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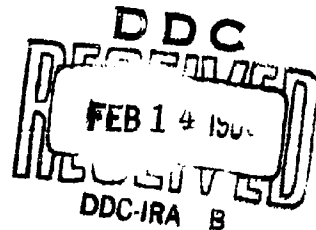
LABORATORY DIAGNOSIS OF TULAREMIA:

I. ISOLATION OF PASTEURELLA TULARENSIS
FROM GASTRIC JUICE.

SUCCESSFUL ISOLATIONS FROM NINE PERSONS
EXHIBITING PULMONIC-
AND TYPHOIDAL-TYPE TULAREMIA

John G. Ray, Jr.
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UNITED STATES ARMY
BIOLOGICAL LABORATORIES
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U.S. ARMY BIOLOGICAL LABORATORIES
Fort Detrick, Frederick, Maryland

TECHNICAL MEMORANDUM 78

LABORATORY DIAGNOSIS OF TULAREMIA:
I. Isolation of Pasteurella tularensis from Gastric Juice.
Successful Isolations from Nine Persons Exhibiting Pulmonic-
and Typhoidal-Type Tularemia

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In conducting the research reported here, the investigators adhered to "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

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ABSTRACT

Laboratory diagnosis of typhoidal or pulmonic tularemia requires the isolation of Pasteurella tularensis from the hospitalized patient. Generally, blood cultures from the patient are used in an effort to isolate this microorganism and, although several authors have reported success by this method, our laboratory has not isolated P. tularensis from blood cultures.

The successful isolation of P. tularensis is reported from cultures made from the gastric juice from five typhoidal and four pulmonic cases of tularemia.

The inoculation of guinea pigs and replicate direct plating of the gastric concentrate is advocated as a standard procedure for tularemia diagnosis.

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I. INTRODUCTION

Early, correct diagnosis of tularemia is essential if the infected patient is to receive the full benefit of chemotherapy^{1,2} with subsequent prevention of possible cardiac³⁻⁶ or cerebrospinal damage.⁷⁻¹¹ Of prime importance in early diagnosis is the suspicion that tularemia may be present because Pasteurella tularensis may remain dormant indefinitely^{3,12} even after apparent clinical recovery.

A. DIAGNOSIS OF TULAREMIA

1. Glandular, Ulceroglandular, and Oculoglandular Tularemia

A typical case of tularemia may cause (i) a lesion on the surface of the body or pharyngeal mucosal membrane,¹³⁻¹⁵ (ii) an inflammation of the conjunctiva^{16,17} in the form of a papule that develops into an ulcer and is usually followed by regional enlargement of the lymph nodes draining the site of infection, and occasionally (iii) enlarged regional lymph nodes without demonstrable primary skin lesions, indicating that the infecting organism apparently penetrated a normal intact skin.

These primary lesions and regional buboes indicate the portal of entry of the infecting organism. The recognition of a tularemia infection in lieu of the case history generally poses no problem. For confirmation, scrapings from the lesion or aspirated lymphatic fluid are cultured on a blood cysteine glucose agar medium and injected into laboratory animals; subsequent isolation of P. tularensis is possible in either case and an accurate diagnosis can be made.

2. Pulmonic and Typhoidal Tularemia

The diagnosis of tularemia and subsequent isolation of P. tularensis in pulmonic and typhoidal cases is not as easy as in the glandular cases. Moss and Weilbaecher¹ reported that these types of tularemia may exist as a blood stream infection from the onset, with or without demonstrable regional lymphadenopathy or local lesions.¹⁷ This is particularly true if (i) there is a prolonged fever of unknown origin or obscure etiology, or (ii) pneumonia for which no reasonable etiological agent such as pneumococci, Friedlander's bacilli, streptococci, or viruses can be demonstrated.

Kennedy¹⁸ was impressed with the lack of clinical recognition of the pulmonic form of the disease because of incorrect or no diagnosis. Pessin¹² collected from the literature 13 incorrect diagnoses of proved cases of fatal tularemia. Tularemia has been confused with primary atypical pneumonia,^{18,19} lobar pneumonia,¹ tuberculosis,¹⁸⁻²⁰ typhoid fever,^{18,19} undulant fever,^{18,19} typhus,^{12,18} diphtheria,¹⁸ chronic malaria,¹⁸ acute rheumatic fever,¹⁸ influenza,¹² encephalitis,¹⁸ poliomyelitis,¹⁸ acute pleuritis,¹⁸ chronic bronchitis,²² and viral pneumonia of known etiology.¹⁴

The symptoms of pulmonary tularemia are variable; on the whole there are two groups of cases: (i) the disease may begin with pulmonary involvement, or (ii) the pulmonary infection may develop during the course of another tularemic-type infection.^{18,25}

In their review of the literature, Blackford and Casey²³ suggested two routes by which *P. tularensis* may reach the lungs, but direct infection of the upper respiratory tract¹²⁻¹⁶ by inhalation appears certain, as evidenced by Permar and MacLachlan,²⁷ Fetterman and Lerner,²⁸ and Overholt et al.,² who have presented illustrative cases in which cutaneous ulceration and regional adenopathy were lacking.

Because of such difficulties, a failure to diagnose or an incorrect diagnosis may evolve unless tularemia is suspected and a continuous effort is made to identify the causative organisms by (i) culture or animal pathology, (ii) the Foshay intradermal test,²⁹ (iii) a roentgenographic study, and (iv) a serological follow-up of the tularensis agglutination titer.

B. THE LABORATORY'S ROLE IN TULAREMIA DIAGNOSIS

In the absence of telltale lesions and regionally located lymphadenopathies, the clinical picture is obscure. Etiological diagnosis must depend upon laboratory measures,^{1,10} that is, the demonstration of the organism by culture or animal inoculation, or development of specific agglutinins in the blood stream.

1. Material for Culture and Animal Inoculations

Pasteurella tularensis has been isolated repeatedly from patients ill with tularemia by animal inoculation and plate cultures of (i) conjunctival scrapings,³⁰ (ii) pus from the nose and throat of a patient with oculoglandular tularemia,³¹⁻³³ (iii) blood,³⁴⁻³⁶ (iv) material from primary lesions of the skin,^{18,20} (v) lymph nodes,^{40,41} (vi) pleural fluid,^{42,43} (vii) ascitic fluid,⁴³ (viii) fluid from the olecranon bursa,³ (ix) spinal fluid,^{7,8} and (x) from sputum specimens.⁴⁴⁻⁴⁶ At autopsy, the organism is found widely distributed in great numbers and most constantly in the regional lymph nodes (axillary, cervical, submaxillary, and bronchial), lungs, liver, spleen, and blood, also in a few cases from the heart and meninges.^{2,10}

2. The Skin Test

The intradermal test suggested by Wherry in 1917, and developed by Foshay²⁹ in 1932, is the earliest available diagnostic aid for determining the presence of tularemia. The reaction may become positive as early as the fourth day of the disease, practically a week before agglutinins appear in the blood. The test is specific and usually reliable, and the reaction becomes negative only when convalescence is complete. Khatenever⁴⁷ also

claims in his experiments in Russia an early diagnosis with tularin as a skin test antigen. However, our experience with previously immunized individuals, and the experience of other laboratories with a nonimmunized group^{9,13} show that Foshay's intradermal test is not always a reliable means of diagnosis.

3. The Chest X-Ray

Roentgenographic studies are especially necessary in pulmonary tularemia when evidence of pulmonic infiltration would further the possibility of isolating from sputum or pleural fluid the causative organism.⁴⁸ Johnson⁴⁸ and Larson⁴⁴ isolated P. tularensis from the sputum of persons who showed no evidence of a pulmonary involvement. This indicates that the presence of the specific organism in the respiratory tract of tularemic individuals is not necessarily associated with the presence of signs or symptoms referable to the respiratory system.

Thus, in the absence of productive sputum or pleural effusion from which cultures are attempted, the chances of isolating P. tularensis are nil, whether or not a pneumonic infiltration can be demonstrated in the chest X-ray film.

4. Serodiagnosis

A serological follow-up on the patient suspected of tularemia needs no explanation, since the majority of correct diagnoses reported in the literature have been based mainly on a diagnostic rise in agglutinin titer. Here the suspicion that the patient has tularemia is paramount to correct diagnosis of this disease. The agglutination test for tularemia should be made in all atypical febrile conditions of the chest.⁴⁹

Although the above evidence indicates correct diagnosis of tularemia, it has been the experience of this laboratory and others^{16,48} that generally, blood cultures are not a reliable means of isolating P. tularensis, especially in nonfulminating cases. Agglutination tests, although often reliable after the second week of illness, cannot be reliable in the early stages of the disease.^{2,18} Furthermore, the absence of diagnostic titers for P. tularensis in severe and fulminating cases does not preclude the presence of tularemia,^{1,28} because their appearance may be delayed or they may even be absent throughout the entire course of the disease. This is especially true in light of the present-day use of broad-spectrum antibiotics.

C. TULAREMIA AND TUBERCULOSIS

It has often been reported that the symptoms of pneumonic-type tularemia closely parallels those of tuberculosis, and early diagnosis is often confused. In view of the roentgenological similarity to tuberculosis^{21,28}

and the same methods of isolation, it appears feasible that an incorrect diagnosis of tuberculosis for tularemia, pneumonic or typhoidal types, might occur.

The histological resemblance of the initial focal necrosis in tularemia to the early tubercle of tuberculosis has been established by Kavanough²⁰ and Blackford.²² It is not surprising, therefore, to find analogous effects produced in the chest by these two diseases. The similarity of the pathological process of the caseous pneumonias associated with tularemia to tubercular pneumonia has been noted by Gundry and Warner;³⁸ Blackford and Archer²⁵ have illustrated the roentgenographic resemblance. Warring and Cullen³¹ stated that tularemia with pleural effusion, in the weeks following the acute onset, closely resembled the wet pleurisy of tuberculosis both clinically and roentgenologically. Diagnosis, especially in the typhoidal type, may be rather difficult "until we can discover better clinical or X-ray means of differentiating TB pleurisy and tularemia with pleural effusion - the isolation of the organism remains the deciding criterion for diagnosis during life in the typhoidal type of case."³¹

Case studies in the appendix of this paper are concerned with the isolation of P. tularensis from another medium often used for the isolation of the tubercle bacillus, the gastric juice. In our first case of typhoidal tularemia, a preliminary diagnosis of tuberculosis was considered. Since there was no production of sputum for laboratory purposes, a Levin tube was passed, and undiluted gastric juice was recovered from which our first successful isolation of P. tularensis was achieved. In the subsequent cases, five were of the typhoidal type in which no apparent respiratory tract involvement could be determined by physical examination and roentgenographic studies; three cases were of the pulmonary or pulmonic-type tularemia, which manifested symptoms of respiratory tract infection.

II. MATERIALS AND METHODS

A. OBTAINING GASTRIC SPECIMENS

Gastric juice was collected from the patient by passing an iced Levin tube either through the nasal passage or through the mouth into the stomach and aspirating the stomach contents with an attached 30-cc syringe. On occasion, a gargle of procaine solution was necessary to stop the gag reflex on passage of the tube. The specimen was placed in a sterile urine bottle after noting the quantity collected. The gastric specimen was immediately transported to the laboratory where the pH was determined. Initially the sample was neutralized with 10% potassium hydroxide. After repeated isolations on various patients, determination of the pH and subsequent neutralization were discontinued because the pH approached neutrality when taken prior to 8 AM, and it was decided not to dilute the specimen of gastric juice.

B. PLATE CULTURING OF GASTRIC JUICE SPECIMEN

Each gastric juice specimen was plated into each of three blood cysteine glucose agar plates by pipetting 0.2 to 0.3 ml per plate with subsequent meticulous streaking with the bacteriological loop or needle to isolate definitive colonies.

The plate medium was made according to the following formula:

Beef Extract	3 grams
Peptone (Bacto)	15 grams
Sodium Chloride	5 grams
Cysteine Monohydrochloride	1 gram
Distilled Water	880 ml

The mixture was brought to pH 7.0 with 5 N sodium hydroxide; 15 grams agar (Bacto) were added, and the medium was autoclaved for 20 minutes at 121 C and 15 pounds pressure. After the medium cooled to 40 C, Seitz-filtered sterile dextrose solution (25 grams dextrose in 80 ml of distilled water) was added and the medium mixed thoroughly. Twenty-five milliliters of packed human red blood cells or 50 ml of human whole blood were added and the medium was poured into petri dishes and allowed to solidify.*

* After initial isolates of P. tularensis were made, 100 to 500 units of penicillin per ml and 0.1 mg per ml of actidione were incorporated in the medium to inhibit growth of gram-positive bacteria and fungi.

After streaking, the plates were incubated at 37 C and observed for not less than 4 days. Isolated colonies of P. tularensis usually started to appear 24 hours after incubation, and by 48 hours could be identified by typical colonial and cellular morphology.

Each P. tularensis gastric juice isolation was serologically typed with a high-titered P. tularensis antiserum (Markham) prior to reporting the positive identification.

The above procedures also applied to the colonial isolation of P. tularensis from infected guinea pig tissue.

C. ANIMAL INOCULATION AND PATHOLOGY

Each of two guinea pigs weighing approximately 300 to 500 grams was inoculated twice, once with 1.0 ml of the gastric juice specimen intraperitoneally and once with 1.0 ml subcutaneously in the inguinal area. The double injection gave a better chance for isolation of the organism. A 5.0-ml syringe with a 20-gauge needle was used for injection. Rectal temperatures of the guinea pigs were taken daily for at least 10 days; temperatures often did not rise above 104.4 F (Table 1). Those temperatures, although above the normal range, are lower than those that result when pure isolated strains of P. tularensis are injected into guinea pigs. Isolated strains generally cause a rapid increase in rectal temperature to 105.5 or 106 F within 2 to 3 days, depending on the number of organisms inoculated.

Most of the guinea pigs from which P. tularensis was isolated died within 4 days postinoculation; upon autopsy the liver and spleen were the most prominently infected tissues. The infected site on the tissue was excised and minced in a mortar by scissors action of two scalpels. A small amount (0.5 ml) of sterile physiological saline was added to the minced infected tissue. Then this was aspirated into a sterile pipette, placed onto the blood cysteine glucose agar plates, and streaked over the surface of the agar with a wire loop in an effort to obtain isolated colonies of P. tularensis.

Contact smears of the infected tissue were made on glass slides and stained by the Gram method. This proved to be a very successful technique, because almost all infective tissue produced typical gram-negative pleomorphic coccobacillary rods, both intra- and extra-cellular.

The direct plating technique of plunging a sterile bacteriological inoculating loop into the infected site and streaking the material on blood cysteine glucose agar plates was often used. Although all methods were equally successful, we prefer the minced tissue technique.

Each resulting isolated P. tularensis colony was serologically typed with high-titered antisera for definite confirmation of the isolation.

TABLE 1. LABORATORY DATA OF BACTERIOLOGICAL FINDINGS

Patient, Male Case Name Age	Blood Specimens		Gastric Juice Specimens					
	Date	Results	Date	pH	Direct Isolation	Guinea Pig Isolation	High Temp	Autopsy Date Tissue Isolation
<u>1956</u>								
1 LAJ 27	15 Oct	Negative	12 Oct	ND ^a /	+	+	103.2	15 Oct
			15 Oct	-	-	-		
			17 Oct	5.0	-	-		
2 RJG 25	16, 17, 25, 26, 29, 31 Oct	Negative Negative Negative	19 Oct	5.5	-	+	104.6	23 Oct
3 DEB 30	7, 8, 9 Nov ^b	Negative	7 Nov	2.0	-	+	104.2	12 Nov
			8 Nov	4.5	+	+	104.4	12 Nov
			9 Nov	6.0	-	+	104.2	13 Nov
4 HPM 27	13, 14, 15, 16 Nov ^b	Negative Negative	15 Nov	7.5	-	-	105.0	24 Nov
			16 Nov	6.0	-	+		
			26 Nov	7.0	-	-		
5 RRR 30	13, 14, 15, 16 Nov ^b	Negative Negative	14 Nov	7.0	-	+	103.4	17 Nov
			15 Nov	2.0	+	-		
			16 Nov	2.0	-	-		
			20 Nov	2.0	-	-		
			26 Nov	7.0	-	-		
<u>1957</u>								
6 EWS 37	27, 28 Feb 1, 4 Mar	Negative Negative	28 Feb	ND	+	+	104.4	4 Mar
			1 Mar	5.0	+	+	105.6	7 Mar
			4 Mar	6.5	-	-		
			6 Mar	2.0	-	-		
7 ENK 22	Omit ^c /		4 Aug	4.5	+	+	103.4	7 Aug
			6 Aug	7.6	+	+	105.6	9 Aug
			9 Aug	7.6	+	+	103.0	13 Aug
			12 Aug	3.5	-	+	106.8	19 Aug
8 MDO 35	Omit		8 Aug	5.0	-	-	102.8	12 Aug
			9 Aug	5.0	-	+		
			12 Aug	4.0	-	-		
9 MED 35	Omit		14 Aug	5.0	-	+	105.0	20 Aug
			17 Aug	2.0	-	-		

a. pH of gastric juice not taken.

b. Twice daily.

c. After repeatedly recording negative results with blood samples, this test was omitted.

d. i.l.n. = inguinal lymph nodes.

III. DISCUSSION

In reviewing the case histories presented in the appendix, it is quite evident that the variability in clinical symptoms could easily prevent correct diagnosis. There are, however, general features typical of all nine of the cases: (i) each infection probably resulted via the respiratory route, (ii) all patients developed symptoms periodically and sporadically, (iii) all exhibited generalized infections, and (iv) all infections were of the typhoid and pulmonic types.

Generally, the urinalyses contributed little to the diagnosis except an occasional albuminuria, and the blood pictures were not remarkable. The range of leukocytosis had been from 6,100 to 14,400 during hospitalization with Case 8 hovering in the normal range; his white blood count (WBC) never exceeded 9,400 throughout illness. In addition, there was evidence of a shift to a high lymphocytic count; the highest was 63%.

There were indications in most of the reported cases that the C-reactive protein responded earlier to the disease process, and it coincided with the therapeutic and clinical recovery when compared with the erythrocyte sedimentation rate (to be published).

In previous tularemia cases, our diagnoses depended mainly on serological techniques; the skin test response and a diagnostic rise in agglutinin titers. However, as shown in Table 2, Cases 5, 7, and 9 did not attain a diagnostic agglutinin rise in titer for P. tularensis or a positive skin test while these patients were hospitalized. The agglutinin titer did rise in Case 5, 30 days after the onset of illness (12 days after discharge from the hospital); in Case 7, it rose at 25 days after onset of illness (the day of discharge); Case 9 did not attain a fourfold rise in titer. Conversely, Case 4 had a diagnostic agglutinin rise in titer on the second day of illness; Case 6 had a positive skin test on the second hospital day (15th day of illness).

The range of positive skin tests among these previously vaccinated patients varied from 9 to 22 days following onset of illness (Table 2); the time required for positive agglutinin diagnostic titers during hospitalization varied from 2 to 25 days. This may indicate that among previously vaccinated individuals the skin test does not appear as early as a diagnostic rise in agglutinin titer and, therefore, might not prove as valuable for a rapid diagnosis. Additional studies are necessary before any conclusions can be drawn, but in only one case was the skin test positive prior to a rise in a diagnostic agglutinin titer.

Evidence that serial roentgenograms can be useful⁴⁸ is adequately shown in Cases 4 and 5, where progressive pulmonic infiltrates were noted and vividly illustrated the course of the disease.

TABLE 2. LABORATORY DATA ON IMMUNIZED PATIENTS WITH TULAREMIA

Case	Name	Patient, Male Age	Tularemia Diagnosis	Date of			Discharge	Agglutinin Titer Range (Hospitalized period)	Skin Test with <i>P. tularensis</i> 1:1000	
				Illness	Hospital	Therapy			Date Administered	Reaction After 48 hr
<u>1956</u>										
1	LAJ	27	Pneumonic with hilar adenopathy	1 Oct	11 Oct	15 Oct	13 Nov	1:80 - 1:2560	22 Oct	Positive
2	RJG	25	Typhoidal with hilar adenopathy	8 Oct	16 Oct	19 Oct	30 Nov	1:80 - 1:2560	22 Oct	Positive
3	DEB	30	Typhoidal	1 Nov	7 Nov	9 Nov	26 Nov	1:160 - 1:1280	7 Nov 18 Nov	Negative Positive
4	HPM	27	Pneumonic	11 Nov	14 Nov	None	28 Nov	1:320 - 1:2560	19 Nov	Positive
5	RER	30	Pneumonic	11 Nov	13 Nov	17 Nov	28 Nov	1:80 - 1:160	19 Nov	Negative
<u>1957</u>										
6 ^{a/}	EMS	37	Pneumonic	13 Feb	27 Feb	28 Feb	25 Mar	1:40 - 1:640	28 Feb	Positive
7 ^{b/}	EMK	22	Typhoidal	2 Aug	3 Aug	7 Aug	26 Aug	1:40 - 1:14	5 Aug 6 Aug 7 Aug 12 Aug 14 Aug 16 Aug	Negative Negative ^{c/} Negative Negative Negative Negative
8	MDO	35	Typhoidal	4 Aug	7 Aug	9 Aug	26 Aug	1:10 - 1:320	7 Aug 24 Aug	Negative Positive
9	MED	35	Typhoidal	12 Aug	13 Aug	13 Aug	22 Aug	1:320 - 1:320	14 Aug 16 Aug 19 Aug 21 Aug	Negative Negative Negative Negative

a. Glassware worker who was not actively engaged in direct laboratory research with *P. tularensis*.

b. Patient relapsed and was readmitted 30 Aug; positive gastric isolation of *P. tularensis* 1 Sept; skin test positive and titer of 1:1280 on 3 Sept.

c. Skin test 1:100 *P. tularensis*.

Unfortunately, the incubation periods of these cases cannot be pinpointed. However, Case 2 came in contact with P. tularensis only 4 days prior to febrile illness; Case 3 possibly was involved in a laboratory accident 13 days prior to his febrile illness; Case 4 probably was implicated in the same laboratory accident, which caused his illness after 25 days; and Case 8 possibly contracted P. tularensis only 5 days prior to the onset of illness. This would give a presumptive range of 3 to 25 days as an incubation period among the previously immunized patients. The wide variation was probably due to the dosage of P. tularensis each case received, and the relationship of this dosage to the host protective mechanism. Case 7 was particularly interesting in this aspect. Because of the early reporting of P. tularensis by isolation in 48 hours from a gastric juice specimen, therapy of tetracycline was initiated and continued for 10 days. The agglutinin titer remained at a 1:40 level throughout the initial hospitalization, and his skin test was consistently negative. His symptoms subsided and he was completely asymptomatic upon discharge. Four days later, he was re-admitted to the hospital, and P. tularensis was again isolated from his gastric specimen. Subsequently, his skin test converted to positive, and his titer for P. tularensis was 1:1280.

This indicated that the therapy of tetracycline had clinically retarded the infection and the protective antibody buildup by eliminating the P. tularensis in the extrinsic body fluids, but did not eliminate these organisms in their intrinsic cellular environment. This subsequently led to the recurrence of the infection after cessation of therapy and subsequent lowering of the antibiotic blood level.

In all cases except Case 4, however, therapy of tetracycline proved beneficial, although there were indications that the patient may have recovered without its use. Generally, the patients became afebrile 24 to 48 hours after tetracycline administration, and serial X-ray films reveal the therapeutic effect on the pulmonic patient. The C-reactive protein tests also showed this correlation. Earlier, Foshay and Pasternack,⁵⁰ Francis,⁵¹ and Ray and Warren⁵² had attested to the effects of streptomycin therapy; Arun⁵³ had revealed success with chlorotetracycline and oxytetracycline. Recently, Overholt et al.⁵ analyzed the treatment of 42 cases of laboratory-acquired tularemia with broad-spectrum antibiotics.

It is evident from Table 1 that blood cultures were not a useful diagnostic tool for us. Thus, Cases 7, 8, and 9 were omitted from attempted blood culture isolation of P. tularensis. However, in the previous cases, blood specimens were taken before, after, and during the same period as the gastric juice specimens, and we were not successful in isolating P. tularensis from the blood even though we utilized the same inoculation media, incubation periods, and laboratory animals. An antibiotic therapeutic blood level could not have interfered with our attainment of positive isolations because blood cultures were taken as early as three days prior to therapy; Case 4 received no therapy during his 15 days of hospitalization.

The cultures from the gastric juice specimens, on the other hand, have been rewarding. P. tularensis was isolated from the gastric juice 2 to 16 days after the onset of illness, and 1 to 6 days after the initiation of therapy (Cases 6, 7, and 9). But of paramount importance has been the reporting to the physician of a positive isolation of P. tularensis from the gastric specimen after 48 hours by direct culture (Case 7). Generally, a positive diagnosis by isolation of the P. tularensis microorganism from the gastric juice specimens occurred within one week after hospitalization of the patient, which was well within his course of illness. This more rapid diagnostic technique has been a valuable aid to our clinical diagnosis of tularemia. Only one case, Case 5, had any abdominal distress during the entire hospitalization of the nine reported cases.

Several interesting observations have been made regarding the animal pathology and the direct culturing of gastric juice specimens from which P. tularensis has been isolated. First, the authors never suspected that P. tularensis could be recovered at the pH range shown in Table 1, especially from a low pH range of 2.0 to 5.0. These isolations were, however, analogous to the in vitro studies of Savel'yera⁵⁴ in determining the bactericidal effect of man's natural gastric juice upon tularemia microorganisms. In addition, King and MacCabe⁵⁵ had previously described a virulence enhancement factor of gastric mucin. We thought that a mucous encapsulation of the tularemia microorganisms protected them in this acid environment, and for longer periods than two hours (the criteria set by Savel'yera). Secondly, P. tularensis was isolated from gastric juice specimens taken from various patients between 7:30 AM and 2:00 PM. However, repeated sampling from the same patient was never attempted in order to establish the viability of P. tularensis in the stomach contents per unit of time.

The reasoning behind using intraperitoneal plus subcutaneous inoculation of the gastric specimen into each of two guinea pigs was that the microorganisms were generally of a low magnitude in the specimen; with this method, 4.0 ml of the specimen could be injected into these animals. This gave a better opportunity to isolate P. tularensis from the sample because a higher proportion of the specimen would be utilized. P. tularensis was isolated from the guinea pig liver, spleen, inguinal lymph nodes, and heart blood (Table 1).

Our laboratory prefers use of guinea pigs in tularemic infections rather than mice or other laboratory animals because of ease of handling and measuring temperatures. Comparative experimental gastric specimen inoculation into rabbits, hamsters, guinea pigs, mice, and rats revealed fewer deaths in guinea pigs because of the presence in the tularemia specimen of other organisms. It appeared that the guinea pig filtered the undesirable microorganisms, permitting P. tularensis to gain control. Mice are especially good when injected with a pure culture of P. tularensis; however, we were losing at least 20% of our mice from extraneous infections

when they were injected with gastric juice specimens. The guinea pig generally is used in all medical hospital laboratories, and can accept a relatively large sample of injected specimen. Rectal temperatures of guinea pigs proved of value also, as shown in Table 1.

It has been our experience with guinea pigs infected by gastric juice containing P. tularensis that the highest average rectal temperature attained was 104.4 F during a 3-day incubation period. This was in direct contrast to our experience using samples from ulceroglandular cases of tularemia or P. tularensis in pure culture, because those injected guinea pigs spiked temperatures of 105.5 to 106.0 F in 3 days. However, these higher temperatures could have been caused by a greater number of organisms present in the latter instances, since the injected material would also have been practically a pure culture with little or no extraneous matter to influence the temperature. High temperatures of 104.6, 105.0, 105.6, and 106.8 F (Table 1) were attained in infected guinea pigs on the 4th, 7th, 7th, and 5th day of illness, respectively.

IV. CONCLUSIONS

Pasteurella tularensis was isolated from the gastric juice of nine patients. Five were diagnosed as tularemia, typhoidal type, and four as tularemia, pneumonic type (pulmonic). These cases were infected by the respiratory route.

The inoculation of guinea pigs with gastric juice concentrate from patients suspected of having tularemia is recommended as a laboratory or research procedure, as is the replicate direct plating of the concentrate on blood cysteine glucose agar, using the isolation techniques described.

This procedure has been a rapid method of diagnosing pulmonic and typhoidal types of tularemia in previously immunized patients.

LITERATURE CITED

1. Moss, E.S.; Weilbaecher, J.O., Jr. 1941. Recent advances in the diagnosis and treatment of tularemia. J. South. Med. Ass. Ala. 34:512-517.
2. Overholt, E.L.; Tigertt, W.D.; Kadull, P.J.; Ward, M.K.; Charkes, N.D.; Rene, R.M.; Salzman, T.E.; Stephens, M. 1961. An analysis of forty-two cases of laboratory-acquired tularemia: Treatment with broad spectrum antibiotics. Amer. J. Med. 30:785-806.
3. Foshay, L.; Mayer, O.B. 1936. Viability of Bacterium tularense in human tissues. J. Amer. Med. Ass. 106:2141-2143.
4. Asgaard, G.N. 1944. Involvement of the heart in tularemia. Minnesota Med. 27:115-121.
5. Foshay, L. 1937. Cause of death in tularemia. Arch. Int. Med. 60:22-38.
6. Kitzmiller, K.V. 1939. Tularemia: A pathological study of the lesions in a case treated with specific antiserum, the patient dying suddenly from intercurrent coronary occlusion. Ann. Int. Med. 12:1375-1381.
7. Haizlip, J.O.; O'Neil, A.E. 1931. A case of meningitis due to Bacterium tularense. J. Amer. Med. Ass. 97:704-705.
8. Glass, G.B.J. 1948. Tularemic meningitis and serous meningitis. Med. Clin. N. Amer. 32:769-778.
9. Bryant, A.R.; Hirsch, E.F. 1931. Tularemia leptomeningitis: Report of a case. Arch. Pathol. 12:917-923.
10. Stuart, B.M.; Pullen, R.L. 1945. Tularemia meningitis. Arch. Int. Med. 76:163-166.
11. David, J.K.; Owens, J.N. 1944. Tularemic meningitis: Report of a case and summary of previously reported cases. Amer. J. Dis. Child. 67:44-48.
12. Pessin, S.B. 1936. Tularemic pneumonia, pericarditis and ulcerative stomatitis. Arch. Int. Med. 57:1125-1144.
13. McGovern, F.H. 1936. Primary tularemic ulcers in pharynx. J. Amer. Med. Ass. 107:1629-1630.
14. Morgan, H.J. 1947. Pleuropulmonary tularemia. Ann. Int. Med. 27:519-528.

15. Anschuetz, R.R. 1941. Tularemia with extensive pharyngitis. Amer. J. Dis. Child. 62:150-153.
16. Goodyear, H.M. 1940. Tularemia: Assimilating symptoms of an acute ethmoid, splenoid infection. J. Med. 21:81-82.
17. Beck, H.G.; Merkel, W.C. 1935. Tularemia: Fatal case of the typhoid form caused by ingestion of rabbit: Autopsy report. Southern Med. J. 28:422-428.
18. Kennedy, J.A. 1942. Pulmonary tularemia. J. Amer. Med. Ass. 118: 781-787.
19. Bunker, C.W.O.; Smith, E.E. 1928. Tularemia, report of four cases, one fatal with autopsy. U.S. Navy Med. Bull. 26:901-911.
20. Kavanaugh, C.H. 1935. Tularemia. Arch. Int. Med. 55:61-85.
21. Warring, F.C.; Cullen, V.F. 1936. Tularemia with pleural effusion. J. Amer. Med. Ass. 107:1365-1367.
22. Blackford, S.D. 1935. Pulmonary manifestations in human tularemia. J. Amer. Med. Ass. 104:891-895.
23. Blackford, S.D.; Casey, C.J. 1941. Pleuropulmonary tularemia. Arch. Int. Med. 67:43-71.
24. Richards, C.G. 1942. Tularemia with pulmonary complications. Ann. Int. Med. 17:78-82.
25. Blackford, S.D.; Archer, V.W. 1937. Roentgen study of a non-fatal case of bilateral tularemic pneumonia treated with specific serum. J. Amer. Med. Ass. 109:264-265.
26. Sante, L.R. 1931. Pulmonary infection in tularemia: Case report. Amer. J. Roentgenol. 25:241-245.
27. Permar, H.H.; MacLachlan, W.W.G. 1931. Tularemic pneumonia. Ann. Int. Med. 5:687-688.
28. Fetterman, G.H.; Lerner, H. 1936. A fatal case of tularemic pneumonia with associated ileitis. J. Lab. Clin. Med. 21:1157-1161.
29. Foshay, L. 1932. Tularemia: Accurate and earlier diagnosis by means of the intradermal test. J. Infect. Dis. 51:286-291.
30. Wherry, W.B.; Lamb, B.H. 1914. Infection of man with Bacterium tularense. J. Infect. Dis. 15:331-340.
31. Freese, H.L.; Lake, S.C.; Francis, E. 1926. Four cases of tularemia (3 fatal) with conjunctivitis. Public Health Rep. 41:369-372.

32. Francis, E. 1942. Oculoglandular tularemia. Arch. Ophthalmol. 28:711-741.
33. Zembrzuski, K. 1955. Forty-two tularemia cases. Pol. Arch. Med. Wewn. 25:377-386.
34. Ransmeier, J.C.; Schant, I.G. 1941. Direct cultivation of Bacterium tularense from human blood drawn during life and at autopsy. Arch. Int. Med. 68:747-762.
35. Francis, E. 1921. The occurrence of tularemia in nature as a disease of man. Public Health Rep. 36:1731-1738.
36. Simpson, W.M. 1928. A clinical and pathological study of forty-eight non-fatal cases and one rapidly fatal case with autopsy occurring in Dayton, Ohio. Ann. Int. Med. 1:1007-1059.
37. Francis, E.; Callender, G.R. 1927. Tularemia: The microscopic changes of the lesions in man. Arch. Path. 3:577-607.
38. Gundry, L.P.; Warner, C.G. 1934. Fatal tularemia: Review of autopsied cases with report of a fatal one. Ann. Int. Med. 7:837-852.
39. Lufkin, N.H.; Evenson, A.E. 1937. Tularemia diagnosed by "routine" blood culture. J. Lab. Clin. Med. 22:346-350.
40. Murphy, J.L. 1930. Tularemia in Michigan: Report of a case. J. Mich. Med. Soc. 29:917-925.
41. Shaw, F.W.; Hunnicutt, T. 1930. Direct isolation of Bacterium tularense from axillary process. J. Lab. Clin. Med. 16:46-48.
42. Gudger, J.R. 1933. Tularemic pneumonia: Report of a fatal case. J. Amer. Med. Ass. 101:1148-1150.
43. Foshay, L. 1940. Tularemia: A summary of certain aspects of the disease including methods for early diagnosis and results of serum treatment in 600 patients. Medicine 19:1-83.
44. Larson, C.L. 1945. Isolation of Pasteurella tularensis from sputum. Public Health Rep. 60:1049-1053.
45. Johnson, H.N. 1944. Isolation of Bacterium tularense from the sputum of an atypical case of human tularemia. J. Lab. Clin. Med. 29:903-905.
46. Ashburn, L.L.; Miller, S.E. 1945. Tularemia: A report of a laboratory infection. Arch. Pathol. 39:388-392.

47. Khatenever, L.M. 1941. Tularin as a preparation of specific and early tularemia diagnostics. Arch. Biol. Nauk. 61:3-12.
48. Overholt, E.L.; Tigertt, W.D. 1960. Roentgenographic manifestations of pulmonary tularemia. Radiology 74:758-762.
49. Stuart, B.M.; Pullen, R.L. 1945. Tularemic pneumonia: Review. Amer. J. Med. Sci. 210:223-236.
50. Foshay, L.; Pasternack, A.B. 1946. Streptomycin treatment of tularemia. J. Amer. Med. Ass. 130:393-398.
51. Francis, E. 1947. Streptomycin in the treatment of tularemia. Trans. Ass. Amer. Phys. 60:181-186.
52. Ray, E.S.; Warren, S. 1952. Tularemic lung abscess: A case report. Amer. Rev. Tuberc. 65:627-630.
53. Arun, N. 1954. Clinical research on cases of tularemia in the region of Antalya. Anadolu Klinigi 20:97-99.
54. Savel'yera, R.A. 1956. The bactericidal effect of natural gastric juice of man upon tularemia microorganisms. J. Microbiol. Epidemiol. Immunobiol. 27:42-47.
55. King, H.K.; MacCabe, A.F. 1949. The Airulives - enhancement factor of gastric mucin. J. Gen. Microbiol. 3:xi-xii.

APPENDIXCASE STUDIES OF NINE PATIENTS INFECTED WITH PASTEURELLA TULARENSIS

A. CASE 1

LAJ, a 27-year-old white male laboratory worker, became ill on 1 October 1956 with night sweats, dysphagia, muscle aches, and pleuritic chest pains in the left axillary region. The following day he developed chilly sensations, but all symptoms subsided except the chest pains. At no time during his illness did he have a cough, malaise, headache, or anorexia. On 8 October his physical examination revealed normal breath sounds but a questionable increase in percussion on the left side of the chest. Chest X-rays showed (i) a distinct round, lobulated enlargement of the left hilar area (2 to 3 cm in diameter), with increased broncho-vascular markings radiating from this enlargement, and (ii) a left pleural effusion. On October 9 he complained of feverishness (oral temperature 100 F) and night sweats. His breath sounds and resistance to percussion at the left base had decreased.

These physical findings persisted on 10 October. On 11 October he was hospitalized.

The patient had a history of working with P. tularensis in the laboratory, and was initially vaccinated in April 1951; his last booster was given in 1952. Since that time, he had maintained a constant but low agglutinin titer for P. tularensis until the time of illness. His physical condition appeared normal except for decreased breath sounds, dullness, and absence of fremitus in the lower left axilla. Small, shoddy cervical nodes were symmetrically distributed in the anterior and posterior triangles. Two nodes (approximately 0.5 cm in diameter), firm and nontender, both movable, also shoddy, were found in the left axilla.

On 8 October, 8 days after the onset of illness, the patient's white blood count (WBC) was 9,200; erythrocyte sedimentation rate (ESR) was 22 mm per hour; C-reactive protein (CRP) was 4+; urinalysis was normal; agglutinin for P. tularensis was 1:80. His previous titer, in July 1956, was 1:40.

When hospitalized on 11 October, his WBC was 12,600, and six consecutive WBC's ranged from 6,450 to 10,100 until his discharge (Table 2). His ESR reached a peak of 35 mm per hour on the date of hospitalization and 8 mm per hour at the discharge date. The CRP was negative on his discharge date. Agglutinin titer for P. tularensis reached 1:640 and 1:1280 on 22 and 26 October, respectively, and 1:2560 on 9 November. Skin test 1:1000 P. tularensis on 22 October was positive in 48 hours.

The chest roentgenograms of 8 October revealed enlarged hilar nodes on the left side but these rapidly diminished during the patient's hospitalization. An X-ray taken on 22 October revealed a clear left costophrenic angle, only minimal enlargement of the left hilar nodes, and increased markings; one on 9 November was completely within normal limits.

At the time of admission, 11 October, the patient's temperature was 102 F, pulse 100, and respirations 24. The chest film revealed possible symptoms of tuberculosis, and because there was no productive sputum, a gastric fluid specimen was obtained on 12 October for culture and inoculation. This material was cultured on blood cysteine glucose agar as well as on Petragnani's medium and inoculated into guinea pigs prior to being digested by the Hanks' method.

P. tularensis was recovered in pure culture from the plated medium and from autopsied guinea pigs' liver, spleen, and inguinal lymph nodes. The pH and volume of this specimen of gastric juice were not recorded. Subsequent isolations from the gastric fluid were attempted on 15 and 17 October without success. A blood culture taken on 15 October was negative.

The patient's condition was therefore diagnosed as tularemia. On 15 October he was administered tetracycline, 2 grams a day for 10 days. Although treatment had begun, the patient's temperature was dropping toward normal; his temperature was never above 99 F after the first 24 hours of therapy. Nevertheless, his pleuritic chest pain continued until 23 October. Following discharge (Table 2),* the patient complained of fatigue and myalgia for approximately one month, but when examined on 26 November he felt normal and his chest film read within normal limits.

Diagnosis: Tularemia, pneumonic with hilar adenopathy.

B. CASE 2

RJG, a 25-year-old negro male laboratory technician (Table 2) became ill with fever and night sweats on 8 October 1956. Moderate anorexia and headache (frontal) developed gradually with peaks of fever reaching 100 to 101 F orally 3 to 4 days prior to hospitalization (16 October). The patient had no cough or chest pains, lost no weight, but did have profuse diaphoresis at night.

RJG came in contact with P. tularensis 4 days prior to febrile illness. Immunization was initiated in February 1953 and from that date no additional booster injections were given because he carried a constant but low agglutinin titer of 1:40 for P. tularensis until his illness. His last agglutinin titer

* Tables referred to in the appendix are those shown in the text.

was 1:40 on 27 June 1956. On 10 October, the third day of illness, the patient's WBC was 6,750; ESR, 13 mm per hour; CRP, 3+; urinalysis was negative; temperature of 97.6 F (oral) and pulse of 72. On 16 October, the ninth day of illness and date of hospitalization, the WBC was 8,250; ESR, 26; CRP, 3+; and agglutinin titer for P. tularensis was 1:80. Chest X-ray films showed no specific change compared with older films; physical examination was entirely within normal limits, but the patient was experiencing a moderate diaphoresis.

Eleven successive WBC's from 18 October to 30 November ranged from 5,850 to 11,500. The patients' ESR reached a peak of 38 mm per hour on 22 October, and was 27 mm per hour when he was discharged, 30 November; the CRP was 4+ and negative, respectively.

RJG's P. tularensis agglutinin titer increased from 1:80 (16 October) to 1:640 on 22 October; 1:1280 on 26 October, and finally 1:2560 on 5 November. Skin test of 1:1000 P. tularensis administered on 22 October was positive 48 hours later; purified protein derivative (PPD) skin tests for tuberculosis, first and second strengths, were negative; and brucellergin skin test was negative.

Serial chest X-rays of lungs on 16, 18, 22, 26, and 29 October, and on 5, 15, 27, and 30 November 1956 evidenced no significant pulmonary involvement.

Results from successive blood cultures taken twice daily and from cultures of gastric specimens, including the pH, are shown in Table 1. Gastric specimens were difficult to obtain. Even with topical anesthesia in the nasopharyngeal passages, no direct tube specimen was produced. However, approximately 10 to 15 ml of mucoid, tenacious fluid, opalescent with a slight greenish tinge were obtained by retching (19 October).

The patient's temperature was 101 F, 102.2 F, and 100.2 F on 16 October, and in the afternoons of 17 and 18 October, respectively. Antibiotic therapy of Chloromycetin, 2 grams per day for 10 days, was initiated on 19 October; then the fever gradually subsided after 48 hours. However, from 24 to 26 October he again had an afternoon temperature from 100 to 101 F. Therapy of penicillin, 300,000 units procaine daily initiated on 26 October reduced his temperature to 99.0 F. From that date his oral and rectal temperatures were considered normal.

On 21 October, a firm, nontender, round mass, 4 by 4 cm, appeared that was not attached to the skin but fixed to the right mandible anterior to the right angle, and involved the submaxillary area as well as the maxilla, over-riding the mandible. X-rays were essentially normal. A slight swelling occurred around the mass, and the submental node increased in size slightly but decreased rapidly with therapy of Chloromycetin and penicillin. The mass was not fluctuant, did not develop heat or redness,

or disturb the floor of the mouth; no alveolar ridge, tonsillitis, pharyngeal or salivary duct inflammation, or definite lesion on the mandible was evident.

The patient was referred to The Johns Hopkins University Hospital, where on 12 November a culture from this excised mass revealed P. tularensis. After the operation, the patient spiked daily elevations of temperature to 101 F for approximately 3 to 4 days. His temperature gradually returned to normal after therapy of tetracycline. The patient was discharged on 30 November 1956.

Diagnosis: Tularemia, typhoidal type, with regional lymph node enlargement over the right mandible.

C. CASE 3

DEB, a 30-year-old white male laboratory technician, had been working with P. tularensis for a month and became ill on 1 November 1956 with fever, stiff neck, pain in the shoulder, and profuse sweating. The following day his physical examination revealed only a boggy pharynx; a throat culture disclosed a heavy growth of alpha-hemolytic streptococci and Neisseria. On the fifth day of illness, DEB's physical examination revealed a few rales at the right posterior axillary line; otherwise, results were negative. His symptoms of fever, oral temperatures of 102 to 103 F in the afternoon, which returned to normal 5 to 8 hours later, and his intermittent frontal headaches became progressively more severe.

The patient's WBC was 12,500; ESR, 34; CRP, 3+; and agglutinin titer for P. tularensis was 1:80. X-ray of the chest revealed no significant changes.

When hospitalized, (Table 2), the patient still had throbbing frontal headaches (especially in the morning) that extended into the temporal areas; pain developed behind his eyes and in back of his legs. He felt weak. The patient's WBC was 10,200; ESR 28 mm per hour; C-reactive protein was 4+; and his urinalysis revealed a trace of albumin.

Repeated WBC's ranged between 7,500 and 13,500. On the 26th day of illness, the patient's ESR was 20 mm per hour, and his C-reactive protein was negative. His P. tularensis agglutinin titer was 1:160, 1:320, 1:640, and 1:1280 on 7, 13, 14, and 19 November, respectively. It reached a peak of 1:2560 on 4 December.

DEB was initially immunized in February 1955, with booster injection series in August 1955. He maintained a titer of 1:80 to 1:320 until 23 October 1956. All skin tests included in this period were negative. Seven chest films taken during his hospitalization were within normal limits.

P. tularensis was isolated from gastric juice specimens cultured directly onto blood cysteine glucose agar plates, and by animal inoculation and pathology (Tables 1 and 2). Results of skin test conversion are shown in Table 2. Blood cultures taken on consecutive days were negative (Table 1).

Therapy of tetracycline, 0.5 gram four times daily continued for 7 days, was initiated on 9 November. The day following initiation of therapy, the patient became completely afebrile, and remained afebrile until discharged. His headache subsided 48 hours after therapy. Repeated physical examinations during hospitalization were completely within normal limits. He was discharged asymptomatic (Table 2).

Diagnosis: Tularemia, typhoidal type.

D. CASE 4

HPM was a 27-year-old negro male who had chilly sensations and a temperature slightly above 99.6 F on 11 November 1956. During the following two days of work, the chilly sensations and low-grade fever continued, so he reported to the hospital.

HPM's oral temperature on 13 November was 101.2 F; WBC was 10,100 with 43% segmented neutrophils, 13% banded neutrophils, 34% lymphocytes, 8% monocytes, 1% basophils, and 1% eosinophils; urinalysis was not abnormal; ESR was 26 mm per hour; P. tularensis agglutinin titer was 1:320. The patient's physical examination, including chest and lungs, was completely negative. He denied fatigue, malaise, headache, cough, or anorexia. Chest X-rays revealed a diffuse, soft density, presumably located in and around the apex of the left lower lobe of the lung.

When HPM was hospitalized (14 November), he appeared neither acutely nor chronically ill; no complaints (chills and fever disappeared); his physical examination was completely within normal limits. A month prior to his illness, the patient had been working with P. tularensis, and he was involved in a laboratory accident.

Because the patient was completely asymptomatic, therapy was not instituted. However, on the third day of illness the chest X-ray revealed an infiltrating lesion in the apex of the left lobe that reached its maximum on the following day, 14 November, and subsequently slowly subsided. On 28 November, there was no evidence of the previous pulmonary infiltrate, and the film was within normal limits. Physical examination on the second and third hospital days revealed rales in the left posterior chest, just medial to the scapula, otherwise the physical examination was always within normal limits. Lymph nodes were unremarkable.

During hospitalization, five white blood counts ranged between 6,200 and 9,100; the ESR reached a peak of 46 mm per hour on 16 November, then declined to 26 mm per hour by 23 November, at which time his CRP was negative. P. tularensis agglutinin titers were 1:320, 1:640, 1:1280, and 1:2560 on 14, 15, 16, and 19 November, respectively.

On the second, third, and thirteenth hospital day, gastric juice specimens were obtained for culture. Results from these cultures, blood cultures, and skin tests are shown in Tables 1 and 2.

This patient was immunized in June 1956, but had not received boosters. His titer on 19 October was 1:80; on 13 November it was 1:320, a fourfold rise in titer, and reached a peak titer of 1:2560 on 19 November. No therapy was instituted.

Diagnosis: Tularemia, pulmonic.

E. CASE 5

RBR, a 30-year-old white male laboratory technician, complained of fever, shaking chills, malaise, anorexia, and chest pain of three days' duration. On 11 November 1956 he developed sneezing, head cold, sore throat, loss of appetite, and a running nose, but did not discontinue work. The following day, sneezing and rhinorrhea ceased but he became feverish, 102 to 103 F orally, and had intermittent shaking chills without excessive sweating. The patient also developed a dull, epigastric pain accentuated by deep inspiration and coughing. He spent most of the day in bed, was very weak, tired easily, showed marked diminution in appetite, and developed slight headache.

On 13 November the latter symptoms persisted; weakness and fatiguability were more severe, so the patient was hospitalized. RBR had worked with P. tularensis, and was first immunized in 1951. Since that time he had a continuous agglutinin titer range of 1:20 to 1:80 that persisted for 4 years. In December 1955, his skin test was positive, and his last agglutinin titer prior to hospitalization was 1:80 on 2 August 1956.

Physical examination upon admission was within normal limits except there was slight tenderness without spasms in the left upper quadrant. RBR appeared moderately to acutely ill with a fever of unknown origin. His WBC was 12,800; ESR, 26 mm per hour; CRP, 2+; urinalysis was normal; chest X-ray was negative to abnormalities.

Seven additional white blood counts in the next 15 days ranged between 7,000 and 10,650. His agglutinin titer was 1:80 on 14 November; 1:160 on 16 November, and remained at 1:160 during hospitalization. Not until 10 December (13 days after discharge) could a significant increase in titer to 1:1280 for P. tularensis be demonstrated.

The patient's sedimentation rate reached a high of 45 mm per hour on 19 November, and was 20 mm per hour on 28 November, at which time his C-reactive protein was negative. On the following day, a roentgenogram of the chest (normal on 13 November) revealed two patchy infiltrates in the left upper and lower middle lung fields, respectively. These lesions progressed to a maximum on 21 November, then continuously regressed until the last chest X-ray on 28 November showed a 1-cm patch of parenchymal involvement in the first interspace, left apex, and left middle lung field at the third interspace.

Results of cultures from gastric and blood specimens, skin tests, and other pertinent data are shown in Tables 1 and 2.

During hospitalization, RBR's temperature reached 102 F rectally on the first 5 hospital days. On 17 November tetracycline was administered, 2 grams per day by mouth, for 7 days. Twenty-four hours after therapy, the patient's temperature was 99.3 F rectally; he was afebrile after that date. The patient's anorexia, malaise, and fatigue disappeared before therapy, but his epigastric pain, left upper quadrant, abdominal tenderness, cough and sputum continued until 4 or 5 days after therapy.

Except for abdominal tenderness to deep palpation, physical examinations during his hospitalization were always within normal limits. He developed a sore throat on the 5th and 6th hospital days, but when discharged on 28 November, after 12 days' hospitalization, he was entirely asymptomatic.

A tularemia 1:1000 skin test performed on the 19th of November was negative.

Diagnosis: Tularemia, pneumonic type.

F. CASE 6

EWS, a 37-year-old white male laboratory worker, developed nasal stuffiness and a throbbing headache, the latter persisting from approximately 13 to 18 February. He was treated for a common cold at this time but on 25 February he developed chilly sensations followed by fever and sweats that persisted intermittently for 3 days. In addition, he experienced severe throbbing frontal headaches, stiffness of the joints, low backache, myalgias, slight dysuria, and chest pain from coughing.

A physical examination on 26 February revealed rales and rhonchi of the lungs in the lower right chest area. His temperature was 102 F, pulse 120; WBC was 10,800; ESR was 31 mm per hour; hematocrit of 47; trace of albumin in the urine; hemoglobin of 14.9 grams per cent; CRP, 4+; and a 1:40 agglutinin titer for P. tularensis. A chest X-ray revealed a soft 1.0-cm patch in the second left interspace next to the hilar area and was compatible with an early bronchopneumonia.

The patient did not work directly with P. tularensis, but cleaned areas and equipment utilized by personnel working with this microorganism.

Successive WBC's ranged from 9,550 to 14,400; the ESR reached a peak of 42 mm per hour on 4 March, and declined to 8 mm per hour on 15 March; C-reactive protein was 4+ on admission and subsequently declined to negative on 15 March; urinalysis was unremarkable except for a 1 March report of 1+ albumin, trace of sugar, and 20 to 25 WBC's with a few clumps, per high power microscopic field. The patient had a titer of 1:40 for P. tularensis when hospitalized 27 February; it increased progressively to 1:80, 1:160, 1:320, and 1:640 on 8, 11, 15, and 25 March, (the day of discharge) respectively.

Initial immunization for P. tularensis began in January 1954, and boosters were given in December 1954, after which time the patient maintained an average titer of 1:80 prior to hospitalization. Skin tests remained negative. However, on EWS's second hospital day, a P. tularensis skin test of 1:1000 produced a positive reaction in 48 hours. A brucella-gin skin test 1:1000, and a PPD (strength 1) skin test were given 28 February, and were negative 2 days afterward. On 4 March, a PPD (strength 2) produced a positive reaction 2 days later.

During serial chest roentgenograms, the left upper lobe infiltrate measured 2 to 3 cm in diameter on 28 February, started decreasing, then on 8 March decreased rapidly until 25 March when only a small irregular infiltrate remained.

Because of the rapid progression of the left lung infiltrate on 28 February, therapy of tetracycline, 2.0 grams per day, was immediately initiated, and continued for 16 days. For the first 2 hospital days, the patient felt fatigue, dysuria, anorexia, and weakness; thereafter these symptoms were absent. Chills, fever, and myalgias were experienced only on the day of admission. Headaches and sweats persisted for 4 and 5 days, respectively, after hospitalization. Nasal stuffiness was a symptom until 6 March, and the patient had a productive cough which gradually disappeared on 3 March. A nonproductive cough persisted until 11 March; then it completely disappeared. On 2 March the patient experienced, for the first time, a dull ache across the left anterior middle chest area from the sternum across the pectoralis muscle to the left axilla. It occurred intermittently, lasted only a few seconds, was not increased by deep inspiration or coughing, and was last observed on 11 March.

Physical examination on each hospital day was unimpressive. During the first 9 days there was a slight inflammation of the nasal mucosa. Otherwise there were no definite clinical findings throughout the hospitalization. Incidental findings were an A-V nodal rhythm by electrocardiogram, which was found on admission and again on 20 March.

Blood cultures taken on 27 and 28 February, and 1 and 4 March were negative both culturally and by animal inoculation. Gastric specimens (Table 1) taken on 28 February and 1 March were positive for P. tularensis by direct culture and animal pathology. Successive gastric specimens on 4 and 6 March were negative.

The patient was discharged from the hospital on 25 March, and except for a head cold, he felt well.

Diagnosis: Tularemia, pneumonic.

G. CASE 7

EMK, a 22-year-old white male bacteriologist was hospitalized 3 August with the chief complaint of headache and weakness of one day's duration. The patient was well until about 5 PM on 2 August 1957, when he experienced a bilateral temporal headache, which persisted and increased by any head motion. He slept poorly at 1- to 1½-hour intervals during the night.

On the morning of 3 August he experienced an onset of fever, sweats, myalgias, and remained near his bed all day. He became generally weak; occasionally his limbs would become "numb" for 10 to 20 minutes. These symptoms gradually increased in severity until he reported to the hospital later that evening.

The patient works with P. tularensis in the laboratory. An immunization against P. tularensis was initiated in March 1957, and boosters were never received prior to his hospitalization. EMK maintained a titer of 1:40 prior to admission; his skin tests were negative.

EMK's past history was noncontributory except that he had had migraine headaches since adolescence. A physical examination given at the time of admittance revealed only minimal infection of the nose and pharynx with no exudate nor pustule formation. His temperature was 103.6 F; pulse, 100; blood pressure, 120/70; he was diaphoresing excessively, and felt warmer than usual. No lymphadenopathy; lungs were clear; heart and abdomen were normal, and neurologically physiological.

The following day, 4 August, his WBC was 7,800; ESR was 4 mm per hour; CRP was 4+; and agglutinin titer for P. tularensis was 1:40.

Successive WBC's ranged from 12,000 on 5 August to 8,350 on 16 August; the ESR reached a high of 44 mm per hour on 9 August, and a low of 13 on 12 August, at which time his CRP was negative. During EMK's hospitalization, he did not have a significant rise in agglutinin titer to P. tularensis in that it remained at the 1:40 level. On the 26th of August while being treated in the outpatient department, his titer reached 1:320, and finally

1:1280 on 3 September. Repeated P. tularensis skin tests on 5, 6, 7, 12, 14, and 16 August, (the 4th, 5th, 6th, 11th, 13th, and 15th days of illness, respectively) were negative.

On 6 August, chest X-rays revealed no evidence of pneumonitis, although the chest film of 7 August revealed a radiodensity near the costochondral junction of the right fifth rib. Serial films of 8 and 9 August demonstrated continual regression, and those of 13 and 15 August showed clear lung fields with normal heart and mediastinal shadows.

Gastric specimens obtained on 4, 6, 9, and 12 August had a pH of 4.5, 7.6, 7.6, and 3.0, respectively. All gastric samples were positive for P. tularensis by animal inoculation and pathology; only the 12 August sample was negative by direct culture isolation (Table 1).

The laboratory reported positive isolation of P. tularensis from gastric juice specimen culture on 6 August, just 48 hours after receiving the 4 August specimen.

Because of a positive isolation, therapy of tetracycline, 2 grams per day, was initiated on 7 August. Prior to therapy the patient's fever appeared to be regressing and his headache had subsided. However, on the date of therapy, the patient spontaneously complained of a left upper posterior chest pain under the scapula, which he described as sharp. It was most noticeable on deep inspiration, and radiated in a horizontal plane to the third thoracic vertebra. The pain was not present when at rest, but at other times persisted as a dull ache. This left subscapular pleuritic pain completely regressed after therapy.

On 9 August, the patient developed a slight bilateral headache and a sore throat. Physical examination revealed infection of the pharynx with a slight amount of yellowish exudate; lungs appeared normal. On 13 August, EMK was entirely asymptomatic upon physical examination, and the chest X-ray was within normal limits. He remained in this condition until discharged on 26 August.

Diagnosis: Tularemia, typhoidal type.

H. CASE 8

MDO, a 35-year-old white male bacteriologist, entered the hospital on 7 August 1957 with complaints of headache, fever, and sore throat. Three days prior to hospitalization his legs felt weak and tired; the following day he experienced soreness in his right second and fifth fingers in the proximal and distal interphalangeal joints, but not the metacarpophalangeal joints, wrist, elbow, or shoulder. Later the second day prior to hospitalization, MDO experienced soreness in the "joints all over my body;" oral temperature at this time was 104 F after having a "flushed" feeling. He then experienced two or three shaking chills over a period of 25 to 30 minutes.

The day prior to admission to the hospital, the above symptoms were repeated except that he had myalgias and arthralgias during the chill-fever period.

MDO had been actively working with P. tularensis for the past year. Immunization against P. tularensis started in May 1952, with boosters in May 1953, after which he maintained a low-grade titer of 1:20 until hospitalization, when his titer was 1:10.

Upon hospitalization, MDO's white blood count was 7,250; ESR of 34 mm per hour; CRP, 3+; an unremarkable urinalysis. Chest X-ray on this date (7 August) revealed a clear lung field with heart and mediastinal shadows normal. Physical examination showed a bilateral maxillary and frontal sinus soreness; pharynx appeared infected with greenish mucopurulent exudate in the posterior pharynx; no pustules were observed.

Serial WBC's during hospitalization ranged from 7,000 to 9,400; ESR reached a peak of 38 mm per hour on 9 August, and declined to 27 on date of discharge (26 August); CRP was negative on date of discharge; urinalysis was unremarkable throughout his hospitalization. Agglutinins for P. tularensis titered 1:10, 1:20, 1:80, and 1:320 on 8, 15, 19, and 26 August, respectively. These agglutinins later increased in titer to 1:640. A 1:1000 P. tularensis skin test of 7 August was negative in 48 hours, but a repeated testing on 24 August was positive after 48 hours. Chest X-rays on 9, 12, 14 August and 3 September revealed no pulmonic infiltrate; lung fields were clear; heart and mediastinal shadows were normal.

Therapy of tetracycline, 2.0 grams per day, was initiated on 9 August. Prior to administration, the patient had a persistent fever, headache, and diaphoresis, especially in the evening. The patient denied headache, fever, or chills after therapy administration, and a gradual decrease of excessive diaphoresis occurred until on 13 August the patient was asymptomatic. Physical examination on 13 August revealed a cleared pharynx with the remainder of the examination clearly within normal limits.

Isolation of P. tularensis from a 9 August gastric juice specimen (Table 1) was obtained from guinea pig inoculation, autopsy, and pathology. Other gastric samples were negative to animal pathology and P. tularensis was not recovered by direct culture from this case's specimens. Blood culture specimens were not obtained from this patient. The patient was asymptotically discharged from hospitalization on 26 August.

Diagnosis: Tularemia, typhoidal.

I. CASE 9

MED, a 35-year-old white male laboratory technician, was hospitalized 13 August 1957 complaining of chills, weakness, sore skin, sensitive joints, headache, and fever of 18 hours' duration. He was in good health until the afternoon of 12 August when he experienced a dull occipital headache that radiated to the vertex; he felt warm in the evening, went to bed, and had several non-shaking chills, developed a sore, dry throat, and a "burning sensation" substernally. His chronic cough was aggravated only slightly; there was no nausea or vomitus.

The patient works with P. tularensis, and was first immunized in 1953; his last titer of agglutinins, 24 June 1957, was 1:20, and a skin test of 1:1000 was negative. He had a booster against tularemia on 29 July. Other history on admission was noncontributory.

Physical examination on hospitalization was not remarkable, and the patient did not appear acutely or chronically ill; slight pharyngeal and soft palate infection; superficial vesicles on all fingers, an erythematous base, some fissuring, but no weeping. No adenopathy, lungs were clear, and heart and mediastinal shadows were normal.

The white blood cell count on admission was 11,450; ESR was 20 mm per hour; agglutinin titer for P. tularensis was 1:320. Consecutive WBC's on 14, 15, 16, 17, 19, 23 August, and 3 September ranged from 8,250 to 13,000. His ESR reached a peak of 28 mm per hour on 14 August, and a low of 15 mm on 23 August. MED's agglutinin titer for P. tularensis remained at 1:320 throughout hospitalization, and finally rose to 1:640 on 10 September. Skin tests of 1:1000 P. tularensis (Table 2) were administered on 14, 16, 19, and 21 August, the 2nd, 4th, 7th, and 9th day of illness respectively, with negative results.

Gastric juice specimens obtained on 14 and 19 August (Table 1) had a pH value of 5.0 and 2.0 respectively. P. tularensis was isolated from autopsied guinea pigs injected with the 14 August specimen. Negative results were obtained by direct culture from both specimens. Blood specimens for culture were not taken.

Serial chest X-rays on 13, 14, 16, and 19 August were completely within normal limits. Because of continuous high fever of 102.4 to 103.8 F on 13 August, tetracycline therapy of 2.0 grams per day was initiated. The following day the patient was feeling better in that the substernal soreness and sore throat had subsided. On 16 August, approximately 60 hours after initiation of therapy, MED was completely asymptomatic. He was discharged from the hospital on 22 August.

Diagnosis: Tularemia, typhoidal.

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13. ABSTRACT <p>Laboratory diagnosis of typhoidal or pulmonic tularemia requires the isolation of <u>Pasteurella tularensis</u> from the hospitalized patient. Generally, blood cultures from the patient are used in an effort to isolate this micro-organism and, although several authors have reported success by this method, our laboratory has not isolated <u>P. tularensis</u> from blood cultures.</p> <p>The successful isolation of <u>P. tularensis</u> is reported from cultures made from the gastric juice from five typhoidal and four pulmonic cases of tularemia.</p> <p>The inoculation of guinea pigs and replicate direct plating of the gastric concentrate is advocated as a standard procedure for tularemia diagnosis.</p>		

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22 March 2001

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FOR THE COMMANDER:

Phyllis M. Rinehart
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