 2% saline suspensions were made respectively with the red blood cells of goat, guinea pig and rabbit. Each of these suspensions was then mixed with the bacterial suspension (1 loopful/ml.). They were incubated for 2 hours and examined for hemolysis. Control examinations were made with *Bacillus subtilis*. The results were as follows:

Bacterial suspension / 2% R.B.C. suspension	Present specimen	*B. subtilis*
Rabbit	no hemolysis	hemolysis
Guinea pig	no hemolysis	hemolysis
Goat	no hemolysis	hemolysis

V. Pathogenicity Tests: A saline suspension was made with the present specimen of bacteria (2 loopful/ml.) Into each of the two white mice, 0.2 c.c. of the suspension was injected intraperitoneally, and into each of the two guinea pigs, 0.5 c.c. were injected subcutaneously. Mice died in 30 hours and guinea pigs died in 3 days. Autopsy on these dead animals revealed enlargement of both liver and spleen. From smears of the heart blood, spleen and liver, Gram positive square ended large bacilli with capsule formation were observed. On culture, same kind of organism was obtained.

VI. Serological examinations: A piece of liver and spleen of the dead guinea-pig was each ground separately and diluted with saline. After boiling for 30 minutes, Ascoli precipitation test was carried out with the filtrate. The filtrates of the liver and spleen of a healthy guinea-pig were used as controls.

Antigen / Sera	Boiled antigens		Boiled organs of healthy animals		Saline
	Liver	Spleen	Liver	Spleen	
Anti-anthrax diagnostic serum for Ascoli test	+	+	—	—	—
Normal rabbit serum	—	—	—	—	—

Conclusion: *Bacillus anthracis* confirmed.

Examined by Hsin Chün M.D.
Chao Lin M.B.

Reported by Hsin Chün M.D.,
Chief Technical Expert of Northeast
Epidemic Prevention Institute.

Date of report: April 15, 1952.

DOCUMENT AA-4

REPORT ON ENTOMOLOGICAL IDENTIFICATION OF PTINUS FUR

Serial No. of specimen: Chien-362-1	Original No. of specimen: Fang-023	Date received: April 6th, 1952

Circumstances of discovery:

Discovered on March 20, 1952, at Liaoyang. Prior to the discovery, the inhabitants of the place had seen a reddish object dropped from the air. Large numbers of insects of this species were discovered immediately afterwards outside houses on the walls and in the fields.

Results of identification:

Scientific name: *Ptinus fur* Linn. **(Coleptera, Ptinidae)**

Common name: Ptinid beetle

Comment: Ptinid beetle is a pest of warehouses. Under natural conditions, they are found mostly in storehouses, granaries, flour mills, or other places for manufacturing or storing animal or plant products. In the present instance, large numbers were suddenly discovered outside houses on the walls, in the fields. This is evidently unusual

Remarks

Identified by:

Liu Chung-lo, B.A., Ph.D., Director of the Entomological Research Institute, Peking College of Agriculture.

Lu Pao-lin, M.S., Assistant Professor of Department of Entomology, Peking College of Agriculture.

Date: April 30, 1952

DOCUMENT AA-5

REPORT ON BACTERIOLOGICAL EXAMINATION OF PTINUS FUR

Serial No.: 362-1

Date received: April 14, 1952.

Kind of specimen: 6 ptinid beetles (*Ptinus fur*)

Source of specimen: Liaoyang

Method of examination: Six ptinid beetles were washed in 5 cc sterile physiological saline. The washing fluid was inoculated on plain agar plate, blood agar plate, S.S. agar plate and cooked meat broth. The beetles were then ground in a sterile mortar with 5 cc of a mixture of serum and meat broth and the suspension was plated on different media as before.

Results of examination: These plates were incubated for 24 hours. On the plain and blood agar plates, 2 kinds of colonies were observed. One was rough surfaced, elevated and grayish white in colour with an irregular edge. The diameter of each colony was 2-3 mm. Smears made from these colonies revealed Gram positive bacilli, with square ends, and lined up in chains.

The other kind of colonies was round in shape, also elevated and greyish white in colour, but the surface was smooth and the edge regular. Smears revealed Gram negative short bacilli. On the S.S. agar plate, there was growth of colorless and transparent colonies. In the cooked meat broth there was a turbid growth with production of gas. Smears revealed both Gram positive and negative bacilli.

I. Gram positive bacilli

From the blood agar plate the bacteria were transferred to serum broth. Observation made 6 hours later revealed no motility. The bacteria were then again inoculated into the following sugar tubes and special media. The results were as follows:

1. Bouillon tube—growth with flocculent sediments.
2. Milk—coagulated.
3. Methylene blue reduction test—negative.
4. Sugar fermentation reactions: Fermentation took place in the tubes containing glucose, maltose and sucrose with production of acid but no gas. There was no fermentation in the tubes containing lactose, mannitol, dulcitol, inositol, xylose, arabinose and salicin.

5. No production of indol or H_2S.

6. Hemolytic test—no hemolysis.

7. Animal inoculations:

A 24 hours' pure culture on agar slant was washed with 5 cc of sterile physiological saline and 0.5 cc of the suspension was injected subcutaneously into a guinea-pig. The animal died in 24 hours. Autopsy revealed the spleen enlarged to about 3 times of its normal size. At the site of injection there was gelatinous exudate. The smears made from the heart blood and local exudate both showed Gram positive encapsulated bacilli. Culture of the heart blood revealed the same kind of organisms.

Meanwhile the bacterial suspension was diluted to 1: 10 and 1:100, and 0.3 c.c. of each preparation was injected into 3 white mice. They all died within 20-30 hours. On autopsy, the spleen was found enlarged to 4 times of its normal size. On the surface of liver and spleen, there were hemorrhagic spots. Direct smears made from the peritoneal fluid, liver, spleen and the heart blood revealed Gram positive encapsulated bacilli with square ends and cultures revealed the same kind of bacilli as before.

A 24 hours' pure culture on an agar slant was again washed with 3 cc of physiological normal saline and 0.5 cc of the suspension was injected subcutaneously into a sheep. The animal died in 44 hours. Direct smears made from the heart blood, meninges, lungs and spleen all showed Gram-positive bacilli with capsule formation. From the bacterial cultures the same kind of organisms was obtained.

8. Ascoli's Test: Positive

II. Gram negative bacilli

(1) Blood agar plate: very small, grayish, elevated colonies of pin-head size. No hemolysis. Edge regular and surface smooth. China blue plate: Transparent, pinkish, well grown.

(2) Semisolid agar: showing motility.

(3) Indol (—), Citrate (+), Liquid base, turbid, Reduction of nitrate (+), Methyl red (—), No liquefaction of gelatin, V. P. test (—).

(4) Intraperitoneal injection (0.5 cc) into a white mouse—no death in 3 days.

(5) Fermentation Reactions:

Glucose, maltose-Production of acid but no gas. Lactose, sucrose, mannitol, xylose, arabinose, dulcitol, salicin and inositol–no fermentation.

Conclusion:

1. *Bacillus anthracis* isolated from *Ptinus fur.*
2. Gram-negative bacilli, not belonging to the "intestinal group."

Examined by
Wang Chin-tung

Reported by
Hsieh Shao-wen (Samuel Zia), M.D.
Professor of Bacteriology
Chang Nai-chu, M.D.
Assistant Professor of Bacteriology
China Union Medical College.

Date of Report: April 23, 1952.

DOCUMENT AA-6

REPORT ON BACTERIOLOGICAL EXAMINATION OF AUTOPSY MATERIAL FROM TIEN CHENG-HO

1. No. of specimen: 168
2. Source of specimen: Antung
3. Date received: April 19, 1952.
4. Kinds of specimen: Heart blood, spleen, lung, kidney and brain of Tien Cheng-ho.
5. Procedure: After receiving the specimens, smear examination, isolation culture and animal inoculation were immediately carried out. The following results were obtained.

(1) Microscopic examination of the stained smear: From the brain, spleen, lung and kidney, large Gram-positive bacilli with square ends were found.

(2) Isolation culture:

(a) The heart blood, spleen, lung, brain and kidney were streaked separately on plain agar slants. After 24 hours incubation at 37°C, large grayish white colonies with uneven edges and rough surfaces were seen. Microscopic examination of stained smears made from these colonies revealed Gram-positive and spore-bearing large bacilli. The morphology of the bacteria in the colonies from various cultures was identical.

(b) The heart blood, spleen, lung, kidney and brain tissue were inoculated into meat broths for enrichment. Microscopic examination of the stained smear on the next day revealed large Gram-positive bacilli. Isolation of culture was made on plain agar plates and morphologically identical bacilli were obtained from various enrichment media.

(c) The brain tissue was made into a 10% emulsion with normal saline and 0.2 ml. of this emulsion was injected subcutaneously into a white mouse. The mouse died 12 hours later.

At autopsy, hemorrhagic spots were seen on the heart, liver and spleen. Microscopic examination of the stained smears made from these organs revealed encapsulated large bacilli. The same kind of bacilli was obtained from the agar slant used for isolation culture.

(3) Ascoli precipitation test:

A piece each of liver and spleen of the dead white mouse was minced separately with 5 ml. of sterile normal saline. Each of the suspension was boiled for 30 minutes and the supernatant clear fluids were used for Ascoli precipitation test against anthrax diagnostic serum. The results were positive.

(4) Pathogenicity to guinea-pigs. The bacteria isolated from the brain tissue was made into a suspension (a slant of culture was mixed with 10 ml. of saline) and 0.5 ml. of this suspension was injected under the abdominal skin of a guinea pig weighing 750 gms. The guinea pig died 95 hours later.

Autopsy findings and bacteriological culture: Gelatinous and bloody exudate were seen at the site of injection. The heart, liver, spleen and lung were congested with hemorrhagic spots. Microscopic examination of the stained smears made from the above mentioned organs revealed Gram-positive encapsulated large bacilli with square ends.

Isolation cultures made with various organs on the agar plates grew large colonies with curled hair-like edge. Microscopic examination of the stained smears revealed Gram positive large bacilli.

6. Conclusion: From the heart blood, brain, spleen, lung and kidney, *Bacillus anthracis* was isolated.

Examined by Sun Ching-ch'ang, Laboratory of Antung Health Bureau.

Date reported: April 25, 1952.

REPORT ON
BACTERIOLOGICAL IDENTIFICATION

1. No. of Specimen: 38040
2. Source of Specimen: A strain of bacteria isolated from the heart blood of Tien Cheng-ho, sent by Laboratory of Municipal Health Bureau of Antung City.
3. Date Received: May 17, 1952.
4. Procedure: The following examinations were done after receiving the specimen:

I. Morphology:

Gram-positive large bacilli with square ends and central spore formation, lined in the form of a long chain. Hanging drop examination revealed no motility of the organism.

II. Cultural Properties:

1. Plain agar plate: The colonies appeared rough and opaque with edge uneven and curled hair-like.

2. Meat broth: Growth with flocculent precipitation. The supernatant fluid clear. No pellicle formation.

3. Blood agar plate: The appearance of colonies was same as those on the plain agar media. No hemolysis.

4. Gelatin: Stab culture after 24 hours incubation produced inverted fir tree-like growth which liquefied gradually several days later.

5. Milk: Coagulated.

III. Biochemical Properties: A pure culture of this strain was used for the following biochemical examinations:

Indol reaction	(—)	Mannitol	(—)
Lactose	(—)	Xylose	(—)
Glucose	(+)	Inositol	(—)
Maltose	(+)	Arabinose	(—)
Sucrose	(+)	Salicin	(—)
Dulcitol	(—)	Hydrogen sulfide	(—)

IV. Hemolytic Reaction:

Three 2% saline suspensions were made respectively with the red blood cells of goat, guinea pig and rabbit. Each of these suspensions was then mixed with a bacterial suspension (1 loopful/ml.), incubated for 2 hours and they were examined for hemolysis. Control examinations were made with *Bacillus subtilis*. The results were as follows:

Bacterial suspension / 2% R.B.C. suspension	Present specimen	Control test with *B. Subtilis*
Rabbit	no hemolysis	hemolysis
Guinea pig	no hemolysis	hemolysis
Goat	no hemolysis	hemolysis

V. Pathogenicity Tests: A saline suspension was made with the present specimen of bacteria (2 loopfuls/ ml.). Into each of the two white mice, 0.2 ml. of the suspension was injected intraperitoneally, and into each of the two guinea pigs, 0.5 ml. was given subcutaneously. Mice died in 18 hours and guinea pigs died in 48 hours. Autopsy on these dead animals revealed enlargement of both liver and spleen. From smears of the heart blood, spleen and liver, Gram positive square ended large bacilli with capsule formation were isolated. On culturing, similar organisms were obtained.

VI. Serological Examinations. A piece each of liver and spleen of the dead guinea-pig was ground separately and diluted with saline. After boiling for 30 minutes, Ascoli precipitation test was carried out with the supernatant clear fluids. The liver and spleen of a healthy animal were used for control.

Antigen / Serum	Boiled antigen		Boiled viscera of healthy animal		Normal saline
	Liver	Spleen	Liver	Spleen	
Anthrax diagnostic precipitation serum	+	+	—	—	—
Serum of healthy rabbit	—	—	—	—	—

Conclusion: *Bacillus anthracis* Confired.

Examined by Cheng Keng, Sc. D.,
Professor of Bacteriology, Department of Veterinary Medicine, College of Agriculture, National Nanking University.

Reported by Hsin Chün, M.D.,
Chief Technical Expert of Northeast Epidemic Prevention Institute.

Date Reported: June 2, 1952.

DOCUMENT AA-7

REPORT ON BACTERIOLOGICAL EXAMINATION OF AUTOPSY MATERIAL FROM CHU CHAN-YUN

1. Specimen No.: 7.
2. Source of specimen: Ssu-ping City.
3. Date received: March 23, 1952.
4. Kinds of specimen: Spleen, lung and liver of Chü Chan-yün, a small piece of each.
5. Procedure:

(1) Microscopic examination of the direct smears made from the liver, spleen and lung revealed large Gram-positive bacilli with square ends and capsule formation.

(2) Isolation culture:

The liver, spleen and lung were streaked separately on plain agar plates. After incubation at 37°C for 24 hours, there grew large colonies with curled hair-like edges. Microscopic examination of the smears made from these colonies revealed large Gram-positive bacilli with centrally placed spores. They showed no motility in hanging drop.

(3) Animal inoculation:

The spleen was made into a 1:10 suspension, 0.2 ml. of which was injected into the abdomen of a guinea pig. The guinea pig. died 24 hours later and on autopsy, its liver and spleen were enlarged, with congestion and hemorrhage. Large Gram positive square-ended bacilli were found in these organs under microscope. were enlarged, with congestion and hemorrhage. Large Gram positive square-ended bacilli were found in these organs under microscope.

(4) Ascoli precipitation test.

A piece of the spleen in the human specimen and a piece each of the liver and spleen of the dead guinea pig were cut with scissors into fragments in physiologic saline. The suspensions were boiled for 20 minutes and the supernatant fluids, after filtration, were tested for precipitation reaction with diagnostic anti-serum for anthrax. The results were all positive.

Conclusion: *Bacillus anthracis* was isolated from the liver, spleen and lung specimens.

Examined by: Chang Yü-chia, Ssu-ping Health Station
Date reported: March 26, 1952.

REPORT ON BACTERIOLOGICAL IDENTIFICATION

1. No. of Specimen: 13042

2. Source of Specimen: A strain of bacteria isolated from the lungs of Chü Chan-yün at Ssu-ping, sent by Health Station of Ssu-ping City.

3. Date Received: May 8, 1952.

4. Procedure: The following examinations were done after receiving the specimen:

I. Morphology:

Gram-positive large bacilli with square ends and central spore formation, lined in the form of a long chain. Hanging drop examination revealed no motility of the organism.

II. Cultural Properties:

1. Plain agar plate. The colonies appeared rough and opaque with edge uneven and curled hair-like.

2. Meat broth: Growth with flocculent precipitation. The supernatant fluid clear. No pellicle formation.

3. Blood agar plate. The appearance of colonies were same as those on the plain agar plate. No hemolysis.

4. Gelatin: Stab culture after 24 hours incubation produced inverted fir tree-like growth which liquefied gradually several days later.

5. Milk: Coagulated.

III. Biochemical Properties: A pure culture of this strain was used for the following biochemical examinations:

Indol reaction	(—)	Mannitol	(—)
Lactose	(—)	Xylose	(—)
Glucose	(+)	Inositol	(—)
Maltose	(+)	Arabinose	(—)
Sucrose	(+)	Salicin	(—)
Dulcitol	(—)	Hydrogen sulfide	(—)

IV. Hemolytic Reaction:

Three 2% saline suspensions were made respectively with the red blood cells from a goat, a guinea pig and a rabbit. Each of these suspensions was then mixed with a bacterial suspension (1 loopful/ml.). They were incubated for 2 hours and examined for hemolysis. Control examinations were made with *Bacillus subtilis*. The results were as follows:

2% R.B.C. suspeusion \ Bacterial suspension	Present specimen	Control test with *B. subtilis*
Rabbit	no hemolysis	hemolysis
Guinea pig	no hemolysis	hemolysis
Goat	no hemolysis	hemolysis

V. Pathogenicity Tests:

A saline suspension was made with the present specimen of bacteria (2 loopfuls/1 ml.). Into each of the two white mice, 0.2 ml. of the suspension was injected intraperitoneally, and into each of the two guinea pigs, 0.5 ml. were given subcutaneously. Mice died in 20 hours and guinea pigs died in 48 hours. Autopsy on these dead animals revealed enlargement of both liver and spleen. From smears of the heart blood, spleen and liver, Gram positive square ended large bacilli with capsule formation were observed. On culture, same kind of organisms was obtained.

VI. Serological Examinations: A piece each of liver and spleen of the dead guinea-pig was ground separately and diluted with saline. After boiling for 30 minutes, Ascoli precipitation test was carried out with the supernatant clear fluids. The liver and spleen of a healthy animal were used for control.

Antigen \ Serum	Boiled antigen		Boiled viscera of healthy animal		Normal saline
	Liver	Spleen	Liver	Spleen	
Anthrax diagnostic precipitation serum	+	+	—	—	—
Serum of healthy rabbit	—	—	—	—	—

Conclusion: *Bacillus anthracis* Confirmed.

Examined by Cheng Keng, Sc.D.,
Professor of Bacteriology, Department of Veterinary Medicine, College of Agriculture, National Nanking University

Reported by Hsin Chün, M.D.,
Chief Technical Expert of Northeast Epidemic Prevention Institute.

Date of Report: May 20, 1952.

DOCUMENT AA-8

REPORT ON BACTERIOLOGICAL EXAMINATION OF AUTOPSY MATERIAL FROM WEI LIU-SHIH

1. No. of specimen: 498
2. Source of specimen: An-shan.
3. Date received: April 16, 1952.
4. Kinds of specimen: Brain tissue of Wei Liu-shih.
5. Procedure: Right after receiving the specimen, isolation culture and animal inoculation were carried out. When suspicious colonies were found, they were taken for pure culture examination and animal inoculation. Result: From the brain tissue, *Bacillus anthracis* was isolated.
6. Examination of the Pure Culture:

   (1) Morphology: Gram positive spore-bearing bacilli with square ends, those from the animal body showed the presence of capsules and those from the culture media were lined up in long chains. No motility.

   (2) Cultural Properties:

   Blood agar plate: Big and rough colonies with uneven edges, non-transparent and non-hemolytic.

   Plain agar slant: Rapid growth. Rough surface. Semi-transparent.

   Bouillon: Flocculent growth at bottom, supernatant fluid clear.

   (3) Biochemical Properties: Milk—coagulated.
   Gelatin liquefied with inverted fir tree-like growth.

   (4) Pathogenicity in animals: White mouse (18 gms.)—Subcutaneous injection of 0.3 ml. Died in 16 hours.

   (5) Immunological Properties: Ascoli precipitation test—positive.
7. Conclusion: *Bacillus anthracis* was isolated from patient's brain tissue.

Examined by Liu Shih-ming,
Technical Expert, Dairen
Health Research Institute.

Reported by Chu Chi-ming, M.B., Ph.D.,
Chief Technical Expert, National Vaccine and Serum Institute, Peking.

Date of report: April 27, 1952.

REPORT ON SUPPLEMENTARY EXAMINATION OF BACILLUS ANTHRACIS 498

1) Biochemical properties: It fermented glucose, maltose, sucrose with production of acid but no gas.

It did not ferment salicin, lactose, mannitol, dulcitol, inositol, xylose and arabinose.

H_2S — negative

Indol — negative

Hemolytic test: No hemolysis of the blood cells of goat, rabbit and guinea pig.

Bacterial suspension / 2% R.B.C. suspension	Present Specimen	Control Specimen (*B. Subtilis*)
Goat	no hemolysis	hemolysis
Rabbit	no hemolysis	hemolysis
Guinea pigs	no hemolysis	hemolysis

2) Pathogenicity test on guinea-pig:

A loopful of the pure culture was mixed with 5 ml. of normal saline. 0.5 ml. of this suspension was injected into each of two guinea pigs subcutaneously. Both animals died in 4 days.

Autopsy findings: At the site of injection, there was gelatinous exudate. The liver and spleen were enlarged. The lungs were congested. There were Gram positive square-ended and encapsulated large bacilli in these organs and B. anthracis was isolated from the heart blood.

3) Liver and spleen of dead guinea pigs were used for Ascoli precipitation test—result positive.

Antigen / Serum	Boiled antigen		Boiled normal tissue control		Saline
	Liver	Spleen	Liver	Spleen	
Anti-anthrax diagnostic serum for Ascoli test	+	+	—	—	—
Normal rabbit serum	—	—	—	—	—

Conclusion: *Bacillus anthracis* confirmed.

Examined by Cheng Keng, Sc.D.,
Professor of Bacteriology, Department of Veterinary Medicine, College of Agriculture,
National Nanking University

Reported by Hsin-Chün, M.D.,
Chief Technical Expert of Northeast Epidemic Prevention Institute.

Date of Report: June 1, 1952

DOCUMENT AA-9

REPORT ON BACTERIOLOGICAL EXAMINATION OF AUTOPSY MATERIAL FROM WANG TZE-PIN

1. No. of specimen: 422.
2. Source of specimen: North District of Shenyang.
3. Date received: March 25, 1952.
4. Kind of specimen: Brain tissue, heart blood and spleen of Wang Tze-pin.
5. Procedure: Right after receiving the specimen, isolation culture was carried out. When suspicious colonies were found, they were taken for pure culture examination and animal inoculation.
   Result: From the heart blood, brain tissue and spleen *Bacillus anthracis* was isolated. No other organism.
6. Examination of the Pure Culture.

   (1) Morphology: Gram positive spore-bearing bacilli with square ends; those from the animal body have capsules and those from the culture media form long chains. No motility.

   (2) Cultural Properties:

   Blood agar plate: Colonies big, rough, non-transparent, not hemolytic and edge uneven.

   Plain agar slant: Growth rapid. Surface not smooth. Semi-transparent.

   Bouillon: Flocculent precipitation at bottom, supernatant fluid clear.

   (3) Biochemical Properties: Milk—coagulated. Gelatin—liquefied with inverted fir tree-like growth.

   (4) Pathogenicity to animals: White mouse (18 gms.)—Subcutaneous injection of 0.3 ml. Died in 36 hours.

   (5) Immunological properties: Ascoli precipitation test positive.
7. Conclusion: From the heart blood, brain and spleen *Bacillus anthracis* isolated.

Examined by Liu Shih-min,
Technical Expert of Dairen Health
Research Institute.

Reported by Chu Chi-ming, M.B., Ph.D.
Chief Technical Expert,
National Vaccine and Serum Institute, Peking.

Date of report: April 8, 1952.

REPORT ON SUPPLEMENTARY EXAMINATION OF *BACILLUS ANTHRACIS* 422

1 Biochemical Properties:

Fermentation tests: It fermented glucose, maltose and sucrose with production of acid but no gas.

It did not ferment salicin, lactose, mannitol, dulcitol, inositol and arabinose.

H_2S—negative

Indol — negative

Heyolytic test: No hemolysis on the blood cells of goat, rabbit and guinea pig:

Bacterial suspension / 2% RBC suspension	*B. anthracis*	*B. subtilis* (control)
Goat	no hemolysis	hemolysis
Rabbit	no hemolysis	hemolysis
Guinea pig	no hemolysis	hemolysis

2 Pathogenicity test on guinea-pig.

A loopful of the pure culture was mixed with 5 ml. of normal saline. 0.5 ml. of this suspension was injected into 2 guinea pigs subcutaneously. Both guinea pigs died in 3 days.

Autopsy findings: At the site of injection, there was gelatinous exudate. The liver and spleen enlarged. The lungs congested. There were Gram positive square-ended and encapsulated bacilli in these organs. *B. anthracis* was isolated from the heart blood.

3 Liver and spleen of dead guinea pigs for Ascoli precipitation test: positive.

Antigen / Serum	Boiled antigen		Boiled viscera of healthy animal		Normal saline
	Liver	Spleen	Liver	Spleen	
Anthrax diagnostic precipitation serum	+	+	—	—	—
Serum of healthy rabbit	—	—	—	—	—

Conclusion: *Bacillus anthracis* confirmed.

Examined by Cheng Keng, Ph.D.,
Professor of Bacteriology, Department of Veterinary Medicine, College of Agriculture,
National Nanking University.

Reported by Hsin Chün, M.D.,
Chief Technical Expert of Northeast Epidemic Prevention Institute.

Date of report: June 1, 1952.

DOCUMENT AA-10

AUTOPSY REPORTS ON FIVE FATAL CASES OF ANTHRAX INFECTION

CASE NO. 1

Name: Chü Chan-yun male age: 55

Residence: Man-ching Station, Ssu-ping City, Liaohsi Province

Occupation: Railway worker (8 yrs.)

Summary of Clinical History:

Present illness: In the morning of March 19th, 1952, he began to have fever, headache, and aching of the extremities. On March 21, he had nausea, vomiting, general malaise, heaviness in the head, pain in the joints and insomnia. In addition, he had cough and the sputum was found to contain encapsulated large Gram positive bacilli. White blood cell count 28000. After admission, he vomited repeatedly. Later he was discovered to have rigidity of neck and mental cloudiness. He died at 2:45 p.m. March 22, 1952.

Date of autopsy: March 23, 1952, 24 hours after death.

Summary of Autopsy Findings:

External examination: Body moderately nourished. Finger nails cyanotic. Superficial lymph glands not enlarged. Skin, no important change.

Peritoneal cavity: No abnormal findings.

Pleural cavity: There is accumulation of about 1000 ml. of fluid in both pleural cavities. Under the left pleura there is ecchymosis.

Pericardial cavity: There is an accumulation of about 100 ml. of fluid.

Heart: No abnormal findings. The heart is in the systolic stage.

Lungs: Show edema and congestion, and there are prominent hemorrhagic spots in the left lower lobe and apex. In the left apex, an old bean-sized tuberculous lesion is found. Hemorrhage in the right lower lung is not marked. The upper lobes of both sides show areas of consolidation. Hilar lymph nodes enlarged.

Liver: Normal in size and color. There is a rice-sized hemorrhagic spot on the upper surface and a millet-sized hemorrhagic spot over the lower surface.

Spleen: Enlarged. Size 20 x 12 x 5 cm. Bluish gray in color.

Stomach and duodenum: Gastric mucosa shows areas of ecchymosis and that of the pyloric region shows hemorrhagic spots. On the duodenal mucosa there are erosions.

Small Intestine: There are 8 ulcerative hemorrhagic areas distributed over the mucosa of the jejunum and ileum. The size of the bigger one is about 1 cm. in diameter and the smaller one is about 0.5 cm. in diameter.

Appendix: There is hemorrhagic inflammatory condition at its tip.

Mesenteric lymph glands: Not enlarged.

Pancreas: There is also an area of ecchymosis, about 2 cm. in diameter.

Bacteriological examinations: *Bacillus anthracis* isolated from both liver and spleen.

Autopsy performed by:

Sung Teh-yü, Director of the Ssu-ping Health Station.
Liu Chan-yuan, Physician of the Ssuping Health Station.

Comment:

From the clinical records, we may conclude that the patient had a severe generalized acute infection. Cough, leucocytosis and the finding of large Gram positive bacilli in the sputum clearly point to anthrax infection of the respiratory tract. Bilateral pleural effusion, hemorrhagic spots on the visceral pleura of the lungs, pericardial effusion, consolidation of the upper lobes of both lungs, enlargement of hilar lymph glands and splenic enlargement establish the diagnosis of pulmonary anthrax.

The isolation of *Bacillus anthracis* from the liver and spleen speaks for septicemia. Clinical symptoms such as loss of consciousness and rigidity of the neck indicate meningitis. The ulcers and hemorrhagic spot in the mucosa of small intestines are probably produced by bacilli swallowed with the sputum, but as the mesenteric glands are not enlarged, it is apparent that the intestinal lesion is not a primary anthrax infection.

Reported by: Wu Tsai-tung, M. B.
Professor of Pathology, Nanking University Medical College.
Li Pei-lin, M. B., Ch. B., Ph. D.
Professor of Pathology, National Medical College, Shenyang.

CASE NO. 2

Name: Wang Tze-pin male age: 47
Residence: Shenyang.
Occupation: Tricycle-rickshaw driver.

Summary of Clinical History:

Present illness: At 10:30 p.m. on March 20, 1952 the patient began to feel general discomfort. On March 21, he rested in bed. His ap-

petite remained good and he was still able to move about. On March 22, he experienced soreness of legs, general malaise, discomfort in the upper abdomen, nausea and headache. In the morning of March 23, he felt better. But at 5 p.m. he experienced constricting sensation all over the whole body and distension over the upper abdomen. He was mentally clear until 6 a.m. March 24, when he gradually developed mental cloudiness with aphasia and restlessness. He was then sent to the First Municipal Hospital at 9 a.m., where he was examined with the following findings:

Temperature 36°C, Pulse imperceptible. Comatose. Pupils dilated with no reaction to light. Bulbar conjunctivae congested. Lips cyanotic. Mouth tightly closed. Heart sounds indistinct. Neck rigid. Kernig's sign positive.

The patient died at 9:35 a.m. on March 25, 1952.

Autopsy was performed 5 hours after death.

Summary of Autopsy Findings:

The body is that of a stout man. Nutritional status good. External examination of the body, including the skin, reveals no abnormal findings. The right pleural cavity contains about 1300 ml. of yellow clear fluid and the left contains about 500 ml. In the pericardial cavity, there is approximately 100 ml. of light yellow clear fluid.

Heart: Weight 340 gm. No important pathological changes.

Lungs: Right lung weighs 490 gms. The visceral pleura is smooth, except the portion near the mediastinum which shows definite gelatinous oedema. On palpation of the lung tissue, no consolidation is found. But on the cut surfaces, thickening of the peribronchial tissues is seen. The interlobular tissue of the lower lobe also shows the same thickenings with edema. The parenchymatous tissue of the lung shows no remarkable change, except for edema of varying degrees. The bronchus and the bronchioles show mild degree of congestion but no erosion or ulcer. The tissues around the bronchus and the lower end of the trachea and the adjacent lymph glands form a hemorrhagic mass measuring about 5 X 3 X 2.5 cm. The surrounding hilar tissue of the lungs is edematous.

Left lung weighs 340 gms. No important pathological change.

Under the microscope, the tissues of the hilum of the right lung and the lymph glands show marked infiltration with polymorphonuclear leucocytes and diffuse hemorrhage. In a small bronchus, the following changes are noted: Desquamation of epithelial cells, edema of bronchial wall and infiltration with polymorphonuclear leucocytes. The lung tissue, besides marked edema, shows no important changes. In the sections stained with Gram method there are large Gram positive bacilli in the wall of the small bronchus as mentioned above and in the neighbouring lymph

glands. In the interlobular tissue of the right lower lung there are marked edema and mild infiltration with polymorphonuclear leucocytes and mononuclear cells.

Intestines: In the upper portion of the jejunum there is a bean-sized hemorrhagic area with an ulceration seen in its center and red discoloration around. On the mucous membrane of the colon, there are also more than ten isolated pea-sized hemorrhagic spots more numerous in the ascending colon. Under microscope, the mucous membrane of the intestine shows fibrin and polymorphonuclear leucocytic infiltration with a number of Gram positive square-ended bacilli. The lymph tissue of the wall of intestine is normal with no polymorphonuclear infiltration. Mesenteric lymph glands normal.

Brain: The dura mater shows no change. The blood vessels on the surface of the brain congested. There is marked diffuse hemorrhage in the subarachnoid space. The vessels at the base of the brain show no sclerotic change. The vessels in the meninges of the spinal cord are congested. Under the microscope, there are diffuse hemorrhages and polymorphonuclear and mononuclear infiltrations in the subarachnoid space. The small vessels inside the brain are congested and in the perivascular tissue there are polymorphonuclear and mononuclear infiltrations and many foci of hemorrhage. The lumen of a blood vessel extending from the meninges into the brain parenchyma is found to be full of bacilli. In the wall of that vessel there is polymorphonuclear leucocytic infiltration with perivascular hemorrhage. In the Gram stained sections many Gram positive square ended bacilli are found in the subarachnoid space.

The *liver, spleen and other organs* show no important changes.

Bacteriological culture: *Bacillus anthracis* was isolated from the heart blood, brain and spleen.

Diagnosis: Hemorrhagic anthrax meningitis, anthrax bronchitis, peribronchitis and interlobular cellulitis of the right lower lobe. Purulent hemorrhagic anthrax lymphadenitis of the hilar lymph glands. Pulmonary edema with pleural effusion. Pericardial effusion. Multiple necrosis and ulcerations of the mucous membrane of the intestine due to anthrax bacillus infection.

Autopsy by Drs. Chu Feng-chun and Wang Hung-lieh, Department of Pathology, National Medical College, Shenyang.

Reexamined and confirmed by:

Wu Tsai-tung M. B.., Professor of Pathology, Nanking University Medical College.

Li Pei-lin, M. B., Ch. B., Ph. D.
Professor of Pathology, National Medical College, Shenyang.

CASE NO. 3A

Name: Wei Liu shih female age: 32
Residence: Anshan

Summary of Clinical History:

Present illness: The patient had repeatedly joined the work of catching and killing insects disseminated by American airplanes before contracting the present illness. In the evening of April 11, 1952, the patient felt slight general discomfort. On April 12, she experienced headache, chilliness and fever, but still carried on her household duties. On April 13, the symptoms increased in severity so that she became bedridden and had cough, chest pain, dyspnea and vomiting. Then, only with great difficulty was she able to go to the Second Combined Clinic in the Tieh-tung District for treatment. After she returned home, the symptoms became progressively worse. On April 14, she was sent to Anshan Municipal Tieh-tung Health Station where she was found in a state of mental confusion, delirium and restlessness, with labored respiration, frequent convulsions, imperceptible pulse, a pale face, cyanotic lips, tightly closed mouth and dilated pupils. Light reflexes of the pupils were sluggish. The neck was very rigid; Kernig's sign positive. Moist rales in the lungs were detected on both sides of the chest. She died at 11:50 p.m. on April 14.

Autopsy performed 21 hours after death.

Summary of Autopsy Findings:

The body is that of a middle-aged woman, normally developed and moderately well-nourished. External examination shows no abnormal findings. Each pleural cavity contains about 1000 ml. of slightly turbid fluid orange in color. The pericardial cavity contains 150 ml. of orange-colored fluid.

Heart: Weight 230 gm. No pathological change.

Lungs: On the back of the lower lobe of the left lung, there is a firm area which measures about 4 X 3 cm. in size. The cut surface appears to be dark red in color, within which there is a fan-shaped area measuring 2 X 1.5 cm. and appearing grayish yellow in color. The bronchial mucosa is slightly congested. Microscopic examination reveals necrotizing pneumonitis and the presence of Gram-positive bacilli. The hilar lymph nodes are markedly enlarged to the size of a pigeon's egg. On section, hemorrhagic lesions can be seen on the cut surface of these lymph nodes. Microscopic examinations reveal hemorrhagic lymphadenitis and the presence of Gram positive square-ended bacilli.

Liver: Congested.

Spleen: Enlarged, weighing 400 gm. It is very soft in consistency. Post-mortem changes are very prominent.

Gastro-intestinal tract: Shows no abnormal findings.

Uterus: Gravid, containing a fetus measuring 15 cm. in length.

Brain and spinal cord: On the surface of the cerebrum in the subarachnoid space there are diffuse hemorrhages. In some areas the sulci and gyri can not be distinguished, being completely covered by blood. There are also diffuse hemorrhages on the surface of cerebellum and the base of the brain. Purulent exudate is not found. Diffuse hemorrhages are also present in the subarachnoid space of the spinal cord. The meningeal vessels are extremely congested. The blood vessels of the base of the brain show no arteriosclerosis or other changes.

Microscopic examinations reveal extremely prominent hemorrhages in the subarachnoid space, being filled with large numbers of red blood cells. There is also infiltration of polymorphonuclear leucocytes and a small number of large mononuclear cells. In Gram-stained slides it is found in the above-mentioned hemorrhagic areas numerous large Gram-positive bacilli, some of which are arranged in the form of bamboo, morphologically identical with *Bacillus anthracis*.

Other organs: No significant changes.

Bacterial culture: *Bacillus anthracis* was isolated from the brain tissue.

Diagnosis: Hemorrhagic anthrax meningitis, necrotizing anthrax pneumonitis of the lower lobe of the left lung, hemorrhagic anthrax lymphadenitis of the hilum of the lungs, edema of the lungs, bilateral pleural effusion, pericardial effusion and acute splenic tumor.

The autopsy was performed by Drs. Chiang Ying-kai and Kuo Cheng-teh, Department of Pathology of the National Medical College, Shenyang.

On reexamination of the pathological material we confirm the above diagnosis.

Reported by:

Wu Tsai-tung, M. B.,
Professor of Pathology, Nanking University Medical College.

Li Pei-lin, M. B., Ch. B., Ph. D.,
Professor of Pathology, National Medical College, Shenyang.

CASE NO. 3B

Name: Wang Shu-chih. Female Age: 23
Residence: Liu-erh-pu, Liaoyang County.
Occupation: Primary school teacher.

Summary of Clinical History:

The patient was healthy in the past, without any chronic illness. After the American planes had dropped insects, she participated in the

work of catching and killing insects. She worked till 10 p.m. every night. Two days before her death (April 6, 1952) she felt dryness in the throat with hoarseness of voice. At times she had headache and pain in her joints. She was still able to carry on her usual work until 9 a.m. on April 8, when she lost her consciousness while she was in the toilet. This was discovered by her colleagues and she was moved to her own room. Her respiration was dyspneic, with white frothy discharge mixed with blood escaping from her mouth. Light reflex lost. Fingers cyanotic. She died at 10:35 a.m. on the same day.

Summary of Autopsy Findings:

Time of autopsy: 24 hours after death.

The body is well developed and well nourished. There is no abnormal findings on the surface of the body.

Chest cavity: There is no excessive fluid in both pleural cavities. The anterior surface of the right lung shows fibrous adhesions. The heart is 290 gms. in weight. The pericardium is smooth and shining. A few hemorrhagic spots are seen on the surface of the left ventricle. The endocardium is stained red by hemoglobin. The valves are normal. Both ventricles slightly dilated. Myocardium is soft.

The anterior surface of the right lung shows fibrous adhesion, while the rest of pleura shows no changes. The posterior portions of both left and right lungs show marked congestion but no definite consolidation.

Microscopically, besides the edema in most of the alveoli of the lungs, there is also polymorphonuclear leucocytic and monocytic infiltration in some of the alveoli of the right upper lobe. The mucous membrane of the bronchioles is detached, and in the lumens of the bronchioles there is cellular exudate. In the Gram stained slides, scattered large Gram positive bacilli are seen. The two ends of the bacteria appear square. Inside some alveoli and vesesls, such large bacilli are also seen, but in areas without inflammatory changes no such bacilli are seen.

Liver: Capsule smooth. Cut surface shows prominent cloudy swelling. A few Gram positive bacilli are also seen under the microscope in the hepatic sinuses.

Spleen: No important changes.

Gastro-intestinal tract and mesenteric lymph glands: No change.

Other organs: Also no remarkable change.

Brain and spinal cord: There is diffuse hemorrhage in the subarachnoid space of both brain and spinal cord. On the coronal section of the cerebrum, hemorrhages are also seen beneath the ependyma of the left and the 4th ventricles. The artery of the base of the brain shows no sclerotic changes.

Microscopically, diffuse hemorrhage can be seen in the subarachnoid space. But the infiltration of the meninges by polymorphonuclear leucocytes is very slight. In Gram stained slides, the small vessels of the brain and meninges contain Gram-positive bacilli.

No bacteriological culture done.

Diagnosis: Acute hemorrhagic anthrax meningitis. Bronchopneumonia of the right upper lung. Pulmonary edema.

Autopsy performed by Drs. Chao Wen-tou and Wang Hung-lieh, Department of Pathology, National Medical College, Shenyang.

The autopsy findings point to acute hemorrhagic anthrax meningitis, bronchopneumonia of right upper lobe and pulmonary oedema. In the pathological sections large Gram positive bacilli with square ends, morphologically identical with *B. anthracis* were found. So the diagnosis of anthrax infection is beyond any doubt.

Reported by: Wu Tsai-tung, M. B.,
Professor of Pathology, Nanking University Medical College.

Li Pei-lin, M. B., Ch. B., Ph. D.,
Professor of Pathology, National Medical College, Shenyang.

CASE NO. 4

Name: Tien Cheng-ho. Male. Age: 44.

Residence: First Section of the Eastern Shuang-shan Village, 5th District of Antung County.

Occupation: Farmer.

Clinical History: In the morning of April 16, 1952, after sending out 3 cartloads of manure into the field, he suddenly experienced chilliness. He immediately returned home and rested. The symptoms at that time were generalized joint pain, fever and mild headache. Food intake was as usual. On April 17, symptoms became worse, he was still able to take food, but vomited twice. On April 18, after dawn, he became unconscious. Both hands were tightly clenched as in spasm. A local doctor named Lin Hou was sent for. Body Temp. was 38.7°C. No definite diagnosis was made. He died on the same day (April 18) at 12, noon.

Gross Findings on Autopsy:

Time of autopsy: April 19, 5:00 p.m. (29 hours after death) at the home of the deceased.

The body is that of a male. Livor mortis over the back prominent. No generalized edema. No enlargement of lymph glands. Pupils 0.5

cm. in diameter. Conjunctivae pale. During the removal of clothes some fresh red fluid comes out from the nostrils. No fluid in the *peritoneal cavity*. Peritoneum normal. *Stomach* markedly dilated. Gastric mucous membrane smooth. Under the serosa of the greater curvature of the stomach, there scatter a number of hemorrhagic areas about the size of finger tips. The surface of *intestine* shows no particular change. *Mesenteric lymph glands* not enlarged.

Liver weighs 1200 gms. Surface smooth and grayish purple, showing postmortem autolysis. Cut surface dark purple with also autolytic changes. *Gall bladder* shows no inflammatory changes. *Spleen* weighs 200 gms. a little bigger than a palm, measuring 7 finger-breadths in length and 5 finger-breadths in width, dark purplish red in color. Surface smooth, cut surface was purplish black. The tissue also shows evidence of autolysis. Each kidney weighs 150 gms. The right *kidney* is 6 finger-breadths in length and 3 finger-breadths in width. Surface smooth and dark red. Cut surface dark purple and the tissue also shows post-mortem autolysis. The left kidney shows similar findings.

In the *pleural cavity*, there is no inflammatory exudate. The parietal pleura shows no adhesions. There is 15 ml. of dark red fluid in the *pericardial cavity*. Epicardium rich in fat. The *heart* is in a relaxed state with a blunt apex. The myocardium also shows postmortem autolysis. No other changes seen. Valves normal. Weight 250 gms. *Lungs* dark bluish gray, and cut surface purplish black showing hemorrhagic changes. The right lung weighs 850 gms. the left lung 700 gms. The hilar lymph glands are about the size of a thumb.

Cranial cavity: Dura mater normal. Under the leptomeninges, there is diffuse hemorrhage. Subarachnoid space filled with blood. Cut surface *of brain* showed no hemorrhagic spots. No inflammatory exudate in the cranial cavity.

In summarizing the above findings, it may be said that the body shows very marked postmortem changes, hemorrhages in subarachnoid space, in serosa of stomach and in the lungs and there is enlargement of the hilum lymph glands of the lungs.

Results of Bacteriological Examination:

(1) Direct smears of the brain tissue, lungs, spleen and kidneys stained with Gram method reveal numerous Gram positive bamboo-like large bacilli.

(2) From bacteriological culture and animal inoculation of the heart blood, brain tissue, lung, liver, spleen and kidney *Bacillus anthracis* was isolated.

Based on the above findings, the diagnosis is that the patient died of anthrax bacillus septicemia.

Examined by

Sung Wei-yi, Superintendent of Liaotung Municipal Hospital.

Kao Chuan-li, Physician of Antung Municipal Hospital.

Wang Chun-lin, Laboratory Technician of Antung Municipal Hospital.

The organism isolated from this patient was reexamined and confirmed as *Bacillus anthracis* by bacteriologists Hsin Chün and Cheng Keng.

The clinical manifestations fit in with the diagnosis of anthrax septicaemia and meningitis. Post-mortem examination revealed lesions of hemorrhagic meningitis. The gnding of *B. anthracis* from direct smear and culture of internal organs would confirm the diagnosis of septicaemia. There was increase of weight of both lungs. Cut surface showed congestion, hemorrhages and oedema. Hilum glands were enlarged. The primary focus was evidently in the lungs and the infection was introduced through the respiratory route.

Reported by

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TABULAR SUMMARY OF 5 CASES OF ANTHRAX INFECTION

	Case number	1	2	3a	3b	4
	Name	Chü Chan-yun	Wang Tze-pin	Wei Liu-shih	Wang Shu-chih	Tien Cheng-ho
	Sex	Male	Male	Female	Female	Male
	Age	55	47	32	23	44
	Occupation	Railway worker	Tricycle-rickshaw driver	House wife	Primary school teacher	Farmer
	Date of onset of disease	March 19 1952 A.M.	March 20 1952 Night	April 11 1952 Night	April 6 1952	April 16 1952
	Date of death	March 22 1952 at 2:45 p.m.	March 25 1952 at 9:35 a.m.	April 14 1952 at 11:50 p.m.	April 8 1952 at 10:35 a.m.	April 18 1952 at 12 noon
	Time of autopsy	24 hrs. after death	5 hrs. after death	21 hrs after death	24 hrs. after death	29 hrs. after death
Clinical manifestations	Fever	+		+		+
	Headache	+	+	+	+	+
	General aching	+	—	—	+	+
	Cough	+	—	+	—	—
	Nausea and vomiting	+	+	+	—	+
	Meningeal irritation	+	+	+	—	+
	Coma	+	+	+	+	+
	Abdominal pain	—	±	—	—	—
Pathological findings	Pneumonia or bronchitis	+	+	+	+	+
	Pulmonary congestion and oedema	+	+	+	+	+
	Enlargement hilum glands	+	+	+	—	+
	Pleural effusion	+	+	+	—	—
	Pericardial effusion	+	+	+	—	—
	Hemorrhagic meningitis		+	+	+	+
	Intestinal ulcer	+	+	—	—	—
	Enlarged mesenteric glands	—	—	—	—	—
	Skin anthrax	—	—	—	—	—

DOCUMENT AA-11

Epidemiological Investigation of Five Fatal Cases of Anthrax Infection

Case No.	1	2	3a	3b	4
Name	Chü Chan-yun	Wang Tze-pin	Wei Liu-shih	Wang Shu-chih	Tien Cheng-ho
Age	55	47	32	23	44
Sex	Male	Male	Female	Female	Male
Residence	Man-ching Station near-by Ssuping	City district of Shenyang	City district of Anshan	Liu-erh-pu Town of Liaoyang Hsien	East Shuang-shan Village, 5th District, Antung Hsien
Occupation	Railway worker	Tricycle-rickshaw driver	House wife	Primary school teacher	Farmer
How infected	During extermination of flies dropped by American airplane	Disease developed after the dissemination by American airplanes of insects and objects in the Shenyang District	During extermination of ptinid beetles dropped by American airplanes	During extermination of ptinid beetles dropped by American airplanes	During disposal of the feather dropped by American planes
Pathological diagnosis	Anthrax of resp. system, (brain and meninges not examined).	Anthrax of resp. system, anthrax meningitis	Anthrax of resp. system, anthrax meningitis	Anthrax of resp. system, anthrax meningitis	Anthrax of respiratory system and anthrax meningitis
History of contact with furs or leather	No	No	No	No	No
History of using new tooth-brush or shaving-brush	Not used	Not used	Not used	Not used	Not used
History of contact with diseased farming animals	Only a donkey in the district of Manching Station, not sick	No farming animal in the neighbourhood			Although he was a farmer, no history of contact
Any domestic animals suffering from anthrax	No	No	No	No	No
Any neighbours suffering from anthrax	Before and after their death, no neighbour suffered from any type of anthrax				
Any manure near their residence	No	No	No	No	No
Disposal of the corpse	By incineration after autopsy				

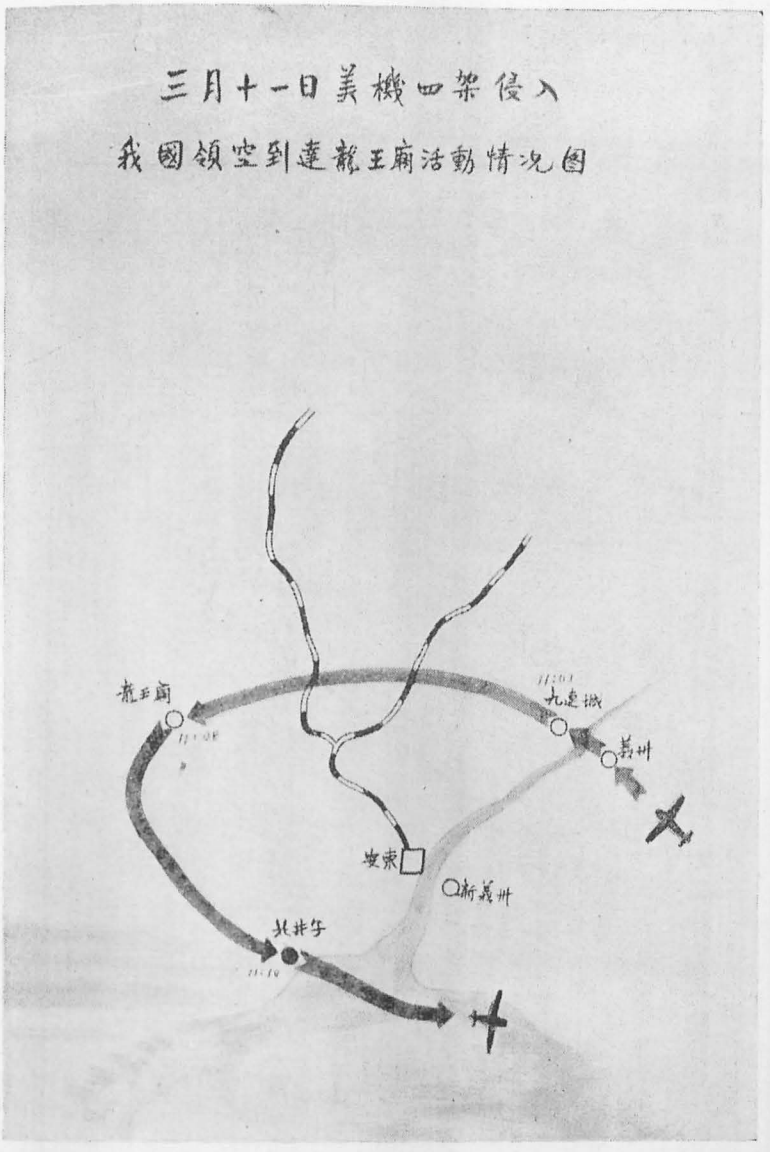


Fig. 1. Chart showing the course of the four American planes intruding over Lung-Wang-Miao and Pei Ching Village on March 11, 1952.

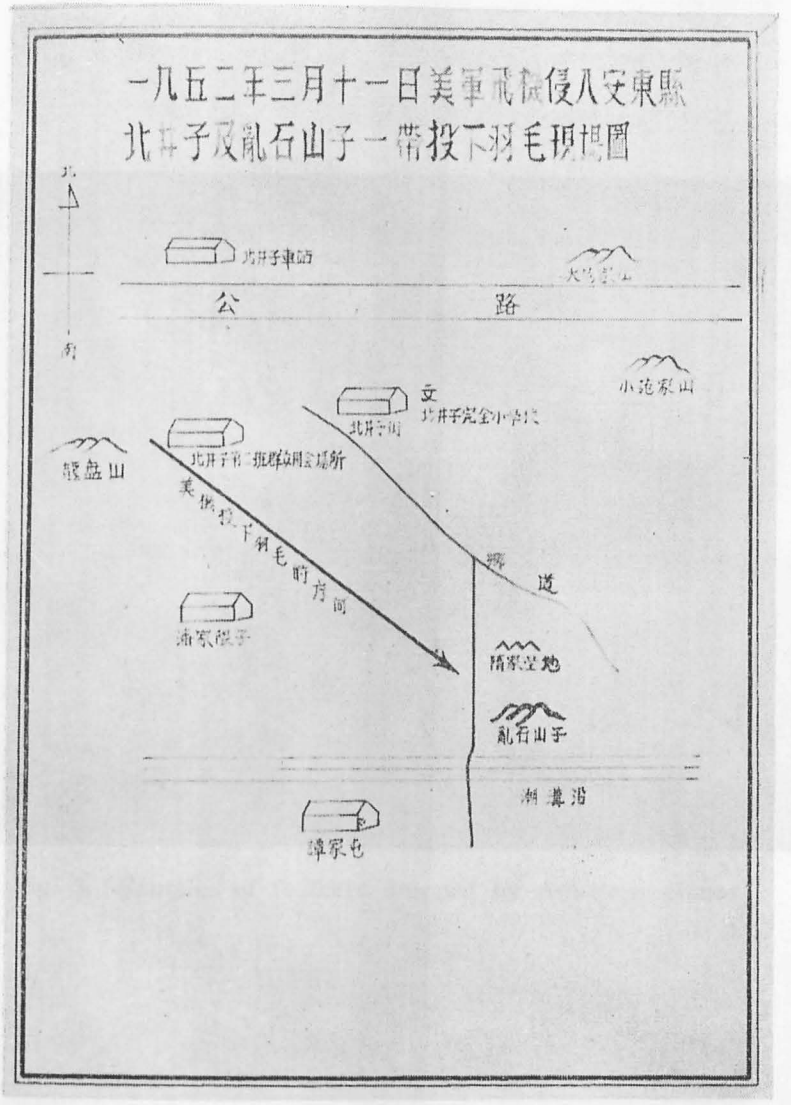


Fig. 2. Chart showing the localities of Pei-Ching-Tzu and Luan-Shih-Shan-Tzu in Antung where American planes dropped feathers on March 11, 1952.

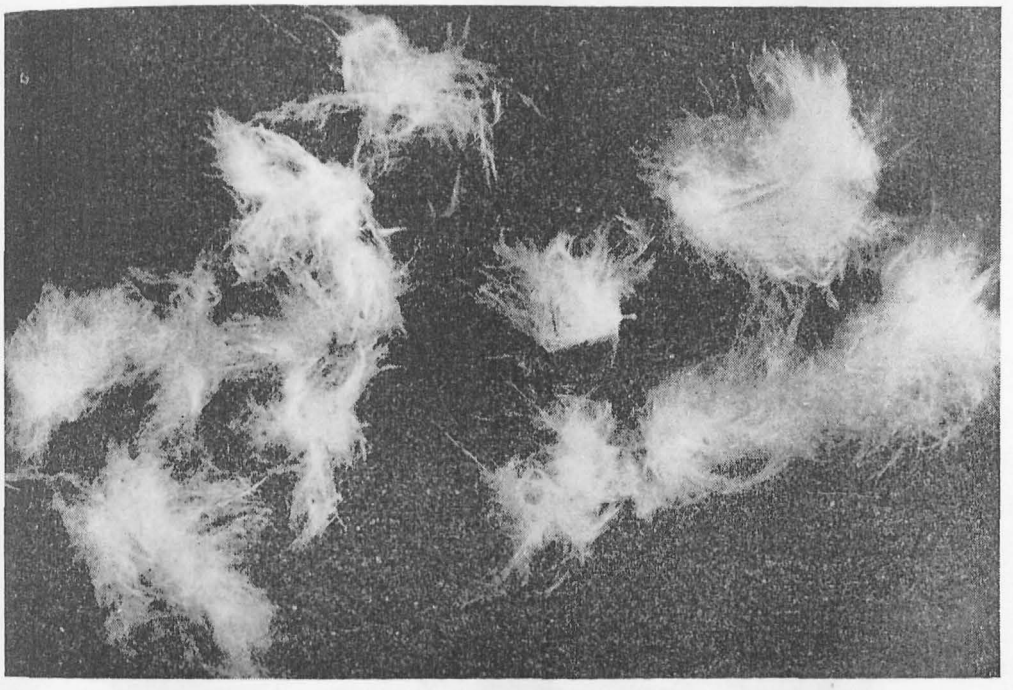


Fig. 3. Samples of feathers dropped by American planes.

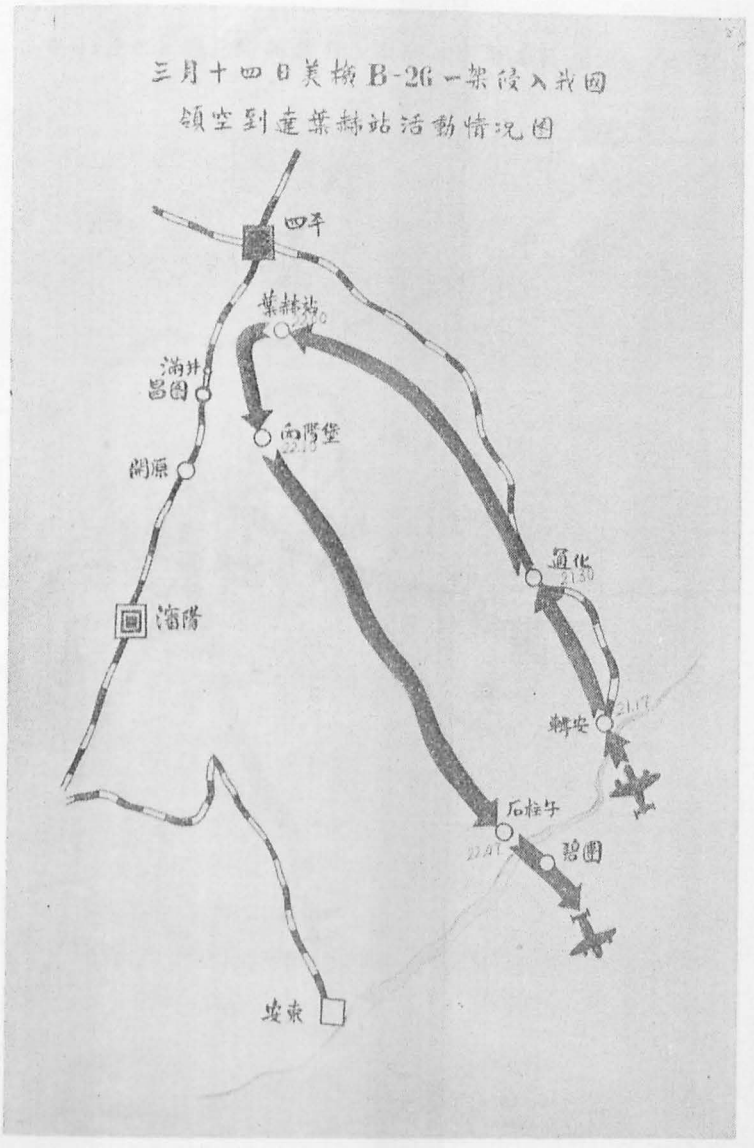


Fig. 4. Chart showing the course of an American plane B-26 intruding over Ssu-Ping and Man-Ching on March 14, 1952.

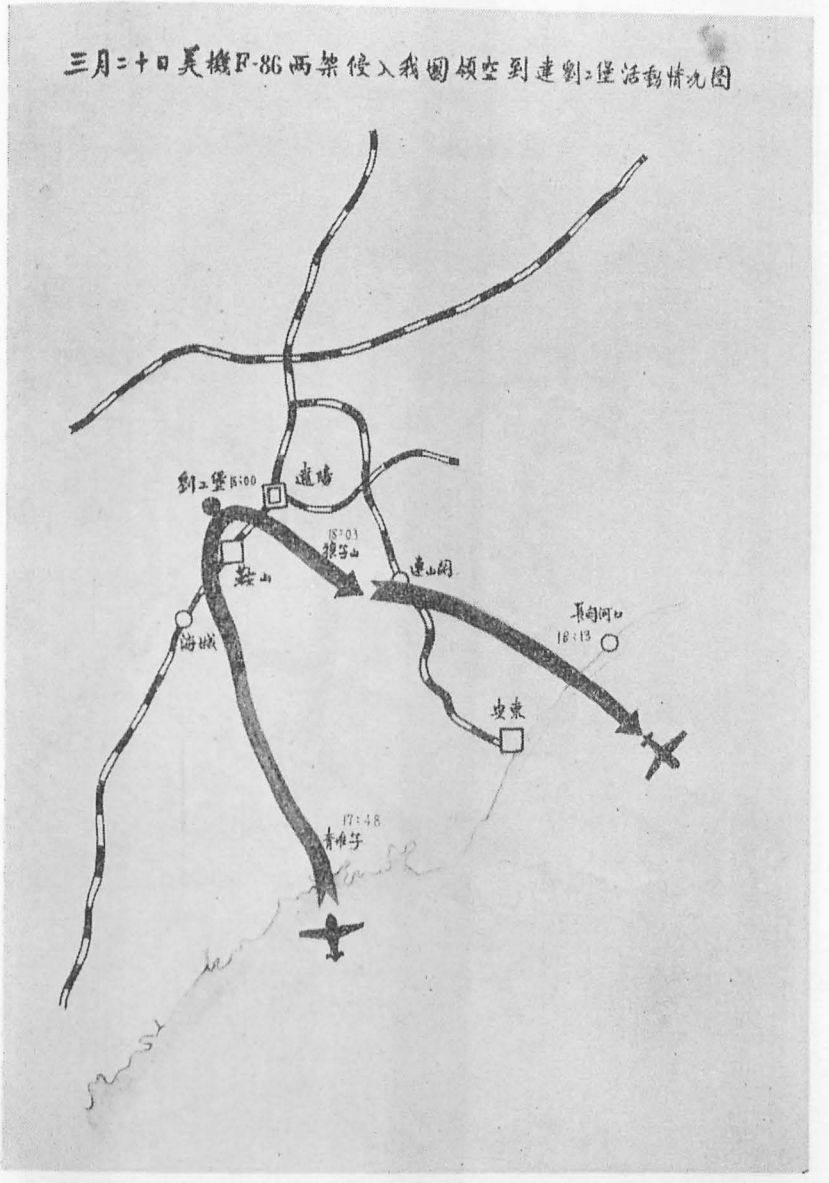


Fig. 5. Chart showing the course of two American F-86 planes intruding over Liu-Erh-Pu on March 20, 1952.

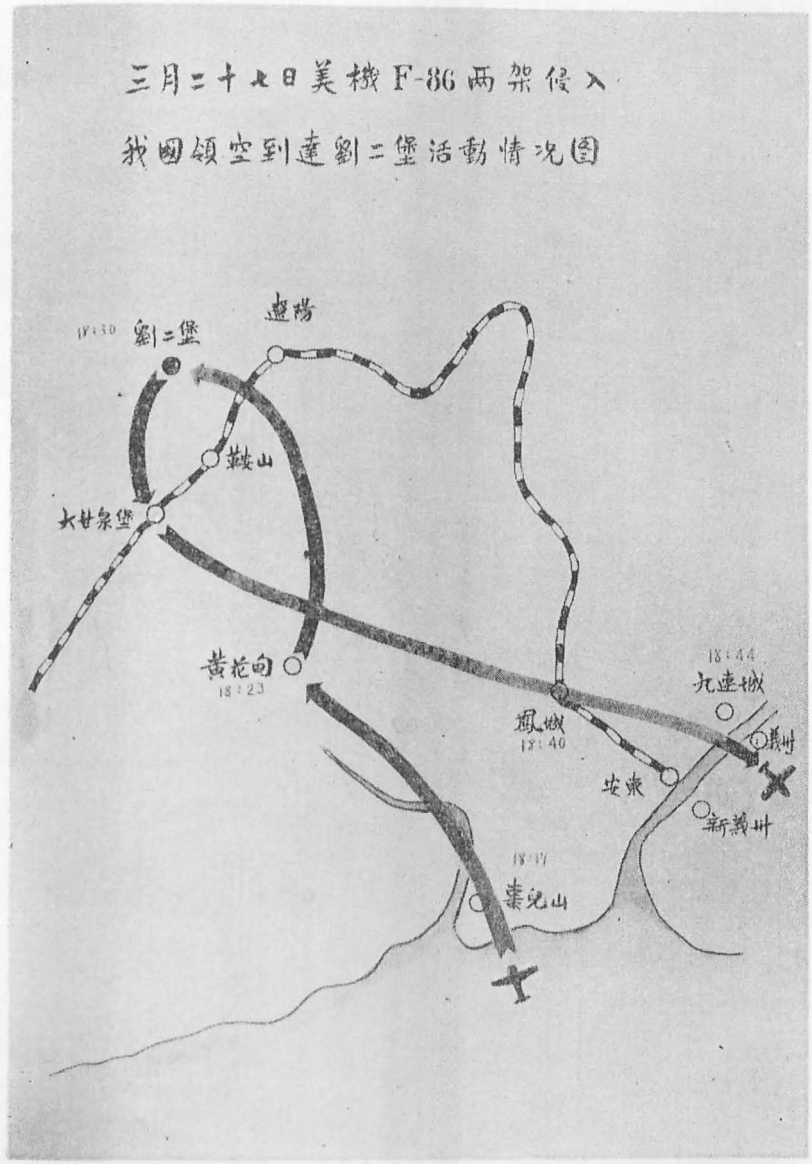


Fig. 6. Chart showing the course of two American F-86 planes intruding over Liu-Erh-Pu on March 27, 1952.

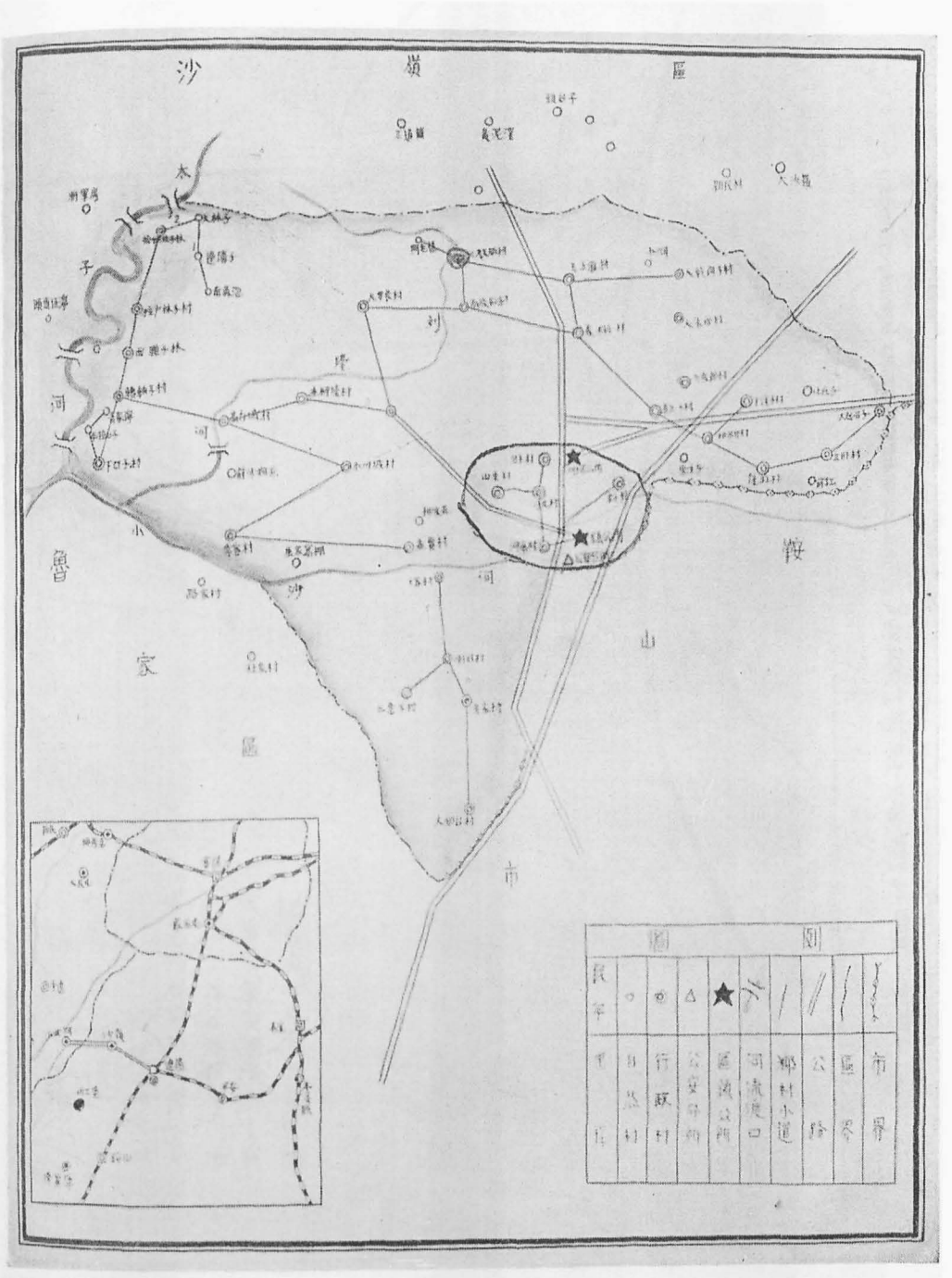


Fig. 7. Map of Liu-Erh-Pu, Liaoyang Hsien.

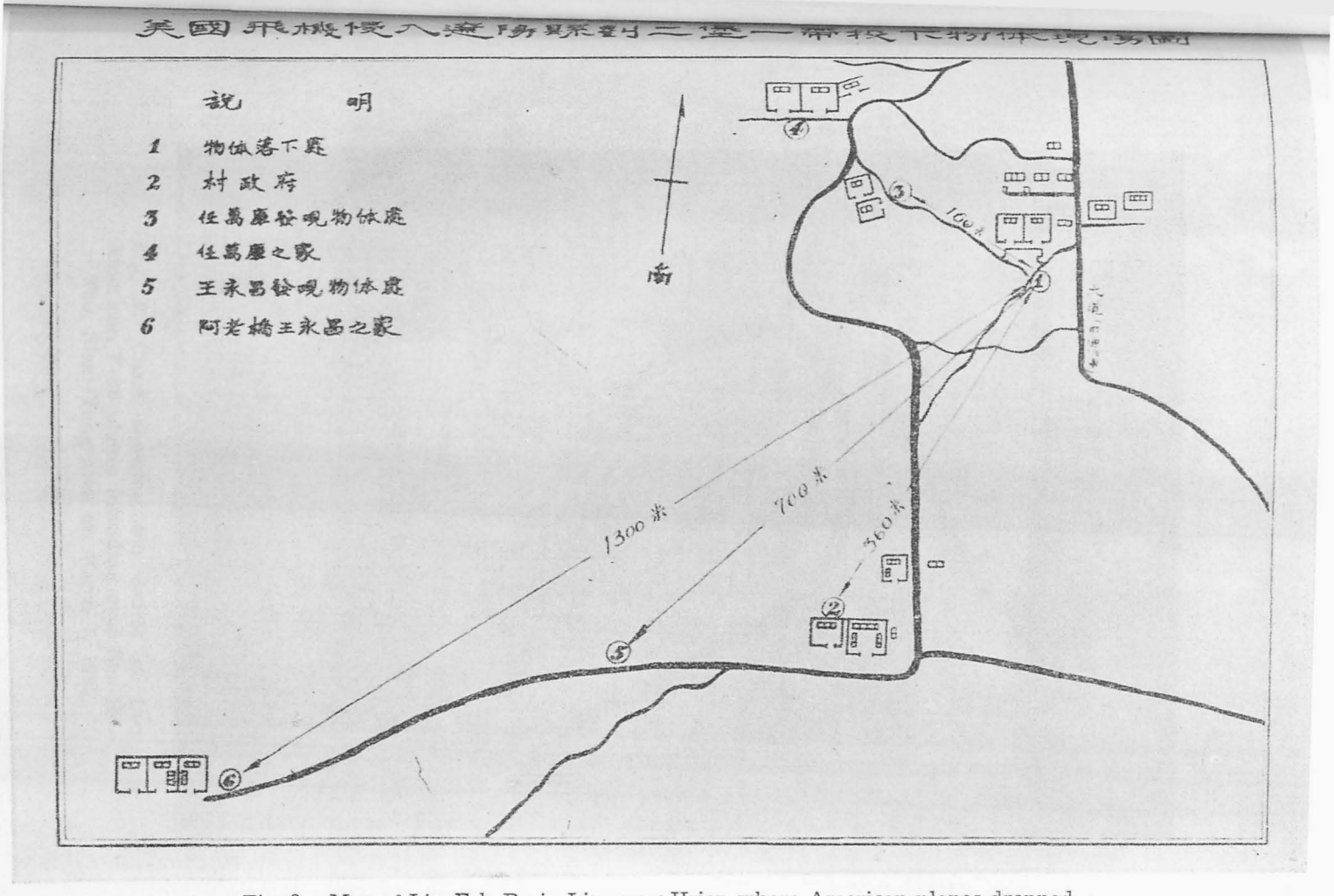


Fig. 8. Map of Liu-Erh-Pu in Liaoyang Hsien where American planes dropped objects. (Explanation: 1. Location where the falling object was seen; 2. Village Government; 3. Location where Jen Wan-Ku discovered the objects; 4. House of Jen Wan-Ku; 5. Location where Wang Yung-Chang discovered the objects; 6. House of Wang Yung-Chang at Ah-Lao-Chiao.

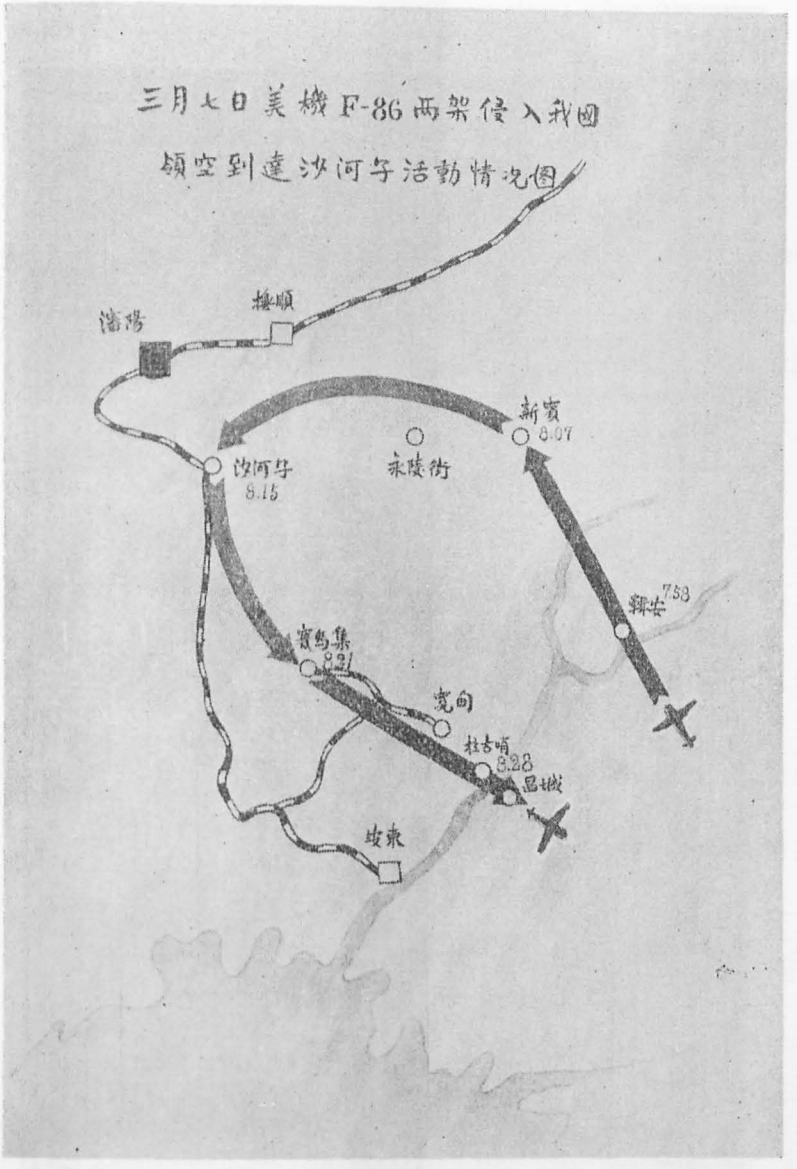


Fig. 9. Chart showing the course of two American F-86 planes intruding over Sha-Ho-Tzu, Shen-Yang area on March 7, 1952.

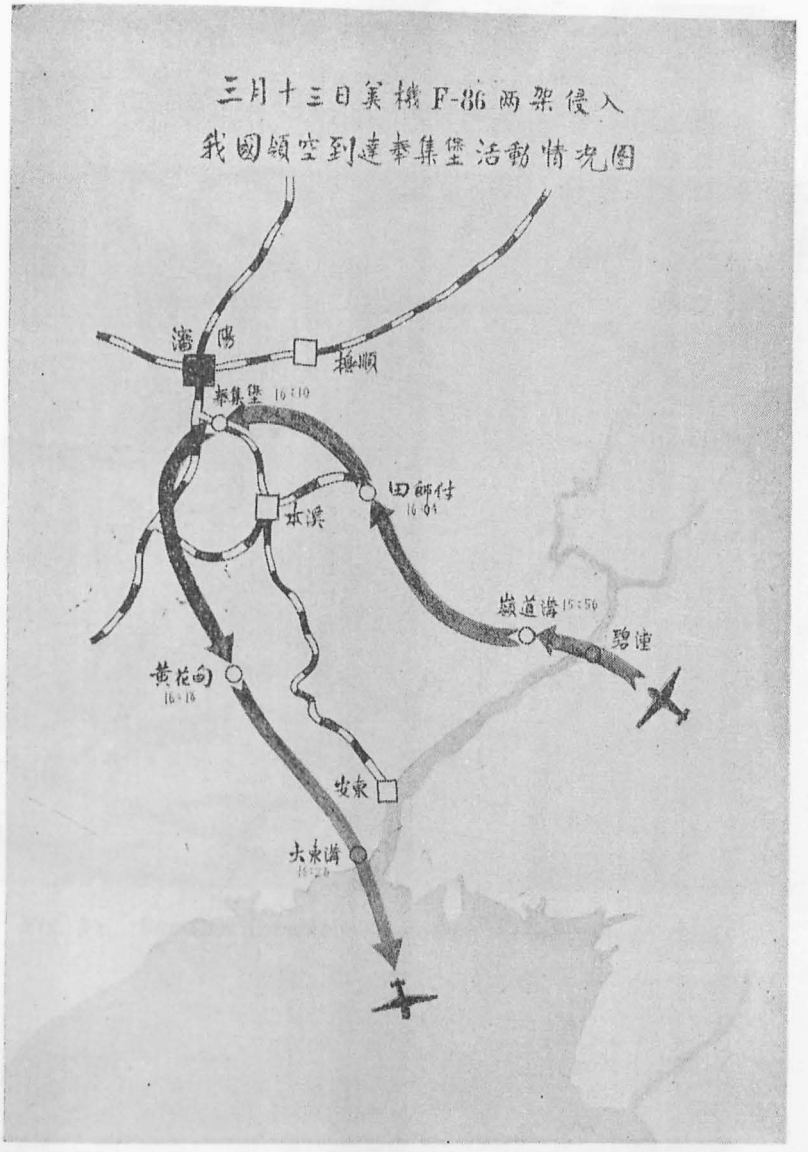


Fig. 10. Chart showing the course of two American F-86 planes intruding over Feng-Chi-Pu, Shen-Yang area on March 13, 1952.

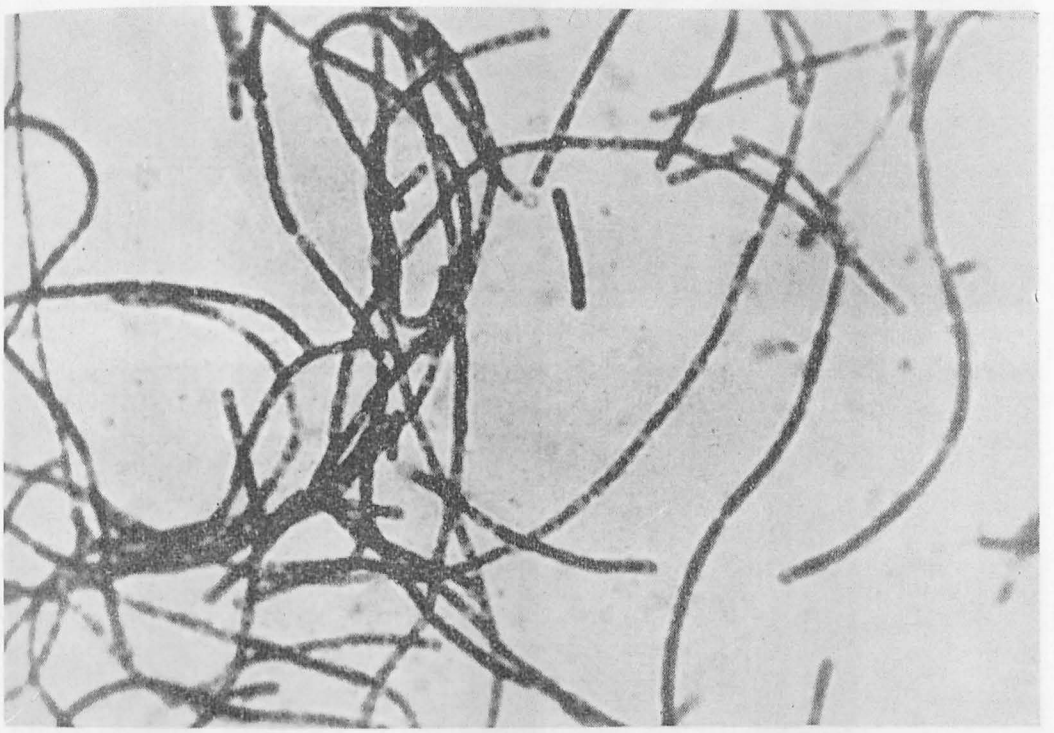


Fig. 11. *Bacillus anthracis*—stained with methylene blue.

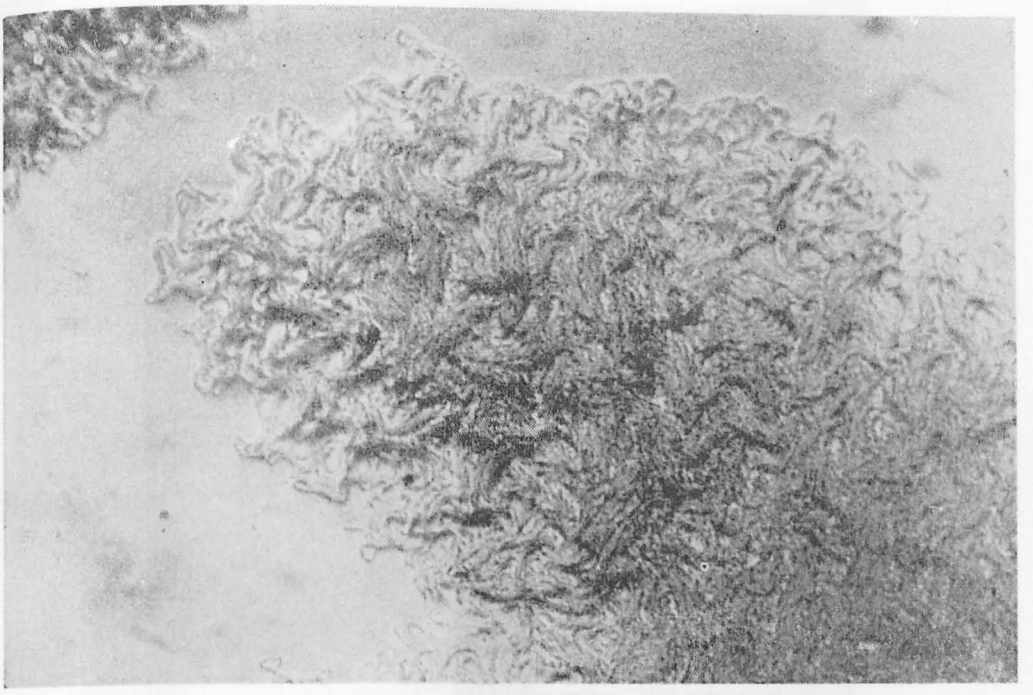


Fig. 12. Colonies of *B. anthracis.*

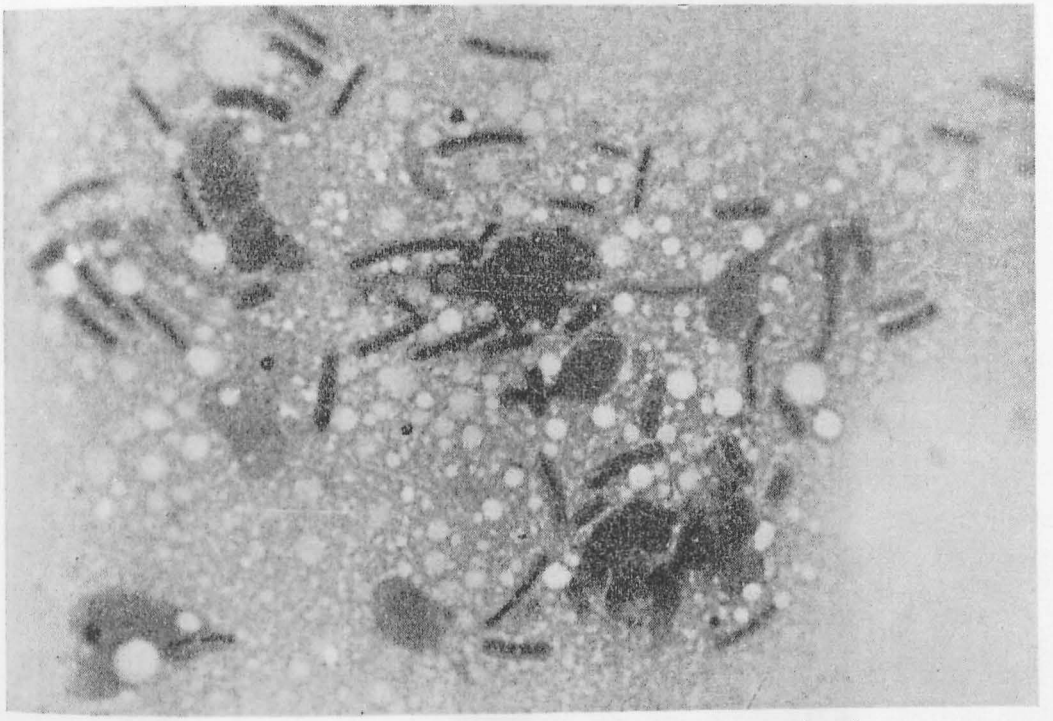


Fig. 13. Smear of the spleen of an infected mouse, showing capsule formation of *B. anthracis.*

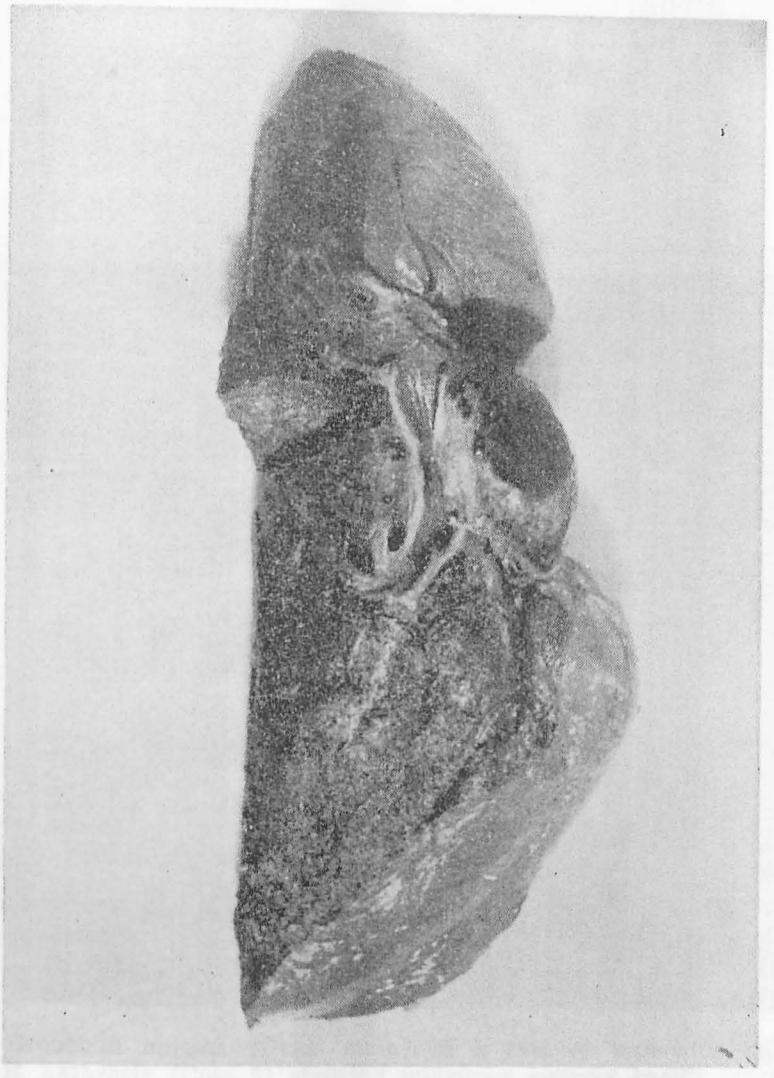


Fig. 14. Cut surface of the left lung: Hemorrhagic lymphadenitis in hilar region due to infection by *B. anthracis* (Case 3A).

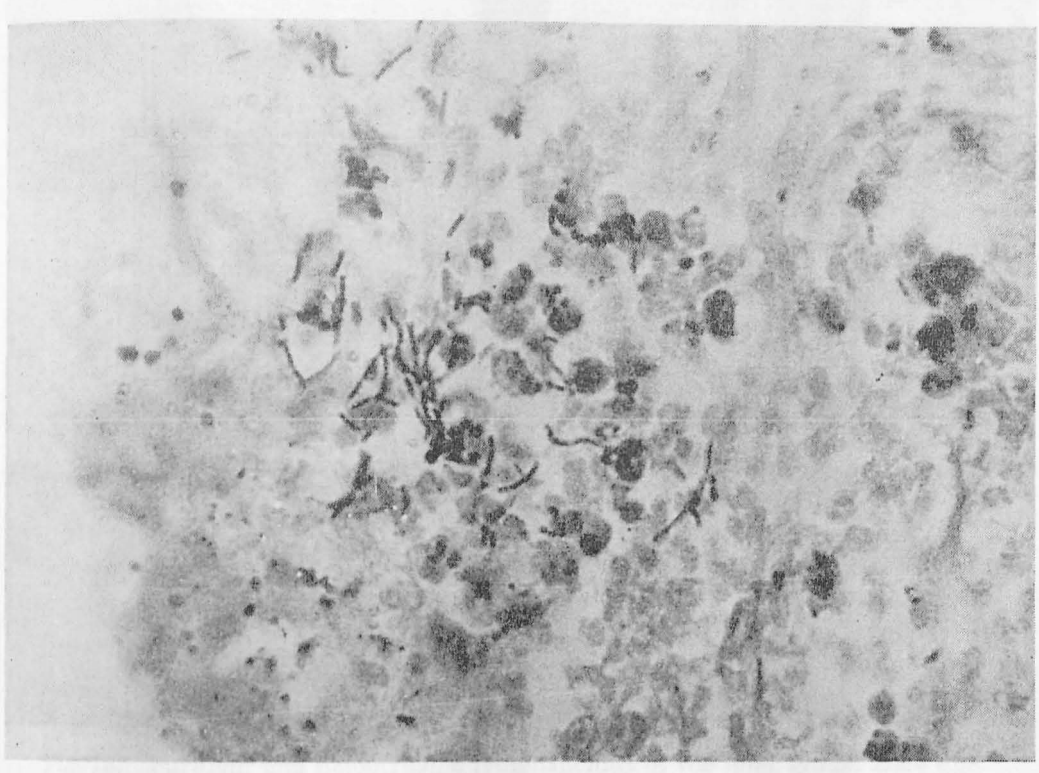


Fig. 15. Bronchial mucosa (Gram stain) of a case of bronchitis due to *B. anthracis* (Case 2) showing numerous anthrax bacilli.

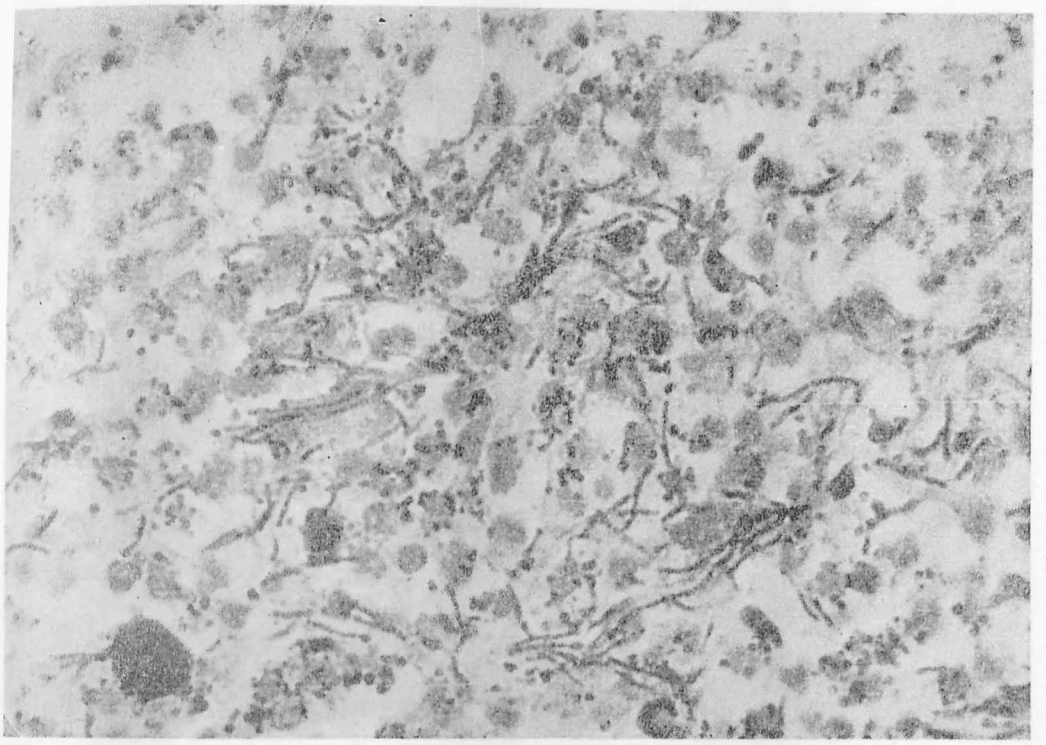


Fig. 16. Purulent and hemorrhagic lymphadenitis of the hilar lymph node due to *B. anthracis* (Gram stain) showing large numbers of anthrax bacilli (Case 2).

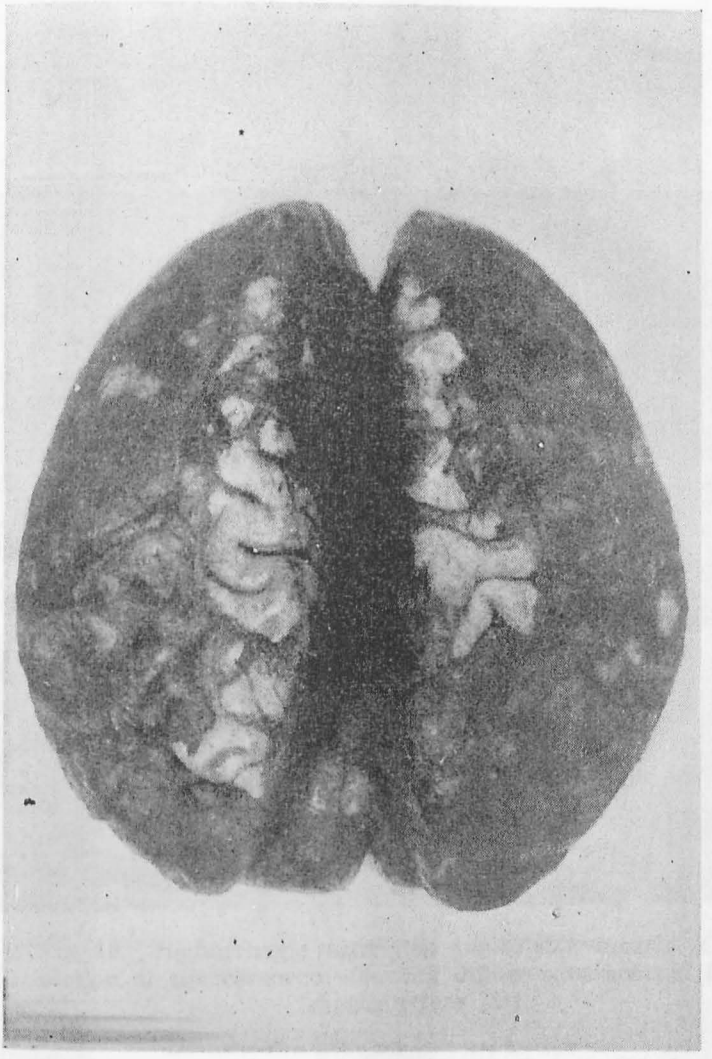


Fig. 17. Hemorrhagic meningitis due to *B. anthracis*. The cerebrum shows extensive and diffuse hemorrhages in the leptomeninges (Case 3A).

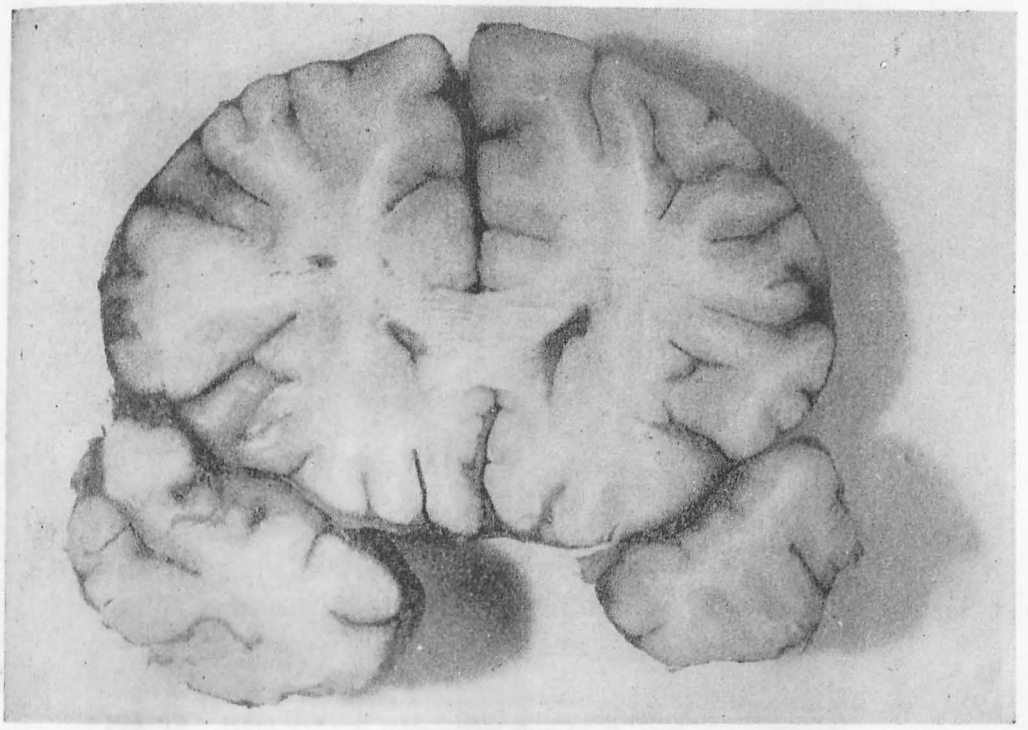


Fig. 18. Hemorrhagic meningitis due to *B. anthracis.* Coronal section of the cerebrum showing diffuse subarachnoid hemorrhages (Case 3B).

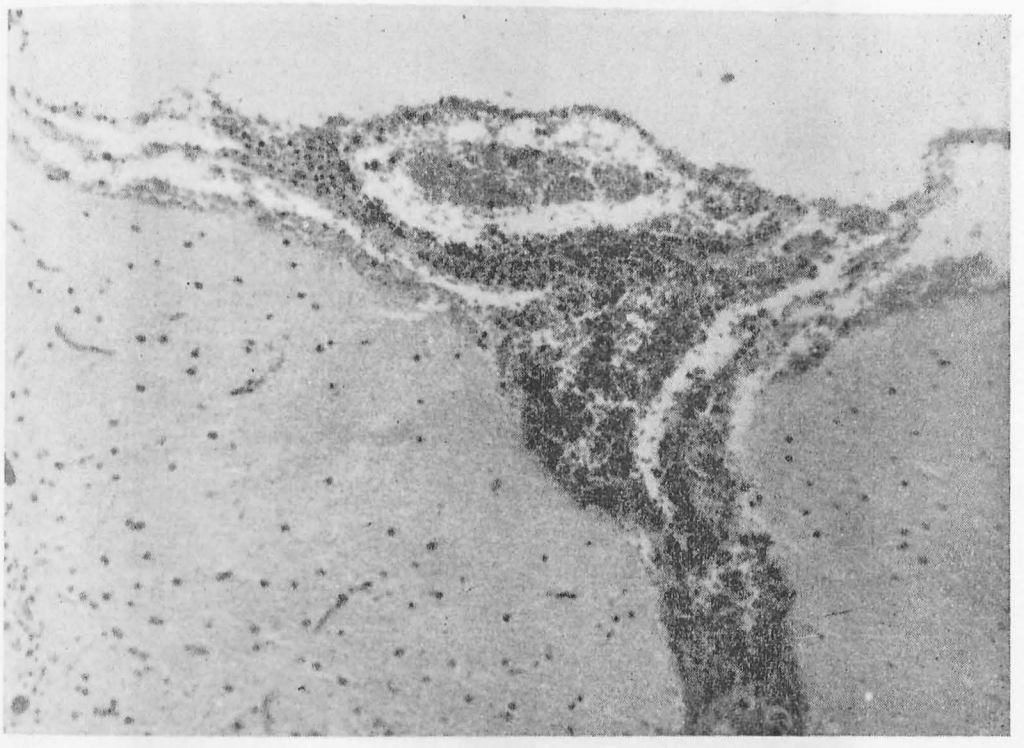


Fig. 19. Hemorrhage meningitis due to *B. anthracis.* Besides diffuse hemorrhage, there is also inflammatory cellular infiltration in the leptomeninges (Case 2).

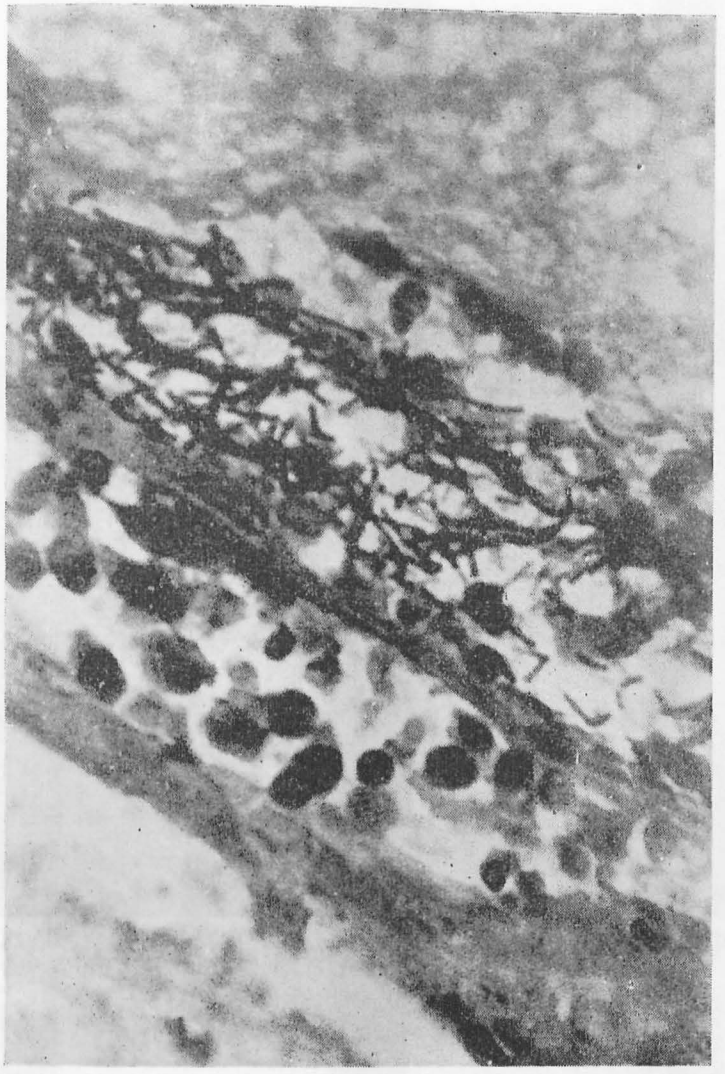


Fig. 20. Hemorrhagic meningitis due to *B. anthracis* (Gram stain). Large numbers of Gram-positive anthrax bacilli in the leptomeninges (Case 2).

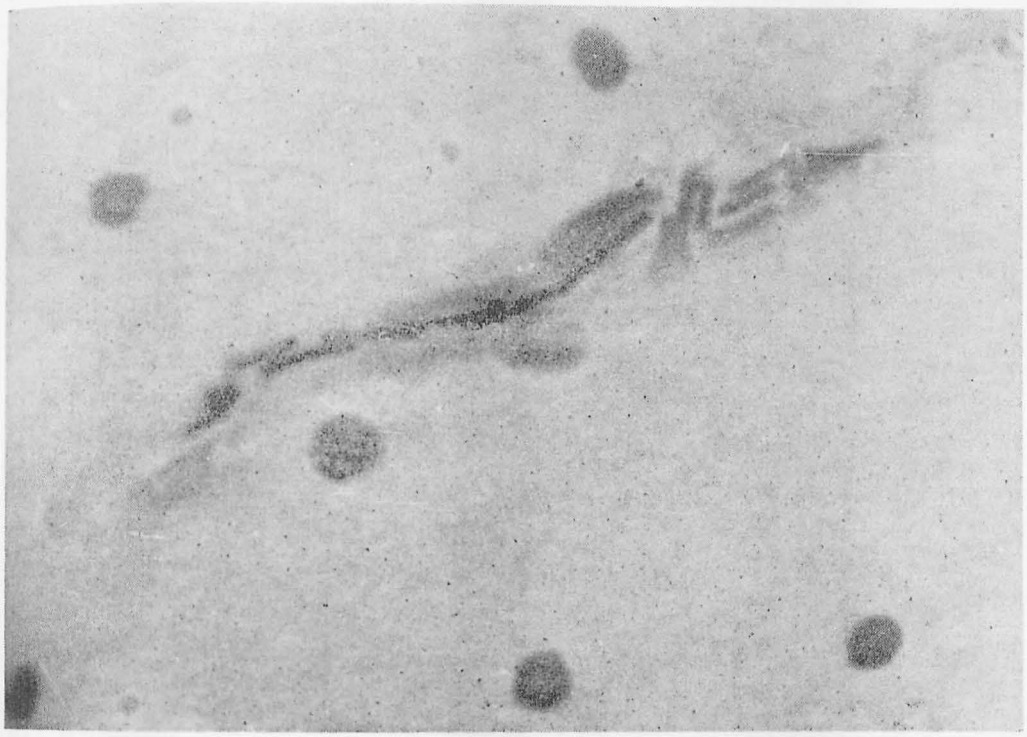


Fig. 21. A small blood vessel in the brain containing *B. anthracis* (Gram stain) (Case 3B).

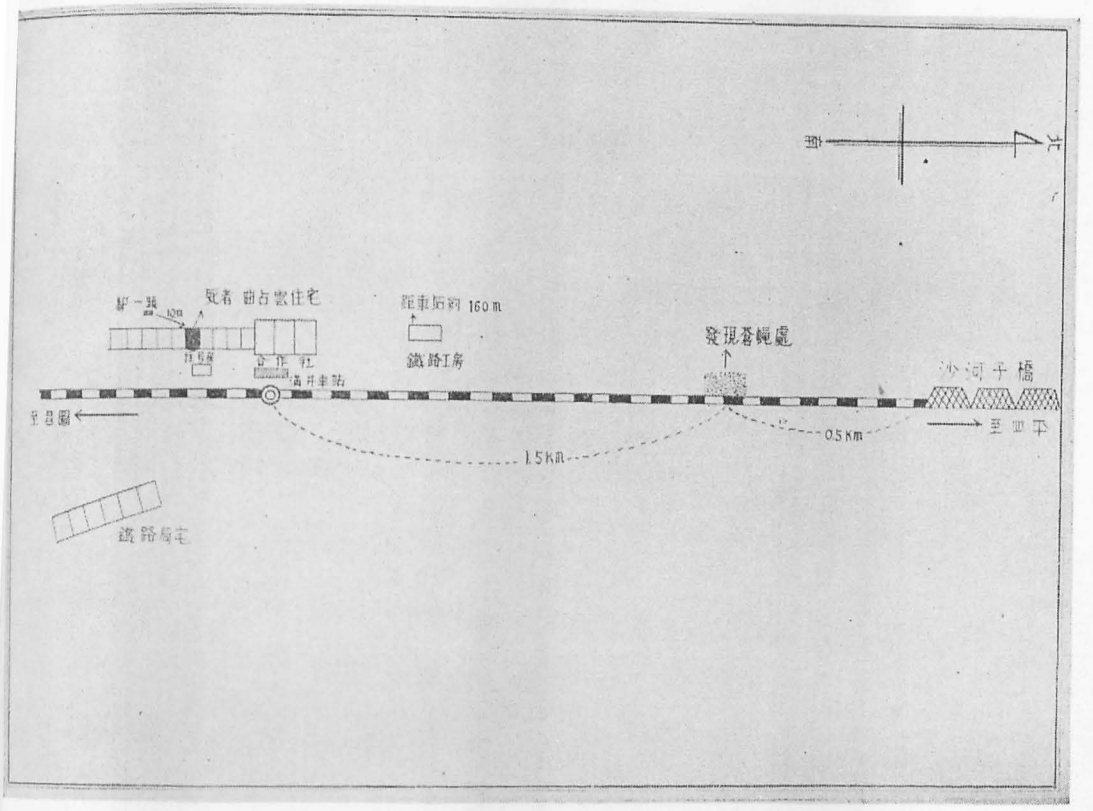
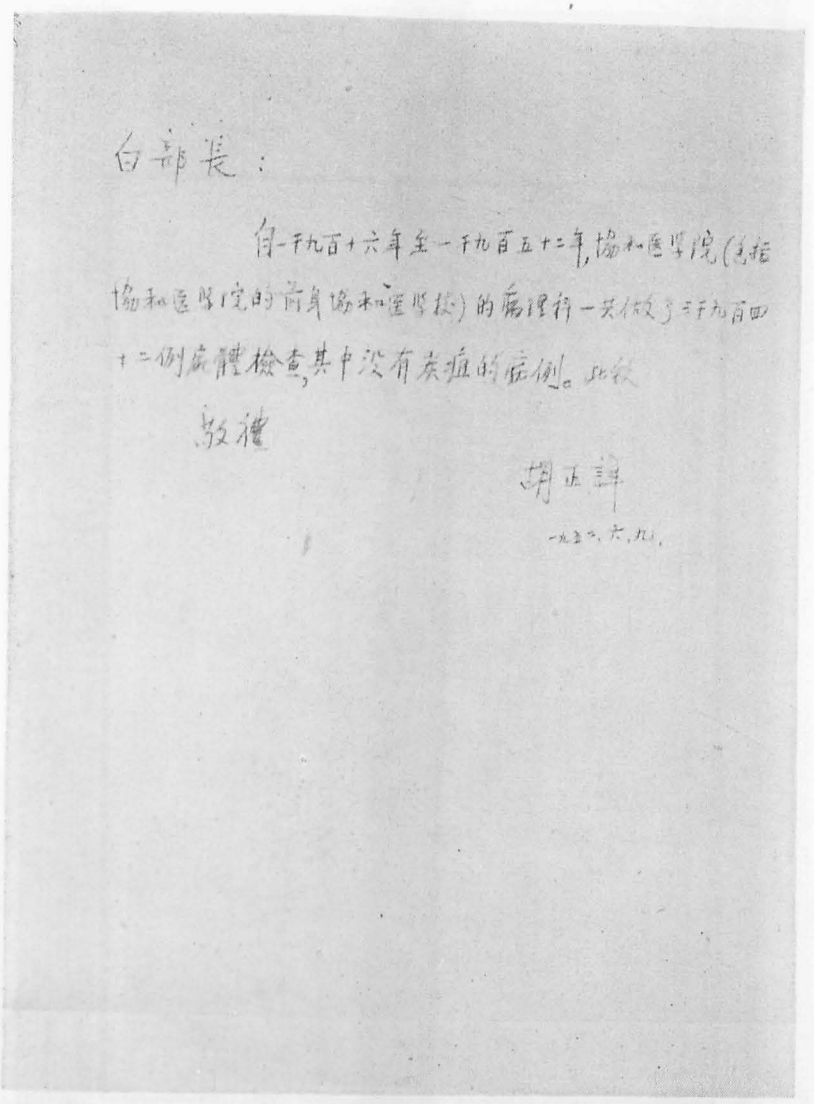


Fig. 22. Plan of Man-Ching Railway Station and its neighbourhood.


白部長：

自一千九百十六年至一千九百五十二年，協和醫學院（先指協和醫學院的前身協和醫學校）的病理科一共做了三千九百四十二例屍體檢查，其中沒有炭疽的症例。此致

敬禮

胡正祥

一九五二、六、九。

Fig. 23. Statement by Prof. C.H. Hu that there has been no case of anthrax among 3942 autopsies since 1916 in the China Union Medical College (formerly P.U.M.C.).

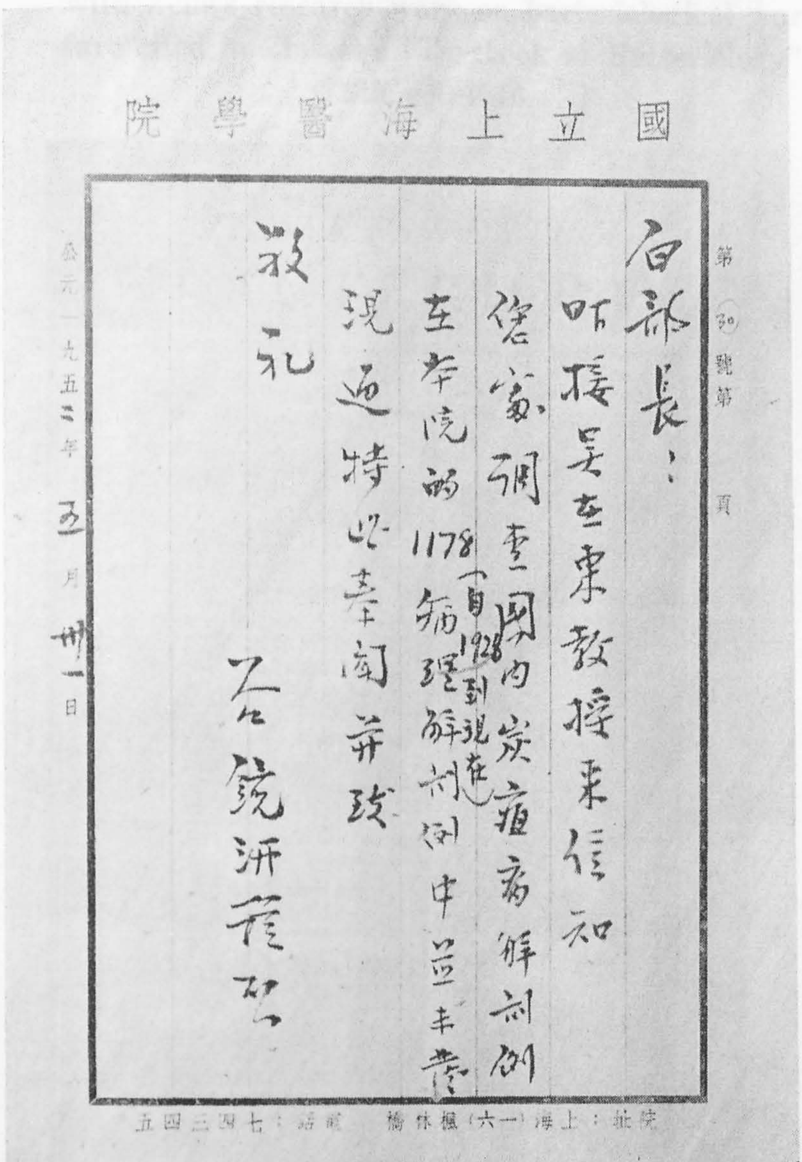
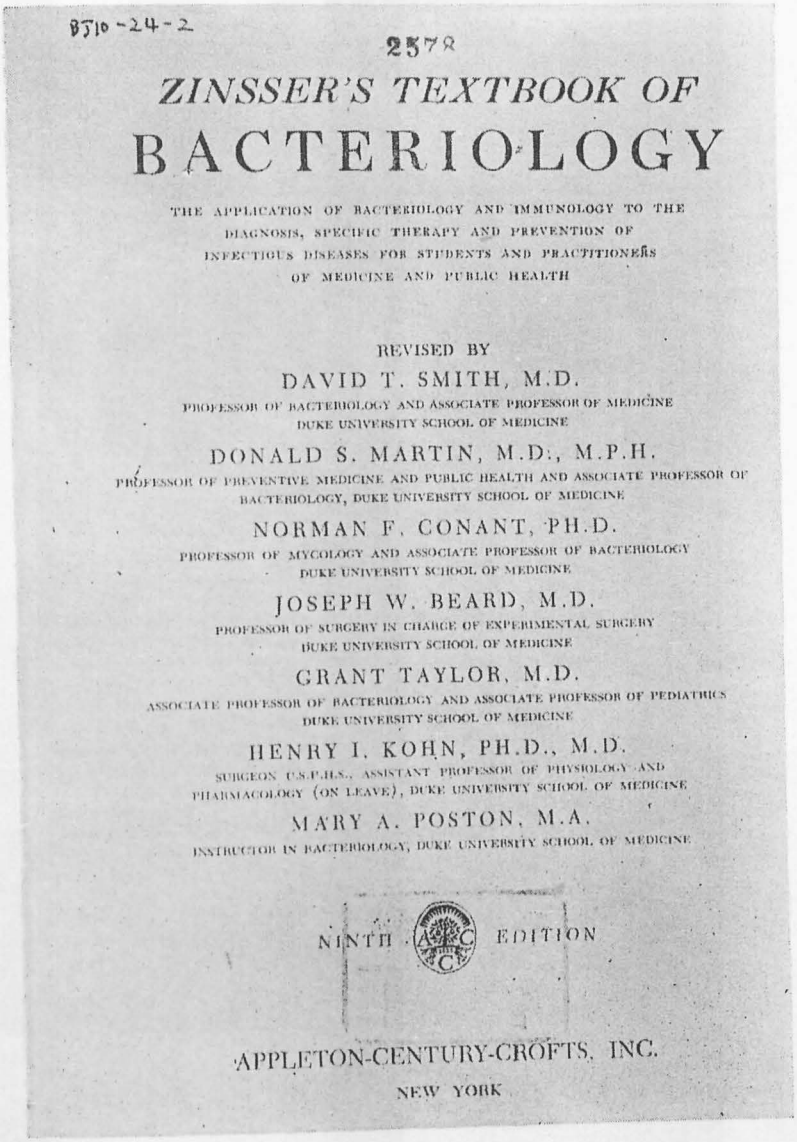


Fig. 24. Statement by Prof. C.Y. Ku that there has been no case of anthrax among 1178 autopsies since 1928 in the Shanghai Medical College.

The following Figs. (25a, 25b, 25c) are concerned with Zelle's research work on bacteriological warfare cited in Zinsser's "Textbook of Bacteriology", 9th. ed., 1946.



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ZINSSER'S TEXTBOOK OF

BACTERIOLOGY

THE APPLICATION OF BACTERIOLOGY AND IMMUNOLOGY TO THE DIAGNOSIS, SPECIFIC THERAPY AND PREVENTION OF INFECTIOUS DISEASES FOR STUDENTS AND PRACTITIONERS OF MEDICINE AND PUBLIC HEALTH

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Fig. 25 a.

phytic, sporulating bacilli; therefore, the test for pathogenicity is essential (Stein, 1941).

Resistance.—Because of its property of spore formation, the anthrax bacillus is extremely resistant to its chemical and physical environment. The vegetative forms themselves are no more resistant than most other nonsporulating bacteria, being destroyed by a temperature of 54° C. in thirty minutes. Anthrax spores may be kept in a dry state for many years without losing their viability (Surmont and Arnould, 1891). While *there are variations in the resistance of different strains of anthrax* spores, all races display an extremely high resistance to heat. Dry heat at 140° C. requires three hours to kill. Live steam at 100° C. kills them in five to ten minutes. Boiling destroys in about ten minutes. Destruction of anthrax spores in furs, hides, and brushes is difficult. Blue (1919) states that for brushes the best method is soaking for four hours in 10 per cent formalin solution at 110° F. Hair and bristles may be sterilized in the autoclave at 15 pounds for three hours, but this ruins many materials.

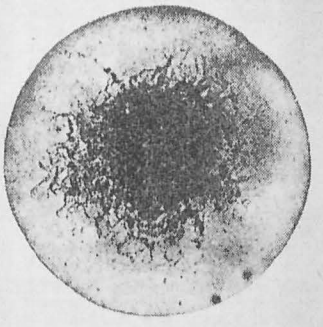

Fig. 114.—Anthrax Colony on Gelatin. (Guenther)

Spores may retain their viability after exposure to 5 per cent carbolic acid for forty days, or may be destroyed by the same solution in two days. Corrosive sublimate, 1:2000, kills most strains in *forty minutes. Direct sunlight* destroys anthrax spores within six to twelve hours.

Experimental anthrax infections in mice have been treated with sulfonamides, penicillin, and streptomycin. When used in maximum doses, sulfonamides saved 5 per cent, penicillin, 58 per cent, and streptomycin, 92 per cent of the *infected animals* (Miller *et al.*, 1946).

Variability.—Virulent anthrax bacilli produce rough (R) colonies. Less virulent or nonvirulent, smooth (S), mucoid (M), and gonidial (G) forms have been described (Gratia, 1924; Nungester, 1929). In connection with the work on bacterial warfare, Zelle and his associates (1946) selected variants that were especially adapted for invasion by the respiratory tract.

The attenuated strains which Pasteur obtained by cultivating the organism at a temperature of 42° to 43° C. were asporogenous. The essential change, however, was not in the loss of the ability to form spores, since some asporogenous races are highly pathogenic. Virulence depends upon the presence of a capsule or the ability of the organism to form one when introduced into the animal body. While separate races of anthrax bacilli may vary considerably in their degree of virulence, a single individual strain remains fairly constant in this respect if dried and preserved upon threads or kept in sealed tubes in a cold, dark place. Virulence is usually, but not always, increased by animal passage.

Fig. 25 b.

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Fig. 25 c.

The following Figs. (26a to 26k) show the reseach works of Zelle and his co-workers on the production of a variant of *B. anthracis* adapted for respiratory infection (J. Infect. Dis. 79, 1946).

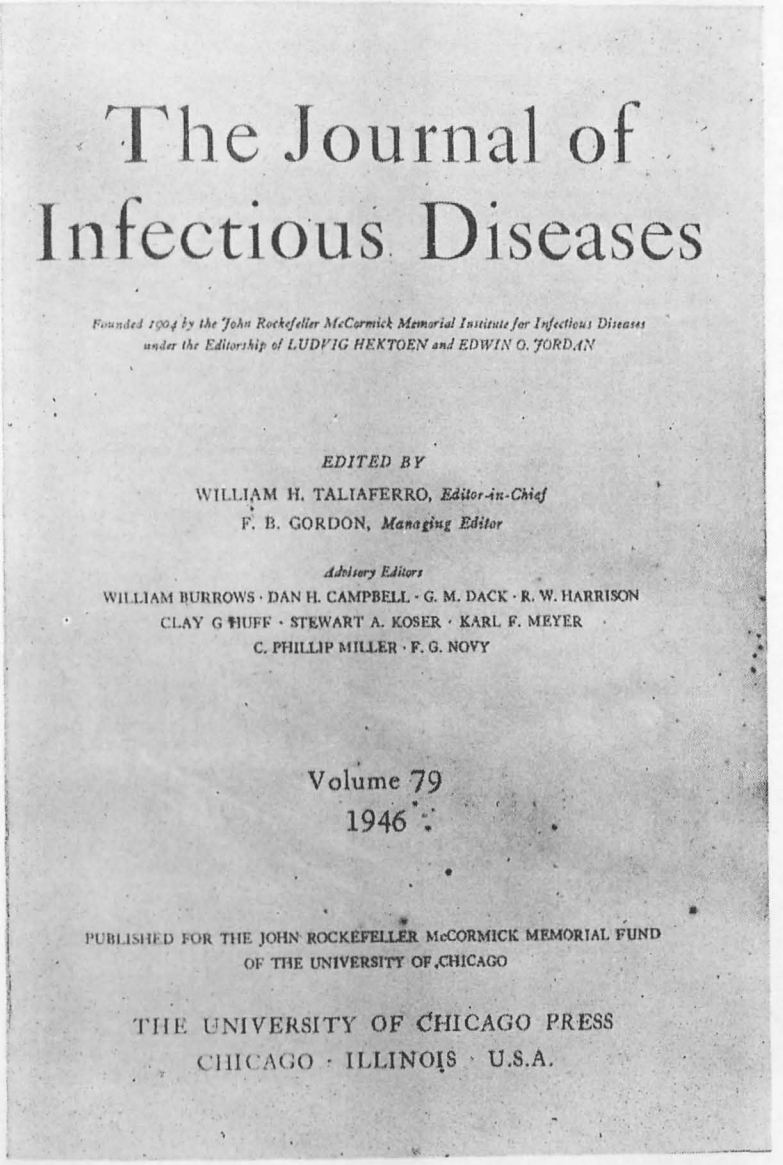

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Fig. 26 a.

RESPIRATORY PATHOGENICITY OF *BACILLUS ANTHRACIS* SPORES

I. METHODS OF STUDY AND OBSERVATIONS ON PATHOGENESIS*

GEORGE A. YOUNG, JR., (CAPT. VC, AUS), M. R. ZELLE, (LT.(J.G.) USNR), AND RALPH E. LINCOLN, (LT. USNR)

INTRODUCTION

In general, variation in pathogenicity of any bacterial species arises from 3 sources: heredity, environment, and interactions between heredity and environment. Studies of some of the factors causing variation in respiratory pathogenicity of *Bacillus anthracis* spores will be presented in this and subsequent papers. The general methods of investigation used throughout the studies and observations on the pathogenesis of respiratory anthrax will be presented in the present paper.

A very complete treatise on anthrax has been presented by Sobernheim[1] which includes over 1,000 specific references as well as mention of a similar number of papers without specific reference. All aspects of the disease including the early and more recent work on respiratory anthrax are considered.

Buchner[2] found it possible to infect mice, guinea pigs, and rabbits by causing these animals to inhale anthrax spores. This was later substantiated by Enderlen[3] who was able to infect sheep in the same manner. This important early work showed that anthrax infection could be established through the normal lung epithelium. However, there was no adequate measurement of the dosage required to produce experimental respiratory infection.

Sanarelli,[4] in an attempt to explain spontaneous anthrax, produced experimental infections via the respiratory route both by inhalation and intranasal instillation. Between 50,000 and 100,000 spores were required to infect rabbits by the latter method. Boquet and Saenz[5] later showed that infection of guinea pigs was possible by intranasal instillations of anthrax spores.

Also of interest in the study of respiratory anthrax is a series of papers by Velu et al.[6–9] These workers concluded that while it was difficult to infect experimental animals with spores alone, anthrax infection of the lung was easily established after the lung had been damaged by inhalation of chlorine gas.

The results in the present paper agree for the most part with those of the earlier workers. However, the method of exposure employed, which permits reasonably accurate measurement of respiratory dosage, and the statistical analysis of the data make it possible to compare the respiratory pathogenicity of different cultures and the suscepti-

Received for publication June 15, 1946.

* Work carried out at Camp Detrick, Frederick, Maryland from December 1943 through August 1945.

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Fig. 26 b.

portion of cloud equivalent to that introduced.

More specifically, a 24×24×36 inch rectangular autoclave without a jacket was equipped with 3 pyrex glass observation windows (fig. 1). One of these windows ⑦ was covered by a flood lamp to illuminate the chamber interior. The other 2 ⑧ were used for observation. Other modifications were openings for pipe lines into the chamber. One of these was an air line to the nebulizers ⑭. Another was a line to a mercury manometer for controlling the pressure of the chamber ⑨. The third opening was for a vacuum line used to withdraw a sample of the cloud for the determination of the spore concentration ④, ⑤, ⑥. Other pipe connections were those which were standard on most commercial autoclaves.

The spores were made airborne by means of 2 nebulizers in parallel near the front of the chamber ⑫. These nebulizers were made from stainless steel with a removable cup in which the spore suspensions were placed. Control of the concentration of spores nebulized into the chamber was obtained by keeping the air pressure in the nebulizer line constant and varying the concentration of the spore suspension. As the size of the air openings was fixed and the pressure constant, the rate of flow through

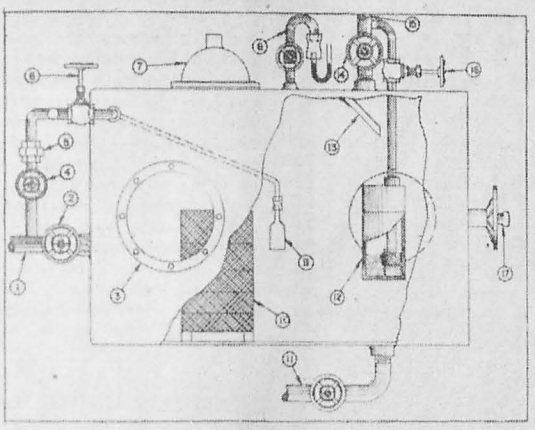

FIG. 1.—Respiratory exposure chamber.

① Vacuum exhaust line.
② Control valve for vacuum exhaust.
③ Observation window.
④ Valve on sample line.
⑤ Union for standard orifice plate.
⑥ Valve on sample line.
⑦ Flood lamp.
⑧ Mercury manometer assembly.
⑨ Cotton sampler.
⑩ Wire mesh animal holder (16 compartments).
⑪ Drain line.
⑫ Nebulizer (one of pair).
⑬ Baffle plate.
⑭ Valve on compressed air line.
⑮ Combination compressed air and steam line.
⑯ Control valve for nebulizers.
⑰ Radial lock for chamber door.

Fig. 26 c.

SUMMARY

An exposure method for producing experimental respiratory anthrax in several species of animals is described. This exposure technic plus statistical treatment of the resulting data makes possible quantitative comparison of the relative pathogenicity of different anthrax spore suspensions and of the comparative susceptibility of animal species.

A description of the histopathology of respiratory anthrax is given. From the essentially negative pathological findings, it is pointed out that anthrax produced by inhalation of anthrax spores is not a specific disease of the lung but rather a systemic disease. There is little or no reaction of lung tissue to anthrax spores in experimental animals exposed to spore clouds when observed until a few hours before death. Terminal bacillemia is accompanied by minimal changes only. These are as follows: Active hyperemia with some hemorrhage caused by increased capillary permeability; presence of bacilli in all vessels and to some extent in the alveolar walls. The original site of invasion of the spores could not be determined histologically.

Invasion of the host by inhaled anthrax spores is shown to occur through the lymphatic system.

Quantitative studies made on the organisms present in the lungs and peribronchial lymph nodes indicate that highly pathogenic spores are more invasive than spores of moderate respiratory pathogenicity in that they are present in greater numbers in the peribronchial lymph nodes and are also more able to persist in the lung itself.

Acknowledgment: The authors wish to express their appreciation for the assistance of A. J. Moses in much of this work.

Fig. 26 d.

RESPIRATORY PATHOGENICITY OF *BACILLUS ANTHRACIS* SPORES

II. GENETIC VARIATION IN RESPIRATORY PATHOGENICITY AND INVASIVENESS OF COLONIAL VARIANTS OF *B. ANTHRACIS*

M. R. ZELLE (LT.(J.G.) USNR), RALPH E. LINCOLN (LT. USNR), AND GEORGE A. YOUNG, JR. (CAPT. VC, AUS)

INTRODUCTION

Variation in morphological and physiological characteristics of bacteria is a common phenomenon. Reviews have been published by Hadley.[1,2] In general, a correlation has been observed between virulence and colony morphology with the smooth colony types exhibiting the greater virulence. However, *Bacillus anthracis* is generally regarded as an exception in that the smooth variants of this species have been found to be less virulent than the normal rough forms. Nungester[3] has reviewed the earlier literature and has classified colonial variants of *B. anthracis* into some 7 categories. Nungester concludes that no or little correlation between colony type and virulence occurs in *B. anthracis* since he observed both virulent and avirulent cultures of the same colonial type. Stein,[4] although not making a point of it, studied cultures of differing pathogenicity but of the same colonial type. The present paper presents evidence bearing on the correlation between colony type and subcutaneous pathogenicity and reports studies of the respiratory pathogenicity and invasiveness of several colonial variants of *B. anthracis*.

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Work carried out at Camp Detrick, Frederick, Maryland from September, 1944, through August, 1945.

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3. Nungester, W. J. 1929, J. Infect. Dis. 44: 73.
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MATERIALS AND METHODS

All cultures studied were derived from the Detrick 25 strain of *B. anthracis*[*] and in all cases were prepared with single vegetative colonies of the desired type as described in the first paper of this series[5] unless otherwise indicated. The cultures were grown in the PPY medium described in the third paper of the series.[6] The composition of the medium is presented below.

1.0% peptiease
0.6% peptone (USP)
0.8% glucose
0.25% plasmolyzed yeast (solids)
0.03 M KH_2PO_4
0.03 M K_2HOP_4
0.00004 $FeSO_4 \cdot 7H_2O$
0.001 M $MnSO_4 \cdot 4H_2O$
0.0002 M $MgSO_4 \cdot 7H_2O$
0.0002 M $CaCl_2 \cdot 6H_2O$

The medium was sterilized at 120 C for 20 minutes, the glucose being sterilized separately and added aseptically to the rest of the medium. Spore suspensions were checked for genetic variation by observing upwards of 250 colonies upon smeared nutrient agar plates. All observations of colony morphology were made with a stereoscopic dissecting microscope at a magnification of 9× utilizing reflected light. The method of exposing animals to respiratory anthrax and methods of statistical treatment have been described in the first paper.[5] The measure of respiratory pathogenicity is defined as the number of spores per liter of cloud (multiplied by 10^{-9}) which will cause 50% mortality among animals exposed to the cloud for 5 minutes and is symbolized as LRE 50 or 50% lethal respiratory exposure. All respiratory tests were made on groups of 16 animals and mortalities recorded for a 7 day period.

[*] Isolated by Dr. D. W. Henderson and Dr. C. E. Venzke following an extensive series of animal passages.

5. Young, G. A., Jr., M. R. Zelle, and R. E. Lincoln 1946, J. Infect. Dis. 79: 233–246.
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Fig. 26 e.

as indicating that respiratory pathogenicity is influenced by numerous genes, some of which affect colony morphology while some do not. Hence, the correlation between colony morphology and respiratory pathogenicity is not perfect since the 2 characteristics may vary independently.

SUMMARY

1. Four colonial variants of *Bacillus anthracis* vary significantly in respiratory pathogenicity for guinea pigs and rats.

2. No significant differences between the 4 variant types were observed in subcutaneous virulence tests on guinea pigs and mice, but significant differences were observed in subcutaneous virulence for rats.

3. Significant differences were demonstrated in the ability of the 4 types to invade through the tissues of the lungs.

4. Fatal respiratory infections with *B. anthracis* spores appear to result from effective invasion by only a very few spores.

5. Significant differences in respiratory pathogenicity were observed between cultures of the same colony type. The magnitude of the differences was equal to that observed between variant types. Hence, it appears that while colony morphology and respiratory pathogenicity of *B. anthracis* are correlated to a certain extent, they may also vary independently.

Fig. 26 f.

RESPIRATORY PATHOGENICITY OF *BACILLUS ANTHRACIS* SPORES

III. CHANGES IN PATHOGENICITY DUE TO NUTRITIONAL MODIFICATIONS

RALPH E. LINCOLN (LT. USNR), M. R. ZELLE (LT. (J.G.) USNR), CHESTER I. RANDLES, (ENS., USNR), JAMES L. ROBERTS, AND GEORGE A. YOUNG, JR. (CAPT. VC, AUS)

Environment may affect the pathogenicity of bacteria by (1) providing conditions under which variant types of cells selectively reproduce with consequent changes in the genetic constitution of the population, (2) by inducing temporary alterations of the protoplasm of the bacterial cell, or (3) by both. Most of the literature dealing with the effect of nutrition of bacteria on pathogenicity is concerned with changes that occur after colonial variation is obviously manifested or after serial transfer for many generations. Changes in pathogenicity after serial transfer are likely to be the result of changes in the genetic constitution of the culture since serial transfer provides ample opportunity for selection of mutant types of different pathogenicity which may or may not differ in colony morphology. For example, McNew[1] has shown that *Phytomonas stewartii* of low virulence became more virulent after growth in a synthetic medium containing inorganic nitrogen. However, he was able to mechanically separate cultures of high virulence from the original culture with low virulence. Thus, the increase in virulence probably was due only to intensive selection. Longley et al.[2] showed that cultivation of either *Phytomonas tumefaciens* or Rhizobia in mediums containing small concentrations of glycine or certain other amino acids resulted in a partial or complete loss of infective activity which was temporary after about 10 transfers and permanent after about 30 transfers. There are other reports in the literature of changes observed in the pathogenicity of bacterial cultures following cultivation upon artificial mediums for varying periods of time. There are also numerous experiments in which changes in pathogenicity were observed after serial passage of bacteria in both plant and animal hosts or by cultivation in the presence of antiseptics. In virtually all such experiments there has been questionable control of genetic changes in the population, so that it is not possible to definitely attribute such changes to temporary alterations in the protoplasm of individual cells or to selection of genetic types of differing pathogenicity. In instances where there was adequate genetic control, changes in virulence following serial host passage could be attributed to selection (Lincoln[3] for *Phytomonas stewartii* in maize and Zelle[4] for *Salmonella typhimurium* in mice). This paper considers the influence of the substrate upon the bacterial cell protoplasm as indicated by changes in pathogenicity of genetically controlled and homogeneous populations of *Bacillus anthracis*.

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Work conducted at Camp Detrick, Frederick, Maryland, from December 1944 through August 1945.

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5. Young, G. A., Jr., M. R. Zelle, and R. E. Lincoln 1946, J. Infect. Dis. 79: 233–246.

METHODS

The procedure for producing spores and the method of testing respiratory pathogenicity have been described in the first paper of this series.[5] The pathogenicity of spore suspensions is re-

Fig. 26 g.

SUMMARY

1. Spores produced in PPY (peptic case plasmolyzed yeast) or DSP (distiller's solubles paste) mediums are of high respiratory pathogenicity; spores produced in amended CSL (corn steep liquor) or in CSL mediums are of lowered respiratory pathogenicity. When 1% CSL is added to PPY or DSP mediums, pathogenicity is decreased. This loss of pathogenicity is about 3-fold.

2. When added to a basal medium in

Fig. 26 h.

conjunction with CSL, no ingredient was found that modified the effect of CSL on pathogenicity. Included in those materials tested were distiller's solubles paste, Puerto Rico invert molasses, dried brewer's yeast, plasmolyzed yeast, "Marmite" yeast, peptiCase, and various peptones.

3. The factor or factors in corn steep liquor causing decreased pathogenicity appear to be water dialyzable. The loss in pathogenicity is not related to the general growth conditions of the culture medium.

4. The change in pathogenicity occurs in less than 11–18 cell generations, is not accompanied by colonial variation, and is non-hereditary.

5. The subcutaneous pathogenicity of spores produced in CSL-free mediums is less than that of spores produced in CSL-containing mediums. However, spores produced in CSL-free mediums are more invasive in respiratory exposures, hence of higher respiratory pathogenicity than spores produced in CSL-containing mediums.

6. The addition of the readily fermentable carbon compounds, sucrose, glycerol, glucose, or potassium lactate to a medium in the absence of "salts" results in a 3-fold loss in pathogenicity. Addition of the non-fermented or slightly fermented carbohydrates, galactose, fructose, and lactose to a medium in which "salts" had been omitted results in about a 2-fold decrease in pathogenicity. The basal medium without "salts" or carbohydrates produces highly pathogenic spores.

7. In mediums containing glucose, $MnSO_4$ is necessary for high pathogenicity. $CaCl_2$, $FeSO_4$, $MgSO_4$, and $ZnSO_4$ had no influence on pathogenicity in the mediums used and did not interact with $MnSO_4$.

8. Corn steep liquor or salts produce their effect on pathogenicity during the growth of the cell and formation of the spore. The addition of these factors to spore suspensions produced in their absence does not alter pathogenicity significantly.

9. The significance of this non-genetic environmentally induced alteration in pathogenicity in relation to pathogenicity of bacterial cultures is discussed.

Fig. 26 i.

RESPIRATORY PATHOGENICITY OF *BACILLUS ANTHRACIS* SPORES

IV. CHEMICAL-BIOLOGICAL SYNERGISMS

GEORGE A. YOUNG, JR. (CAPT. VC, AUS) AND M. R. ZELLE, (LT. (J.G.) USNR)

The influence of a variety of chemical agents on the course of the disease anthrax has been previously reported. Most of these studies were made in an attempt to establish evidence of therapeutic value for the chemicals used in the treatment of animals infected with anthrax. However, there are several papers which show the variety of substances which will adversely affect the course of the disease. In this respect, Cadeac[1] ascertained that previously infected dogs died suddenly from anthrax following injections of as little as 0.0005 g of mercuric bichloride. Neri and Miceli[2] found that the natural resistance of rabbits to No. 1 and No. 2 spore vaccine was lowered by repeated sublethal injections of lead acetate. Likewise, Sanarelli[3] showed that the injection of arsenic, quinine, lactic acid, sodium nucleate, glucose, peptone, sodium hyposulfite, blood, distilled water, or cultures of living or dead colon bacilli was capable of lowering natural resistance so that fatal infections could be established with sublethal injections of anthrax spores. The adjuvants were used in concentrations small enough to cause no apparent damage to the host. Similarly, Hruska[4] demonstrated that the glucosides, digitonin and saponin, when injected in minimal concentrations lowered resistance sufficiently to allow infection with No. 2 Pasteur vaccine in previously immunized rabbits. More recently, Velu et al.[5] have shown that chlorine predisposes mice and guinea pigs to respiratory anthrax infection.

In this paper, experiments are described in which a variety of chemicals, largely salts of heavy metals, have been used to lower the natural resistance to anthrax infection established by respiratory exposure. The significance of the synergistic effects observed will be discussed in relation to the nature of natural resistance to respiratory infection with *B. anthracis* spores.

METHODS

The methods used in these studies were essentially the same as those employed previously.[6] The chemicals used were nebulized from varying concentrations of solution from 1 of the 2 nebulizers in the exposure chamber, the other being used to produce the cloud of anthrax spores. Chemical concentrations were determined by sampling known volumes of the cloud formed in the chamber by means of cotton samplers. Quantitative colorimetric analyses were made on the trapped chemicals. Since the cotton samplers also trapped the spores, quantitative plate counts were made from the dilute chemical solution to determine the concentration of the anthrax spores suspended in the chamber cloud.

As described in the first paper of the series,[6] respiratory exposure was measured in terms of RE which is the average number of spores (multiplied by 10^{-4}) suspended in a liter of cloud for a 5-minute exposure. The respiratory exposure or RE of the chemical agents is taken as the average

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5. Velu, H., P. Soulie, and B. Bellocq 1941, Bull. Acad. Med. 125: 159.
6. Young, G. A., Jr., M. R. Zelle, and R. E. Lincoln 1946, J. Infect. Dis. 79: 243–255.

Fig. 26 j.

SUMMARY

Respiratory exposure to mixed aerosols of salts of heavy metals and *Bacillus anthracis* spores in concentrations which would have no lethal effect if used separately causes fatal anthrax infections in mice, guinea pigs, and rats. This relationship between the chemicals and anthrax spores is truly synergistic. A hypothesis is presented attributing the synergism to chemical inactivation of the enzyme systems, mainly those which are dependent upon the presence of free sulfhydryl groups for their activity.

It is pointed out that studies of the physiological effects of chemical compounds which exhibit synergistic relations with pathogenic organisms may be of value in furthering the understanding of the nature of natural resistance to specific diseases.

The authors wish to thank Charles Frazier for making the chemical determinations and for suggestions related to the chemical problems encountered.

Fig. 26 k.

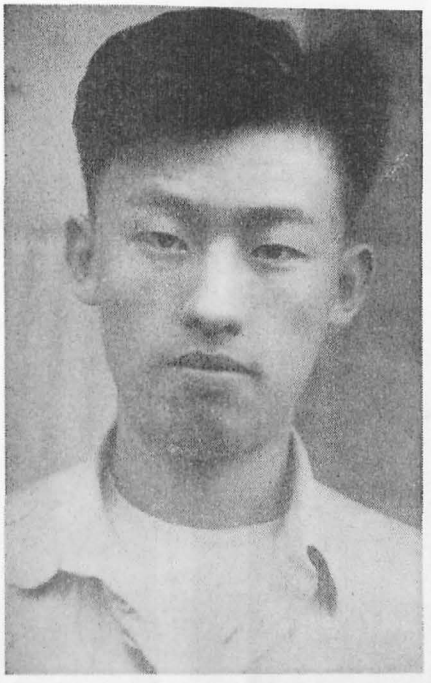


Fig. 27. Witness Liu Chi-An.

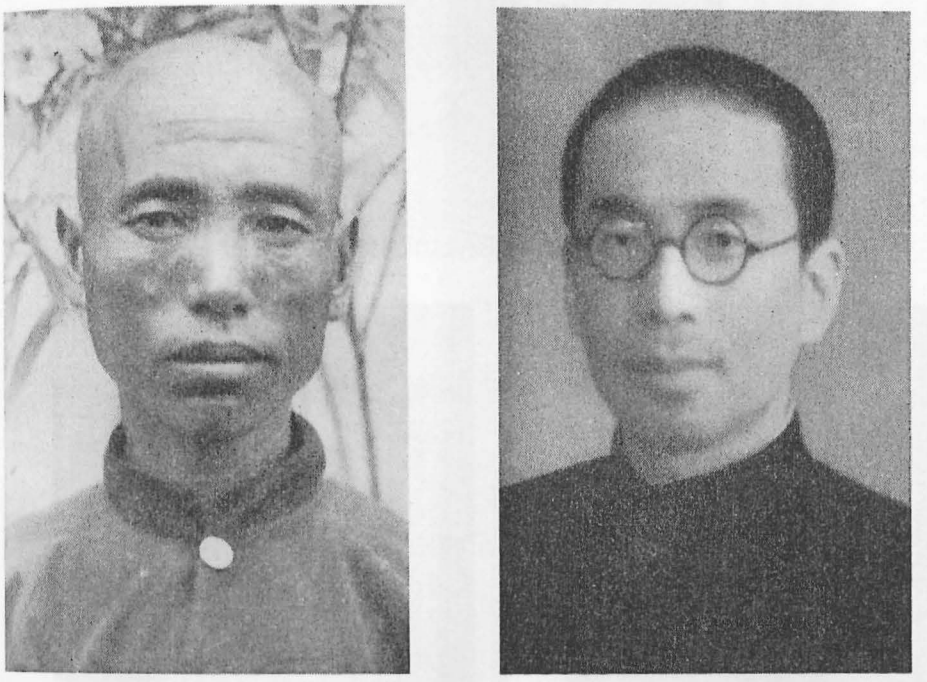
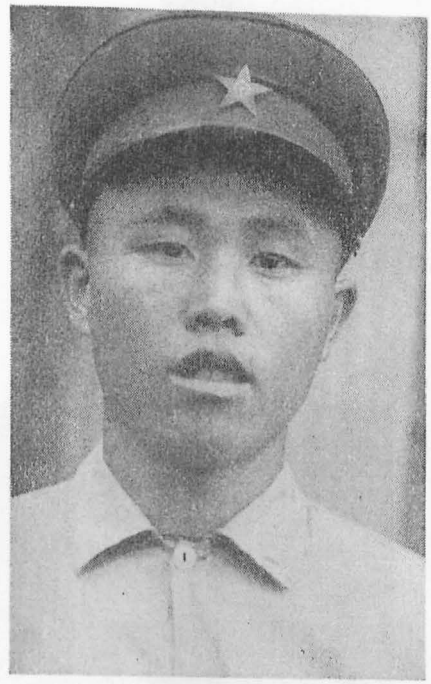


Fig. 28. Witnesses Wang Chiao-Ping (left), Sung Wei-I (right).



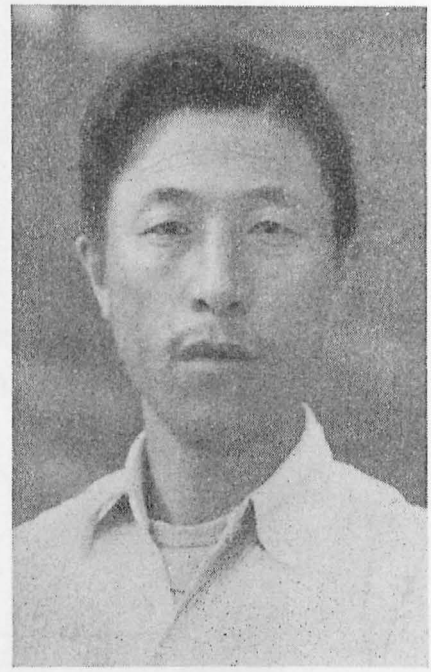


Fig. 29. Witnesses Liu Chung-Kuo (upper), Sung Teh-Yu (lower left), Pan An-Ying (lower right).

Fig. 30. Witness Wei Hung-Chin.

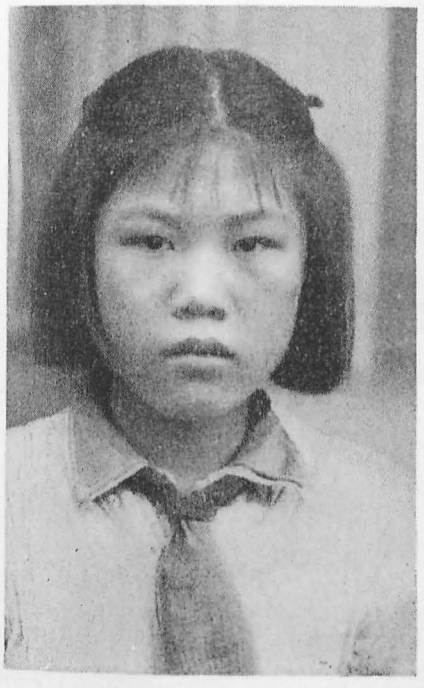


Fig. 31. Witnesses Ho Ming-Chia (upper), Liu Ching (lower left), Chao Yü-Chin (lower right).

Fig. 32. Witness Old Mrs. Liu.