TREASURY DEPARTMENT
UNITED STATES PUBLIC HEALTH SERVICE

HYGIENIC LABORATORY—BULLETIN No. 130
MARCH, 1922

# TULARÆMIA Francis 1921

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By EDWARD FRANCIS

II. EXPERIMENTAL TRANSMISSION OF TULARÆMIA BY FLIES OF THE SPECIES CHRYSOPS DISCALIS
By EDWARD FRANCIS and BRUCE MAYNE

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# TULARÆMIA Francis 1921.[1]

## I. THE OCCURRENCE OF TULARÆMIA IN NATURE AS A DISEASE OF MAN.[2]

By EDWARD FRANCIS, Surgeon, United States Public Health Service.

Tularæmia is a specific infectious disease due to *Bacterium tularense* and is transmitted from rodents to man by the bite of an infected blood-sucking insect or by the handling and dissecting of infected rodents by market men [3] or laboratory workers.[4]

As observed in Utah in the months of June, July, and August, the disease is initiated by the bite of an insect, most probably the blood-sucking fly, *Chrysops discalis*, which previously has bitten a jack rabbit infected with *Bacterium tularense*. Following the fly bite on some exposed surface of the body (neck, face, hands, or legs), the onset is sudden, with pains and fever; the patient is prostrated and is confined to bed; the lymph glands which drain the bitten area become tender, inflamed, and swollen, and commonly suppurate, requiring incision. The fever is of a septic type, lasting from three to six weeks, and convalescence is slow.

Probably two dozen cases occurred in Millard County, Utah, in each of the years 1917, 1918, 1919, and 1920. The first case known to have terminated fatally was reported by the writer in 1919.[5] The chief interest in tularæmia as a disease of man arises from the disability which accompanies the illness; a disabling illness which overtakes the farmer in the busy season of midsummer, causing two or three months of sickness in the harvest season, is a serious matter. Tularæmia is a disease of the rural population, particularly attacking persons who work in the field. It occurs during the seasonal prevalence of the fly (*Chrysops discalis*) in a community where jack

[1] The name tularæmia is based on the specific name *Bacterium tularense*, plus *æmia*, from the Greek, and has reference to the presence of this bacterium in the blood, on the analogy of leukæmia or bacteræmia, etc. The names thus far used for this disease are strictly vernacular and do not lend themselves to international usage as easily as a name in Latin form. Accordingly, the name tularæmia is proposed as a technical international name.

[2] Reprinted from Public Health Reports 1921, vol. 36, pp. 1731–1738.

[3] Wherry, W. B., and Lamb, B. H.: Infection of Man with Bacterium Tularense, J. Infect. Dis. 15: 331–340, 1914. Vail, D. T.: Bacillus Tularense Infection of the Eye, Ophth. Rec. 23: 487, 1914. Sattler, Robert: Bacillus Tularense Conjunctivitis, Arch. Ophth. 44: 265, 1915. Wherry, W. B.: A New Bacterial Disease of Rodents Transmissible to Man, Pub. Health Rep. 29: 3387–3390, 1914. Lamb, Frederick W.: Conjunctivitis Tularensis with Report of a Case, Ophth. Rec. Vol. 26, pp. 221–226, 1917.

[4] Lake, G. C., and Francis, Edward: Six Cases of Tularæmia Occurring in Laboratory Workers, Pub. Health Rep. 37: 392–413 (Feb. 22), 1922.

[5] Deer-Fly Fever: A Disease of Man of Hitherto Unknown Etiology. By Edward Francis, Surgeon, United States Public Health Service. Public Health Reports, Sept. 12, 1919, pp. 2061–2062.

rabbits are dying from an epizootic of plaguelike disease of rodents.[6] The reservoir of infection is in the sick and dying jack rabbits.

### SEVEN CASES OF TULARÆMIA IN MILLARD COUNTY, UTAH.

A brief summary is given below of seven cases of tularæmia which the writer investigated clinically and culturally in Millard County, Utah, one in 1919 and six in 1920:

#### CASE 1.

R. S., male; 52 years of age; farmer; residence 7 miles southeast of Delta, Utah; patient of Dr. H. L. Charles, Delta.

*July 23, 1919.*—Patient was taken sick while mowing alfalfa; went to bed with fever, pains in head, neck, and right shoulder; wife saw a small sore on right side of neck posteriorly, but paid very little attention to it; patient remained in bed from this date until death.

*July 26.*—Temperature was 101° F. at 3 p. m. During the night of July 26 was sleepless on account of pain in head and right side of neck.

*July 27.*—Temperature normal at 1 p. m. A sore on right side of neck, posteriorly, showed a black center ⅛ inch in diameter and surrounded by a yellow zone ⅛ inch wide, which probably resulted from a fly bite, although patient did not recall having been bitten. Behind the right ear was a very tender and somewhat swollen area. No enlargement of axillary glands or of glands of left side of neck. Drew 65 c. c. of blood from median basilic vein for inoculation of animals.

*July 28.*—Temperature at noon, 100.5° F.

*July 30.*—Temperature at noon, 99.8° F. The appearance of the bite unchanged. Right cervical glands palpable, size of peas. Over the right mastoid, a swelling which fluctuates and is very tender and painful.

*August 1.*—Temperature 99° at 10 a. m., 101° at 6 p. m. Pain in neck has been severe during past two days. Some pus has exuded from the site of bite. Glands palpable. Swelling over mastoid very tender.

*August 2.*—Temperature a. m., 99°; p. m., 101°; pulse, 65. The black center of the bite has sloughed out and a few drops of pus exuded. A very tender swollen gland is palpable. Complains of great pain beneath the outer end of the right clavicle.

*August 3.*—Temperature a. m., 100°; p. m., 101°.

*August 4.*—Temperature a. m., 99°; p. m., 98.6°.

*August 5.*—Temperature a. m., 99°; p. m., 100°.

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[6] A plaguelike disease of rodents. By George W. McCoy, Passed Assistant Surgeon, United States Public Health Service. Public Health Bulletin No. 43, April, 1911.

*Bacterium tularense*, the cause of a plaguelike disease of rodents. By George W. McCoy and Charles W. Chapin, Passed Assistant Surgeons, United States Public Health Service. Public Health Bulletin No 53, January, 1912.

*August 6.*—Temperature a. m., 99.2; p. m., 100°. Opened the abscess over the mastoid and got about 2 c. c. of pus, which was injected into animals. Drew 20 c. c. of blood from left median basilic vein, which was used for animal inoculations. Much pain in neck; patient said he had a chill yesterday.

*August 7.*—Temperature p. m., 101.6°.

*August 8.*—Temperature noon, 98.6°. The bite is the site of a hole which is exuding a little pus.

*August 18.*—Terminated fatally.

CASE 2.

W. E. C., male; age, 50 years; farmer; residence, 5 miles west of Holden, Utah; patient of Dr. John E. Fuhrer, Fillmore, Utah.

*June 16, 1920.*—Patient was taken sick while mowing alfalfa; he noticed a stinging and burning sensation above the left ear, but did not know whether or not he had been bitten; had headache and backache; had a chill and felt weak.

*June 17–22.*—Patient remained on farm unable to do any work.

*June 23.*—Went to Fillmore, Utah, to consult Dr. John E. Fuhrer, who reported a temperature of 100.6° F.; pulse, 76; white blood cells, 14,000. Examination showed a small crust in the hair of the left temple, about 1 inch above and 2 inches in front of the left ear, about the diameter of a match stick. The tissues were swollen and tender, and the lymph glands behind the ear and at the angle of the jaw were enlarged and very tender. Patient remained in bed from this date.

*June 25.*—Blood taken from median basilic vein for animal inoculations.

*June 27.*—Temperature, 99° F.; pulse, 76.

*June 30.*—Temperature, 98.6°.

*July 2.*—Temperature, 98.6°.

*July 17.*—Incised gland behind left ear and evacuated pus, which was used for animal inoculations.

*July 21.*—Incised gland at angle of left jaw and evacuated pus.

*July 23.*—Incised skin in front of left ear and evacuated pus. Site of bite on temple in hair has sloughed, leaving an ulcer three-quarters of an inch in diameter.

This patient recovered after an illness of about three months.

CASE 3.

J. T. G., male; age, 48 years; farmer; residence 7 miles northwest of Holden, Utah; patient of Dr. John E. Fuhrer.

*June 27, 1920.*—Patient became sick in the field while spading dirt about an irrigation ditch. First noticed a painful lump at the angle of the right jaw. Quit work and went to Holden with aching sensation through his body.

*June 29.*—Called Dr. John E. Fuhrer, who was the first to find the site of the bite, which was in the hair of the right temple, 1 inch above and 2 inches in front of the right ear. The patient had entirely overlooked the site of the bite, all of his attention having been directed to the painful gland at the angle of the jaw.

*July 3.*—Patient in bed with clothes on. The site of the bite has a black necrotic center. Glands at the angle of right jaw and behind the ear are much swollen, tender, and painful. Drew 30 c. c. blood from median basilic vein for animal inoculations.

Recovery was complete after an illness lasting about 10 weeks.

CASE 4.

M. S., male; age, 16 years; farmer; worked 1 mile west of Holden, Utah; patient of Dr. John E. Fuhrer.

*June 23, 1920.*—Bitten on the posterior surface of right ear while in the hay field.

*June 24.*—Had headache and felt badly and went to bed. Noticed a lump behind the right ear.

*July 3.*—The boy has been in bed most of the time for the past nine days. Temperature, 103°; pulse, 110. There is a punched out ulcer one-quarter of an inch in diameter on the posterior surface of the right ear. There is an enlarged gland behind the right ear over the mastoid, which is beginning to soften. Incised the gland and evacuated the pus, which was used to inoculate guinea pigs.

The patient recovered after an illness of about six weeks.

CASE 5.

Mrs. McK, female; age, 41 years; residence, 4 miles west of Holden, Utah, in the country; patient of Dr. W. B. Hamilton, of Delta, Utah.

*June 16, 1920.*—Patient was taken sick; she was not conscious that she had been bitten by a fly nor does she know whether a fly bit her or not.

*July 2.*—A suppurating gland located behind the right ear was incised and the pus used for inoculation of animals. About 30 c. c. of blood was drawn from the median basilic vein for the inoculation of animals. The site of the fly bite is plainly seen in the edge of the hair of the neck on the right side as a small scar.

Case recovered after a protracted illness lasting about three months.

CASE 6.

C. F., male; age, 30 years; resident of Meadows, Utah, 9 miles southwest of Fillmore; patient of Dr. John E. Fuhrer.

*July 21, 1920.*—First noticed glandular swelling under the right ear. The bite is apparent on the posterior surface of the right ear; did not know he was bitten at the time.

*August 7.*—Incised post-auricular gland on the right side, from which some bloody pus was obtained for the inoculation of laboratory animals.

The duration of illness was about six weeks; ended in recovery.

TABLE 1.—*Bacteriological confirmation of seven human cases of tularæmia in Millard County, Utah.*

| Case. | Sex. | Age (years). | Occupation. | Date of onset. | Site of insect bite. | Site of suppurating lymph glands. | Blood from median basilic vein injected into guinea pigs. | Pus from suppurating lymph glands injected into guinea pigs. | Results in inoculated animals and in cultures from inoculated animals. |
|---|---|---|---|---|---|---|---|---|---|
| 1. R. S..... | Male.... | 52 | Farmer..... | July 23, 1919 | Right side of neck, posteriorly. | Right posterior auricular area. | July 27 and Aug. 6.... | Aug. 6 | Typical for *Bacterium tularense.* |
| 2. W. E. C..... | ...do..... | 50 | .....do..... | June 16, 1920 | Left temple in hair.... | Left posterior auricular area; in front of left ear; at angle of jaw. | June 25..... | July 17 | Do. |
| 3. J. T. G..... | ...do..... | 48 | .....do..... | June 27, 1920 | Right temple in hair.. | Right posterior auricular area; at angle of right jaw. | July 3..... | ..... | Do. |
| 4. M. S..... | ...do..... | 16 | .....do..... | June 23, 1920 | Posterior surface of right ear. | Right posterior auricular area. | ..... | July 3 | Do. |
| 5. McK..... | Female.. | 41 | Wife of farmer..... | June 16, 1920 | In hair of neck, right side. | .....do..... | July 2..... | July 2 | Do. |
| 6. C. F..... | Male.... | 30 | (?)..... | July 21, 1920 | Posterior surface of right ear. | .....do..... | ..... | Aug. 7 | Do. |
| 7. Jackson..... | Female.. | 7 | Village resident... | Aug. 26, 1920 | Posterior surface, lower third of right thigh. | Right inguinal region.. | ..... | Sept. 9 | Do. |

CASE 7.

Jackson, female; age, 7 years; resident of Hinckley, Utah; patient of Dr. H. L. Charles, Delta, Utah.

*September 9, 1920.*—Patient came to the doctor's office with a bubo of the right inguineal region, which was fluctuating. By incision there was readily obtained some pus, which was used for the inoculation of laboratory animals. The site of the bite was on the posterior surface of the lower third of the right thigh, on that bare area so commonly seen above the stocking when a small girl bends forward; the bite had the appearance of a punched-out ulcer about a quarter of an inch in diameter.

Patient recovered.

## TULARÆMIA IN JACK RABBITS.

The coexistence in the same locality of tularæmia in man and in jack rabbits was proved by the writer in June, July, and August, 1920, in Millard County, Utah. During this period, *Bacterium tularense* was isolated from 17 jack rabbits and 6 human cases.

A survey of jack rabbits for evidence of tularæmia was conducted throughout a stretch of irrigated farming country 60 miles in length, extending from Sugarville, Utah, which is approximately 35 miles northwest of Delta, to Fillmore, which is approximately 25 miles southeast of Delta. This survey began May 28 at Sugarville and ended June 18 at Fillmore. A total of 556 jack rabbits were shot and immediately dissected on the ground, examination being directed to their lymph glands, spleen, and liver. When the spleen and liver were considered suspicious, specimens were taken to the laboratory and rubbed on the abraded skin of the abdomen of a guinea pig; and in case of death of the guinea pig with typical lesions, cultures were made from the spleen and liver of the guinea pig. Twenty-three jack rabbits were found sufficiently suspicious by gross examination in the field to warrant inoculation of guinea pigs with their spleens and liver. Of these 23 jack rabbits, 17 were proved to be infected with *Bacterium tularense* in the laboratory by subinoculation in animals and by cultures; three jack rabbits which failed of confirmation in the laboratory were found dead in the field, and it is presumed that their infection had died before they reached the laboratory.

The jack rabbit survey, which ended June 18, outlined the district of most heavily infected jack rabbits and located it west of Holden. At this time no human cases had yet been reported for 1920, although it developed a few days later that two cases had had their onset on June 16 and that they lived west of Holden. The third and fourth cases of the season developed on June 23 and June 27; both worked west of Holden. All four cases occurred in the heart of the jack-rabbit-infected district, 5 miles west of Holden, where, on June 16, we located five infected jack rabbits and 26 jack rabbit carcasses.

TABLE 2.—*Jack rabbits found infected with Bacterium tularense in Millard County, Utah.*

| Date found. | Sex, age, or condition. | How taken. | Condition of spleen. | Condition of liver. | Condition of lymph glands of groin and axilla. | Where found. | "Positive" means confirmed by sub-inoculations and sub-cultures. | Remarks. |
|---|---|---|---|---|---|---|---|---|
| 1920. | | | | | | | | |
| June 1 | | Found dead | Spotted | | | 2 miles south of Abrahams | Positive | |
| June 7 | Pregnant | Shot running | do | Spotted | Negative | Near Sugarville | do | |
| June 11 | do | Found dead | do | do | do | Maney ranch | do | |
| June 14 | Female | Shot running | do | do | do | 2 miles northwest of McCornick. | do | |
| Do | Pregnant | do | Negative | Few spots | do | do | Negative | |
| Do | Male | do | do | Spotted | do | do | Positive | |
| June 15 | do | Found dead | Spotted | do | do | 2 miles southwest of McCornick. | do | 12 dried rabbit carcasses seen 2 miles southwest of McCornick, June 15. |
| Do | Adult | do | do | do | do | do | | No specimens taken. |
| Do | Young | Shot running | Shattered | do | do | do | Positive | |
| Do | Pregnant | do | Spotted | do | do | do | do | |
| Do | Male adult | Found dead | 3 spots | Negative | do | do | Negative | |
| June 16 | Adult | Dead in road | | | | | | |
| Do | Pregnant | Shot running | Spotted | Spotted | do | 8 miles northwest of Holden | Positive | |
| Do | Adult | Found dead | do | | do | 5 miles west of Holden | do | 26 dried carcasses of jack rabbits seen 5 miles west of Holden, June 16. |
| Do | Female | Shot running | do | Spotted | do | do | do | |
| Do | Pregnant | do | do | do | do | do | do | |
| Do | Young | Found dead | do | 3 spots | do | do | Negative | |
| Do | Young female | Shot running | Negative | Spotted | do | do | Positive | |
| June 18 | Young | do | Spotted | do | do | 2 miles northwest of Fillmore | do | 18 dried carcasses of jack rabbits seen 2 miles northwest of Fillmore, June 18. |
| Do | Adult | Dead in road | Large, no spots | do | do | do | Negative | |
| Do | Young | Shot running | Spotted | do | do | do | Positive | |
| Do | do | Found dead | do | do | do | do | do | |
| July 16 | | do | do | Negative | do | Blue Lake, 7 miles southwest of Hinckley. | do | |

### TULARÆMIA IN GROUND SQUIRRELS (*Citellus mollis*) IN UTAH.

During the jack rabbit survey referred to above, extending from Sugarville to Fillmore, 277 ground squirrels (*Citellus mollis*) were shot. These animals were immediately dissected on the ground for evidence of tularæmia. The livers and spleens of three were considered suspicious by gross examination and were brought to the laboratory for confirmation by guinea pig inoculations and cultures. One, which was shot June 2, 1 mile southwest of Abrahams, and had a typical spleen, proved positive, i. e., the spleen was rubbed on the abraded skin of the abdomen of a guinea pig and inoculated subcutaneously into another guinea pig; both pigs died with typical lesions of tularæmia, from which a culture of *Bacterium tularense* was obtained. The other two squirrels failed of confirmation in the laboratory.

### ACKNOWLEDGMENTS.

In response to a request from Dr. T. B. Beatty, State health commissioner of Utah, to the Surgeon General of the United States Public Health Service for the detail of an officer to investigate this new disease, the writer was directed to proceed to Salt Lake City for the purpose of conferring with Commissioner Beatty and to make a study of tularæmia. Following a conference with Commissioner Beatty at Salt Lake City on July 23, 1919, and acting upon his recommendation, the writer proceeded to Delta, Utah, for conference with Dr. H. L. Charles.

Through the courtesy of Mr. W. C. Henderson, acting chief of the Bureau of Biological Survey, Department of Agriculture, specimens of *Citellus mollis* were determined.

## II. EXPERIMENTAL TRANSMISSION OF TULARÆMIA BY FLIES OF THE SPECIES CHRYSOPS DISCALIS.[1]

By EDWARD FRANCIS, Surgeon, and BRUCE MAYNE, Associate Sanitarian, United States Public Health Service.

A study of tularæmia in Millard County, Utah, in 1920, by one of us (Francis) proved the coexistence in the same locality of human cases of this disease and of a fatal epidemic in jack rabbits, both due to *Bacterium tularense*. Further studies showed a much greater prevalence of *Chrysops discalis* (a blood-sucking fly) in these infected localities than in noninfected localities. It was well known that *Chrysops discalis* bites man. Popular belief had connected the occurrence of human cases of tularæmia with the bites of *Chrysops discalis*. No data were at hand, nor did we elicit any, bearing on the question of whether *Chrysops discalis* bites jack rabbits in nature, but we assume that they do and especially if the jack rabbits are in sick or dying condition and thus oblivious to biting flies. A prerequisite to the conveyance of the infection from jack rabbit to man by a blood-sucking fly is the presence of the causative organism in the rabbit's

[1] Reprinted from Public Health Reports 1921, vol. 36, pp. 1738–1746.

peripheral blood. McCoy[2] had already shown that the heart's blood of animals experimentally infected with *Bacterium tularense* was infective even after great dilution when injected into fresh laboratory animals. Francis had isolated this organism from the peripheral blood of two human cases. The bacteræmia characteristic of the disease thus afforded the necessary condition for transference by a blood-sucking fly.

We decided to test the question of whether *Chrysops discalis* was capable by its bite of carrying the infection of *Bacterium tularense* from an infected laboratory animal to a healthy laboratory animal. The experiments which we are about to report show that specimens of *Chrysops discalis* which have first bitten infected guinea pigs and tame rabbits in a laboratory can by their subsequent bites convey that infection to healthy guinea pigs and tame rabbits which they are allowed to bite. We therefore draw the conclusion that *Chrysops discalis* is capable of carrying the infection of *Bacterium tularense* in nature from infected jack rabbits to man.

## EXPERIMENTAL TRANSMISSION BY THE FLY (*Chrysops discalis*).

The specimens of *Chrysops discalis* used were female adult insects, captured in nature on horses near Blue Lake, Utah, a locality in which three human cases of tularæmia (diagnosed clinically) occurred in 1920 and in which one jack rabbit was found infected with *Bacterium tularense*. Our transmission experiments are therefore open to the criticism that the *Chrysops discalis* which we used had an opportunity of becoming infected in nature before being brought into captivity in the laboratory. If this were true, however, it would only give added weight to the evidence of the agency of *Chrysops discalis* as a transmitter of tularæmia.

Each fly under experiment was confined singly at all times within a lantern globe, the ends of which were covered with cloth gauze of coarse mesh. Biting was permitted by applying the end of the globe to the animal's skin, the fly biting through the meshes of the gauze. When not actually biting, the flies were kept in the cold room at a temperature of approximately 15° to 20° C.

The experiments were conducted in the summer of 1920, at Delta, Utah, a town 150 miles south of Salt Lake City. The guinea pigs and rabbits used in these experiments were shipped from Washington, D. C., to Delta. In the transmission experiments we used, four human strains of *Bacterium tularense* which one of us (Francis) had isolated in 1920 from four human cases of tularæmia in Utah.

Table 3 shows the essential data connected with 11 successful transmissions of *Bacterium tularense* from infected laboratory animals to healthy laboratory animals through the agency of the bites of *Chrysops discalis*. Twenty-seven unsuccessful attempts were made which are not reported in this paper. The unsuccessful experiments

[2] A Plague-like Disease of Rodents (Public Health Bulletin No. 43). By George W. McCoy, Passed Assistant Surgeon, United States Public Health Service. 1911.

TABLE 3.—*Successful transmission of tularæmia in the laboratory from inoculated animals to healthy animals by the bites of Chrysops discalis.*

| Number of experiment. | Inoculated animal which flies were allowed to bite and thus become infected. | Length of time between inoculation of animal and death of same. | Length of time between the biting of the infected animal by the fly and death of animal. | Number of flies which bit infected animal and then bit healthy animal. | Number of times that infected flies bit healthy animal. | Length of time between biting infected animal and biting healthy animal. | Dates on which infected flies bit healthy animal. | Healthy animals which the infected flies were allowed to bite. | Results. "Positive" means death from tularæmia. |
|---|---|---|---|---|---|---|---|---|---|
| | | *Days.* | *Hours.* | | | | 1920. | | |
| 1 | Rabbit | 6 | 2½ | 1 | 1 | Few seconds | Aug. 14 | Guinea pig | Positive; died Aug. 21. |
| 2 | Guinea pig No. 8 | 4 | 2–5 | 6 | 6 | do | Aug. 25 | Guinea pig No. 10. | Positive; died Aug. 30. |
| 3 | Rabbit C | 4½ | 33, 31, 30, 9, 8, 6 | 6 | 6 | do | Aug. 25 or 26 | Guinea pig No. 11. | Positive; died Sept. 1. |
| | Rabbit G | 4 | 22, 8, 2, 1, ¼ | 5 | 5 | do | do | do | |
| 4 | Guinea pig No. 9 | 5 | 22–24 | 3 | 3 | do | Aug. 30 or 31 | Guinea pig No. 13. | Positive; died Sept. 6. |
| | Guinea pig No. 10 | 5 | [1] 5 | 1 | 1 | do | do | do | |
| | Guinea pig No. 11 | 6, 7 | 17–43 | 11 | 11 | do | do | do | |
| | Guinea pig No. 12 | 4 | [1] 15 | 1 | 1 | do | do | do | |
| 5 | Rabbit No. 3 | | | 22 | 340 | do | July | Guinea pig No. 5 | Positive; died Aug. 2. |
| 6 | Rabbits | | | 20 | 348 | do | August | Rabbit No. 8 | Positive; died Aug. 8. |
| 7 | do | | | 51 | 207 | One hour | August | Rabbit No. 9 | Do. |
| 8 | do | | | 23 | 33 | 3 hours | Aug. 8 | Rabbit No. 10 | Positive; died Aug. 15. |
| | | | | 31 | 55 | do | Aug. 9 | do | |
| | | | | 26 | 51 | do | Aug. 10 | do | |
| | | | | 34 | 74 | do | Aug. 11 | do | |
| | | | | 36 | 73 | do | Aug. 12 | do | |
| 9 | Guinea pig No. 13 | 6, 7 | 60, 60, 36 | 3 | 3 | 3 days | Sept. 5, 7, 8, 9, or 10. | Rabbit | Positive; died Sept. 16. |
| | Guinea pig No. 14 | 7 | 3 | 1 | 1 | do | do | do | |
| | Guinea pig No. 15 | 5 | 8 | 3 | 3 | do | do | do | |
| | Guinea pig (yellow) | | | 3 | 3 | 5 hours | do | do | |
| 10 | Rabbit McK | | 3 | 29 | 29 | 24 hours | Aug. 13 | Rabbit No. 11 | Positive; died Aug. 23. |
| | Rabbit G | 5 | 51 | 39 | 39 | do | Aug. 14 | do | |
| | do | 5 | 27 | 45 | 45 | do | Aug. 15 | do | |
| | Rabbit S | | 26 | 25 | 25 | do | Aug. 16 | do | |
| | Rabbit McK | 4 | 48 | 37 | 37 | do | Aug. 17 | do | |
| | Rabbit C | 4 | 30 | 38 | 38 | do | Aug. 18 | do | |
| | Rabbit | | | 35 | 35 | do | Aug. 19 | do | |
| 11 | Guinea pigs 7, 10, 11, 13; eight rabbits. | | | 24 | 41 | 4–16 days | Sept. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 13. | Rabbit | Positive; died Sept. 14. |

[1] Minutes.

were conducted according to the same methods as were the 11 successful ones. No specific reason can be given for the failure of any experiment. In this connection it is very interesting to note Experiment No. 1, in which, out of five flies which fed at the same time on the same infected rabbit, only one was found to be either infective or infected. The other four were found to be neither infective nor infected.

EXPERIMENT NO. 1.

In this experiment a single fly by a single bite caused the death of a guinea pig with typical lesions due to *Bacterium tularense*, the interval which elapsed between biting the infected rabbit and the healthy guinea pig being only a few seconds and the interval between biting the healthy guinea pig and the death of this animal being seven days.

Flies Nos. 1, 2, 3, 4, and 5, fed August 14, 1920, during 5, 5, 6, 6, and 10 minutes, respectively, to partial engorgement on the clipped skin of the region of the crest of the ilium of a rabbit six days after its inoculation and two and one-half hours before death; and then, after an interval of only a few seconds, each fly bit to engorgement one of five guinea pigs, on the clipped skin on the region of the crest of the left ilium, for the period of 15, 15, 15, 6, and 25 minutes, respectively. The guinea pigs all remained well except the one which was bitten by fly No. 5; this one died August 21. At autopsy there were the typical lesions due to *Bacterium tularense* in the left inguinal gland, pelvic gland, liver, and spleen. A piece of the spleen of the guinea pig was rubbed on the scarified shaven abdomen of another guinea pig, causing death in four days with typical buboes, spleen, and liver.

Inasmuch as only one of the five flies infected its guinea pig, the infectivity of all of the flies was tested as follows; the flies were dissected and the stomach contents of each were injected subcutaneously into a guinea pig.

*Fly No. 1.*—Stomach contents injected August 16, two days after biting the infected donor. Guinea pig remained well.

*Fly No. 2.*—Stomach contents injected August 16, two days after biting the infected donor. Guinea pig remained well.

*Fly No. 3.*—Stomach contents injected August 17, three days after biting the infected donor. Guinea pig died August 21. Negative.

*Fly No. 4.*—Stomach contents injected August 17, three days after biting the infected donor. Guinea pig remained well.

*Fly No. 5.*—Stomach contents injected August 18, four days after biting the infected donor. Guinea pig died August 23, with typical lesions of spleen, liver, and lymph glands due to *Bacterium tularense.*

EXPERIMENT 2.

In this experiment 6 flies, by 6 bites (1 each) caused the death of guinea pig No. 10 with typical lesions of tularæmia, the interval

between the biting of the infected guinea pig and the healthy guinea pig being only a few seconds, and the time between the biting of the healthy guinea pig and the death of same being 5 days. The 6 flies fed to partial engorgement on August 25 during 2, 7, 12, 2, 9, and 14 minutes, respectively, on the ear of infected guinea pig No. 8, 4 days after inoculation and 2 to 5 hours before death; and then, after an interval of only a few seconds, they fed to engorgement on guinea pig No. 10, upon the clipped skin of the region of the crest of the ilium, for the periods of 20, 3, 14, 2, 10, and 16 minutes, respectively. Guinea pig No. 10 died August 30. At autopsy the glands and liver were apparently negative, but the spleen, being suspicious, was used to inoculate two guinea pigs subcutaneously. These guinea pigs died September 2 with typical inguinal and pelvic glands and typical livers and spleens. The virus was passed through three subsequent generations in guinea pigs, these animals dying with typical lesions on Sept. 6, 10, and 16. The method of inoculation was that of rubbing spleen tissue on the shaven, abraded skin of the abdomen of the guinea pig.

EXPERIMENT 3.

In this experiment 11 flies, by 11 bites (1 each), caused the death of guinea pig No. 11, with typical lesions of tularæmia, the interval between the biting of the infected rabbits and the healthy guinea pig being only a few seconds, and the interval between biting the healthy guinea pig and the pig's death being 6 or 7 days. Six flies fed to partial engorgement (3 on Aug. 25 and 3 on Aug. 26) for 4, 4, 5, 6, 5, and 5 minutes, respectively, on the ear of infected rabbit C, 33, 31, 30, 9, 8, and 6 hours, respectively, before its death. Five other flies fed to partial engorgement (1 on Aug. 25, and 4 on Aug. 26) for 2, 10, 5, 1, and 16 minutes, respectively, on the ear of infected rabbit G, 22, 8, 2, 1, and $\frac{1}{4}$ hours, respectively, before its death. After an interval of only a few seconds, the 11 flies fed to engorgement on guinea pig No. 11, on being applied to the clipped skin of the region of the crest of the right ilium for the periods of 42, 10, 40, 3, 4, 25, 5, 24, 20, 1, and 16 minutes, respectively. Guinea pig No. 11 died September 1, with typical lesions of the right inguinal gland, spleen, and liver. There was a slight local reaction at the site of the fly bites, consisting of a little redness and thickening of the skin. The infection was carried through two subsequent generations in guinea pigs by rubbing spleen tissue on the shaven abraded skin of the abdomen of guinea pigs. The pigs died September 5 and 12 with typical lesions of tularæmia.

EXPERIMENT 4.

In this experiment, 16 flies by 16 bites (1 each), caused fatal tularæmia in guinea pig No. 13, the interval between the biting of the infected guinea pigs and the healthy guinea pig being only a few seconds and the time between biting the healthy guinea pig and the death of same being 6 or 7 days. Sixteen flies fed to partial engorgement for an average period of 7 minutes on the ears of infected guinea pigs Nos. 9, 10, 11, and 12, the pigs being in the latter stages of the disease, and after an interval of only a few seconds they fed to engorgement for an average period of 7 minutes on the clipped skin of the region of the crest of the right ilium of guinea pig No. 13. This pig was found dead September 6 with typical inguinal glands on the right side only (left inguinal glands negative) and typical liver and spleen; the site of the fly bites showed a slight pale thickening of the skin.

The infection was carried through two subsequent generations in guinea pigs by rubbing spleen tissue on the clipped, abraded skin of the abdomen of guinea pigs. The pigs died with typical lesions September 10 and 15.

EXPERIMENTS 5 AND 6.

In experiment No. 5, 22 flies by 340 bites, caused the death of guinea pig No. 5, with typical tularæmia, and in experiment No. 6, 20 flies by 348 interrupted bites, caused the death of rabbit No. 8. These were the first transmission experiments which we performed; they were preliminary, and therefore large numbers of bites were employed, the object being to determine whether the fly acted even remotely in the role of the carrier of the infection. The method of fly biting in these experiments differed from that recorded in experiments 1, 2, 3, and 4, in that the flies were never allowed to bite longer than from 30 to 60 seconds. For instance, a given fly was allowed to bite from 30 to 60 seconds on the clipped skin of the region of the crest of the ilium of the infected animal and then was immediately applied for from 30 to 60 seconds to the clipped skin of the region of the crest of the ilium of the healthy animal, and this was repeated, the fly alternately biting the infected and healthy animal until it reached engorgement. Another fly would then be taken through the same process. This method of biting accounts for the large numbers of bites recorded in experiments 5 and 6, each fly biting the healthy animal an average of 16 times for an average of about 45 seconds each time.

Guinea pig No. 5 was dead August 2. The site of the fly bites showed hemorrhagic points on the underside of the skin; three inguinal glands were caseous on the side of the fly bites, but the inguinal glands on the opposite side were negative; pelvic glands

were caseous; liver and spleen were typical. The infection was carried over by rubbing a piece of spleen on the clipped, abraded skin of the abdomen of a guinea pig and a rabbit. These animals died with typical lesions on August 5 and 6.

Rabbit No. 8 was dead August 8. The site of the fly bites was negative; inguinal and axillary glands were negative; pelvic glands, substernal glands, liver, spleen, and lungs showed typical lesions of tularæmia. The infection was carried over by rubbing a piece of spleen on the clipped, abraded skin of the abdomen of a guinea pig and rabbit. These animals died with typical lesions August 12 and 13.

EXPERIMENTS 7, 8, AND 10.

In Experiments Nos. 7, 8, and 10 the interval between the biting of the infected animal and the healthy animal was extended to 1 hour, 3 hours, and 24 hours, respectively. Large numbers of flies were used and they bit a great number of times. In these experiments, as in Experiments Nos. 5 and 6, the flies were interrupted in their biting, being allowed to bite only from 30 to 60 seconds at a time and being made to bite alternately the infected and the healthy animal on the clipped skin in the region of the crest of the ilium. Each fly bit the infected and healthy animals in Experiment No. 7 an average of about four times each; in Experiment No. 8 an average of about two times each; and in Experiment No. 10 only once. Transmission was successful in the three experiments, rabbits Nos. 9, 10, and 11 dying with typical lesions of tularæmia. Rabbit No. 9 showed no enlargement of the inguinal or axillary glands, but showed typical lesions of the pelvic glands, liver, and spleen. The virus was carried over to another rabbit and a guinea pig by rubbing the spleen on the abraded surface of the abdomen of those animals. Death followed in 6 days, with typical lesions of the inguinal and pelvic glands, liver, and spleen.

Rabbit No. 10 showed no enlargement of the inguinal glands, but showed typical pelvic glands, liver, and spleen. The infection was carried over to a guinea pig by rubbing the spleen on the abraded skin of the abdomen of the pig, resulting in death in 5 days, with typical lesions of the inguinal and pelvic glands, liver, and spleen. Rabbit No. 11 showed no enlargement of inguinal or axillary glands, but the pelvic glands on the right side were typical, as were also the liver and spleen. The local reaction of the skin at the site of the fly bites over the crest of the right ilium was marked; the skin for an area 1 inch in diameter was raised and thickened, but perfectly movable over the fascia covering the muscle; and on section, this skin was pale, thick, and membranous.

EXPERIMENT 9.

In Experiment No. 9, 10 flies, by 10 bites (1 each), caused fatal tularæmia in a rabbit, the intervals between the biting of the infected guinea pigs and the healthy rabbit being 5 to 72 hours. The average time of biting the infected animals on the ear, to partial engorgement, was 5 minutes, and the average time of biting the healthy rabbit, to complete engorgement, on the clipped skin of the area of the crest of the ilium, was 8 minutes. At autopsy the rabbit showed typical inguinal and pelvic glands, spleen, and liver. The infection was carried over to another animal by rubbing a piece of the spleen on the abraded skin of the abdomen, resulting in death in 4 days, with typical lesions of the inguinal, pelvic, and axillary glands and the liver and spleen.

EXPERIMENT 11.

In this experiment, 24 flies, by 41 bites, caused fatal tularæmia in a rabbit, the intervals between the biting of the infected guinea pigs and rabbits and the biting of the healthy rabbit being 4 to 16 days. The average time of feeding the flies to partial engorgement on the ears of the infected animals was 5 minutes and the average time of feeding the flies to full engorgement on the clipped skin on the region of the crest of the ilium of the healthy rabbit was 9½ minutes. The rabbit died with typical lesions of the spleen, liver, and lungs, and showed a local lesion of the skin, consisting of a papule one-fourth inch in diameter on the right hip, posterior to the crest of the ilium, which was movable, hard, and which, on section, was hard, white, and not broken down. The pelvic glands on the right side were much enlarged and caseous, whereas those on the left side were negative, as were also the inguinal glands on both sides.

The infection was carried over for two generations in rabbits by rubbing a portion of the spleen on the abraded skin of the abdomen, both animals dying with typical lesions of inguinal and pelvic glands, liver, and spleen after 5 and 6 days, respectively.

LENGTH OF TIME THAT CHRYSOPS DISCALIS WILL REMAIN INFECTED.

This question was answered in two ways: First, by noting among the 11 experiments in which tularæmia was successfully transmitted by *Chrysops discalis* the longest interval which elapsed between the bite which infected the fly and the subsequent bite by which that fly infected a healthy animal, 4 days being the longest successful interval noted; second, by injecting infected flies subcutaneously into guinea pigs, the flies having been kept for various lengths of time after becoming infected by biting an infected animal.

Flies which had bitten infected animals were kept at an average temperature of 15° to 20° C., and on each succeeding day from 1 to 15 days one or more flies were killed, their wings and legs discarded, and the entire fly was ground in a mortar with normal saline solution and the suspension injected subcutaneously into a guinea pig.

The longest interval of time that such a fly remained infected, as evidenced by the death of the guinea pig with typical lesions of tularæmia, was 14 days.

In all, 44 injections were made of 99 flies which had been kept for various periods after biting infected animals. The following table gives the results. Up to 5 days the flies remained quite constantly infected. The longer the flies were kept, the less tendency they showed to be infected. This would indicate probably that the virus does not multiply within the fly, but that *Chrysops discalis* acts merely in a mechanical way as a transmitter of tularæmia.

TABLE 4.—*The length of time that Chrysops discalis remained infected as shown by injection of flies into guinea pigs.*

| Number of days between biting the infected animals and being injected into a healthy guinea pig. | Number of flies injected into 1 guinea pig. | Results. ("Positive" means death of guinea pig with typical lesions of tularæmia.) | Number of days between biting the infected animals and being injected into a healthy guinea pig. | Number of flies injected into 1 guinea pig. | Results. ("Positive" means death of guinea pig with typical lesions of tularæmia.) |
|---|---|---|---|---|---|
| 1 | 3 | Negative. | 8 | 2 | Positive. |
| 2 | 4 | Positive. | 8 | 2 | Negative. |
| 2 | 1 | Do. | 8 | 2 | Positive. |
| 2 | 3 | Do. | 8 | 1 | Do. |
| 2 | 8 | Do. | 9 | 1 | Negative. |
| 2 | 3 | Do. | 9 | 1 | Do. |
| 2 | 2 | Do. | 9 | 1 | Positive. |
| 2 | 1 | Negative. | 10 | 4 | Do. |
| 2 | 1 | Do. | 10 | 2 | Negative. |
| 3 | 2 | Positive. | 10 | 5 | Do. |
| 3 | 5 | Do. | 10 | 6 | Do. |
| 3 | 3 | Do. | 10 | 1 | Do. |
| 3 | 1 | Do. | 10 | 1 | Do. |
| 3 | 1 | Negative. | 12 | 2 | Do. |
| 4 | 4 | Positive. | 12 | 1 | Do. |
| 4 | 1 | Do. | 12 | 1 | Do. |
| 4 | 2 | Do. | 12 | 1 | Do. |
| 4 | 1 | Do. | 12 | 1 | Do. |
| 5 | 4 | Do. | 13 | 2 | Do. |
| 5 | 3 | Negative. | 14 | 2 | Positive. |
| 5 | 1 | Positive. | 15 | 1 | Negative. |
| 6 | 4 | Do. | 15 | 1 | Do. |

ACKNOWLEDGMENT.

We are indebted to Dr. J. M. Aldrich, of the division of insects of the National Museum, for determining specimens of *Chrysops discalis*.

## III. EXPERIMENTAL TRANSMISSION OF TULARÆMIA IN RABBITS BY THE RABBIT LOUSE, HÆMODIPSUS VENTRICOSUS (DENNY).[1]

By Edward Francis, Surgeon, and G. C. Lake, Passed Assistant Surgeon, United States Public Health Service.

The experiments here reported show that the common rabbit louse, *Hæmodipsus ventricosus* (Denny), when taken from rabbits which have died with the typical lesions of tularæmia and placed in the hair of healthy rabbits causes the death of the latter with typical tularæmia.

Experiments which we conducted in the Hygienic Laboratory between February 3 and May 16, 1921, showed that healthy tame rabbits in contact with rabbits inoculated intraperitoneally with heart's blood of infected rabbits died typically from tularæmia. Forty-three such positive results were obtained. The conditions of contact were that one inoculated rabbit and two healthy rabbits were confined in each compartment, the diameter of which was about 18 inches. These compartments were glass aquarium jars, galvanized-iron garbage cans, or well-ventilated wire cages. In determining the means of transmission in these cases, consideration was given to insects and to the infectivity of nasal secretions and urine.

### INFECTIVITY OF NASAL SECRETIONS OF RABBITS.

It was found that the nasal washings from infected rabbits, when dropped into the nares of healthy rabbits or injected subcutaneously into guinea pigs, produced the disease. Of 17 specimens of nasal washings obtained from 17 infected rabbits, 9 were infective, as shown by the death from tularæmia of healthy rabbits into whose nares these washings were dropped; and of 24 specimens of nasal secretions obtained from 24 infected rabbits, 21 were infective, as shown by the death from tularæmia of healthy guinea pigs into which these washings were injected subcutaneously.

### INFECTIVITY OF URINE OF RABBITS.

Four specimens of urine, two from rabbits dead of tularæmia and two from rabbits sick with the disease, injected subcutaneously into guinea pigs, caused the death of the pigs with typical lesions of tularæmia. A fifth specimen from a rabbit dead of tularæmia gave negative results. The amounts injected varied from 0.05 to 5 c. c. Precautions were taken to prevent the presence of blood in the specimens.

It was found impossible, however, to infect four rabbits and two guinea pigs by mixing with their food large quantities of nasal washings or urine from infected rabbits, although they ate the mix-

[1] Reprinted from Public Health Reports 1921, vol. 36, pp. 1747–1753.

ture readily. This latter result made it doubtful whether the 43 positive results referred to above were due either to droplet infection or urine.

A constant watch for fleas has been kept with the result that only three were found in the laboratory during the eight months period ending in July, 1921. McCoy and Chapin had reported one successful experiment on the transmission of the infection from squirrel to squirrel by fleas (*C. acutus*).

### INFESTATION OF RABBITS WITH LICE.

No systematic search was made for lice upon rabbits in the Hygienic Laboratory until early in May, when one was accidentally found. Since that time some lice have been found on every rabbit that has been carefully examined. Usually only a few are present, and careful search is required to find them; but occasionally they are present in large numbers. Most of them are found over the lumbar region, either on the skin or clinging to the butt ends of the hair.

The presence of blood-sucking lice immediately opened the question as to whether the large number of successful contact infections obtained between February 3 and May 16 may not have been due in part or entirely to this cause. It was therefore decided (1) to conduct experiments to determine whether the louse could readily carry the infection from rabbit to rabbit and (2) to repeat the contact experiments, using only carefully deloused animals. The results of (1) are quite conclusive and are the subject of this paper. The results of (2) will be reported later.

### EXPERIMENTAL TRANSMISSION BY LICE.

Experiments upon the agency of lice in the transmission of tularæmia were carried out as follows:

As soon as possible after the death of an infected rabbit its hair was pulled out over the lumbar and sacral regions, and, since the lice cling to the butts of the hairs, the butts were clipped off with a scissors and transferred to a glass jar. In most instances the infested hair was immediately transferred to the hair of a healthy rabbit, but in other instances an interval of one, two, or three days was allowed to elapse between the removal of lice from the infected rabbit and their transfer to a healthy rabbit. The louse-infested hair was applied to the hair of the lumbar region of a healthy rabbit and overlaid with two layers of gauze, the margins of which were held down by adhesive tape to hold the hair in place. The gauze and adhesive were removed on the following morning, care being taken not to injure the animal's skin in loosening the tape, which

was done by cutting off a little of the hair. The rabbits were placed at once in thoroughly cleaned tall ash cans, a single rabbit to each can, and a ring of vaseline was placed around the inside, about 6 inches from the top.

GUINEA PIGS IN CONTACT WITH RABBITS.

In 22 cases a healthy guinea pig was placed in the can with the infested rabbit in order to determine whether the guinea pig would develop tularæmia from contact with the rabbit. These guinea pigs all remained negative with the exception of five which were in contact with rabbits infested with lice removed from rabbit S 62 R; four of these died in from seven to nine days with typical lesions of tularæmia; the fifth died with typical lesions after 26 days. These five guinea pigs were all searched at the moment of death for rabbit lice, their hair being pulled out and examined. On one there were five dead rabbit lice; on another, there were six dead rabbit lice; on the other three guinea pigs none could be found. Of the 17 guinea pigs which remained well, 11 were in contact with infested rabbits which died typically of tularæmia, and six were in contact with infested rabbits which failed to contract tularæmia.

STRAINS OF TULARÆMIA USED.

The strains of tularæmia used in these experiments were five strains isolated by Francis in 1920 from human cases in Utah, and one California ground squirrel strain isolated by Passed Assistant Surgeon W. T. Harrison in San Francisco in 1920.

RESULTS.

The following results were obtained:

*First series.*—In this series, transmission of tularæmia to healthy rabbits was effected by the transfer to them of lice removed from rabbits dead after intraperitoneal inoculation with heart's blood from infected animals. Sixteen healthy rabbits were thus infested, the number of lice used for each rabbit varying from 28 to several hundred. Eleven of the 16 died with typical tularæmia. In these 11 cases the interval which elapsed between the removal of lice from the infected rabbit and their application to the healthy rabbit was at most only a few hours, and the intervals which elapsed between the infestation of the healthy rabbits and their deaths varied from 8 to 25 days, the average being 11.7 days. Five rabbits of this series remained negative. In two of these, intervals of 24 hours and two days, respectively, were allowed to elapse between the removal of lice from the infected rabbits and their application to the healthy rabbits. No explanation is offered for the three negative ones in which no such interval was interposed.

*Second series.*—Transmission of tularæmia was effected by lice transferred from the first series of louse-infected rabbits to healthy rabbits. Seven healthy rabbits were thus infested, the number of lice used for each rabbit varying from 70 to 300. Six of the seven rabbits died with typical tularæmia. In four of these six cases, the interval which elapsed between the removal of lice from the infected rabbit and their application to the healthy rabbit was less than three hours; in the fifth case (R L 18) the interval was two days, during which time the lice were allowed to feed on a healthy rabbit, R L 15, which died of pneumonia on the second day. In the sixth case (R L 23) the interval was three days.

*Third series.*—Transmission of tularæmia was effected by lice transferred from the second series of louse-infected rabbits to healthy rabbits. Four rabbits were thus infested. The first died with typical lesions 18 days after infestation; the second died with typical lesions 13 days after infestation; the third died 13 days after infestation (and in this case only 17 lice were applied and an interval of 3 days had elapsed between the removal of the lice from the infected rabbit and their application to the healthy rabbit); the fourth at the time of this report, 30 days after infestation, is still well, only four lice having been used for the infestation.

*Fourth series.*—Lice were transferred from louse-infected rabbits of the third series to healthy rabbits. Two rabbits were thus infested and both are still well (at the time of this report) on the twenty-ninth and thirtieth days, respectively, after infestation. One of them was infested with 20 lice which had been kept 3 days after removal from the infected rabbit before application to the healthy rabbit; the other was infested with 20 lice 3 hours after removal from the infected rabbit.

TABLE 5.—*Transmission of tularæmia in rabbits by Hæmodipsus ventricosus.*

| Series. | Number of experiment. | Infected animals from which lice were removed. | | | Interval which elapsed between removal of lice from infected animals and transfer to healthy animal. | Healthy animal to which lice were transferred. | Number of days between infestation with lice and death of rabbit. | Date of death of infested rabbit. | Result. "Positive" means death from tularæmia. |
|---|---|---|---|---|---|---|---|---|---|
| | | Number of animal. | Date found dead from tularæmia. | Approximate number of lice removed. | | | | | |
| First series: Transmission was effected by lice transferred from inoculated rabbits to healthy rabbits. | 1 | SF 34 R | May 28, 1 p. m | 36 | Less than 15 minutes | RL 1 | 15 | June 12 | Positive. |
| | | McK 66 R | May 29 | 4 | do | do | | | |
| | 2 | S 59 R | May 22 | 15 | do | RL 2 | 9 | May 31 | Do. |
| | | S 60 R | May 27, 3 p. m | 13 | do | do | | | |
| | 3 | C 65 R | May 27, 1 p. m | 80 | About 2 hours | RL 3 | | | Negative, July 27. |
| | 4 | S 62 R | June 7 | 180 | From 1 to 3 hours | RL 6 | 8 | June 15 | Positive. |
| | | do | do | 180 | do | RL 7 | 16 | June 23 | Do. |
| | | do | do | 180 | do | RL 8 | 11 | June 18 | Do. |
| | | do | do | 180 | do | RL 9 | 9 | June 16 | Do. |
| | | do | do | 180 | do | RL 10 | 9 | do | Do. |
| | | do | do | 180 | do | RL 11 | 8 | June 15 | Do. |
| | 5 | SF 36 R | June 8, 2 p. m | 100 | Not over 2 hours | RL 12 | | | Negative, July 27. |
| | 6 | J 57 R | June 6 | 70 | About 1 hour | J 58 R | 11 | June 17 | Positive. |
| | 7 | McK 67 R | June 5 | 500 | Less than 3 hours | RL 13 A | 25 | June 30 | Do. |
| | 8 | SF 37 R | June 10, 1 p. m | 300 | 2 to 3 hours | RL 13 | 8 | June 18 | Do. |
| | 9 | C 67 R | June 13, 1 p. m | 200 | Not over 3 hours | RL 20 | | | Negative, July 27. |
| | 10 | UF 1 | June 19 | 25 | 24 hours | RL 21 | | | Do. |
| | 11 | G 14 R | do | 200 | 2 days | RL 22 | | | Do. |
| Second series: Transmission was effected by lice transferred from louse-infected rabbits of first series to healthy rabbits. | 12 | RL 2 | May 31 | 50 | 2 to 3 hours | RL 4 | 11 | June 11 | Positive. |
| | | do | do | 50 | do | RL 5 | 15 | June 15 | Do. |
| | 13 | RL 6 | June 15 | 180 | 1 to 4 hours | RL 15 | 2 | June 17 | Negative.[1] |
| | | RL 11 | do | 130 | do | do | | | |
| | | | | | 2 days during which lice were on RL 15. | RL 18 | 9 | June 26 | Positive. |
| | 14 | RL 9 | June 16 | 120 | Not over 3 hours | RL 16 | 26 | July 12 | Do. |
| | | RL 10 | June 16, 9 a. m | 160 | do | do | | | |
| | 15 | J 58 R | June 17 | 70 | Not over 2 hours | RL 19 | 11 | June 28 | Do. |
| | 16 | RL 8 | June 18 | 150 | 3 days | RL 23 | 18 | July 16 | Do. |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Third series: Transmission was effected by lice transferred from second series of louse-infected rabbits to healthy rabbits. | 17 | RL 4 | June 11 | 100 | Not over 2 hours | RL 14 | 18 | June 29 | Positive. |
| | 18 | RL 5 | June 15 | 200 | Not over 3 hours | RL 17 | 13 | June 28 | Do. |
| | 19 | RL 18 | June 26, 6 a. m | 30 | 3 days, after which 17 remained alive. | RL 25 | 13 | July 12 | Do. |
| | 20 | RL 19 | June 28, 6 a. m | 4 | About 1 hour | RL 24 | | | Negative, July 27. |
| Fourth series: Lice transferred from third series of louse-infected rabbits to healthy rabbits. | 21 | RL 17 | June 28, 6 p. m | 47 | 3 days, after which 20 lice remained alive. | RL 27 | | | Do. |
| | 22 | RL 14 | June 29 | 20 | About 3 hours | RL 26 | | | Do. |

[1]Died of pneumonia, June 17, on second day; his lice were then transferred to RL 18.

## SUMMARY.

The transmission of tularæmia was effected in 20 out of 29 attempts through the agency of the common rabbit louse (*Hæmodipsus ventricosus*), by the transfer of lice from rabbits dead of tularæmia to the hair of healthy rabbits, the intervals elapsing between infestation of the healthy rabbits and their deaths varying from 8 to 26 days, the average being 12½ days. The intervals between the removal of lice from the infected animals and their application to the healthy animals were in all successful attempts not over three hours, with three exceptions, in which the interval was 2, 3, and 3 days, respectively.

Transmission of tularæmia was effected through three successive series of rabbits by transfer of lice to each succeeding series from the preceding series.

## CONCLUSION.

The practical importance of this experimental transmission of tularæmia from infected rabbits to healthy rabbits by the rabbit louse *Hæmodipsus ventricosus* is that it offers an explanation of the means by which the infection is kept alive throughout the year in the jack rabbits of Utah. Proof is at present complete that these jack rabbits are infested with lice, four specimens of *Hæmodipsus ventricosus* having been received from that source in July, 1921.

*Acknowledgment.*—Through the courtesy of Dr. L. O. Howard, Chief of the Bureau of Entomology, Department of Agriculture, the determination of specimens of *Hæmodipsus ventricosus* was made by Dr. H. E. Ewing, of that Bureau.

## IV. TRANSMISSION OF TULARÆMIA BY THE BEDBUG, CIMEX LECTULARIUS.[1]

By Edward Francis, Surgeon, and G. C. Lake, Passed Assistant Surgeon, United States Public Health Service.

The experiments here reported show that the bedbug *Cimex lectularius*, which commonly infests beds and bites human beings, is capable of transmitting tularæmia from an infected to a healthy white mouse. Two distinctly different methods of transmission were successful. In one method we followed the usual procedure of first allowing the insects to feed on an infected animal and then to feed on a healthy animal. In the other method we followed the first half of the usual procedure, but the second half was reversed; i. e., the mouse ate the infected bug instead of the infected bug biting the mouse. Following the usual method, transmission was successful in 10 experiments in which the intervals which elapsed between biting the infected mice and biting the healthy mice were a few seconds, 18 hours, 7 days, 15 days, and 71 days. Following the unusual method, transmission was successful in 55 experiments in which the intervals which elapsed between the bug's biting the infected mouse and the mouse's eating the infected bugs varied uniformly from 6 hours to 100 days; this latter limit promises to be still further extended and to keep pace with the natural length of life of a bedbug.

### METHODS EMPLOYED.

White mice and guinea pigs were the experimental animals used. Infection was due to strain (S) of *Bacterium tularense* isolated in 1920 from a human case of tularæmia in Utah. The bedbugs were collected from cracks in the wooden cages in which the laboratory stock of guinea pigs and white mice are bred. The bugs were kept on hand from one to three weeks before attempting to infect them. They were confined at all times to test tubes. They sucked an infecting meal of blood either from the tail of an inoculated white

[1] Reprinted from Public Health Reports 1922, vol. 37, pp. 83-95.

mouse, the tail being poked into the tube through a hole in the gauze which covered the mouth of the tube, or they sucked through the gauze, the tube being inverted and held on the infected mouse's abdomen.

The white mice which were used for infecting the bugs were inoculated subcutaneously on the back with the heart's blood of a dead infected mouse.

Bugs were applied to a sick mouse between 72 and 96 hours after inoculation, since four days was the maximum life of a white mouse inoculated subcutaneously.

The tubes containing the bugs were always kept at room temperature, which during the summer was practically that of the outside air, but after cool weather arrived in October it was that of the ordinary steam-heated laboratory, which averaged 22° C.

A tube contained not over 40 bugs and was supplied with a strip of filter paper on which the bugs rested and deposited their feces. The white mice were kept in glass battery jars, one mouse to each jar.

### TRANSMISSION FOLLOWING BEDBUG BITES.

Ten white mice died with typical lesions of tularæmia following bedbug bites. Their histories are summarized at the head of the table and given more in detail in their appropriate places in the body of the table. Experimental transmission by biting is largely dependent upon interrupted feeding. An engorged bug will not bite. A partially engorged bug will bite. Forced interruption of a bug's meal of blood on the infected animal conduces to a completion of that meal on the healthy animal. The shorter the interruption the greater the likelihood of transmission. The lengths of interruption in our experiments were (1) a few seconds, (2) 18 hours, (3) 48 hours, (4) no interruption.

(1) *Interruption of a few seconds.*—The bugs were allowed to feed 2½ minutes on the infected mouse and then, after only a second's interval, they were allowed to feed for 2½ minutes on the healthy mouse; they fed in this manner alternately on the infected and healthy mice during 15 minutes, thus permitting of three insertions of the proboscis in each of the two animals. It is not probable that all of the bugs made six insertions, but certainly each bug made multiple insertions. In this method transmission was probably accomplished purely by a grossly contaminated proboscis.

During an experiment the infected mouse and the healthy mouse were tied to a board, abdomens upward. The abdomen of the infected mouse was shaved three days previously, but the abdomen of the healthy mouse was unshaved and unclipped. The bugs were contained in a glass tube, the mouth of which was covered with gauze. The mouth of the tube was held alternately in contact with

the two abdomens, the bugs biting through the gauze. No evidences of defecation were noted on the hair of the healthy mice.

Results: Five sets of bugs, averaging 25 bugs to each set, bit four infected mice, and then, after an interval of only a few seconds, bit 5 healthy mice according to the above-mentioned interrupted method of feeding. Transmission occurred in all instances. The intervals which elapsed between biting the healthy mice and their deaths from tularæmia were 3½, 4½, 4½, 5½, and 5½ days, the average being 4½ days. There were no unsuccessful attempts at transmission when the period of interruption was only a few seconds.

(2) *Interruption of 18 hours.*—The bugs were allowed to feed on the abdomen of the infected mouse for only 2½ minutes, after which they were set aside for 18 hours, at the end of which time they were applied for 12½ minutes in contact with the tail of a healthy mouse and allowed to feed to full engorgement. For this purpose the tail of the healthy mouse was poked through a hole in the gauze which covered the mouth of the tubes. No evidences of defecation were noted on the tails of the healthy mice while being bitten by the bugs.

Results: Three sets of bugs, averaging 70 bugs to each set, fed for 2½ minutes on three infected mice, and then after an interval of 18 hours, fed to engorgement on three healthy mice. Transmission occurred in two instances, the intervals which elapsed between biting the healthy mice and their deaths from tularæmia being 4½ and 5½ days. The mouse bitten by the third set of bugs remained well. (See table, Lots 262, 263, and 264.)

(3) *Interruption of 48 hours.*—Five sets of bugs, averaging 54 bugs to each set, fed for 2½ minutes on 5 infected mice and then, after an interval of 48 hours, fed to full engorgement on 5 healthy mice. Transmission occurred in no instance. (See table, Lots 272 to 284.)

(4) *Noninterrupted feeding.*—This method of transmission varied from the foregoing methods in that the bugs' first meal was a full meal. They were allowed to feed on the abdomen or tail of the infected mouse to full engorgement without interruption.

Having sucked one full meal of infected blood, all subsequent feedings took place on healthy mice and were for the purpose of determining whether infection of healthy mice would follow the bites of infected bugs.

The second, third, and fourth feedings took place approximately on the third, sixth, and tenth days after the infecting meal. Subsequent feedings occurred approximately every 10 days throughout the life of the bugs.

While feeding on healthy mice, the bugs were applied in contact with the tails of these mice for periods of one hour, thus not only permitting them to feed to engorgement, but encouraging them to defecate on the tails. For this purpose the tail of the healthy mouse

was kept poked for one hour into a slender glass tube containing the bugs. The bugs avoid resting on the tail of the mouse if possible; they even prefer glass to a mouse's tail as a resting place. In order to compel them to rest on the tail, the caliber of the glass tube was just enough larger than the diameter of the tail to permit the passage of the bugs along the tail, and, moreover, the tube was held in a vertical position so that the bugs were forced to rest on the tail of the mouse. The result was that the bugs deposited their feces principally on the tail so that the points of biting must have become overlaid with bug feces in some instances.

Results: Ten lots of bugs (Nos. 230 to 258), varying from 16 to 140 bugs, but averaging 88 bugs to the lot, fed to engorgement on 10 infected mice, and then, after intervals of from 3 to 110 days, fed to engorgement during one hour on the tails of 23 healthy mice, feeding an average of five times on each healthy mouse. Transmission occurred in 3 out of 23 mice. The intervals which elapsed between biting the infected mice and biting the 3 healthy mice were 7, 15, and 71 days, respectively, and the intervals between biting the 3 healthy mice and their deaths from tularæmia were 6, 5, and 7 days, respectively; the number of bugs employed in the three transmissions were 28, 24, and 14, respectively.

TRANSMISSION OF TULARÆMIA DUE TO EATING INFECTED BEDBUGS.

Transmission experiments with bedbugs and white mice which do not take into account the mouse's habit of eating bedbugs will be full of errors. White mice readily attack and eat living bedbugs; they eat dead bedbugs with equal readiness. If the bugs are infected with *Bacterium tularense*, the mouse is almost certain to die of tularæmia. One of our white mice confined with 45 infected living bugs within a small glass jar free from bedding or other hiding places for the bugs, ate all the bugs in less than an hour, being at the rate of one bug a minute. A mouse ate 15 living infected bugs over night, the mouse and bugs being loose in a glass battery jar containing bedding composed of coarse screenings of sawdust. Two healthy mice, dropped into a large jar containing cut hay and small wooden boxes and a plentiful supply of bugs which had been infected 21 days previously, died of tularæmia 3½ and 5½ days after entering the jar. Presumably the mice contracted the infection from eating the bugs rather than from the bites of the bugs.

Experiments in other fields of research designed to demonstrate transmission by fleas often have permitted the unrestricted presence of rodents and fleas in the same container. Such a procedure in bug-mouse experiments would leave the experimenter totally in the dark on the question of whether transmission resulted from the bedbug's biting the mouse or the mouse's eating the bedbug.

We conducted 72 bug-eating experiments, 55 of which were successful. The infected bugs came from 10 lots (Nos. 230 to 258). These bugs had fed once to engorgement on infected mice and were fed subsequently every 3 to 10 days on healthy mice. These healthy mice served to test the infectiveness of the bug bites. Some of the bugs were found dead every morning, while others were dying and consequently almost motionless. These infected dead or dying bugs were fed to mice, thereby infecting the mice. The same lots of bugs, therefore, were used in the bug-biting and bug-eating experiments.

Bugs which were to be eaten were dropped, together with a white mouse, into a bottomless glass cylinder 4 inches in diameter which rested on a blotter; the purpose of the blotter was to absorb the urine. The mouse remained until he had eaten the bugs, which in some instances was overnight. The mouse was then transferred to a glass battery jar, commonly used as a mouse cage, each mouse occupying a separate jar.

*Summary.*—Seventy-two white mice ate dying or dead bugs from each of 10 lots of infected bugs. Of this number, 55 died from tularæmia and 17 remained well. The number of bugs offered to and eaten by each mouse varied from 1 to 10, the average being 3. The average length of time which elapsed between eating infected bugs and death of the mice from tularæmia was 4½ days. Of 20 mice which each ate one infected bug, 14 died acutely from tularæmia. The average length of time from the date of infection of the 14 bugs until they were eaten was 65 days. Three white mice which each ate a bug infected 100 days previously died five, four, and five days later from tularæmia.

*Bacterium tularense* suffers no apparent diminution of virulence by reason of long residence in the bedbug.

It is the intention to continue these eating experiments throughout the life of the bugs, which promises to be long.

### INFECTIVITY OF FRESH BEDBUG FECES.

While transmission experiments with our 10 lots of infected bugs were in progress, the infectivity of the bug feces was frequently tested. The strips of filter paper on which the bugs habitually rested served also for the deposition of feces. When the strips were renewed, the old ones were soaked in saline solution, rubbed in a mortar, strained through gauze, and the suspension was either injected subcutaneously into a guinea pig or mixed with corn meal and fed to a white mouse. From each of ten lots of infected bugs two samples of feces were collected about 10 days apart and between 17 and 58 days after the date of infection of the bugs. Both samples from a given lot of bugs were fed at the time of collection to the same mouse. Ten mice

which were thus fed with fresh feces from 10 lots of infected bugs remained well.

Forty-one samples of feces, representing about three samples from each of the 10 lots of infected bugs, were injected, while fresh, subcutaneously into 41 guinea pigs. At least one sample in each of the 10 lots was collected 35 days or more after the date on which bugs were infected, and in one instance the sample was collected 250 days after the date of infection. The 41 guinea pigs all died acutely from tularæmia.

The two sets of experiments show in marked contrast the invariable susceptibility of the guinea pig's subcutaneous tissue to the infected bedbug feces in question, and the invariable resistance of the mouse's stomach to the same material.

*Summary.*—The fresh feces of bedbugs which were infected with *Becterium tularense* by sucking the blood of infected white mice and which were fed every 10 days thereafter on the blood of healthy white mice, contained virulent organisms of this infection at all times and did so up to 250 days after the date of infection of the bugs, and probably will do so throughout the life of the bugs.

### INFECTIVITY OF DRIED FECES OF BEDBUGS.

Feces deposited by our 10 lots of infected bugs on filter papers, between 46 and 75 days after the dates of infection of the bugs, were subsequently set aside and allowed to remain on those filter papers in a dried condition at an average room temperature of 20° C. unexposed to direct light. At the end of 20 days of drying, the filter papers were soaked in saline solution, ground in a mortar, and the pooled suspension of feces was injected subcutaneously into two guinea pigs, causing their deaths within six days with typical lesions of tularæmia.

Feces deposited on filter papers by 8 lots of infected bugs (see table, Lots 262 to 284) between 18 and 70 days after the dates of infection of the bugs, were subsequently kept under the conditions described above for 25 days, at the end of which time the pooled suspension was injected subcutaneously into a guinea pig, causing its death 8 days later with typical lesions of tularæmia.

### TRANSMISSION TO GUINEA PIGS.

The foregoing experiments relate to transmission to white mice. A few attempts were made on transmission to guinea pigs by infected bedbugs. Bugs from 6 of our 10 lots of infected bugs bit 6 guinea pigs, respectively. The guinea pigs were exposed to the bugs under the following conditions.

A healthy guinea pig and infected living bugs were dropped into a bottomless glass cylinder, 6 inches in diameter, which rested on a blotter; the purpose of the blotter was to absorb the urine. Guinea pig and bugs remained in the cylinder overnight. The guinea pigs never showed any tendency to eat the bugs. The bugs were plainly seen to feed to engorgement on the feet of the guinea pigs. They never crawled over the guinea pig's feet but approached only close enough to insert their outstretched proboscides.

The bugs were counted into the cylinder in the evening and counted out in the morning. The evening and morning counts always tallied, with one exception. The guinea pigs all remained well, with the exception of one (see Lot 258). This one was the guinea pig in whose cylinder we failed to recover one bug one morning, 47 having been counted in and only 46 having been counted out. In this case the blotter had been chewed to small bits. The presumption is that the guinea pig unintentionally swallowed one infected bug in chewing its blotter, because it died 7 days later from tularæmia and showed five typical cervical buboes and a chain of typical lymph glands in the mesentery, in addition to the ordinary typical lesions of the spleen and liver.

In order to test the susceptibility of guinea pigs to infection by eating, five lots of 2 bugs each were fed to engorgement on 5 infected mice and 24 hours later were fed to five guinea pigs, respectively, each pair of bugs being ground in a mortar with normal saline solution and applied to a small piece of bread which the guinea pigs readily ate. One of the five guinea pigs died seven days after eating the two infected bugs and showed the typical lesions of tularæmia; the other four remained well. This is taken as strong evidence that the one guinea pig which died from tularæmia after confinement with 46 infected bedbugs contracted its infection from the accidental ingestion of the one missing bug rather than from the bites of the bugs.

In another experiment pooled fresh feces from our 10 lots of infected bugs were fed on bread to a guniea pig with negative results.

TABLE 6.—*Transmission of tularæmia by bedbugs of the species Cimex lectularius.*

| Various lots of bugs: each lot was infected by biting a separate mouse. | *Infectivity of bug feces:* Number of days after date of infection of bugs when their fresh feces were (1) injected into a guinea pig or (2) fed to a white mouse. "Positive" means death from tularæmia. | *Transmission due to eating infected bugs:* Length of time after date of infection of bugs when dying or dead bugs were eaten by a white mouse. "Positive" means death from tularæmia. | *Transmission following bug bites:* Length of time after date of infection of bugs when bugs were allowed to bite a healthy white mouse or guinea pig. "Positive" means death from tularæmia. | Remarks. |
|---|---|---|---|---|
| | (The 10 "Positives" following bedbug bites are assembled in brief at the right of the table for ready reference.) | | Few seconds; 25 bugs bit abdomen of mouse. Positive.<br>Few seconds; 30 bugs bit abdomen of mouse. Positive.<br>Few seconds; 12 bugs bit abdomen of mouse. Positive.<br>Few seconds; 30 bugs bit abdomen of mouse. Positive.<br>Few seconds; 30 bugs bit abdomen of mouse. Positive.<br>18 hours; 62 bugs bit tail of mouse. Positive.<br>18 hours; 67 bugs bit tail of mouse. Positive.<br>7 days; 28 bugs bit tail of mouse. Positive.<br>15 days; 24 bugs bit tail of mouse. Positive.<br>71 days; 14 bugs bit tail of mouse. Positive. | See Lot No. 244.<br>See Lot No. 248.<br>See Lot No. 248.<br>See Lot No. 250.<br>See Lot No. 252.<br>See Lot No. 263.<br>See Lot No. 264.<br>See Lot No. 258.<br>See Lot No. 258.<br>See Lot No. 234. |
| Lot No. 244. 80 bugs infected Sept. 15, 1921. | (1) Feces injected:<br>13 days. Positive.<br>23 days. Positive.<br>43 days. Positive.<br>(2) Feces fed:<br>22 days. Negative.<br>35 days. Negative. | 6 hours; fed 2 dying bugs to mouse 82. Positive.<br>6 hours; fed 2 dying bugs to mouse 83. Positive<br>2 days; fed 6 dead bugs to mouse 84. Positive.<br>35 days; fed 5 dying bugs to mouse 85. Positive.<br>44 days; fed 5 dead bugs to mouse 86. Negative.<br>55 days; fed 5 dead bugs to mouse 87. Positive.<br>67 days; fed 2 dead bugs to mouse 99. Positive.<br>74 days; fed 1 dead bug to mouse 100. Positive. | Few seconds; 25 bugs bit abdomen of mouse 60. Positive.<br>8 days; 16 bugs bit tail of mouse 5. Negative.<br>13 days; 30 bugs bit tail of mouse 5.<br>15 days; 28 bugs bit tail of mouse 5.<br>18 days; 26 bugs bit tail of mouse 5.<br>23 days; 22 bugs bit tail of mouse 6. Negative.<br>27 days; 20 bugs bit tail of mouse 6.<br>35 days; 18 bugs bit tail of mouse 6.<br>39 days; 16 bugs bit tail of mouse 6.<br>54 days; 14 bugs bit tail of mouse 6.<br>74 days; 4 bugs bit tail of mouse 6.<br>84 days; 3 bugs bit tail of mouse 6. | Reported above. |
| Lot No. 248. 140 bugs infected Sept. 19, 1921. | (1) Feces injected:<br>20 days. Positive.<br>30 days. Positive.<br>40 days. Positive.<br>(2) Feces fed:<br>22 days. Negative.<br>35 days. Negative. | 6 hours; fed 2 dying bugs to mouse 10. Positive.<br>6 hours; fed 2 dying bugs to mouse 11. Negative.<br>6 hours; fed 2 dying bugs to mouse 12. Negative.<br>6 hours; fed 2 dying bugs to mouse 13. Negative.<br>31 days; fed 9 dead bugs to mouse 14. Negative.<br>32 days; fed 10 dying bugs to mouse 15. Negative.<br>37 days; fed 10 dead bugs to mouse 16. Positive.<br>84 days; fed 6 dead bugs to mouse 101. Negative. | Few seconds; 30 bugs bit abdomen of mouse 8. Positive.<br>Few seconds; 12 bugs bit abdomen of mouse 9. Positive.<br>2 days; 20 bugs bit tail of mouse 14. Negative.<br>8 days; 30 bugs bit tail of mouse 14.<br>11 days; 25 bugs bit tail of mouse 14.<br>14 days; 24 bugs bit tail of mouse 14.<br>18 days; 22 bugs bit tail of mouse 14.<br>20 days; 21 bugs bit tail of mouse 14.<br>30 days; 50 bugs bit tail of mouse 15. Negative.<br>40 days; 36 bugs bit tail of mouse 15.<br>50 days; 24 bugs bit tail of mouse 15.<br>8 days; 48 bugs bit feet of guinea pig 2. Negative.<br>11 days; 45 bugs bit feet of guinea pig 2.<br>13 days; 43 bugs bit feet of guinea pig 2. | Reported above.<br>Do. |

TABLE 6.—*Transmission of tularæmia by bedbugs of the species Cimex lectularius*—Continued.

| Various lots of bugs: each lot was infected by biting a separate mouse. | *Infectivity of bug feces:* Number of days after date of infection of bugs when their fresh feces were (1) injected into a guinea pig or (2) fed to a white mouse. "Positive" means death from tularæmia. | *Transmission due to eating infected bugs:* Length of time after date of infection of bugs when dying or dead bugs were eaten by a white mouse. "Positive" means death from tularæmia. | *Transmission following bug bites:* Length of time after date of infection of bugs when bugs were allowed to bite a healthy white mouse or guinea pig. "Positive" means death from tularæmia. | Remarks. |
|---|---|---|---|---|
| Lot No. 250. 90 bugs infected Sept. 23, 1921. | (1) Feces injected:<br>17 days. Positive.<br>26 days. Positive.<br>36 days. Positive.<br>(2) Feces fed:<br>17 days. Negative.<br>31 days. Negative. | 6 hours; fed 2 dying bugs to mouse 18. Positive.<br>6 hours; fed 2 dying bugs to mouse 19. Positive.<br>6 hours; fed 2 dying bugs to mouse 20. Positive.<br>28 days; fed 8 dead bugs to mouse 21. Positive.<br>30 days; fed 5 dying bugs to mouse 22. Positive.<br>33 days; fed 5 dead bugs to mouse 23. Positive.<br>36 days; fed 5 dead bugs to mouse 24. Positive.<br>56 days; fed 1 dead bug to mouse 88. Positive.<br>67 days; fed 1 dead bug to mouse 102. Negative. | Few seconds; 31 bugs bit abdomen of mouse 17. Positive.<br>3 days; 45 bugs bit feet of guinea pig 3. Negative.<br>6 days; 43 bugs bit feet of guinea pig 3.<br>9 days; 41 bugs bit feet of guinea pig 3.<br>14 days; 35 bugs bit tail of mouse 25. Negative.<br>17 days; 33 bugs bit tail of mouse 25.<br>26 days; 14 bugs bit tail of mouse 25.<br>36 days; 8 bugs bit tail of mouse 25.<br>46 days; 7 bugs bit tail of mouse 25.<br>56 days; 6 bugs bit tail of mouse 25.<br>66 days; 4 bugs bit tail of mouse 25.<br>76 days; 4 bugs bit tail of mouse 25. | Reported above. |
| Lot No. 252. 86 bugs infected Sept. 22, 1921. | (1) Feces injected:<br>16 days. Positive.<br>27 days. Positive.<br>37 days. Positive.<br>(2) Feces fed:<br>17 days. Negative.<br>27 days. Negative. | 1 day; fed 2 dying bugs to mouse 26. Positive.<br>1 day; fed 2 dying bugs to mouse 27. Positive.<br>1 day; fed 2 dying bugs to mouse 28. Positive.<br>27 days; fed 4 dead bugs to mouse 29. Positive.<br>30 days; fed 5 dying bugs to mouse 30. Negative.<br>35 days; fed 6 dead bugs to mouse 31. Positive.<br>44 days; fed 3 dead bugs to mouse 32. Positive.<br>57 days; fed 1 dead bug to mouse 89. Positive.<br>77 days; fed 1 dying bug to mouse 91. Positive. | Few seconds; 30 bugs bit abdomen of mouse 33. Positive.<br>5 days; 45 bugs bit tail of mouse 34. Negative.<br>7 days; 41 bugs bit tail of mouse 34.<br>10 days; 36 bugs bit tail of mouse 34.<br>16 days; 34 bugs bit tail of mouse 35. Negative.<br>20 days; 32 bugs bit tail of mouse 35.<br>27 days; 28 bugs bit tail of mouse 36. Negative.<br>37 days; 17 bugs bit tail of mouse 36.<br>47 days; 9 bugs bit tail mouse 36.<br>57 days; 9 bugs bit tail of mouse 36<br>67 days; 8 bugs bit tail of mouse 36.<br>77 days; 7 bugs bit tail of mouse 36. | Reported above. |
| Lot No. 258. 130 bugs infected Sept. 29, 1921. | (1) Feces injected:<br>7 days. Positive.<br>24 days. Positive.<br>41 days. Positive.<br>(2) Feces fed:<br>19 days. Negative.<br>29 days. Negative. | 6 hours; fed 2 dying bugs to mouse 49. Positive.<br>12 hours; fed 2 dying bugs to mouse 50. Positive.<br>2 days; fed 2 dying bugs to mouse 51. Positive.<br>27 days; fed 8 dead bugs to mouse 52. Positive.<br>29 days; fed 5 dead bugs to mouse 53. Positive.<br>32 days; fed 4 dead bugs to mouse 54. Positive.<br>34 days; fed 2 dead bugs to mouse 55. Negative.<br>36 days; fed 1 dying bug to mouse 56. Positive. | 36 hours; 47 bugs bit feet of guinea pig 6. Positive.<br>3 days; 40 bugs bit feet of guinea pig. 6.<br>7 days; 28 bugs bit tail of mouse 63. Positive.<br>15 days; 24 bugs bit tail of mouse 64. Positive.<br>19 days; 20 bugs bit tail of mouse 65. Negative.<br>29 days; 20 bugs bit feet of mouse 65.<br>40 days; 13 bugs bit tail of mouse 65.<br>15 days; 58 bugs bit tail of mouse 66. Negative. | Reported above.<br>Do. |

| | | | | |
|---|---|---|---|---|
| | | 36 days; fed 1 dying bug to mouse 57. Positive.<br>36 days; fed 1 dying bug to mouse 58. Positive.<br>37 days; fed 1 dead bug to mouse 59. Negative.<br>40 days; fed 3 dead bugs to mouse 61. Positive.<br>54 days; fed 1 dying bug to mouse 92. Positive. | 19 days; 47 bugs bit tail of mouse 66.<br>29 days; 35 bugs bit feet of mouse 66.<br>40 days; 28 bugs bit feet of mouse 66.<br>50 days; 25 bugs bit tail of mouse 66.<br>60 days; 16 bugs bit tail of mouse 96. Negative.<br>70 days; 16 bugs bit tail of mouse 97. Negative. | |
| Lot No. 230. 112 bugs infected Sept. 1, 1921. | (1) Feces injected:<br>28 days. Positive.<br>30 days. Positive.<br>33 days. Positive.<br>38 days. Positive.<br>44 days. Positive.<br>54 days. Positive.<br>71 days. Positive.<br>80 days. Positive.<br>90 days. Positive.<br>100 days. Positive.<br>110 days. Positive.<br>120 days. Positive.<br>(2) Feces fed:<br>48 days. Negative.<br>58 days. Negative. | 16 days; fed 45 living bugs to mouse 78. Positive.<br>28 days; fed 20 living bugs to mouse 79. Positive.<br>53 days; 4 dying bugs to mouse 80. Positive.<br>57 days; fed 5 dead bugs to mouse 81. Positive.<br>71 days; fed 2 dead bugs to mouse 82. Positive.<br>83 days; fed 1 dead bug to mouse 93. Positive.<br>100 days; fed 1 dying bug to mouse 94. Positive.<br>103 days; fed 1 dead bug to mouse 105. Negative. | 28 days; 38 bugs bit tail of mouse 1. Negative.<br>30 days; 38 bugs bit tail of mouse 1.<br>28 days; 28 bugs bit tail of mouse 2. Negative.<br>30 days; 28 bugs bit tail of mouse 2.<br>33 days; 33 bugs bit tail of mouse 2.<br>44 days; 28 bugs bit tail of mouse 3. Negative.<br>50 days; 27 bugs bit tail of mouse 3.<br>54 days; 21 bugs bit tail of mouse 3.<br>69 days; 16 bugs bit tail of mouse 3.<br>80 days; 15 bugs bit tail of mouse 3.<br>90 days; 14 bugs bit tail of mouse 3.<br>100 days; 12 bugs bit tail of mouse 3. | |
| Lot No. 234. 60 bugs infected Sept. 8, 1921. | (1) Feces injected:<br>1 day. Positive.<br>6 days. Positive.<br>10 days. Positive.<br>14 days. Positive.<br>20 days. Positive.<br>30 days. Positive.<br>50 days. Positive.<br>(2) Feces fed:<br>36 days. Negative.<br>46 days. Negative. | 43 days; fed 5 dying bugs to mouse 77. Negative.<br>67 days; fed 1 dead bug to mouse 95. Negative.<br>100 days; fed 1 dead bug to mouse 103. Positive.<br>100 days; fed 1 dead bug to mouse 104. Positive. | 1 day; 60 bugs bit tail of mouse 4.<br>3 days; 34 bugs bit tail of mouse 4.<br>6 days; 30 bugs bit tail of mouse 4.<br>10 days; 27 bugs bit tail of mouse 4.<br>20 days; 26 bugs bit tail of mouse 4.<br>31 days; 25 bugs bit tail of mouse 4.<br>36 days; 24 bugs bit tail of mouse 4.<br>42 days; 21 bugs bit tail of mouse 4.<br>46 days; 18 bugs bit tail of mouse 4.<br>61 days; 15 bugs bit tail of mouse 4.<br>71 days; 14 bugs bit tail of mouse 4. Positive.<br>80 days; 14 bugs bit tail of mouse 5. Negative.<br>90 days; 14 bugs bit tail of mouse 5. | Reported above. |
| Lot No. 247. 16 bugs infected Sept. 19, 1921. | (1) Feces injected:<br>21 days. Positive.<br>30 days. Positive.<br>40 days. Positive.<br>(2) Feces fed:<br>26 days. Negative.<br>35 days. Negative. | 15 days; fed 3 dead bugs to mouse 75. Positive.<br>51 days; fed 1 dead bug to mouse 76. Positive. | 2 days; 14 bugs bit feet of guinea pig 1. Negative.<br>7 days; 14 bugs bit feet of guinea pig 1.<br>10 days; 13 bugs bit feet of guinea pig 1.<br>13 days; 13 bugs bit feet of guinea pig 1.<br>18 days; 7 bugs bit tail of mouse 7. Negative.<br>21 days; 7 bugs bit tail of mouse 7.<br>30 days; 7 bugs bit tail of mouse 7.<br>40 days; 5 bugs bit tail of mouse 7.<br>50 days; 5 bugs bit tail of mouse 7.<br>60 days; 4 bugs bit tail of mouse 7.<br>70 days; 4 bugs bit tail of mouse 7.<br>80 days; 3 bugs bit tail of mouse 7. | |

TABLE 6.—*Transmission of tularæmia by bedbugs of the species Cimex lectularius*—Continued.

| Various lots of bugs; each lot was infected by biting a separate mouse. | *Infectivity of bug feces:* Number of days after date of infection of bugs when their fresh feces were (1) injected into a guinea pig or (2) fed to a white mouse "Positive" means death from tularæmia. | *Transmission due to eating infected bugs:* Length of time after date of infection of bugs when dying or dead bugs were eaten by a white mouse. "Positive" means death from tularæmia. | *Transmission following bug bites:* Length of time after date of infection of bugs when bugs were allowed to bite a healthy white mouse or guinea pig. "Positive" means death from tularæmia. | Remarks. |
|---|---|---|---|---|
| Lot No. 254. 44 bugs infected Sept. 25, 1921. | (1) Feces injected:<br>20 days. Positive.<br>35 days. Positive.<br>(2) Feces fed:<br>30 days. Negative.<br>40 days. Negative. | 1 day; fed 3 dead bugs to mouse 37. Positive.<br>1 day; fed 3 dead bugs to mouse 38. Positive.<br>31 days; fed 5 dying bugs to mouse 39. Negative.<br>35 days; fed 1 dead bug to mouse 40. Negative.<br>53 days; fed 1 dead bug to mouse 98. Negative. | 3 days; 28 bugs bit feet of guinea pig 4. Negative.<br>5 days; 24 bugs bit feet of guinea pig 4.<br>8 days; 23 bugs bit feet of guinea pig 4.<br>16 days; 30 bugs bit tail of mouse 41. Negative.<br>20 days; 21 bugs bit tail of mouse 41.<br>29 days; 14 bugs bit tail of mouse 41.<br>34 days; 9 bugs bit tail of mouse 41.<br>44 days; 8 bugs bit tail of mouse 41.<br>54 days; 6 bugs bit tail of mouse 41.<br>64 days; 6 bugs bit tail of mouse 41.<br>74 days; 6 bugs bit tail of mouse 41. | |
| Lot Nos. 255–256. 126 bugs infected Sept. 26, 1921. | (1) Feces injected:<br>14 days. Positive.<br>24 days. Positive.<br>35 days. Positive.<br>(2) Feces fed:<br>15 days. Negative.<br>27 days. Negative. | 1 day; fed 3 dying bugs to mouse 42. Positive.<br>1 day; fed 3 dying bugs to mouse 43. Positive.<br>27 days; fed 14 dead bugs to mouse 44. Positive.<br>30 days; fed 5 dying bugs to mouse 45. Positive.<br>40 days; fed 4 dead bugs to mouse 46. Positive.<br>54 days; fed 1 dead bug to mouse 90. Positive. | 3 days; 50 bugs bit feet of guinea pig 5. Negative.<br>6 days; 46 bugs bit feet of guinea pig 5.<br>3 days; 50 bugs bit tail of mouse 47. Negative.<br>6 days; 50 bugs bit tail of mouse 47.<br>12 days; 48 bugs bit tail of mouse 48. Negative.<br>14 days; 45 bugs bit tail of mouse 48.<br>28 days; 17 bugs bit tail of mouse 48.<br>42 days; 6 bugs bit tail of mouse 48.<br>52 days; 6 bugs bit tail of mouse 48.<br>62 days; 5 bugs bit tail of mouse 48.<br>72 days; 5 bugs bit tail of mouse 48. | |
| Lot No. 263. 62 bugs infected Oct. 4, 1921. | Feces injected:<br>35 days. Positive. | | 18 hours; 62 bugs bit tail of mouse 67. Positive. | Reported above. |
| Lot No. 264. 62 bugs infected Oct. 4, 1921. | 35 days. Positive. | | 18 hours; 67 bugs bit tail of mouse 68. Positive. | Reported above. |

| | | | | |
|---|---|---|---|---|
| Lot No. 262. 80 bugsinfected Oct. 2, 1921. | 35 days. Positive. | | 18 hours; 80 bugs bit tail of mouse 69. Negative. | |
| Lot No. 272. 66 bugsinfected Oct. 11, 1921. | 30 days. Positive. | | 48 hours; 66 bugs bit tail of mouse 70. Negative. | |
| Lot No. 275. 60 bugsinfected Oct. 15, 1921. | 25 days. Positive. | | 48 hours; 60 bugs bit tail of mouse 71. Negative. | |
| Lot No. 276. 39 bugsinfected Oct. 15, 1921. | 25 days. Positive. | | 48 hours; 39 bugs bit tail of mouse 72. Negative. | |
| Lot No. 283. 79 bugsinfected Oct. 21, 1921. | 2 days. Positive. | | 48 hours; 79 bugs bit tail of mouse 73. Negative. | |
| Lot No. 284. 27 bugsinfected Oct. 21, 1921. | 2 days. Positive. | | 48 hours; 27 bugs bit tail of mouse 74. Negative. | |

*Summary.*—An average of 35 infected bedbugs from each of 6 of our 10 lots of infected bugs were exposed with 6 healthy guinea pigs, respectively, during approximately the third, sixth, and tenth nights after the date of infection of the bugs. Although freely bitten by the bugs, only 1 of the guinea pigs contracted tularæmia, and in this instance infection is believed to have taken place from the ingestion of a missing infected bug (see Lot No. 258).

*Acknowledgment.*—The determination of specimens of *Cimex lectularius* were made by Dr. H. E. Ewing, of the Bureau of Entomology, Department of Agriculture.

## GENERAL SUMMARY.

The common bedbug *Cimex lectularius* transmitted tularæmia from infected to healthy mice in 10 instances, in which the intervals which elapsed between biting the infected and biting the healthy mice were a few seconds, 18 hours, 7 days, 15 days, and 71 days. The exact parts played by bites and by feces in the 10 transmissions are impossible of determination.

White mice readily eat living and dead bugs.

White mice which eat infected bugs usually contract tularæmia. Of 20 white mice which each ate one infected bug, 14 died acutely from tularæmia. The average length of time from the date of infection of the 14 bugs until they were eaten was 65 days. Three white mice which each ate a bug infected 100 days previously died 5, 4, and 5 days later from tularæmia.

Guinea pigs apparently do not eat bugs intentionally.

Guinea pigs bitten by infected bugs failed to contract tularæmia, with one exception; in the latter instance the guinea pig probably ate one infected bug unintentionally and thereby contracted the infection.

The fresh feces of bedbugs which were infected with *Bacterium tularense* by sucking the blood of infected white mice, and which were fed every 10 days thereafter on the blood of healthy white mice, contained virulent organisms of this infection at all times and did so up to 250 days after the date of infection of the bugs.

Feces of infected bedbugs deposited on filter papers at least 46 days after the dates of infection of the bugs and subsequently dried for 20 days contained virulent organisms of *Bacterium tularense* at the end of that time.

In spite of the last two preceding paragraphs, the fresh feces of infected bugs have always failed to infect white mice or guinea pigs which ate those feces.

*Bacterium tularense* suffered no apparent diminution of virulence by reason of long residence in bedbugs. This virulence was manifested by acute death from tularæmia within seven, five, five, and six days, respectively, in cases of—

(1) A mouse which was bitten by bugs infected 71 days previously;

(2) A mouse which ate a bug infected 226 days previously;

(3) A guinea pig which was injected with fresh feces of bugs infected 250 days previously; and

(4) A guinea pig injected with bug feces which had been deposited on filter paper 76 days after the date of infection of the bugs and which had subsequently dried on filter paper at an average room temperature of 20° C. for 26 days.

## V. TRANSMISSION OF TULARÆMIA BY THE MOUSE LOUSE POLYPLAX SERRATUS (BURM.).[1]

By EDWARD FRANCIS, Surgeon, and G. C. LAKE, Passed Assistant Surgeon, United States Public Health Service.

In two experiments healthy white mice were placed in contact in glass aquarium jars with white mice which had been inoculated subcutaneously with diluted heart's blood of mice dead from tularæmia. Not only did the inoculated mice die from tularæmia but the healthy mice contracted the disease and died with typical lesions.

### TRANSMISSION BY CONTACT.

One of the experiments just cited lasted 15 days, from June 24 to July 9.

On the first day of this experiment, 24 healthy mice and 2 mice inoculated two days previously were introduced into a glass aquarium jar 18 inches in diameter. As soon as an inoculated mouse died it was replaced by another inoculated mouse in the third day of the disease; thus the jar was kept constantly supplied throughout the experiment with two inoculated mice in the later stages of the disease. By the fifteenth day of the experiment all of the 24 healthy mice had died, 16 having died with the typical condition of the spleen due to *Bacterium tularense*. Nine of the 16 spleens were rubbed on the shaved abraded skin of 9 guinea pigs, all of which died with the typical lesions of tularæmia. Of the 16 mice referred to, 1 died on the fifth day of the experiment, 4 died on the seventh day, 3 on the eighth day, 1 on the ninth day, 1 on the tenth day, 1 on the eleventh day, 1 on the twelfth day, 2 on the thirteenth day, 1 on the fourteenth day, and 1 on the fifteenth day. Eight of the 24 mice died from causes other than tularæmia, 4 having died during the first three days of the experiment, and 4 having died later in the experiment.

In a similar experiment, 4 inoculated white mice and 26 healthy white mice were introduced into a glass aquarium jar on August 20, after which date no additional mice were added. The four inoculated mice died within the first five days, with typical lesions of tularæmia. On September 15 the last remaining healthy mouse died with typical lesions of the spleen, which was rubbed on the abraded skin of a guinea pig and caused its death on the sixth day from tularæmia. During the interval of 25 days between August 20 and September 15, the 26 healthy mice all died, the majority of them showing typical lesions of tularæmia.

After the death of the last mouse in the aquarium jar and its removal therefrom, the jar was left undisturbed for eight days. At the expiration of this time eight healthy mice were introduced into the jar. They remained well, thus showing the absence of any residual infection.

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[1] Reprinted from Public Health Reports 1922, vol. 37, pp. 96–101.

Throughout these two contact experiments we searched the mice and the bedding for parasites and tested the infectivity of the urine of infected mice, and also noted whether dead infected mice had been mutilated by contacts, our object being to determine the mode of transmission in these experiments. The several possible factors concerned in the transmission will now be considered.

### INFECTIVITY OF MOUSE URINE.

Throughout our experiments we kept going in white mice strain "S" of *Bacterium tularense* by subcutaneous inoculations of the heart's blood of a recently dead infected mouse into a healthy mouse. Urine voided naturally by living infected mice was collected between 72 and 96 hours after inoculation, since death quite regularly occurred by the end of 96 hours.

The urine collected from four mice was injected subcutaneously into 4 guinea pigs, respectively. The guinea pigs all died acutely with typical lesions of tularæmia. The amounts of urine injected were 10 gtts., 12 gtts., ½ c. c., and ½ c. c. The infectivity of similar samples of mouse urine was also tested by feeding the urine to white mice. The first mouse ate 12 gtts. of such urine mixed with corn meal. The second mouse ate ½ c. c. urine mixed with corn meal on six consecutive occasions spaced about a week apart, thus consuming about 3 c. c. Both mice remained well. We concluded that urine, though infected, was not an agent of transmission in nature.

### CANNIBALISM AND INFECTION.

White mice mutilate and sometimes completely eat their dead comrades. While we made no observation on whether a healthy mouse will contract the infection by eating a mouse dead from tularæmia, we did feed six white mice with a small amount of the liver of a rabbit which had just died with typical lesions of tularæmia. All the mice died within five days: One died on the third day, two on the fourth, and three on the fifth day. In each instance the mouse's spleen was rubbed on the shaved, abraded skin of a guinea pig, causing acute death with typical lesions of tularæmia. In our contact experiments, infected mice, when found dead, occasionally showed evidence of mutilation by their comrades.

### INFESTATION OF WHITE MICE WITH LICE AND MITES.

Examination of the mice revealed the presence of the blood-sucking louse *Polyplax serratus* (Burm), the blood-sucking mite *Liponyssus isabellinus*, and two species of non-blood-sucking mites. No other parasites were found either on the mice or in the hay used for bedding.

The lice were present in variable numbers; some mice had none; two had about 60 each; 30 lice was considered a large number for a

single mouse; about 15 were commonly found on a mouse. The mites were found in much smaller numbers; most mice had none; 20 was the largest number found on a mouse; 9 was the next largest number found; the number on a mouse hardly ever exceeded 4 or 5.

### EXPERIMENTAL TRANSMISSION BY THE MOUSE LOUSE, POLYPLAX SERRATUS.

Experiments were planned to determine definitely the rôle of the mouse louse as an agent in the transfer of the infection from infected mice to healthy mice. Mice were inoculated subcutaneously with diluted blood taken from the heart of a mouse dead from tularæmia, and upon the death of the inoculated mouse his hair was pulled out, transferred to a sheet of white paper and examined for lice. The hair was well teased apart with needles, and any moving object was readily seen in the white hair on the white background. A glance at any moving object with a hand lens was sufficient to exclude the possibility of the parasite being other than a louse. The few hairs to which a louse was clinging were transferred to a clean petri dish, and thus the lice were collected into a pile of very few hairs. The small pile of hairs was then picked up with a forceps and transferred to beneath the hair of the back of a healthy white mouse which was being held. The lice almost instantly left the pile of hairs and disappeared among the hairs of the living mouse. Mice to which lice were thus transferred were placed separately in clean jars for observation.

The time which elapsed between the removal of lice from a dead mouse and their transfer to a healthy mouse probably never exceeded an hour. No effort was made to learn how long lice would remain infected. We did, however, remove from three mice three lots of lice, numbering about 25 to the lot, and kept them on hair in petri dishes at room temperature in August; they were all dead by the end of 48 hours. The time which elapsed between the death of a mouse and the removal of its lice was likewise not definitely determined, although it never exceeded 18 hours. An effort was made to remove the lice as soon as possible. Lice were collected in the morning from mice which died during the previous night, and lice were collected during the day from mice dying during the day.

These transmission experiments, as conducted, excluded the entrance of several possible factors which might have operated to make the contact experiments successful, namely, the agency of the blood-sucking mites, and the eating of infected mice, or possibly infected secretions or excretions, by healthy mice.

The strain of *Bacterium tularense* (S) used was one isolated from a human case in Utah in the summer of 1920.

*First series.*—In this series, transmission of tularæmia to healthy mice was effected by the transfer to them of lice removed from mice dead after subcutaneous inoculation with diluted heart's blood of

infected mice. Eleven healthy mice were thus infested, the number of lice used for each mouse varying from 10 to 43. Nine of the 11 died with typical lesions of the spleen due to tularæmia, and, moreover, the spleen in each instance, when rubbed on the shaved, abraded skin of a guinea pig, caused the death of this animal with the typical lesions of the disease. Two mice of this series remained negative; 1 had been infested with 10 lice and the other with 33 lice.

*Second series.*—Transmission of tularæmia was effected in the second series by the transfer of lice from louse-infected mice of the first series to healthy mice. Six healthy mice were thus infested, the number of lice transferred to each mouse varying from 5 to 25. Three of the six mice died with typical lesions of the spleen due to tularæmia, and the spleen in each instance, when rubbed on the shaved, abraded skin of the abdomen of a guinea pig caused the death of this animal with the typical lesions of the disease. Three mice of this series remained well; they had been infested with 5, 9, and 20 lice, respectively.

### INFECTIVITY OF THE MITE LIPONYSSUS ISABELLINUS.

Ten mites of the species *Liponyssus isabellinus* were collected while on the ends of the hairs about to leave the body of a white mouse dead from tularæmia after subcutaneous inoculation with infected blood. The mites were rubbed in a mortar with saline solution and the suspension was injected subcutaneously into a white mouse, causing its death in 4½ days, with typical lesions of tularæmia. A portion of the spleen of the latter mouse was used for subcutaneous injection of one guinea pig, while another portion was rubbed on the shaved abraded skin of another guinea pig; both guinea pigs died acutely with typical lesions of tularæmia. Had these blood-sucking mites been present in sufficient numbers, transmission experiments would have been conducted along the lines followed in the louse transmission.

### FAILURE OF ATTEMPTS TO RENDER MICE LOUSE-FREE AND MITE-FREE.

We endeavored to render white mice free from parasites. Nicotine sulphate in water (1:1000), 95 per cent alcohol, undiluted kerosene, and undiluted gasoline were used. A lousy mouse dipped into either of these agents suffered considerable toxic effects; he was rendered apparently free from parasites for a few days, but if examined 7 to 10 days later, he was again lousy. These agents did not kill the eggs. We failed to find a delousing agent into which a mouse could be dipped four or five times at intervals of a week without causing death or injury to the mouse. Had we been able to render our mice lice-free and mite-free we would have conducted with them a contact experiment similar to our two contact experiments with the expectation that transmission to contacts would not occur.

## THE ABSENCE OF LICE FROM HOUSE MICE.

An observation was made on the occurrence of lice on the ordinary house mouse found in various parts of the laboratory. The mice were caught during the night in snap traps and were collected each morning and examined. The hair of 56 mice were pulled out and searched for lice and mites. Only 1 louse and 16 mites were found. This louse was the rat louse *Polyplax spinulosa* (Burm). No specimen was found of the mouse louse *Polyplax serratus* (Burm) so commonly found on our white mice.

The apparent absence of lice from house mice has a bearing on the question of whether tularæmia would become epizoötic in house mice if by chance infected ones got at large.

The susceptibility of "gray" mice to the infection has already been shown by McCoy.[2] In an experiment of ours, house mouse No. 1, inoculated subcutaneously with the heart's blood of an infected white mouse, died with typical lesions of tularæmia. House mouse No. 2, inoculated with the heart's blood of No. 1, died with typical lesions of tularæmia. The infection was similarly carried over to No. 3. The spleen of No. 3, when rubbed on the shaved, abraded skin of a guinea pig caused its death with typical lesions of tularæmia.

TABLE 7.—*Transmission of tularæmia in white mice by the mouse louse Polyplax serratus.*

| Series. | Infected mice from which lice were removed. | | | Healthy mouse to which lice were transferred. | Length of time between infestation with lice and death of mouse. | Results: "Positive" means death of a guinea pig with typical lesions of tularæmia, the guinea pig having been rubbed on the shaved abraded skin of the abdomen with the spleen of the louse-infected mouse. |
|---|---|---|---|---|---|---|
| | No. of mouse. | Date of death. | Number of lice transferred from infected mouse to healthy mouse. | | | |
| | | | | | *Days.* | |
| First series: Transmission of tularæmia by lice, transferred from inoculated mice to healthy mice. | SM156 | July 2, 1921 | 15 | Mouse No. 1 | 5 | Positive. |
| | SM158 | do | 14 | do | | |
| | SM155 | July 3, 1921 | 3 | Mouse No. 2 | 9 | Do. |
| | SM160 | do | 5 | do | | |
| | SM161 | do | 15 | do | | |
| | SM162 | do | 10 | do | | |
| | SM163 | July 4, 1921 | 15 | Mouse No. 3 | 9 | Do. |
| | SM164 | do | 12 | do | | |
| | SM165 | do | 15 | Mouse No. 4 | 6 | Do. |
| | SM166 | do | 8 | do | | |
| | DC6 | do | 6 | do | | |
| | DC2 | July 5, 1921 | 30 | Mouse No. 5 | 7 | Do. |
| | DC2 | do | 30 | Mouse No. 6 | 9 | Do. |
| | SM172 | July 6, 1921 | 30 | Mouse No. 7 | 5 | Do. |
| | SM173 | do | 13 | do | | |
| | SM176 | July 7, 1921 | 15 | Mouse No. 8 | 6 | Do. |
| | SM178 | do | 12 | Mouse No. 9 | 12 | Do. |
| | SM181 | July 9, 1921 | 20 | Mouse No. 10 | | Negative. |
| | SM185 | do | 13 | do | | |
| | DC11 | July 10, 1921 | 10 | Mouse No. 11 | | Do. |
| Second series: Transmission of tularæmia was effected by lice transferred from louse-infected mice of first series to healthy mice. | 1 | July 7, 1921 | 14 | Mouse No. 20 | 5 | Positive. |
| | 7 | July 11, 1921 | 25 | Mouse No. 21 | 7 | Do. |
| | 5 | July 12, 1921 | 20 | Mouse No. 22 | | Negative. |
| | 2 | do | 5 | Mouse No. 23 | | Do. |
| | 8 | July 13, 1921 | 12 | Mouse No. 24 | 7 | Positive. |
| | 9 | July 19, 1921 | 9 | Mouse No. 25 | | Negative. |

[2] A plaguelike disease of rodents. By George W. McCoy, Passed Assistant Surgeon, United States Public Health Service. Public Health Bulletin No. 43, April, 1911.

SUMMARY.

The transmission of tularæmia was effected in 12 out of 17 attempts through the agency of the mouse louse (*Polyplax serratus*) by the transfer of lice from white mice dead of tularæmia to healthy white mice, the intervals elapsing between infestation of the healthy mice and their deaths varying from 5 to 12 days, the average being 7¼ days. The number of lice transferred in the 12 successful attempts varied from 12 to 43, the average being 25. The intervals which elapsed between the deaths of infected mice and the transfer of their lice to healthy mice varied from a few minutes to 18 hours. Transmission of tularæmia by lice was thus effected to two series of mice, the first series being infected by lice removed from inoculated mice and the second series being infected by lice removed from the louse-infected mice of the first series.

When inoculated mice were dropped into a jar in contact with lousy healthy mice, the infection killed off all the healthy mice in 25 days. Transmission in this case was probably due to lice.

Blood-sucking mites of the species *Liponyssus isabellinus* removed from an infected white mouse were crushed and injected subcutaneously into another white mouse causing its death from tularæmia.

The urine of infected white mice was infective for guinea pigs when injected subcutaneously into the latter. Similar urine failed to infect white mice when fed to them on corn meal.

The mouse louse *Polyplax serratus* commonly found on our white mice was absent from 56 house mice caught in snap traps in the laboratory.

*Acknowledgment.*—The determinations of specimens of lice and mites were made by Dr. H. E. Ewing, of the Bureau of Entomology, Department of Agriculture.

## VI. CULTIVATION OF BACTERIUM TULARENSE ON MEDIUMS NEW TO THIS ORGANISM.[1]

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The only culture mediums reported heretofore for the cultivation of *Bacterium tularense* are coagulated hen's egg yolk, originally used by McCoy and Chapin,[2] and hen's ovomucoid with a trace of yolk, recommended by Wherry and Lamb.[3] All attempts at cultivation on other laboratory mediums had failed.

The writer now reports the cultivation of this organism on (1) serum glucose agar, (2) glucose blood agar, (3) blood agar, (4) each of the foregoing mediums plus a piece of fresh, sterile rabbit spleen.

It is a common practice in laboratories to adapt an organism to growth on ordinary mediums after original isolation from man or animals has been accomplished by cultivation on some special medium. The mediums which are here reported for *Bacterium tularense* were used for original isolations of this organism from animals, and therefore the question of acquired adaptability to a new medium brought about by previous cultivation on a special medium is not involved.

The strains used for this cultural work were three human strains from Utah and one ground-squirrel strain from California, all obtained in 1920. These strains were originally obtained by the inoculation of human or squirrel tissue into guinea pigs, and they were subsequently passed for many generations through guinea pigs or rabbits, always by subinoculation of infected tissues. Original isolation from animals of pure cultures of these strains was accomplished by use of the proposed mediums; the resultant cultures have never been on egg medium either before or since their first isolation.

### COMPOSITION OF MEDIUMS.

(1) *Serum glucose agar.*—Beef infusion containing 1 per cent peptone and 1½ per cent agar adjusted to a reaction having a $p_H$ of 7.6 is kept on hand in stock. When needed, the stock agar is melted and brought to 45° C. in a water bath, at which temperature there is added 1 per cent glucose from a sterile 50 per cent solution of glucose and 5 per cent sterile horse serum. This is immediately tubed, slanted, and incubated 48 hours to insure sterility.

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[1] Reprinted from Public Health Reports 1922, vol. 37, pp. 102-115.

[2] Bacterium tularense, the Cause of a Plague-like Disease of Rodents. By George W. McCoy and Charles W. Chapin, Passed Assistant Surgeons, United States Public Health Service. Public Health Bulletin No. 53, January, 1912.

Further Observations on a Plague-like Disease of Rodents with a Preliminary Note on the Causative Agent, Bacterium tularense. By George W. McCoy and Charles W. Chapin, Passed Assistant Surgeons, United States Public Health Service. The Journal of Infectious Diseases, vol. X, No. 1, January, 1912, pp. 61-72.

[3] Infection of Man with Bacterium tularense. By William B. Wherry and B. H. Lamb. Journal of Infectious Diseases, 1914, vol. 15, p. 331.

(2) *Glucose blood agar.*—This is the same as (1) except that 5 per cent defibrinated rabbit blood is substituted for the horse serum.

(3) *Blood agar.*—This is the same as (2) except that no glucose is added.

(4) *Mediums (1), (2), and (3) plus spleen tissue.*—A spleen is removed from a healthy rabbit and under sterile precautions is cut into pieces of about 3 mm. diameter. One piece is rubbed on the slanted surface of each tube of a portion of mediums (1), (2), and (3) and the piece of spleen is left remaining on the surface of each slant just above the water of condensation. After 48 hours' incubation the tubes, if sterile, are ready for inoculation.

### COAGULATED HEN'S EGG YOLK.

At this place it will be well to give also the composition of the coagulated hen's egg yolk described by McCoy and Chapin. Fresh eggs are scrubbed with a brush in soap and water, if fecal matter is present on the shells, and then placed in a wire basket. The basket containing the eggs is dipped into 95 per cent alcohol for a few seconds, after which time it is withdrawn and the small amount of alcohol which still remains on the basket and eggs is ignited in order to remove the alcohol and help sterilize the shells.

While one person with clean hands holds an egg, grasping it at each end, an assistant strikes the shell in its middle with a sterile knife with sufficient force to crack the shell. The whites are separated from the yolks by decanting from one half of the shell to the other, thus allowing the whites to drain away while the yolks are saved and collected in a sterile beaker.

The volume of yolks is measured in a sterile graduate and to this is added sterile normal saline solution in the proportion of 40 per cent saline solution to 60 per cent egg yolk. Mix thoroughly. Tube in sterile test tubes, using a sterile funnel.

Place the tubes in metal racks constructed so as to allow one-half-inch space between the tubes for circulation. Heat the racked tube, in a slanting position for the first half hour at 70° C., and for the second half hour at 72° C. A uniform temperature is best maintained for this purpose in a water-jacketed chamber. The chamber should contain about a half inch of water above which the racks of tubes are exposed in the moist, heated air. After coagulation, paraffined sterile cork stoppers are substituted for the cotton plugs and the tubes are incubated upright for three or four days to insure against a slow-growing contamination.

Instead of the jacketed chamber one may, with patience, use an Arnold steam sterilizer, a board having been placed at the bottom to protect the tubes from the direct steam.

The finished medium should be soft; that is, the surface of the slant should yield slightly when pressed with a platinum loop, and to that end the medium should not be overheated. A glazed surface results from overheating. The tubes should be stored in the cold room, unexposed to the light. The water of condensation in a batch of medium which grew the organism very well showed a reaction having a $p_H$ of 6.8. No titration or adjustment of reaction has been done on batches of this medium used for routine cultivation of the organism in the laboratory.

### CULTIVATION ON SERUM GLUCOSE AGAR.

Serum glucose agar was successfully used (1) for original isolation of strains from the spleens of infected rabbits, (2) for second and third isolations of strains from the spleens of infected guinea pigs, (3) for subcultures (without the addition of a piece of fresh, sterile rabbit spleen), and (4) for subcultures (with the addition of a piece of fresh, sterile rabbit spleen).

(1) *Original isolation of strains.*—Human strains "J" and "G" and ground-squirrel strain "S F" were isolated on serum glucose agar by planting a piece of infected rabbit spleen on serum glucose agar in each instance. (See Table 8, and Table 10, animals 1, 3, and 4.) These human strains had been carried over from July 3 and September 9, 1920 (the dates on which they left the humans), to April 11, 1921 (the date on which they were cultured), in laboratory animals, i. e., guinea pigs and rabbits. These human strains having been carried over exclusively by animal passages for 9 and 7 months, respectively, original isolations were made on serum glucose agar. The ground-squirrel strain had been carried over from May, 1920 (the date on which it left a California ground squirrel), to April 10, 1921 (the date on which it was cultured), in laboratory animals, guinea pigs, and rabbits. This California ground-squirrel strain having been carried over exclusively by animal passages for 11 months, original isolation was made on serum glucose agar.

On April 10 and 11, 1921, each of the three strains was inoculated on a serum glucose agar slant by transferring to the surface of the medium a piece about 3 mm. in diameter, taken under sterile precautions, from the spleen of a rabbit dead from tularæmia; the piece of spleen was rubbed over the surface of the medium as forcibly as the consistency of the latter would permit and then left to remain on the solid medium just above the water of condensation. The inoculated tubes were placed at 37° C. for eight days, at the end of which time they were observed for the first time and found to have a growth which was not examined microscopically, but the tubes were replaced in the incubator under the impression that the growths

were contaminations. The tubes remained at 37° for a month longer without observation, at the end of which time they were given another examination preliminary to discarding. The presence of the growth saved the tubes from the discard and they remained another month at room temperature, when, on June 23 and August 23, the growths were examined microscopically and found to simulate *Bacterium tularense.*

Confirmation of these original cultures was obtained when subcultures in the fifth generation on glucose blood agar plus a piece of fresh, sterile rabbit spleen caused acute death with typical lesions of tularæmia in a set of guinea pigs which had been rubbed on the shaved abraded skin of the abdomen with those subcultures.

Further confirmation was obtained when cultures derived from the spleens of the above set of guinea pigs caused acute death with typical lesions of tularæmia in a second set of guinea pigs, the second set having been rubbed on the shaved abraded skin of the abdomen with subcultures in the fifth generation on glucose blood agar plus a piece of fresh sterile rabbit spleen. (For details see Table 8.)

(2) *Second and third isolations.*—The two sets of guinea pigs just referred to not only gave confirmation to the identity of the original cultures which were isolated on serum glucose agar but afforded opportunities for the second and third isolations of those strains from animals on serum glucose agar as follows: On the death of a guinea pig of either set a piece of its spleen was planted on a tube of serum glucose agar and incubated at 37° C. Ten tubes were thus planted from 10 guinea pigs. (See Table 10, animals 5 to 15.) Five tubes showed growth and five showed no growth.

The five growths therefore constituted second and third isolations of our three human strains and one ground squirrel strain on serum glucose agar.

(3) *Subcultivation on serum glucose agar.*—Plain serum glucose agar was used for subcultures as follows: Starting with a piece of infected spleen of a rabbit or guinea pig, this was planted on a tube of serum glucose agar and the resultant growth was transferred for its second generation to a tube of serum glucose agar. Thirteen serum glucose agar tubes were thus inoculated with six cultures having an antecedent history as indicated. Four of the tubes developed growth after an average of six days and nine failed to grow. (See Table 10, footnotes 4, 5, and 6.) The four growths constituted subcultivation on serum glucose agar.

(4) *Subcultivation on serum glucose agar* (plus a piece of fresh sterile rabbit spleen).—Subcultivation from serum glucose agar to serum glucose agar plus a piece of fresh sterile rabbit spleen was successful in 14 out of 27 attempts; the average time which elapsed before the appearance of growth on the 14 tubes was 2½ days.

Three cultures were thus carried for the third to sixth generations on serum glucose agar plus a piece of fresh sterile rabbit spleen, the first generation in each case having been obtained on a tube of serum glucose agar inoculated with a piece of spleen of an infected guinea pig. (See Table 10, animals 5, 6, and 12.) The fifth generation of culture 1 was injected subcutaneously into a guinea pig, causing its death in three days with typical lesions of tularæmia. The sixth generation of culture 1 was rubbed on the shaved abraded skin of a guinea pig and caused its death on the twelfth day with typical lesions of tularæmia. (See Table 8, footnote 2.) The sixth generations of cultures 2 and 3 when injected subcutaneously each failed to kill a guinea pig.

*Summary.*—Serum glucose agar *per se* is a poor medium for the cultivation of *Bacterium tularense* because subcultures from serum glucose agar to serum glucose agar grew in only 30 per cent of the instances. Serum glucose agar is a fair medium for the cultivation of *Bacterium tularense* if the surface of the medium is provided with a piece of fresh spleen tissue. Such tissue may be supplied in either of two ways.

If the inoculating material is in substance a piece of fresh tissue, as is the case when a piece of the spleen of an infected rabbit or guinea pig is planted on a serum glucose agar tube, growth may be expected in 48 per cent of the tubes. Growth did occur in 11 out of 23 such attempts, the average time before the appearance of growth being 8½ days.

If the inoculating material is a culture, as is the case in subcultivation, and a piece of fresh sterile rabbit spleen has been supplied to the surface of the serum glucose agar tubes, growth may be expected in 51 per cent of the tubes. Growth did occur in 14 out of 27 such attempts, the average time which elapsed before the appearance of growth on the 14 tubes being 2½ days.

It was noted, however, that the growth of *Bacterium tularense* on serum glucose agar under all conditions tends to become scanty and its virulence tends to become either diminished or lost.

### CULTIVATION ON GLUCOSE BLOOD AGAR.

Glucose blood agar was successfully used (1) for original isolation of strains from the spleens of infected rabbits, (2) for second and third isolations of strains from the spleens, liver, and heart's blood of infected guinea pigs, (3) for subcultures (without the addition of a piece of fresh sterile rabbit spleen), and (4) for subcultures (with the addition of a piece of fresh sterile rabbit spleen).

(1) *Original isolation of strains.*—Human strains "J" and "S" were isolated on glucose blood agar by planting a piece of infected

rabbit spleen on a glucose blood agar tube in each case. (See Table 10, animals 1 and 2.)

These human strains had been maintained in the laboratory in guinea pigs and rabbits from September 9 and July 3, 1920 (the dates on which they left the humans), to April 11 and 13, 1921, the dates on which they were first cultured; they had not been on any culture medium during that period. On April 11 and 13 these strains were inoculated on glucose blood agar slants by transferring to the surface of the medium a piece about 3 mm. in diameter taken under sterile precautions from the spleen of a rabbit dead from tularæmia. The tubes were incubated at 37° and not observed for 8 days, at the end of which time growth was present in case of the "J" strain, but no notation was then made of growth of "S" strain, although it was noted at a later date. As in the case of original cultures on serum glucose agar these cultures remained unobserved in the incubator for one month longer, when, on May 24, strain "J" was subcultured and it was subcultured next again on June 22; whereas strain "S" went from April 13 to July 4 before the first subculture was made.

Confirmation of the original cultures was obtained when subcultures in the fifth generation on glucose blood agar, plus a piece of fresh, sterile rabbit spleen caused acute death with typical lesions of tularæmia in a set of guinea pigs which had been rubbed on the shaven, abraded skin of the abdomen with those subcultures.

Further confirmation was obtained when cultures derived from the spleens of the above set of guinea pigs caused acute death with typical lesions of tularæmia in a second set of guinea pigs, the second set having been rubbed on the shaved, abraided skin of the abdomen with subculture in the fifth generation on glucose blood agar, plus a piece of fresh, sterile rabbit spleen. (For details see Table 9.)

(2) *Second and third isolations.*—The two sets of guinea pigs just referred to not only gave confirmation to the identity of the original cultures which were isolated on glucose blood agar, but afforded opportunity for the second and third isolations of these strains from animals on glucose blood agar as follows: On the death of a guinea pig of either set, a piece of its spleen was planted on a tube of glucose blood agar and incubated at 37° C. Thirteen tubes were thus planted from 13 guinea pigs, including liver and heart's blood in two instances. (See Table 10, animals 5 to 15.) All of the 13 tubes showed growth after an average of a little less than four days.

These 13 growths therefore constituted second and third isolations of these two human strains on glucose blood agar.

(3) *Subcultivation on glucose blood agar.*—Plain glucose blood agar was used for subculture as follows: Starting with a piece of infected spleen of a rabbit or guinea pig, this was planted on a tube of glucose

blood agar and the resultant growth was transferred for its second generation to a tube of glucose blood agar. Five glucose blood agar tubes were inoculated with five cultures having an antecedent history as indicated. None of the tubes developed any growth. (See Table 10, animals 21 to 25.)

Four cultures grown for two or three generations on glucose blood agar, plus a piece of fresh sterile rabbit spleen, were subsequently transferred for two or three generations to glucose blood agar; the transfer was accompanied by a falling off in the abundance of growth. Moreover, a falling off in virulence also took place because the last generation of each culture was rubbed on the shaven, abraded skin of one or two guinea pigs, with the result that one guinea pig died acutely on the seventh day, two died tardily on the twelfth day, one died subacutely on the twenty-third day, and two guinea pigs, vaccinated with the fourth culture, remained well. (See Table 8, footnote 1, and Table 9, footnotes 1, 2, and 3.)

(4) *Subcultivation on glucose blood agar* (plus a piece of fresh, sterile rabbit spleen).—Fifteen cultures which had been isolated by planting a piece of the spleen of an infected rabbit or guinea pig on either glucose blood agar or serum glucose agar, with or without the addition of a piece of fresh, sterile rabbit spleen were subcultured from the second to the fifth generations on glucose blood agar plus a piece of fresh sterile rabbit spleen; the fifth generation in each instance was rubbed on the shaven abraded skin of a guinea pig causing its death acutely with typical lesions of tularæmia. (See Tables 8 and 9, and Table 10, footnote 5.)

This medium was successful in 88 out of 103 attempts at subcultivation of the organism; the average time which elapsed before the appearance of growth on the 88 tubes was two days; growth appeared before the end of 24 hours in 61 of the 88 tubes.

*Isolation of cultures* from animal tissues to glucose blood agar plus a piece of fresh sterile rabbit spleen was successful in 21 out of 27 attempts. The animal tissues consisted of a piece of the spleen of 19 infected rabbits and guinea pigs, a piece of the liver of one infected guinea pig, the heart's blood of two infected guinea pigs, and the heart's blood of five infected white mice. The average of time before the appearance of growth on the 21 tubes was five days. (See Table 10.)

*Summary.*—Glucose blood agar *per se* is not a good medium for the cultivation of *Bacterium tularense*. The plain glucose blood agar is a good medium only when the infected material with which it is inoculated is in substance a piece of fresh tissue as represented by a piece of the infected spleen of a rabbit or guinea pig. This was the case when 25 rabbits or guinea pigs dead from tularæmia each furnished a piece of spleen which was inoculated on a tube of glucose

blood agar. Growth appeared after an average of 4⅓ days on 21 tubes; no growth appeared on 4.

Subcultures were made on 56 tubes of glucose blood agar; growth appeared after an average of three days on 26 tubes and failed to appear on 30. The growth, when subcultured on plain glucose blood agar became scanty and of lowered virulence.

The falling off in growth and virulence which accompanied the transfer of cultures to plain glucose blood agar is accounted for by the absence of a piece of fresh sterile rabbit spleen from the medium.

On the other hand, glucose blood agar plus a piece of fresh sterile rabbit spleen is a good medium, both for the isolation of *Bacterium tularense* from animals and for subcultivation. This medium was successful in 21 out of 26 attempts at isolation of the organism from the tissues of 26 animals. This medium was successful in 88 out of 103 attempts at subcultivation of the organism. Fifteen subcultures each in the fifth generation on this medium were rubbed on the shaven abraded skin of 15 guinea pigs, causing acute death in each instance with typical lesions of tularæmia, thus indicating no loss of virulence after subcultivation on this medium.

### CULTIVATION ON BLOOD AGAR.

Blood agar was used (1) for second and third isolation of strains from rabbits and guinea pigs and (2) for subcultures.

(1) *Second and third isolations.*—No attempt was made at original isolation of our strains from animals on blood agar, but second and third isolations of these strains from animals were accomplished on blood agar. (See Table 10, animals 5, 8, 10, 11, and 13.)

The organism was isolated on a blood agar slant which had been inoculated with a piece of the spleen of animal No. 5 dead after vaccination with the fifth generation of a culture which was *originally isolated* on serum glucose agar and subsequently grown for four generations on glucose blood agar plus a piece of fresh sterile rabbit spleen. Practically the same statement can be made concerning blood agar cultures obtained from animals 8, 10, 11, and 13.

Isolation on blood agar slants was also accomplished from the spleens of animals Nos. 21, 22, 23, 24, and 25 (see Table 10, all of which had been inoculated with the heart's blood of Mouse 206. For one year previous to Mouse 206 the strain had had a continuous passage through mice, tame rabbits, and guinea pigs, back to human case "S" in Utah except that once during that year (from May 27 to June 6, 1921) the strain was carried on coagulated egg yolk for ten days.

(2) *Subcultivation.*—Subcultures on blood agar failed to grow in 13 out of 15 attempts; growth appeared on two tubes after three and six days, respectively.

*Summary.*—For the isolation of *Bacterium tularense* from infected animals, this medium was successful in 9 out of 15 attempts; growth appeared on the nine tubes after an average of seven days. The success of this medium for isolation from infected animals was undoubtedly due to the transfer to the medium of a piece of fresh tissue as represented by the piece of infected spleen of rabbit or guinea pig with which the tubes were inoculated. For subcultivation of this organism, blood agar *per se* is a poor medium, growth having been obtained only two times out of 15 attempts at subcultivation on this medium.

TABLE 8.—*Serum glucose agar used for original isolation of human strains "J" and "G" and squirrel strain "SF" of Bacterium tularense. Subcultures made on glucose blood agar and serum glucose agar, each being supplemented by a piece of fresh sterile rabbit spleen.*

| Human strain "J." (See Table 10, animal No. 1.) | Human strain "G." (See Table 10, animal No. 3.) | California ground squirrel strain "SF." (See Table 10, animal No. 4.) |
|---|---|---|
| 1921. | 1921. | 1921. |
| Apr. 11. Piece of infected rabbit spleen planted on serum glucose agar. | Apr. 11. Piece of infected rabbit spleen planted on serum glucose agar. | Apr. 10. Piece of infected rabbit spleen planted on serum glucose agar. |
| June 23. Subcultured on glucose blood agar plus a piece of fresh sterile rabbit spleen. | June 22. Subcultured on glucose blood agar plus a piece of fresh sterile rabbit spleen.[1] | Aug. 23. Subcultured on glucose blood agar plus a piece of fresh sterile rabbit spleen. |
| July 4. Subcultured on above medium. | July 4. Subcultured on above medium. | Aug. 25. Subcultured on above medium. |
| July 9. Subcultured on above medium. | July 10. Subcultured on above medium. | Aug. 26. Subcultured on above medium. |
| July 14. Subcultured, fifth generation. | July 14. Subcultured, fifth generation. | Aug. 28. Subcultured, fifth generation. |
| July 18. Vaccinated a guinea pig with a loop of 4-day culture of the fifth generation. | July 18. Vaccinated guinea pig with a loop of a 4-day culture of the fifth generation. | Aug. 29. Subcultured, sixth generation. |
| July 25. Guinea pig dying, chloroformed, planted pieces of its spleen on 5 mediums. (See Table 10, animal No. 7.) | July 25. Guinea pig dead, planted pieces of its spleen on 6 media. (See Table 10, animal No. 12.)[2] | Aug. 31. Subcultured, on above medium. |
| Aug. 21. Subcultured from glucose blood agar to glucose blood agar plus a piece of fresh sterile rabbit spleen. | Aug. 6. Subcultured from glucose blood agar plus a piece of fresh sterile rabbit spleen to same medium. | Sept. 6. Subcultured on above medium. |
| Aug. 31. Subcultured on above medium. | Aug. 21. Subcultured to same medium. | Sept. 14. Subcultured on above medium. |
| Sept. 2. Subcultured on above medium. | Aug. 23. Subcultured to same medium. | Sept. 2. Vaccinated one guinea pig with a loop of a 4-day culture of the sixth generation. |
| Sept. 3. Subcultured, fifth generation. | Aug. 24. Subcultured, fifth generation. | Sept. 5. Injected another guinea pig subcutaneously with a loop of an 8-day culture of the fifth generation. |
| Sept. 5. Vaccinated a guinea pig with a loop of 48-hour culture of the fifth generation. | Aug. 26. Vaccinated a guinea pig with a loop of 48-hour culture of the fifth generation. | Sept. 12. Both guinea pigs dead with typical lesions of tularæmia. Vaccinated a guinea pig with the spleen of the guinea pig which had been injected subcutaneously. |
| Sept. 11. Guinea pig died with lesions of the lymph glands, spleen and liver typical of tularæmia. | Sept. 1. Guinea pig dead with typical lesions of tularæmia. | Sept. 19. Guinea pig died with typical lesions of tularæmia. |

[1] This culture of June 22 being in the second generation was also subcultured for the third, fourth, and fifth generations on glucose blood agar without the addition of a piece of fresh sterile rabbit spleen. The fifth generation was then vaccinated on two guinea pigs on July 23. Both guinea pigs were dead Aug. 4 with typical lesions of tularæmia.

[2] On July 25 one piece of spleen was planted on serum glucose agar.

Aug. 21. Subcultured on above medium.
Sept. 1. Subcultured on serum glucose agar plus a piece of fresh sterile rabbit spleen.
Sept. 9. Subcultured on above medium.
Sept. 11. Subcultured, fifth generation.
Sept. 14. Subcultured, sixth generation.
Sept. 14. Guinea pig injected subcutaneously with 3-day culture of the fifth generation.
Sept. 17. Guinea pig dead with typical lesions of tularæmia.
Sept. 18. Vaccinated a guinea pig with a loop of 4-day culture of the sixth generation.
Sept. 30. Guinea pig died with typical lesions of tularæmia.

TABLE 9.—*Glucose blood agar used for original isolation of human strains "J" and "S" of Bacterium tularense. Subcultures on glucose blood agar plus a piece of fresh sterile rabbit spleen.*

| Human strain "J." (See Table 10, animal No. 1.) | Human strain "J." (See Table 10, animal No. 1.) | Human strain "S." (See Table 10, animal No. 2.) |
|---|---|---|
| 1921. | 1921. | 1921. |
| Apr. 11. Piece of infected rabbit spleen planted on glucose blood agar. | Apr. 11. Piece of infected rabbit spleen planted on glucose blood agar. | Apr. 13. Piece of infected rabbit spleen planted on glucose blood agar. |
| May 24. Subcultured on glucose blood agar plus a piece of fresh sterile rabbit spleen. | June 22. Subcultured on glucose blood agar plus a piece of fresh sterile rabbit spleen. | July 4. Subcultured on glucose blood agar plus a piece of fresh sterile rabbit spleen. |
| June 22. Subcultured on above medium. | June 24. Subcultured on above medium.[2] | July 10. Subcultured on above medium.[3] |
| July 10. Subcultured on above medium.[1] | July 3. Subcultured on above medium. | July 14. Subcultured on above medium. |
| July 14. Subcultured fifth generation. | July 9. Subcultured on above medium. | July 18. Vaccinated a guinea pig with a loop of four-day culture of the fourth generation. |
| July 18. Vaccinated a guinea pig with a loop of a 4-day culture of the fifth generation. | July 14. Subcultured sixth generation. | July 25. Guinea pig dying, chloroformed, planted pieces of its spleen on 7 mediums. (See Table 10, animal No. 8.) |
| July 27. Guinea pig dying, chloroformed, planted pieces of its spleen on 7 mediums. (See Table 10, animal No. 5.) | July 18. Vaccinated a guinea pig with a loop of a 4-day culture of the sixth generation. | Aug. 6. Subcultured from glucose blood agar plus a piece of fresh sterile rabbit spleen to same medium. |
| Aug. 8. Subcultured from glucose blood agar plus a piece of fresh sterile rabbit spleen to same medium. | July 31. Guinea pig dying, chloroformed, planted pieces of its spleen on 7 mediums. (See Table 10, animal No. 6.) | Aug. 21. Subcultured to same medium. |
| Aug. 14. Subcultured to same medium. | Aug. 6. Subcultured from glucose blood agar plus a piece of fresh sterile rabbit spleen to same medium. | Aug. 23. Subcultured to same medium. |
| Aug. 21. Subcultured to same medium. | Aug. 21. Subcultured to same medium. | Aug. 24. Subcultured fifth generation. |
| Aug. 23. Subcultured to same medium. | Aug. 23. Subcultured to same medium. | Aug. 26. Vaccinated a guinea pig with a loop of 48-hour culture of the fifth generation. |
| Aug. 24. Subcultured sixth generation. | Aug. 24. Subcultured fifth generation. | Sept. 1. Guinea pig dying chloroformed, planted pieces of its spleen on 7 mediums. (See Table 10, animal No. 9.) |
| Aug. 26. Vaccinated a guinea pig with a loop of a 48-hour culture of the sixth generation. | Aug. 26. Vaccinated a guinea pig with a loop of a 48-hour culture of the fifth generation. | Sept. 6. Subcultured from glucose blood agar plus a piece of fresh sterile rabbit spleen to same medium. |
| Sept. 9. Guinea pig dying, chloroformed, showed typical lesions of tularæmia. | Sept. 9. Guinea pig dying, chloroformed, showed typical lesions of tularæmia. | |

[1] This subculture planted July 10 being in the fourth generation was also subcultured for the fifth and sixth generations on glucose blood agar without the addition of a piece of fresh sterile rabbit spleen. This sixth generation was on July 23 vaccinated on a guinea pig which on Aug. 15 was dying and was chloroformed; it showed the typical lesions of the subacute type of tularæmia.

[2] This subculture planted June 24 being in the third generation was also subcultured for the fourth, fifth, and sixth generations on glucose blood agar without the addition of a piece of fresh sterile rabbit spleen. This sixth generation was on July 23 vaccinated on two guinea pigs which remained well.

[3] July 14. The subculture planted July 10 being in the third generation was also subcultured on July 14 on glucose blood agar without the addition of a piece of fresh sterile rabbit spleen.
July 17. Subcultured on above medium.
July 23. Vaccinated a guinea pig with a loop of 6-day culture of the fifth generation.
July 30. Guinea pig dying, chloroformed, planted pieces of its spleen on 8 mediums. (See Table 10, animal No. 10.)
Aug. 7. Subcultured from blood agar to blood agar.
Aug. 21. Subcultured on glucose blood agar plus a piece of fresh sterile rabbit spleen.
Aug. 23. Subcultured on the above medium.
Aug. 24. Subcultured fifth generation.
Aug. 26. Vaccinated a guinea pig with a loop of 48-hour culture of fifth generation.
Sept. 1. Guinea pig dying, chloroformed, planted pieces of its spleen on 7 mediums. (See Table 10, animal No. 11.)

TABLE 10.—*Comparative value of several mediums for the isolation of Bacterium tularense from the spleens and heart's blood of 29 infected animals.*

| No. of animal. | Either a piece of the spleen or the heart's blood of infected animals was planted. | Date planted. | Mediums on which pieces of infected tissue were planted. | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Coagulated egg yolk. | Glucose blood agar slant plus a piece of fresh sterile rabbit spleen. | Glucose blood agar slant. | Blood agar slant. | Serum glucose agar slant. | Plain agar slant. | Glucose fermentation tube. |
| | (A) ORIGINAL ISOLATIONS OF BACTERIUM TULARENSE. | 1921. | | | | | | | |
| 1 | Human strain "J" from spleen of rabbit J45R | Apr. 11 | Good growth. | | Growth after 8 days.[1] | | Growth after 8 days.[1] | No growth. | No growth. |
| 2 | Human strain "S" from spleen of rabbit S51R | Apr. 13 | Good growth after 48 hours. | | Growth [1] | | No growth | do | Do. |
| 3 | Human strain "G" from spleen of rabbit GCR3 | Apr. 11 | Growth | | No growth | | Growth after 8 days.[1] | do | Do. |
| 4 | California ground squirrel strain "SF" from spleen of rabbit SF25R. | Apr. 10 | Growth, second day. | | do | | Growth after 9 days.[1] | do | Do. |
| | (B) SECOND AND THIRD ISOLATIONS OF ABOVE STRAINS. | | | | | | | | |
| 5 | Spleen of guinea pig dead after vaccination [2] with subculture from animal No. 1. | July 27 | Growth, third day. | About 100 colonies, fifth day.[1] | About 50 colonies, fourth day.[3] | 5 colonies, fifth day.[4] | Growth, fifth day.[5] | No growth. | No growth. |
| 6 | do | July 31 | Growth at end of 24 hours. | About 100 colonies, fourth day.[1] | do.[3] | | 3 colonies, fourth day.[5] | do | Do. |
| 7 | do | July 25 | Contaminated. | Contaminated. | Pure culture, fifth day.[1] | Contaminated. | Contaminated. | | |
| 8 | Spleen of guinea pig dead after vaccination [2] with subculture from animal No. 2. | do | Growth. | Good growth, fifth day.[1] | Good growth, fifth day.[6] | Growth, fifth day.[6] | No growth | No growth. | Do. |
| 9 | Spleen of guinea pig dead after vaccination [2] with subculture from animal No. 8. | Sept. 1 | No growth | Good growth, third day.[4] | Good growth, third day. | No growth | do | do | Do. |
| 10 | Spleen of guinea pig dead after vaccination [2] with subculture from animal No. 2. | July 30 | do | do.[1] | do.[4] | Good growth, third day.[4] | do | do | Do. |

[1] Subcultured on glucose blood agar plus a piece of fresh sterile rabbit spleen from second to fifth generations; the fifth generation was rubbed on the shaved abraded skin of a guinea pig causing its death acutely with typical lesions of tularæmia.

[2] "Vaccination" means that the shaved abraded skin was rubbed with a culture or with a piece of infected tissue.

[3] Subcultures on this same medium for the second, third, and fourth generations grew well.

[4] A subculture on this same medium for the second generation showed growth.

[5] Subcultured on serum glucose agar for the second and third generations and on serum glucose agar plus a piece of fresh sterile rabbit spleen for the fourth to seventh generations; the sixth and seventh generations injected subcutaneously failed to kill a guinea pig.

[6] A subculture on this same medium for the second generation failed to grow.

TABLE 10.—*Comparative value of several mediums for the isolation of Bacterium tularense from the spleens and heart's blood of 29 infected animals*—Continued.

| No. of animal. | Either a piece of the spleen or the heart's blood of infected animals was planted. | Date planted. | Mediums on which pieces of infected tissue were planted. | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Coagulated egg yolk. | Glucose blood agar slant plus a piece of fresh sterile rabbit spleen. | Glucose blood agar slant. | Blood agar slant. | Serum glucose agar slant. | Plain agar slant. | Glucose fermentation tube. |
| | (B) SECOND AND THIRD ISOLATIONS OF ABOVE STRAINS—Continued. | | | | | | | | |
| 11 | Spleen of guinea pig dead after vaccination [2] with subculture from animal No. 10. | Sept. 1 | Good growth, third day. | Good growth, third day. | Good growth, third day. | 1 dozen colonies, ninth day. | 1 dozen colonies, ninth day. | No growth. | No growth. |
| | Heart's blood of guinea pig No. 11 | do | No growth | 25 colonies, third day. | | | | do | Do. |
| 12 | Spleen of guinea pig dead after vaccination with subculture from animal No. 3. | July 25 | Growth | Good growth, fifth day.[1] | Good growth, fifth day.[3] | | 30 colonies after 21 days.[7] | do | Do. |
| 13 | Spleen of guina pig dead after vaccination with spleen of guinea pig dead after subcutaneous injection with subculture from animal No. 4. | Sept. 19 | Growth at end of 24 hours. | Growth at third 48 hours. | Growth at end of 48 hours. | Growth at end of 5 days. | Growth at end of 4 days. | Contaminated. | Do. |
| 14 | Spleen of guinea pig dead after subcutaneous injection with cultures grown on glucose blood agar. | July 28 | No growth | 1 dozen colonies, fifth day.[1] | 50 colonies, third day.[4] | No growth | No growth | No growth. | Do. |
| | Heart's blood of guinea pig No. 14 | do | do | Growth around tissue, ninth day.[4] | Growth, seventh day.[3] | do | do | do | Do. |
| 15 | Spleen of guinea pig dead after subcutaneous injection with cultures grown on glucose blood agar plus a piece of fresh sterile rabbit spleen. | July 24 | Growth, second day. | Good growth, second day.[2] | Good growth, second day.[9] | | | do | |
| | Liver of guinea pig No. 15 | do | do | do.[1] | do.[4] | | | do | |
| | (C) ISOLATIONS FROM VARIOUS SOURCES. | | | | | | | | |
| 16 | Heart's blood of white mouse SM206 inoculated subcutaneously with heart's blood of mouse. | July 26 | No growth | Growth, sixth day.[3] | | | | No growth. | |
| 17 | Heart's blood of white mouse SM207 inoculated subcutaneously with heart's blood of mouse. | do | do | Growth eighth day.[8] | | | | do | |
| 18 | Heart's blood of white mouse SM214 inoculated subcutaneously with culture on egg. | Aug. 14 | Good growth. | No growth | | | | do | |
| 19 | Heart's blood of white mouse SM215 inoculated subcutaneously with culture on egg. | do | Good growth, second day. | Growth, seventh day.[3] | | | | do | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 20 | Heart's blood of white mouse SM217 inoculated subcutaneously with heart's blood of mouse. | July 17 | .....do..... | No growth.... | ..... | ..... | ..... | .....do..... | |
| 21 | Spleen of rabbit RL20 inoculated subcutaneously with heart's blood of mouse SM206. | July 26 | Good growth, third day. | Good growth, sixth day.[3] | Growth, fourth day.[6] | No growth.... | Good growth, sixth day.[6] | .....do..... | |
| 22 | Spleen of rabbit RL26 inoculated subcutaneously with heart's blood of mouse SM206. | July 29 | Good growth, sixth day. | .....do.[4]..... | Good growth, third day.[6] | Good growth, ninth day.[6] | No growth.... | .....do..... | No growth. |
| 23 | Spleen of rabbit RL3 inoculated subcutaneously with heart's blood of mouse SM206. | July 30 | Growth, second day. | No growth.... | Growth, third day.[6] | Growth, eighth day.[6] | Good growth, seventh day.[6] | .....do..... | |
| 24 | Spleen of rabbit RL24 inoculated subcutaneously with heart's blood of mouse SM206. | ...do.... | .....do..... | .....do..... | .....do.[6]..... | Growth, third day.[6] | No growth.... | .....do..... | Do. |
| 25 | Spleen of rabbit GCR inoculated subcutaneously with heart's blood of mouse SM206. | July 29 | No growth.... | .....do..... | Growth, fourteenth day.[6] | Growth, seventeenth day.[6] | .....do..... | .....do..... | Do. |
| 26 | Spleen of guinea pig vaccinated with spleen of mouse 30. | ...do.... | Good growth, fifth day. | Good growth, ninth day. | No growth.... | No growth.... | .....do..... | .....do..... | Do. |
| 27 | Spleen of guinea pig vaccinated with spleen of mouse 21. | Aug. 5 | Good growth, second day. | Good growth, seventh day.[1] | .....do..... | ..... | .....do..... | .....do..... | Do. |
| 28 | Spleen of guinea pig vaccinated with spleen of mouse C4. | Aug. 14 | Good growth, third day. | Good growth, fourth day.[1] | Growth, fourth day.[3] | ..... | Growth, twelfth day.[6] | .....do..... | Do. |
| 29 | Spleen of guinea pig vaccinated with spleen of mouse 31. | Aug. 30 | Good growth, fifth day. | No growth.... | Growth, seventh day. | No growth.... | No growth.... | .....do..... | Do. |
| | Total number of "growths" on each medium.. | ..... | 23 | 21 | 22 | 9 | 11 | | |
| | Total number of "no growths" on each medium | ..... | 8 | 6 | 4 | 6 | 12 | | |
| | Percentage of "growths" on each medium..... | ..... | 74.2 | 77.7 | 84.6 | 60 | 48 | | |

[1] Subcultured on glucose blood agar plus a piece of fresh sterile rabbit spleen from second to fifth generations; the fifth generation was rubbed on the shaved abraded skin of a guinea pig causing its death acutely with typical lesions of tularæmia.

[2] "Vaccination" means that the shaved abraded skin was rubbed with a culture or with a piece of infected tissue.

[3] Subcultures on this same medium for the second, third, and fourth generations grew well.

[4] A subculture on this same medium for the second generation showed growth.

[6] A subculture on this same medium for the second generation failed to grow.

[7] Subcultured on serum glucose agar for the second generation and on serum glucose agar plus a piece of fresh sterile rabbit spleen for the third to sixth generations; the sixth generation rubbed on the shaved abraded skin of the guinea pig caused its death with typical lesions of tularæmia.

[8] A subculture on coagulated egg yolk grew well.

## CONCLUSION.

The view heretofore held that *Bacterium tularense* will grow only on a culture medium containing egg yolk is no longer tenable.

The present paper contains reports of the growth of this organism in subcultures on serum glucose agar, glucose blood agar, and blood agar. Growth on the above mediums *per se* is, however, scanty and of lowered virulence.

But these mediums take on an exalted value for the cultivation of this organism when supplied with a piece of fresh tissue; this tissue may be supplied either by the piece of spleen of the infected rabbit or guinea pig with which the medium is inoculated or a piece of fresh sterile spleen of a rabbit may be transferred to the medium, thereby preparing it to grow a subculture with which it may subsequently be inoculated.

The success of the cultural experiments here reported can not be ascribed to adaptation from a special medium to an ordinary medium because our mediums were employed for original isolations of the strains. The work here reported is with strains of *Bacterium tularense* which have never been on egg medium either before or since isolation; the only exception to this statement is contained in the very limited work done on animals 16 to 29 of Table 10, in which the strain had had a few days' cultivation on coagulated egg yolk as exemplified by the statement at the bottom of page 51.

From the data presented in Table 10 there appears to be very little difference between the efficiency of glucose blood agar plus a piece of fresh rabbit spleen, and coagulated egg yolk. I am however of the opinion that coagulated egg yolk, carefully prepared, is still the best medium for routine isolation and cultivation of *Bacterium tularense*.

*Acknowledgment.*—I am indebted to Maj. G. R. Callender, Medical Corps, United States Army, Curator Army Medical Museum, for making the photomicrographs.

## EXPLANATION OF PLATE.

FIG. 1.—*Bacterium tularense*, rod form, 48-hour culture on coagulated egg yolk. Stained with aniline gentian violet. × 3200 (approximate).

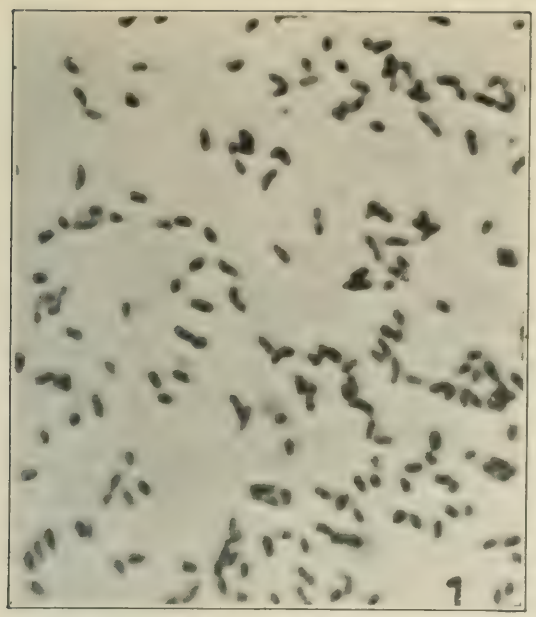
FIG. 2.—*Bacterium tularense*, coccus form, 18-day culture on coagulated egg yolk. Stained with aniline gentian violet. × 3200 (approximate).

FIG. 3.—*Bacterium tularense*, rod form, heart's blood of guinea pig. Red corpuscles in field. × 2500 (approximate).

FIG. 4.—*Bacterium tularense*, coccus form, heart's blood of rabbit. Red corpuscles in field. × 3400 (approximate).

FIG. 5.—*Bacterium tularense*, smear of guinea pig's spleen. × 3200 (approximate).

FIG. 6.—Same as figure 5. × 2500 (approximate).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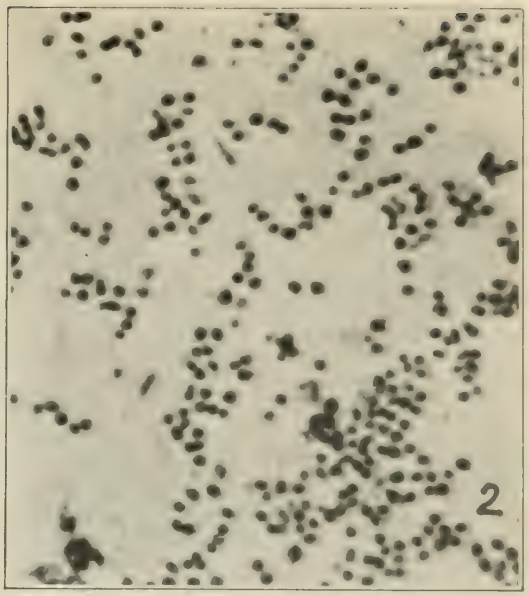
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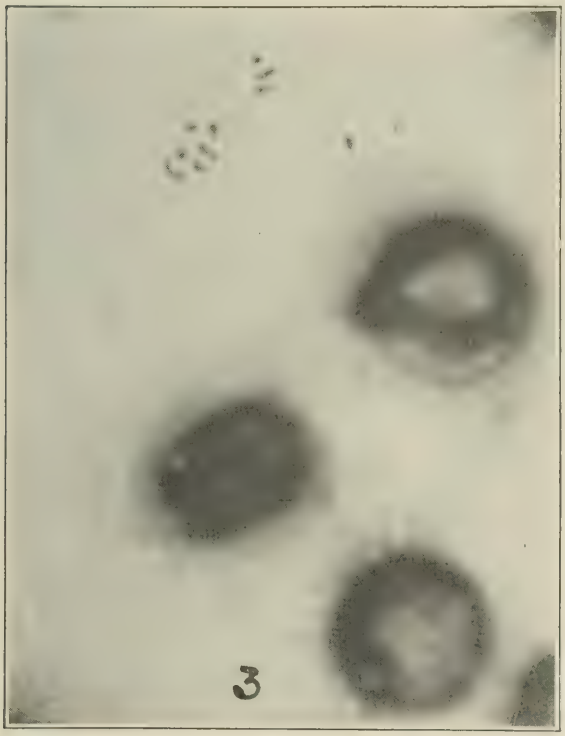
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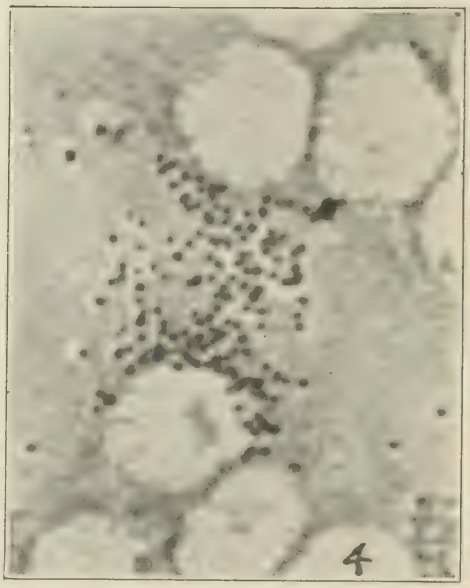
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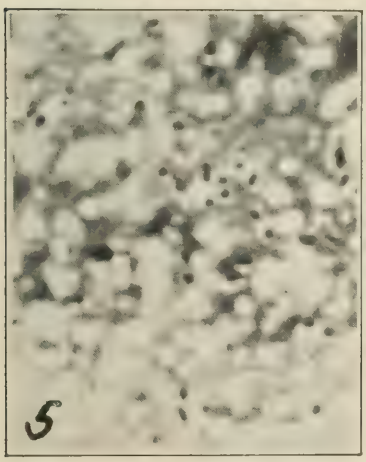
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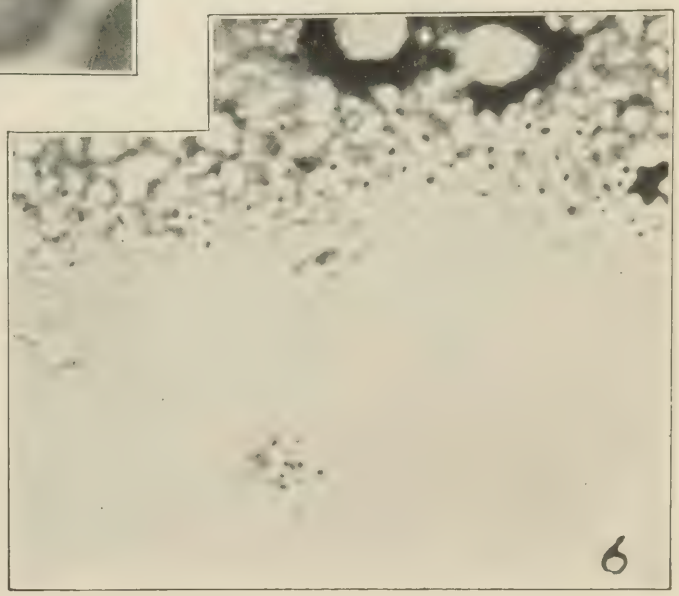
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## VII. SIX CASES OF TULARÆMIA OCCURRING IN LABORATORY WORKERS.[1]

By G. C. Lake, Passed Assistant Surgeon, and Edward Francis, Surgeon, United States Public Health Service.

All of the men, six in number, who have been intimately connected during the past two years with the laboratory investigations of tularæmia, which the Public Health Service has been conducting, have contracted this disease. Such a record of morbidity among investigators of a disease is probably unique in the history of experimental medicine. Fortunately, there were no fatalities. Two of the men contracted the disease in the field laboratory in Utah, where they were compelled to work under primitive conditions; the other four contracted the infection in the Hygienic Laboratory at Washington, D. C. Two of the men were physicians, with years of experience in working with infectious diseases and materials; one was a highly trained scientist; and the other three were experienced laboratory assistants.

Before discussing the diagnosis of tularæmia in these laboratory cases we will first summarize the picture presented by seven known cases of this disease which have occurred by natural infection in Utah. All seven had a sudden onset of illness with fever, closely following an insect bite, which became the site of suppuration and which was accompanied by a consequent unilateral suppurative lymphadenitis of the glands, which immediately drained the bitten area. The constitutional disturbance was severe, as indicated by febrile attacks which lasted from three to six weeks and which were followed by slow convalescence. *Bacterium tularense* was isolated from the suppurating lymph glands in five cases and from the blood in two. Serological tests were positive for complement fixation and agglutination, using antigens composed of cultures of *Bacterium tularense*. In an endemic focus no second attacks have come to our attention, although this subject was not especially investigated.

In reaching the diagnosis of tularæmia in the six infections contracted in the laboratory, the evidence will be considered in comparison with that of the seven infections contracted in nature in Utah, under the following heads: (1) Clinical evidence, (2) serological tests, (3) epidemiologic evidence, (4) absence of local lesions and the portal of entry of the infection, and (5) absence of *Bacterium tularense* from the blood.

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[1] Reprinted from Public Health Reports 1922, vol. 37, pp. 392–413

CLINICAL EVIDENCE. (See Appendix A.)

The laboratory cases all had a sudden onset, with high fever, which, after remitting about the third day almost to normal, immediately became high again and then fell gradually to normal at the end of about three weeks (see Charts 1 and 2). A lack of other significant constitutional disturbances or physical signs was noted. A slow convalescence extended over about two months, and recovery took place without complications.

SEROLOGICAL TESTS. (See Appendix B.)

Complement fixation and agglutination tests made on the serums of the six laboratory cases on several occasions, from January, 1921, to October, 1921, were all positive. The shortest interval after the onset of the disease before the serum was tested was 13 days; the longest interval from the date of illness was more than two years. Serums from two of our laboratory cases were found positive by comparison with serums from four known cases of tularæmia from which Francis had isolated the organism in Utah. These two serums served as positive controls in the tests made on the other laboratory cases. In all 66 negative control serums, for the most part from nonfebrile patients hospitalized in Washington, were used. Two or three of the latter gave some degree of positive complement fixation action but were negative by the agglutination test. We wish to point out that the control serums preferably should have been from patients in the febrile stages of well-known diseases, but such cases were not available. Seven of the 66 negative control serums were from laboratory personnel coming in casual contact with infected animals; these were completely negative. The control on Case 6 was unique and the most perfect one that could be obtained; his serum was tested on two occasions by complement fixation and agglutination during his exposure to the laboratory infection, but before the onset of his illness, and was negative by both tests, whereas after his illness it became strongly positive by both tests on two occasions.

EPIDEMIOLOGIC EVIDENCE.

The entire laboratory personnel (six) who have been employed continuously in handling or dissecting rodents infected with the Utah strains of *Bacterium tularense* have contracted febrile attacks which lasted approximately three weeks and were followed by slow convalescence. These attacks developed on the seventh, seventeenth, thirtieth, forty-third, eightieth, and ninety-eighth days, respectively, of such employment. Case 1 developed a second attack two years and five months after the first attack. Three of the cases have continued their work after recovery for many months in the same manner as before their illness without developing a second attack.

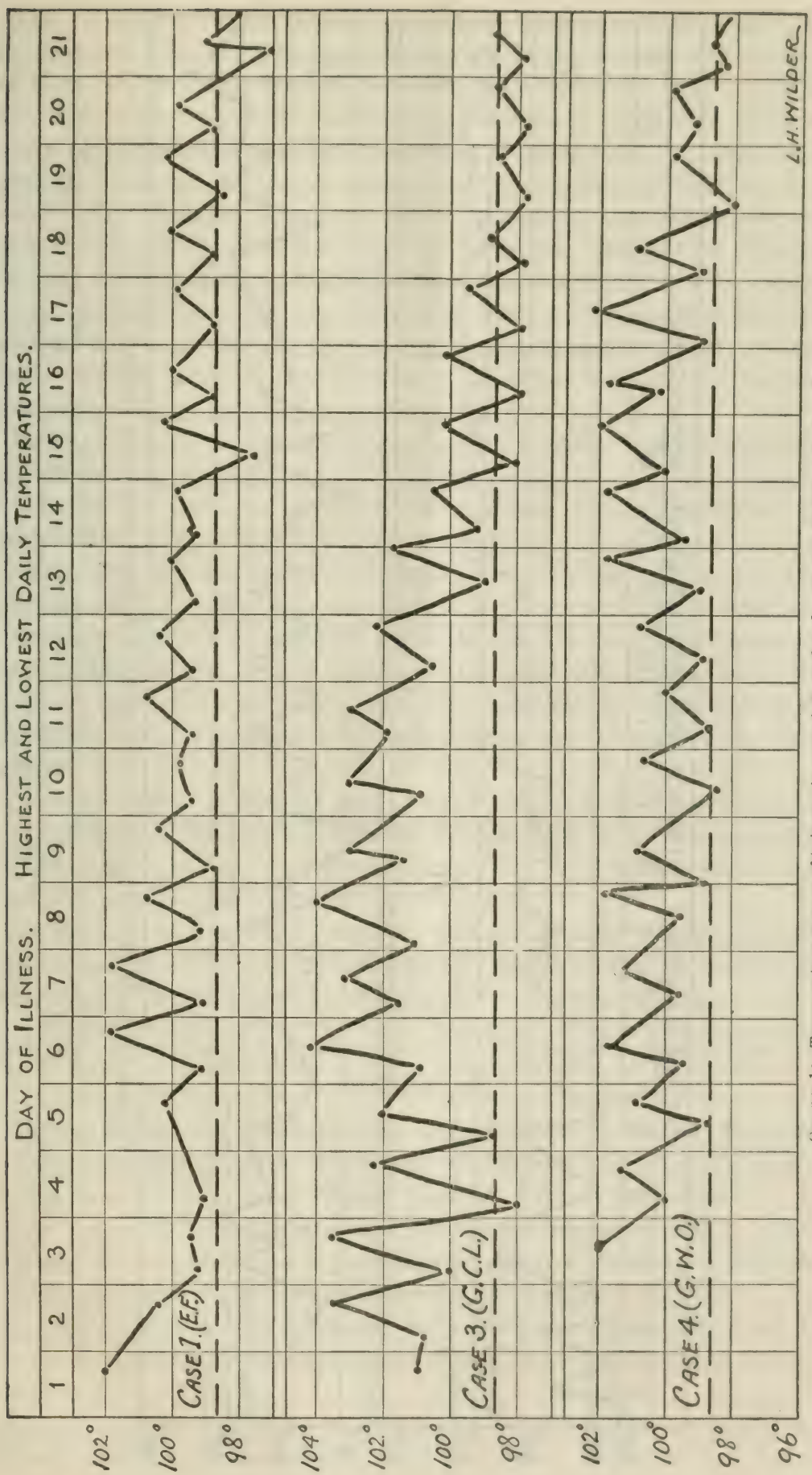


CHART 1.—Temperature curves of tularæmia cases 1, 3, and 4, developing in laboratory workers.

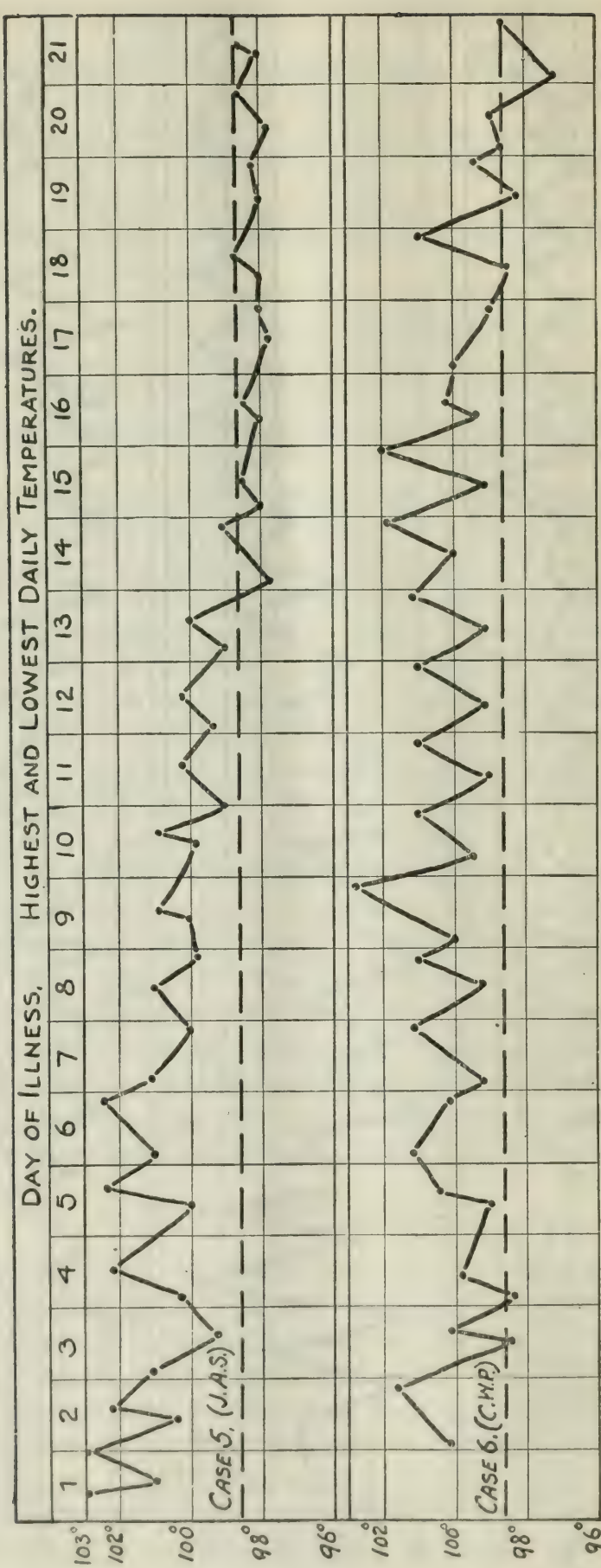


CHART 2.—Temperature curves of tularæmia cases 5 and 6, developing in laboratory workers.

The laboratory room in which animals were inoculated, dissected, and handled after autopsy was so located in the middle of the building that it was freely used as a passageway by other workers on the same floor and by general laboratory attendants. None of the laboratory personnel thus coming into casual contact with the work developed the disease, although several either worked with cultures or occasionally inoculated an animal. During the period when four cases developed in the laboratory in those who handled or dissected rodents, there was a remarkably low sick rate among the other personnel of the laboratory, numbering about 100, none of whom developed a febrile attack. Moreover, no infections occurred among fresh stock animals kept in the laboratory in cages adjacent to infected animals.

Infected insects whose bites have been followed by transmission of the infection to animals are: The bloodsucking fly, *Chrysops discalis;* the stable fly, *Stomoxys calcitrans;* the bedbug, *Cimex lectularius;* the squirrel flea, *Ceratophyllus acutus;* the rabbit louse, *Hæmodipsus ventricosus;* and the mouse louse, *Polyplax serratus.* Of the insects enumerated, only the first four are known in our experience to bite man.

*Chrysops discalis* can not be excluded as a factor in the transmission to Cases 1 and 2, who contracted the infection while working in the field laboratory in Utah, but can positively be excluded in the four cases which contracted the infection in the laboratory in Washington.

*Stomoxys calcitrans* might have been a factor in the Cases 1 and 2, developing in Utah, but three of the Washington cases developed during the season of minimal prevalence of this fly, during which time none was seen in the laboratory.

No fleas or bedbugs were seen in connection with the infected animals either in Utah or in Washington. None of the cases had any knowledge of being bitten by the insect carriers enumerated.

### ABSENCE OF LOCAL LESIONS AND THE PORTAL OF ENTRY OF THE INFECTION.

The six laboratory cases (except second attack of Case 1) furnished no local lesions indicating the portal of entry of the infection and no involvement of superficial lymph glands. This is in contrast to the human cases of tularæmia which contracted the infection in nature in Utah, all of whom had a pronounced lesion at the site of infection (insect bite) and a consequent pronounced lymphadenitis of the adjacent glands; but it is in harmony with numerous observations on the disease in animals, both by natural infection and laboratory inoculation.

Francis has recently shown that the infection traversed the unclipped, unshaved, unabraded, and unrubbed skins of five guinea

pigs when spleen juice of infected guinea pigs was gently placed on the skin of these animals after turning aside the hair on their backs. The experimental guinea pigs all wore a stiff collar 1½ inches wide, which served to sufficiently immobilize the head to prevent ingestion of the infected material. The animals all died acutely. The local lesion consisted of a pale papule surrounded by slight congestion. The secondary lymph glands were caseous, and the spleen and liver showed the typical lesions of the disease.

White mice injected with blood subcutaneously or bitten by infected bedbugs may die from the infection and yet show almost no appreciable lesion at the site of infection or in the superficial lymph glands.

*Bacterium tularense* was isolated at autopsy by guinea pig inoculations from the spleens of 17 jack rabbits infected in nature in Utah, in which an absence of involvement of the inguinal and axillary glands of the jack rabbits was noted.

In transmission experiments conducted in Utah upon rabbits and guinea pigs, instances were noted of the absence of a lesion at the site of an infected *Chrysops discalis* bite or in the adjacent lymph glands, whereas the liver or spleen showed typical lesions.

We have noted instances of the absence of involvement of the subcutaneous glands of guinea pigs after subcutaneous injection with infected bedbug feces which had dried 20 days on a filter paper; yet the guinea pigs died acutely with lesions of the spleen and liver typical of tularæmia.

In view of the facts stated in preceding paragraphs, consideration must be given to the skin of the hands as a possible portal of entry of the infection in laboratory workers, even in the absence of a local lesion or lymphadenitis.

On the other hand, in the second attack which Case 1 of our series developed two years and five months after his first attack, there was a papule on the finger from which *Bacterium tularense* was isolated by guinea pig inoculation. There was also a secondary lymphadenitis involving the epitrochlear and axillary glands of the same arm, but an absence of constitutional symptoms.

### ABSENCE OF BACTERIUM TULARENSE FROM THE BLOOD.

The blood of three of the laboratory cases, taken during the febrile stage, was injected intraperitoneally into guinea pigs, but with negative results. The absence of the organism from the blood in these cases as shown by guinea pig inoculations is taken as an indication of the mildness of the attacks. Of the two cases in Utah in which the organism was isolated from the blood, one terminated fatally and the other was very sick. The presence of the organism in the blood

probably indicates a grave condition in which the patient's resistance has given way. Laboratory animals which uniformly die from the infection show the organism with great constancy in the blood in the later stages.

## UNRECOGNIZED CASES OF TULARÆMIA.

Known foci of this infection in rodents have been reported from California, Utah, and Indiana. The known insects capable of transmitting the infection in animals are two species of biting flies, one species of fleas, two species of lice, and the common bedbug. There are probably other foci and other transmitting insects in the United States. The most practical method of search for unrecognized cases of this disease is the routine testing of specimens of blood collected from various parts of the country for complement fixation and agglutination using an antigen consisting of *Bacterium tularense.* Our laboratory cases show well-marked antibodies to this antigen for many months after recovery.

As an instance of unrecognized cases of tularæmia, we wish to refer to the work of McCoy and Chapin,[2] of the Public Health Service, who discovered *Bacterium tularense* in 1912 as the cause of a plaguelike disease of rodents in the California ground squirrels.

They reported at that time complement fixation and agglutination to *Bacterium tularense* antigens not only in the case of serums of naturally or artificially immune animals but also in the case of 2 out of 11 human serums tested. The two positive human serums were from Dr. C. W. Chapin and a laboratory attendant, both of whom were extensively engaged in handling or dissecting infected rodents in the laboratory.

Dr. Chapin now states that shortly previous to testing his own serum in 1912 he had had a febrile attack which kept him off duty for about three weeks and which was unaccompanied by glandular enlargement or other local lesion. No absence from duty on the part of the laboratory attendant can now be recalled by Dr. Chapin.

In the light of present knowledge it seems certain that what to McCoy and Chapin was a puzzling circumstance (the presence of antitularense amboceptors in the serums of two laboratory workers) was the proof of two unrecognized human cases of tularæmia.

## SUMMARY AND CONCLUSIONS.

All of the persons (six) who have been intimately engaged during the past two years in the laboratory in handling or dissecting rodents infected with the Utah strains of *Bacterium tularense* have suffered an attack of tularæmia.

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[2] McCoy, G. W., and Chapin, C. W., *Bacterium tularense*, the cause of a plaguelike disease of rodents. Public Health Bulletin, No. 53, 1912, p. 21.

The diagnosis in each of the six cases rests upon the occurrence of a febrile period lasting about three weeks, positive serum reactions for agglutination and complement fixation to antigens composed of *Bacterium tularense*, and the absence of febrile attacks in 100 other persons in the laboratory coming in casual contact with the infected rodents.

Consideration must be given to the skin of the hands as a possible portal of entry of the infection in laboratory workers even in the absence of a local lesion or lymphadenitis.

A second attack has recently occurred in Case 1 of the above series, two years and five months after his first attack. The second attack was associated with evident cracks on the fingers, on one of which there developed an inflammatory papule which was soon followed by enlarged, painful, and tender lymph glands in the epitrochlear and axillary regions of the corresponding side, but without fever or other constitutional disturbance. *Bacterium tularense* was isolated from the papule by guinea pig inoculation.

The absence of constitutional symptoms in the second attack, although there was a local lesion and consequent lymphadenitis, is accounted for by the persistence of immune bodies acquired by the first attack.

Unrecognized cases of tularæmia probably occur in the known foci of infection in the United States, some of which may have febrile attacks without local lesions, while some may have local lesions and a secondary regional lymphadenitis without very notable constitutional disturbance.

Routine serological tests for agglutination especially and for complement fixation using antigens composed of *Bacterium tularense* would probably not only detect cases in known foci of infection but would bring to light unknown foci. Positive serological reactions are known to persist for two years after an attack. Light might be thrown upon the etiology of some fevers of undetermined origin.

A warning is sounded against unwarranted indifference to an infection which, in our experience, has claimed all of those who have persistently worked with it in the laboratory.

*Acknowledgment.*—Through the courtesy of the United States Naval Hospital, Washington, D. C., Case 3 was treated in that institution on the service of Lieut. Commander J. J. O'Malley, Medical Corps, United States Navy, to whom we are indebted for clinical data on this case.

To Dr. J. J. Bateman, Passed Assistant Surgeon (R.), United States Public Health Service, we are indebted for clinical data on Cases 4, 5, and 6, who were treated in United States Public Health Service Hospital No. 32, Washington, D. C.

## Appendix A.

### BRIEF CLINICAL REPORTS OF SIX LABORATORY CASES OF TULARÆMIA.

#### CASE 1.

*First attack.*—E. F., male, age 49, physician, began investigations of tularæmia in Delta, Utah, July 23, 1919. His exposure differs from the other cases to be reported in that in addition to exposure to laboratory animals he took blood and pus on two occasions from a human case which terminated fatally. On the thirtieth day of his investigation, August 23, 1919, E. F. became ill in the late afternoon, feeling tired and weak and having a temperature of 102.2°. With the exception that his temperature (see temperature curve, Chart 1, Case 1) almost reached normal on the third and fourth days, at which time he felt slightly improved; his fever continued until the twenty-fourth day. During the first 12 days of his illness he packed up his laboratory equipment and animals in Utah with great difficulty and proceeded with them to Washington, D. C., and after his arrival made a futile attempt to continue work. The next 14 days he spent in the hospital lying on the bed, but not confined to the bed.

The temperature was highest, 102.2°, on the first day and showed a steady decline to normal on the twenty-fourth day. The departure of the patient from the hospital on the twenty-eighth day was attended with some forced exercise, which resulted in a secondary rise of temperature which lasted four days, after which it remained normal.

The second month was spent in a hotel, lying on the bed most of the time. The third month was one of slow convalescence.

Throughout the illness there was an absence of localized pain or tenderness, except that on the sixteenth day of illness a sore throat developed on the right side, manifested by redness of the anterior pillar of the fauces without involvement of the tonsil. Practically the only complaint was that of languor, or weakness, and a desire to remain quiet on the bed.

Blood: White cell count on the fifteenth day, 13,600; white cell count on the twenty-first day, 8,650.

Agglutination tests for typhoid, paratyphoid A and B, on the twenty-first day were negative.

Serological tests for tularæmia, made January 20, April 29, June 15, and September 30, 1921, were all positive. (See Tables 11, 12, 14, and 15.)

*Second attack.*—Following recovery from the first attack, this patient continued handling and dissecting infected guinea pigs, rabbits, and white mice in the laboratory for two years without using gloves. During this time infected material frequently got on his hands, but was washed off. In the first part of January, 1922, he

handled formaldehyde excessively in preparing specimens for preservation in Kaiserling solutions, and very evident cracks appeared on the fingers of both hands. In spite of this he autopsied infected animals without gloves.

On January 14 the right index finger showed on the inside of the first phalanx, near its upper end, a red, tender papule at the site of a recent crack. That night attention was directed to enlarged, tender lymph glands located in the right epitrochlear and right axillary regions.

From January 15 to 20 the glands mentioned were painful, tender, and noticeably enlarged on inspection and palpation. There was a red flush of the skin overlying the glands and at the outer border of the right biceps muscle, but no red streaks were noted on the hand, forearm, or arm.

On January 21 no redness could be noted, and the glands were not painful, but were still tender and enlarged on palpation.

The temperature was taken daily throughout the attack, but was never above normal. No notable constitutional disturbance was observed and the patient continued work as usual. On the seventh day the white blood cells numbered 7,500, and 30 c. c. of blood taken from the median basilic vein were injected intraperitoneally into six guinea pigs with negative results.

On the second day of the attack the papule on the finger was incised, but no pus was noted. The escaping blood was injected subcutaneously on the right side of the abdomen of a guinea pig. The papule was swabbed with iodine and dressed with wet bichloride of mercury dressings for the next five days, during which time no pus was noted in the wound.

The guinea pig was dying on the fifth day after injection and was chloroformed. It showed a severe local reaction at the site of injection and typical gray granular caseation of the right inguinal, right retroscapular, and retropancreatic lymph glands. Its liver and spleen were studded over the surface with small foci or granules of necrosis. Portions of the lymph glands and spleen of the guinea pig were rubbed on the shaved, abraded skin of the abdomen of two healthy guinea pigs, causing acute death with the typical lesions of tularæmia.

CASE 2.

B. M., male, age 37, scientific expert, stated that on July 20, 1920, seven days after beginning work in the field laboratory at Delta, Utah, which work brought him into intimate contact with laboratory animals suffering with, or dead from, tularæmia, he became ill rather suddenly. His chief symptoms at the time were headache, backache, shifting pains involving especially the chest, knees, and elbows, and

fever. His temperature was not taken at the time, but from July 22 to July 31 it ranged from 100° in the morning to 103° in the evening, after which it reached normal in the morning and was no longer taken. Malaria was suspected, but examinations of the blood for parasites were negative. He remained at work most of the time, although barely able to get about. The shifting pains persisted for about a month, during which time there was some loss of appetite and gastro-intestinal disturbance, with a weight loss of 15 pounds. On August 19 he took 10 days' sick leave, during which he spent most of the time lying on the bed. After this he returned to duty, but stated that it was three or four months before he was able to perform his work without undue fatigue, and that for more than a year afterwards he has been troubled with pains in the back.

On June 14, 1921, the patient happened to be at the Hygienic Laboratory, and a sample of his serum was obtained, which was tested for tularæmia antibodies, by both the complement fixation and agglutination reactions. In both tests the results were positive. (For protocols of tests see Tables 14 and 15. Temperature curves are not given, as complete temperature records were not kept.)

CASE 3.[3]

G. C. L., physician, age 37, engaged in experimental investigations of tularæmia at the Hygienic Laboratory, Washington, D. C., was in good health up to October 23, 1920 (43 days after beginning this work), when, after putting in a full day at the laboratory, he suddenly became ill in the evening. He was compelled to go to bed because of weakness and dizziness, and a few minutes later had a fairly severe chill, after which the temperature was found to be 101°. (See temperature curve, Case 3.) The temperature, which was quite irregular, gradually became higher, reaching 104.2° on the sixth day. There was a remission to 98° the morning of the fourth day, at which time the patient got up with the intention of going to work, but suddenly became dizzy and weak and had to go back to bed. On the eighth day he was taken to the hospital, where the temperature, after reaching 103° for the next three days, gradually began to fall, reaching normal on the seventeenth day, and, with the exception of a slight rise a few days later, remained normal. The pulse was fairly rapid, ranging from 80 to 98, and remained high for some time after the fever dropped. The blood pressure, taken on several occasions, was normal. During the first two weeks there was a moderately severe rhinitis, the secretions being at times blood tinged, and on two occasions a slight epistaxis occurred. There were no pains at any time, only a desire to be quiet and sleep a great deal, and occasionally there was slight nausea. Repeated physical examinations were

[3] This case is also reported by Lieut. Commander J. J. O'Malley, Medical Corps, U. S. Navy, in the Journal of the American Medical Association, 1922, vol. 78, p. 1018.

practically negative. The treatment was absolute rest in bed and careful feeding and nursing. He was discharged from the hospital November 29, having lost only 15 pounds in weight.

After returning home the patient spent a month resting most of the time. Temperature of about 100° was noted several times during the first 10 days at home. By the end of the month he could walk a half mile without much fatigue. The only special symptoms were the development at different times of localized hyperesthetic areas of the skin (the sensation being that of a mild burn, but with no visible lesion), and an attack of mild tympanitis lasting more or less continuously, except while patient slept, for about 48 hours.

He returned to work January 1, but for the first month usually went home at noon and spent most of the afternoon in bed. It was late in the spring before he had regained a condition approximating normal health. Transient pains in the calves of the legs, gradually becoming milder and occurring less frequently, have persisted for more than a year.

*Laboratory examinations made on Case 3.*

October 26: White cell count, 12,000; nasal secretions blood tinged injected into guinea pigs with negative results.

October 28: Blood culture for typhoid negative.

October 30: Blood culture for typhoid negative. Widal positive for typhoid, negative for paratyphoid A and B. (Patient had received three injections of single typhoid vaccine late in 1914.) Inoculation of a guinea pig with 5 c. c. blood intraperitoneally resulted negatively.

October 31: White cell count, 8,300, red cells, 5,900,000, differential not significant.

October 30 to November 20: Several examinations of urine and feces for *B. typhosus* were made with negative result.

January 20, April 29, May 11, June 15, and September 30, 1921: Serological tests for tularæmia were all positive. (See Tables 11 to 16.)

CASE 4.

G. W. O., male, age 36, laboratory assistant in connection with investigations with experimental tularæmia. On April 9, 1921, after having been engaged in this work 98 days, he was taken suddenly ill. He had not felt well in the forenoon and at 3 p. m., while at work, was suddenly seized with a sharp pain over the right shoulder, radiating downward with the spine and localizing near the twelfth dorsal vertebra. On reaching home, only a short distance away, the pain radiated to the lumbar region and later to the muscles and joints of the legs. The pains continued to shift, at times involving the eyeballs, superciliary ridges, and occipital regions. He remained at home for a week, continuing to have shifting pains and temperature, which, after dropping to normal during the forenoon of the fourth day, gradually became higher, accompanied by a feeling of increasing

weakness. During this time physical examination was practically negative. On the seventh day he was taken to the hospital, where a carefully conducted and complete physical examination was negative, except that the areas in which he complained of pain were sensitive or tender, namely, occipital region, muscles of neck, superciliary ridges, and vertebral border in the lumbar region.

Treatment was absolute rest in bed and symptomatic. Temperature (see Chart 1, Case 4) reached normal on the twenty-first day. Patient continued to have pains in the head, muscles, and joints until the sixteenth day. During the febrile stage his pulse range was from 70 to 80, reaching 90 April 22. After the febrile stage, the average was about 75. (This patient normally has a slow pulse, which now averages about 66, when sitting.)

*Laboratory examinations Case 4.*

Feces: Examined for *B. typhosus* with negative result on May 4, 11, and 14.

Blood: White cell count 6,400 on April 15; 8,770 on April 28, when red cells were 5,000,000, differential about normal.

Blood cultures: Made April 15 and April 23, designed to show the presence of *Bacterium tularense*, *B. typhosus*, streptococcus, etc., on the following mediums: Glucose blood agar slants and plates, 1 per cent glucose agar, special egg medium of McCoy and Chapin, Levinthal's cooked blood agar, and in graded amounts into a series of tall test tubes, each containing 50 c. c. of bouillon (great care being taken not to jar the tubes and disturb the filamemts of fibrin). No growth was obtained on any of the mediums.

Inoculations of 7 guinea pigs April 15, and 5 guinea pigs April 23, each receiving intraperitoneally 4 c. c. of blood plus 4 c. c. of saline, all resulted negatively for tularæmia.

Immunological tests: Widal was slightly positive for *B. typhosus*, negative for paratyphosus *A* and *B*. (Patient had received three injections of single typhoid vaccine in November, 1913.) Agglutination and complement fixation tests for tularæmia, April 29, May 11, June 15, August 5, and September 30, 1921, were all positive. (See Tables 12 to 16.)

CASE 5.

J. A. S., male, age 29, succeeded G. W. O. (Case 4) as laboratory assistant with the tularæmia investigation. On April 28, 1921, the 17th day of his exposure to infected animals, after working till 11 a. m., he complained of not feeling well and of being chilly. Temperature, taken at once, was 103°, pulse 100, respirations 24; otherwise physical examination was negative. During the next half hour, while waiting for the ambulance to take him to the hospital, he had a fairly severe chill. History taken on his admission to hospital shows that he complained of headache, shifting pains in the muscles and joints, weakness, and anorexia. Physical examination was negative, except that the areas in which he complained of pain were found to be either hypersensitive or tender, and that there was a slight impair-

ment of resonance over the right scapular region. Blood pressure was normal.

Patient's temperature (see Chart 2, Case 5), after dropping almost to normal on the third day, continued high until the sixth day, after which there was a gradual drop to normal on the thirteenth day. This was the only one of our cases who was taken immediately to the hospital on the onset of symptoms, which may account for the shorter febrile stage. His pulse range during the febrile stage was from 80 to 100, and during the next two weeks about 80, after which it dropped to 70.

He complained of headache and muscular pains a great deal during the first few days, and, to some extent, for the first two weeks. He was discharged May 29, 18 days after his temperature became normal, and remained at home gradually improving until July 4, when he was almost instantly killed in a railway accident. A complete post-mortem examination failed to show any evidence of lesions of tularæmia either active or healed. All the organs and tissues were normal except for the crushing injuries produced by the accident.

*Laboratory examinations made on Case 5.*

Feces: Negative for *B. typhosus*, May 5, 11, and 14.

Blood: Cultures made on April 28, as was done in Case 4, except that in addition fermentation tubes were used, all negative.

Inoculations intraperitoneally of 7 guinea pigs on April 28, and 5 more guinea pigs May 10, each receiving 4 c. c. of defibrinated blood plus 4 c. c. of saline, gave negative results for tularæmia.

Immunological tests: Agglutination and complement fixation tests for tularæmia, made on May 10 and June 15, 1921, were positive. (See Tables 13, 14, and 15.)

CASE 6.

C. W. P., male, age 29, succeeded J. A. S. (Case 5) as laboratory assistant in tularæmia investigations. On July 17, 1921, 80 days after beginning this work, he felt a severe pain in his left elbow just after going to bed. This pain lasted only a few minutes and was followed by a chill lasting about 10 minutes. The following day he felt weak, had no appetite, had a headache of moderate severity, and was in this condition when first examined at his home, July 19. A partial physical examination conducted at the time revealed nothing of importance except temperature 101.8°, pulse 100, respiration normal. He was taken to the hospital the same afternoon, where a complete physical examination was also practically negative.

Examination of temperature curve (Chart 2, Case 6) shows that on the mornings of the third and fourth days patient's temperature reached normal. At this time he said that he did not feel sick enough to stay in bed. After that his temperature began to rise and remained fairly high (highest 102.7° on July 26) until the six-

teenth day, after which it began to fall, becoming normal on the twentieth day. His pulse during the febrile stage was variable, ranging from 80 to 100; after the febrile stage it averaged about 80. He complained of nothing at any time except weakness and occasionally some nausea. He was discharged August 21, 14 days after his temperature became normal. He remained at home slowly convalescing until October 1. For the next month he worked in the laboratory during the forenoons and rested most of the afternoons. Since that time he has been on duty full time. His only complaint since going to work has been that of a dull pain in the left side, which at first bothered him a great deal, but which has now almost entirely disappeared.

*Laboratory examinations made on Case 6.*

Blood: White cells 7,300, red cells 5,000,000, differential unimportant July 19.

Inoculations of 7 guinea pigs on July 22 and of 5 pigs August 5, each with 4 c. c. of defibrinated blood plus 4 c. c. of saline intraperitoneally, gave negative results for tularæmia.

Immunological tests for tularæmia made August 5 were positive. (See Table 16.) Further tests made September 30 by both agglutination and fixation methods were also positive. (Protocols not given.)

## Appendix B.

### SEROLOGICAL REPORTS.

#### DISCUSSION OF TABLE 11.

On January 20, 1921, complement fixation tests were made (1) to determine whether serums collected after recovery from naturally infected human cases of tularæmia would give a definite reaction with *Bacterium tularense* antigen; (2) to determine whether serums from human cases 1 and 3 originating in the laboratory would react positively; and (3) to determine whether serums from control persons, presumably uninfected, would fail to react.

The serums from the naturally infected cases definitely known to be tularæmia were collected by Francis September 28, 1920, and were from cases from which he had isolated *Bacterium tularense* (see Public Health Reports, vol. 36, No. 30, July 29, 1921, pp. 1731–1738). These serums were heated 30 minutes at 56° C. at time of collection and preserved by adding an equal amount of glycerin. Serum from laboratory Case 1 was obtained January 19, 1921, about 17 months after the onset of illness; serum from laboratory Case 3 was obtained January 19, 1921, about three months after the onset of illness. The control serums used in this test were from samples sent in for routine Wassermann tests.

The antigens used were saline suspensions of *Bacterium tularense* made by washing off the 72-hour growth on egg medium slants with small amounts of saline, care being taken to avoid breaking the

TABLE 11.—*Complement fixation tests of serums from four known positive cases of tularæmia, two of the laboratory cases here reported, and nine negative human controls. Saline suspensions of Bacterium tularense of squirrel, rabbit, and human origin were used as antigens. Tests were made Jan. 20, 1921.*

| Serum. | Serum dilutions. | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | California squirrel strain antigen. | | | | | | Utah rabbit strain antigen. | | | | | | Human strain G. antigen. | | | | | | No-antigen controls. | | | | | |
| | No serum. | 1:10 | 1:20 | 1:60 | 1:180 | 1:540 | No serum. | 1:10 | 1:20 | 1:60 | 1:180 | 1:540 | No serum. | 1:10 | 1:20 | 1:60 | 1:180 | 1:540 | No serum. | 1:10 | 1:20 | 1:60 | 1:180 | 1:540 |
| Utah human cases: [1] | | | | | | | | | | | | | | | | | | | | | | | | |
| C | − | 4+ | 4+ | 4+ | + | − | − | 4+ | 4+ | 4+ | 4+ | − | − | 4+ | 4+ | 4+ | 4+ | 4+ | − | 4+ | 3+ | − | − | − |
| G | − | 4+ | 4+ | 4+ | 4+ | 3+ | − | 4+ | 4+ | 4+ | 4+ | 4+ | − | 4+ | 4+ | 4+ | 4+ | − | − | 4+ | 4+ | − | − | − |
| McK | − | 4+ | 4+ | 4+ | 3+ | 2+ | − | 4+ | 4+ | 4+ | 3+ | − | − | 4+ | 4+ | 4+ | 2+ | − | − | 4+ | + | − | − | − |
| S | − | 4+ | 4+ | 4+ | 4+ | 2+ | − | 4+ | 4+ | 4+ | 4+ | 2+ | − | 4+ | 4+ | 4+ | 4+ | + | − | 4+ | + | − | − | − |
| Laboratory human cases: [2] | | | | | | | | | | | | | | | | | | | | | | | | |
| Case 1 | − | 3+ | 3+ | 4+ | 4+ | 3+ | − | 3+ | 2+ | 3+ | 3+ | + | − | 3+ | 3+ | 3+ | 3+ | − | − | − | − | − | − | − |
| Case 3 | − | 4+ | 4+ | 4+ | 4+ | 4+ | − | 4+ | 4+ | 4+ | 4+ | 4+ | − | 4+ | 4+ | 4+ | 4+ | 3+ | − | − | − | − | − | − |
| Negative controls: [3] | | | | | | | | | | | | | | | | | | | | | | | | |
| 7610 | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| 7570 | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| 7609 | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| 7560 | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| 7561 | − | 3+ | + | − | − | − | − | 3+ | 3+ | 3+ | − | − | − | 3+ | + | − | − | − | − | 2+ | + | − | − | − |
| 7622 | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| 7566 | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| 7564 | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| 7530 | − | − | − | − | − | − | − | 3+ | 3+ | 3+ | + | − | − | + | + | − | − | − | − | − | − | − | − | − |

[1] These serums were obtained in Utah on Sept. 28, 1920, from four recently recovered cases of tularæmia which had received their infection in nature and from which *Bacterium tularense* had been isolated during their illness. The serums were heated at time of collection for 30 minutes at 56° C. and subsequently preserved by adding an equal amount of glycerin. In the lower dilutions of the no-antigen controls, these four serums were anticomplementary.

[2] Serums from laboratory cases 1 and 3 were taken on the day preceding the test, about 17 months and 3 months, respectively, after the onset of illness.

[3] These control serums were from samples sent in for Wassermann tests.

surfaces, and then heating the suspensions for 30 minutes at 54° C. No preservative was added. Three separate antigens were prepared; one was from a strain isolated from a ground squirrel by Passed Asst. Surg. W. T. Harrison in California in May, 1920, and the other two were isolated by Francis in Utah, one from a jack rabbit and one from a typical human case (G), whose serum was also used in the tests.

Tests had previously been made of other antigens prepared as above to determine whether fixation occurred with pooled Wassermann positive and pooled Wassermann negative serums, with negative results. The antigens used in this test had been titrated to determine suitable units for use in these experiments.

The results obtained are shown in Table 11. It will be noted that very definite positive reactions were obtained with the four known positive serums and also with the serums from laboratory Cases 1 and 3 against all three antigens used. It is unfortunate that higher dilutions were not added, particularly in the series with the California strain antigen, so that the positive serums would be carried out to extinction of fixation; but even the old Utah serums, which were anticomplementary (see the no-antigen controls) in 1: 20 dilutions, fixed complement in 1: 540 dilution with at least one of the antigens. The nine control serums used gave negative results, with the exception of 7561, which can well be explained by the degree of anticomplementary effect present, and 7530, which gave a fairly strong fixation with one antigen and practically no fixation with the other two antigens. No more of serum 7530 was available for further tests. The positive serums all reacted definitely with suspensions of *Bacterium tularense* of squirrel, rabbit, and human origin, suggesting that the organism from these three sources is the same.

DISCUSSION OF TABLE 12.

On April 29, 1921, agglutination tests were made to determine (1) whether serums from laboratory Cases 1 and 3, positive by the complement fixation test in Table 11, would be positive by the agglutination test, and (2) whether serum from laboratory Case 4, taken on the thirteenth day of his illness, contained agglutinins. Six serums from hospital patients suffering with mild disorders unrelated to tularæmia were used as controls.

The antigen used was prepared from human strain G, in the same manner as described in the discussion of Table 11, except that the suspension was heated 30 minutes at 56° C. and then preserved by the addition of 0.3 per cent tricresol. This antigen, designated G–32, was sealed in glass ampules and used in all the subsequent tests for agglutination and complement fixation.

TABLE 12.—*Agglutination tests of serums from laboratory Cases 1, 3, and 4, and six human controls, using Bacterium tularense antigen G 32. Test made Apr. 29, 1921.*

| Serum. | Date serums were obtained. | Serum dilutions. | | | | | | | Remarks. |
|---|---|---|---|---|---|---|---|---|---|
| | | No serum. | 1:10 | 1:20 | 1:50 | 1:100 | 1:500 | 1:1000 | |
| Laboratory cases: | | | | | | | | | |
| Case 1 | Jan. 19 | − | + | + | + | + | − | − | Serum not heated. |
| Case 3 | ...do..... | − | + | + | + | + | − | − | Do. |
| Case 1 | Jan. 24 | − | + | + | + | − | − | − | Heated 56° ½ hour. |
| Case 3 | ...do..... | − | + | + | + | + | − | − | Do. |
| Case 3 | Apr. 27 | − | + | + | + | + | − | − | Serum not heated. |
| Case 4 | Apr. 22 | − | + | + | + | − | − | − | Do. |
| Control serums: | | | | | | | | | |
| No. 1 | Apr. 26 | − | − | − | − | − | − | − | Heated 56° ½ hour. |
| No. 2 | ...do..... | − | − | − | − | − | − | − | Do. |
| No. 3 | ...do..... | − | − | − | − | − | − | − | Do.. |
| No. 4 | ...do..... | − | − | − | − | − | − | − | Do. |
| No. 5 | ...do..... | − | − | − | − | − | − | − | Do. |
| No. 6 | ...do..... | − | − | − | − | − | − | − | Do. |

The results (Table 12) show that serum from laboratory Case 4, taken on the thirteenth day of illness, was positive. Serums, both unheated and heated, from laboratory Cases 1 and 3, which had been kept in the ice box over three months, but in neither case with preservative, gave about the same degree of positive reaction as fresh serum from laboratory Case 3. The controls failed to give any agglutination.

TABLE 13.—*Comparison of complement fixation and agglutination tests made on laboratory Cases 3, 4, and 5. Test made May 11, 1921. Antigen used, G 32.*

| Serum. | Date collected. | Complement fixation. | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Serum dilutions. | | | | | | | | |
| | | [1] 1:10 | 1:20 | 1:50 | 1:100 | 1:200 | 1:500 | 1:1000 | 1:2000 | 1:4000 |
| Laboratory cases: | | | | | | | | | | |
| Case 3 | May 10 | − | 4+ | 4+ | 4+ | 4+ | 4+ | − | − | − |
| Case 4 | ...do..... | − | 4+ | 4+ | 4+ | 4+ | 2+ | − | − | − |
| Case 5 | ...do..... | − | 4+ | 4+ | 4+ | 4+ | − | − | − | − |
| Control serums: [2] | | | | | | | | | | |
| A | ...do..... | − | − | − | − | − | − | − | − | − |
| No. 1 | ...do..... | − | − | − | − | − | − | − | − | − |
| No. 2 | ...do..... | − | − | − | − | − | − | − | − | − |

| Serum. | Date collected. | Agglutination. | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Serum dilutions. | | | | | | | |
| | | 1:10 | 1:20 | 1:50 | 1:100 | 1:200 | 1:500 | 1:1000 | 1:2000 |
| Laboratory cases: | | | | | | | | | |
| Case 3 | May 10 | 2+ | 2+ | 2+ | 2+ | − | − | − | − |
| Case 4 | ...do..... | 2+ | 2+ | 2+ | 2+ | + | − | − | − |
| Case 5 | ...do..... | + | 2+ | 2+ | 2+ | + | − | − | − |
| Control serums: [2] | | | | | | | | | |
| A | ...do..... | − | − | − | − | − | − | − | − |
| No. 1 | ...do..... | − | − | − | − | − | − | − | − |
| No. 2 | ...do..... | − | − | − | − | − | − | − | − |

[1] No antigen in 1:10 dilution.

[2] Control serum A was from laboratory case 6 of our series 13 days after he began work with infected animals, but 67 days before he developed the disease.

## DISCUSSION OF TABLE 13.

On May 11, 1921, tests were made by both the complement fixation and agglutination reactions (1) to compare the results of these two methods and (2) to determine whether serum from laboratory Case 5 (onset of illness Apr. 27, 1921) was positive. Serums from laboratory Cases 3 and 4, already found positive by previous tests, served as positive controls, and serums from three other men in the laboratory served as negative controls. Control serum A was from the man who later was the sixth laboratory case of our series. He had been working with animals infected with *Bacterium tularense* since April 28, 1921, but did not contract the disease until July 17, 1921. The results show that serum from laboratory Case 5 was positive on the fourteenth day of his illness. Both tests gave satisfactory results. The control serums were negative throughout.

TABLE 14.—*Complement fixation test on serums of 5 laboratory cases of tularæmia, using 35 control serums. Antigen used, G 32. Test made June 15, 1921.*

| Serum. | Serum dilutions (no antigen in 1 : 10 dilution). | | | | | | | | Results. |
|---|---|---|---|---|---|---|---|---|---|
| | 1 : 10. | 1 : 20. | 1 : 40. | 1 : 100. | 1 : 200. | 1 : 400. | 1 : 1,000. | 1 : 2,000. | |
| Laboratory cases: | | | | | | | | | |
| Case 1 | − | 4+ | 3+ | + | + | − | − | − | Positive. |
| Case 2 | − | 4+ | 4+ | 3+ | 3+ | + | − | − | Do. |
| Case 3 | − | 4+ | 4+ | 4+ | 4+ | 4+ | − | − | Do. |
| Case 4 | − | 4+ | 4+ | 4+ | 4+ | 4+ | + | − | Do. |
| Case 5 | − | 4+ | 4+ | 4+ | 3+ | 2+ | − | − | Do. |
| Control serums: | | | | | | | | | |
| A [1] | − | − | − | − | − | − | − | − | Negative. |
| No. 1 [2] | − | − | − | − | − | − | − | − | Do. |
| No. 2 [3] | − | − | − | − | − | − | − | − | Do. |
| No. 3 | − | − | − | − | − | − | − | − | Do. |
| No. 4 | − | − | − | − | − | − | − | − | Do. |
| No. 5 | − | − | − | − | − | − | − | − | Do. |
| No. 6 [4] | 4+ | + | − | − | − | − | − | − | Negative (Ac).[7] |
| No. 7 [5] | − | − | − | − | − | − | ..... | ..... | Negative. |
| No. 8 | − | − | − | − | − | − | ..... | ..... | Do. |
| No. 9 | − | − | − | − | − | − | ..... | ..... | Do. |
| No. 10 | − | 4+ | 4+ | 4+ | 3+ | − | ..... | ..... | Positive.[8] |
| No. 11 | − | − | − | − | − | − | ..... | ..... | Negative. |
| No. 12 | − | − | − | − | − | − | ..... | ..... | Do. |
| No. 13 | − | + | + | 2+ | + | − | ..... | ..... | Positive (?).[8] |
| No. 14 | − | − | − | − | − | − | ..... | ..... | Negative. |
| No. 15 | − | − | − | − | − | − | ..... | ..... | Do. |
| No. 16 | − | − | − | − | − | − | ..... | ..... | Do. |
| No. 17 | − | − | − | − | − | − | ..... | ..... | Do. |
| No. 18 [6] | + | 4+ | 4+ | 4+ | + | − | ..... | ..... | Positive.[8] |
| No. 19 | − | − | − | − | − | − | ..... | ..... | Negative. |
| No. 20 | − | − | − | − | − | − | ..... | ..... | Do. |
| No. 21 | − | − | − | − | − | − | ..... | ..... | Do. |
| No. 22 | − | − | − | − | − | − | ..... | ..... | Do. |
| No. 23 | − | − | − | − | − | − | ..... | ..... | Do. |
| No. 24 | − | − | − | − | − | − | ..... | ..... | Do. |
| No. 25 | + | + | + | − | − | − | ..... | ..... | Negative (Ac).[7] |
| No. 26 | − | − | − | − | − | − | ..... | ..... | Negative. |
| No. 27 | − | − | − | − | − | − | ..... | ..... | Do. |
| No. 28 | − | − | − | − | − | − | ..... | ..... | Do. |
| No. 29 | − | 3+ | 2+ | + | − | − | ..... | ..... | Positive (?). |
| No. 30 | + | − | − | − | − | − | ..... | ..... | Negative (Ac).[7] |
| No. 31 | + | + | − | − | − | − | ..... | ..... | Do. |
| No. 32 | − | − | − | − | − | − | ..... | ..... | Negative. |
| No. 33 | − | − | − | − | − | − | ..... | ..... | Do. |
| No. 34 | 2+ | 2+ | 3+ | 4+ | 4+ | 4+ | ..... | ..... | Positive (?) Ac.[7] |

[1] Control serum A was taken 48 days after C. W. P. began work with infected animals. He developed tularæmia 32 days after this test.

[2] Control serum 1 is from C. W. C., who had a probable attack of tularæmia more than 10 years ago.

[3] Control serums 2–5 were from other members of laboratory staff who have been slightly exposed to infection.

[4] Control serum 6 was a high titre rabbit antityphoid serum.

[5] The remainder of the serums are from two large local Government hospitals.

[6] Control serum 18 was from a case of typhoid fever (in which *B. typhosus* was isolated), which, at this time, showed a positive Widal.

[7] Ac=anticomplementary.

[8] See Table 15.

DISCUSSION OF TABLE 14.

On June 15, 1921, serums from the five laboratory cases which had occurred up to that time were tested by the complement fixation method in comparison with (1) serums of several of the laboratory personnel, including serum A, from C. W. P., who, 32 days after this test, contracted tularæmia and became laboratory Case 6 of our series; (2) a serum from an immunized rabbit with high antityphoid titre, and a serum from a known case of typhoid with a positive Widal; and (3) 27 serums from ordinary hospital cases from two Government hospitals. The serums were collected on the day preceding the test and were not heated. The serums of the five laboratory cases were positive; Case 1 in dilutions up to 1 in 200; Cases 2, 3, and 5 in dilutions up to 1 in 400; Case 4 in dilutions up to 1 in 1,000.

The serum of Case 1 was taken 22 months after the attack of tularæmia. Of the 35 control serums, 27 were completely negative. Four, Nos. 6, 25, 31, and 34, can be classed as probably negative on account of being anticomplementary or reacting only in dilutions too low to be regarded as significant. The remaining four serums, Nos. 10, 13, 18, and 27, may be regarded as more or less positive, as the first three of them reacted in dilutions as high as our weakest positive control. These three, Nos. 10, 13, and 18, were therefore further tested by the agglutination method (see Table 15, with discussion). There was none of No. 29 remaining or it also would have been tested.

There is a possibility that some of the questionable positives with the complement fixation test would have been avoided had the serums been heated.

TABLE 15.—*Agglutination tests to determine whether control serums 10, 13, and 18, found positive by the complement fixation test (see Table 14), would be negative by agglutination. Antigen used, G 32. Test made June 16, 1921.*

| Serum. | Serum dilutions. | | | | | | Results. |
|---|---|---|---|---|---|---|---|
| | 1:10 | 1:20 | 1:40 | 1:100 | 1:200 | 1:400 | |
| Laboratory cases: | | | | | | | |
| Case 1 | 4+ | 4+ | 3+ | + | − | − | Positive. |
| Case 2 | 4+ | 4+ | 3+ | 2+ | − | − | Do. |
| Case 3 | + | 2+ | 4+ | 4+ | 2+ | − | Do. |
| Case 4 | 4+ | 4+ | 4+ | 4+ | 3+ | − | Do. |
| Case 5 | + | 3+ | 4+ | 3+ | 2+ | − | Do. |
| Control serums: | | | | | | | |
| A | − | − | − | − | − | − | Negative. |
| No. 1 | − | − | + | 2+ | − | − | Slightly positive (?). |
| No. 2 | − | − | − | − | − | − | Negative. |
| No. 3 | − | − | − | − | − | − | Do. |
| No. 4 | − | − | − | − | − | − | Do. |
| No. 5 | − | − | − | − | − | − | Do. |
| No. 10 | − | − | − | − | − | − | Do. |
| No. 13 | − | − | − | − | − | − | Do. |
| No. 18 | − | − | − | − | − | − | Do. |

DISCUSSION OF TABLE 15.

On June 16, 1921, serums Nos. 10, 13, and 18, which were found more or less positive by the complement fixation test on the preceding day and have been referred to in the discussion of Table 14, were submitted to the agglutination test. All serums tested were remaining portions of serums tested on the previous day; serums from laboratory Cases 1–5 served as positive controls; serums from controls 1–5 served as negative controls. The positive controls all reacted positively; the negative controls all reacted negatively, with the exception that control serum 1 gave some agglutination in the third and fourth dilutions, but not in the first two dilutions; the serums under investigation, Nos. 10, 13, and 18, all reacted negatively.

This result tends to confirm some previous observations (not recorded here) which we have made that the agglutination test is more reliable in that it is more specific than the complement fixation test for the detection of *Bacterium tularense* antibodies.

TABLE 16.—*Agglutination test made Aug. 5, 1921, on serum of laboratory Case 6 taken on the nineteenth day of his illness. This patient had furnished negative control serum A 51 and 87 days previously. (See Tables 13, 14, and 15.) All serums taken Aug. 5 and heated 30 minutes at 55° C. before using. Antigen used, G 32.*

| Serums. | Serum dilutions. | | | | | | | Results. |
|---|---|---|---|---|---|---|---|---|
| | No serum. | 1:20 | 1:40 | 1:80 | 1:200 | 1:400 | 1:800 | |
| **Laboratory cases:** | | | | | | | | |
| Case 6 | − | 2+ | 3+ | 2+ | 2+ | + | − | Positive. |
| Case 3 | − | 2+ | 2+ | 2+ | + | − | − | Do. |
| Case 4 | − | 3+ | 3+ | 2+ | + | − | − | Do. |
| **Control serums:** | | | | | | | | |
| No. 1 | − | − | − | − | − | − | − | Negative. |
| No. 2 | − | − | − | − | − | − | − | Do. |
| No. 3 | − | − | − | − | − | − | − | Do. |
| No. 4 | − | − | − | − | − | − | − | Do. |
| No. 5 | − | − | − | − | − | − | − | Do. |
| No. 6 | − | − | − | − | − | − | − | Do. |

DISCUSSION OF TABLE 16.

On August 5, 1921, agglutination tests (see Table 16) were carried out on serum of laboratory Case 6, 19 days after the onset of his illness. This serum, as well as those from positive controls (Cases 3 and 4) gave definitely positive results. Control serum A (see Tables 13, 14, and 15) was from the laboratory attendant who became laboratory Case 6 of our series. The tests show that his serum reacted negatively on the thirteenth and forty-eighth days of his exposure to infected laboratory animals; but, having contracted the infection on the eightieth day, his serum reacted positively to the agglutination test 19 days after the onset of illness. His serum, shown to be definitely positive by this test, was subsequently tested October 1 by both the

complement fixation and agglutination tests (protocols not given) in comparison with three positive and nine negative control serums, all taken on the same date and heated 30 minutes at 55° C. His serum was at this time somewhat more strongly positive than that of the positive controls. The negative controls remained negative throughout.

The serological tests in Case 6 are particularly significant in that they were negative before his illness and positive afterwards, the same antigen being used in all the tests.

TABLE 17.—*Agglutination test made June 30, 1922, to determine the degree of persistence of agglutination in the known laboratory cases and to test for agglutination in two suspected cases of tularæmia occurring in market men.*

| Serums. | Length of time since onset of tularæmia. | Serum dilutions. | | | | Results. |
|---|---|---|---|---|---|---|
| | | 1 : 25 | 1 : 50 | 1 : 100 | 1 : 200 | |
| Laboratory cases: | | | | | | |
| Case 1 | 2 years 10 months | 0 | 0 | 0 | 0 | Negative. |
| Case 3 | 1 year 8 months | 3 | 1 | 0 | 0 | Positive. |
| Case 4 | 1 year 3 months | 3 | 1 | 0 | 0 | Do. |
| Case 6 | 1 year | 2 | 0 | 0 | 0 | Do. |
| Market men: | | | | | | |
| New cases— | | | | | | |
| E. N.[1] | 6 months | 4 | 3 | 1 | 0 | Do. |
| Mrs. W.[2] | do | 3 | 3 | 1 | 0 | Do. |
| Normal serums: | | | | | | |
| No. 1 | | 0 | 0 | 0 | 0 | Negative. |
| No. 2 | | 0 | 0 | 0 | 0 | Do. |
| No. 3 | | 0 | 0 | 0 | 0 | Do. |
| No. 4 | | 0 | 0 | 0 | 0 | Do. |
| No. 5 | | 0 | 0 | 0 | 0 | Do. |
| No. 6 | | 0 | 0 | 0 | 0 | Do. |
| Typhoid fever | | 0 | 0 | 0 | 0 | Do. |
| Antiplague | | 0 | 0 | 0 | 0 | Do. |

[1] Patient of Dr. J. Lawn Thompson, Washington, D. C.
[2] Patient of Dr. Lucius G. Gage, Charlotte, N. C.

### DISCUSSION OF TABLE 17.

The table embodies the retesting of serums of four laboratory cases of tularæmia, 1, 3, 4, and 6, discussed in the preceding tables, the testing of serums of two suspected cases of tularæmia occurring in market men, the testing of six normal human serums, one typhoid human serum, and one antiplague horse serum.

The serums of the known laboratory cases reacted positively except in Case 1 in which 2 years and 10 months had elapsed since his first attack. It is interesting to note that the serum of Case 1 which had reacted positively in all preceding tests had now become entirely negative in spite of a second local attack of tularæmia which this case experienced in January, 1922. The absence of fever and constitutional manifestations during the second attack probably accounts for the absence of an increase of agglutinins following the second attack.

The serums of the two suspected cases of tularæmia reacted positively. One of these (Case E. N.) was a market man who skinned

rabbits in the market and manipulated their internal organs extensively in November and December, 1921, at which time he suffered an attack of fever and prostration, accompanied by marked painful enlargement of the axillary glands of one arm, lasting over a month. Strangely, he diagnosed his own case, calling it "rabbit fever," and stated that the condition was well known among market men. The other suspected case (Mrs. W.) while cleaning some quail on December 27, 1921, stuck a sharp point of a wing bone into the middle finger of her left hand, after which she turned to a pan full of rabbits and manipulated them. This was followed by an attack of fever which lasted about a month and was accompanied by suppuration of the glands at the elbow and adenitis of the axilla, and marked scarring at the site of infection on the finger.

Six control serums taken for routine Wassermann tests, one serum from a recent case of typhoid fever from which *B. typhosus* had been isolated and which had given a positive Widal agglutination, and one sample of antiplague serum prepared from a horse, all reacted entirely negatively.

The serums (excepting that of Mrs. W., which was collected two and one-half months before the test) were all collected on the day before the test and were heated to 55° C. for 30 minutes on the day of the test. The antigen was the pooled 48-hour growth on serum glucose cystine agar of three strains of *Bacterium tularense*, "J", "G", and "S", isolated from human cases in Utah in 1920, made up in buffered sodium chloride solution ($p_H$ 7.0) with a density of 1,000 ppm. (500 ppm. in final suspension). Two antigens were pooled in equal quantities for the test, one of which had been heated at 60° C. for 30 minutes and preserved with phenol, while the other was unheated and preserved with trikresol.

This test was made by Assistant Bacteriologist Alice C. Evans.

## CULTIVATION OF BACTERIUM TULARENSE ON THREE ADDITIONAL MEDIUMS NEW TO THIS ORGANISM.[1]

By EDWARD FRANCIS, Surgeon, United States Public Health Service.

The writer now reports the cultivation of *Bacterium tularense* on (1) beef infusion agar containing 0.02 per cent of an amino-acid (cystine), (2) beef infusion agar plus a piece of fresh sterile rabbit spleen, and (3) Loeffler's blood serum coagulated at 70° C.

The mediums here reported were used for the subcultivation of strains of *Bacterium tularense* which had had their original isolations from animals 12 months previously and had been carried continuously during the year on artificial mediums other than egg yolk, except that about 8 months previously they were carried for one or two passages through guinea pigs.

The strains used for this cultural work were human strains of "J", "G", and "S" and ground squirrel strain "S. F.", which were the subject of previous cultural studies. (See pp. 44–58.)

### COMPOSITION OF MEDIUMS.

(1) *Cystine agar.*[2]—Beef infusion agar containing 1 per cent peptone and 1 per cent agar adjusted to a reaction having a $p_H$ of 7.6 is kept on hand in stock. When needed, there is added to the stock agar 0.02 per cent of cystine, and this is placed for 15 minutes at the temperature of streaming steam in an Arnold sterilizer to melt the agar and to sterilize the cystine, after which the medium is tubed, slanted, and incubated 24 hours to insure sterility.

(2) *Plain agar plus a piece of fresh sterile rabbit spleen.*—The spleen is removed from a healthy rabbit and under sterile precautions is cut into pieces of about 3 mm. diameter. One piece is rubbed on the slanted surface of each beef infusion agar slant and the piece of spleen is left remaining on the surface of each slant just above the water of condensation; one piece of spleen is planted as a control into a fermentation tube containing glucose beef infusion broth. The tubes are incubated 24 to 48 hours, and, if sterile, are ready for inoculation.

### CULTIVATION ON CYSTINE AGAR.

Cultures grown upon cystine agar are now in their fourteenth consecutive generation, having been carried over every 48 hours. Growth in the fifth generation was rubbed on the shaved abraded skin of the abdomen of a guinea pig, causing its death on the sixth day with typical lesions of tularæmia. At the moment of death the spleen was removed and, under sterile precautions, was seared and cut into pieces about 3 mm. in diameter. One piece was planted on the

[1] Reprinted from Public Health Reports 1922, vol. 37, pp. 987–989.

[2] Later work has shown that the abundant growth of *Bacterium tularense* desired for agglutination and complement fixation tests and for immunization experiments is best obtained by increasing the amount of cystine in this medium to 0.1 per cent, by adding 1 per cent of glucose, and finally by the addition of 5 per cent sterile horse serum at a temperature of 45° to 50° C. before tubing, thus making serum glucose cystine agar.

slanted surface of each of three cystine agar tubes, two plain agar tubes, and two serum glucose agar tubes by rubbing the tissue over the surface of the medium and then allowing it to remain just above the water of condensation. One piece was planted into each of two fermentation tubes containing glucose beef infusion broth as a control. The heart's blood was planted on cystine agar slants, plain agar slants, and into fermentation tubes containing glucose bouillon broth. All tubes were incubated at 37° C. The cultures on the infected guinea pig's spleen and heart's blood will now be discussed.

(1) *Growth from guinea pig's spleen.*—Growth appeared on the third day on all seven of the tubes of slanted medium planted with infected spleen, but the fermentation tubes are still clear on the thirteenth day. The growth on the seven tubes had the morphology of *Bacterium tularense*, was Gram negative, and was subcultured as follows: Cystine agar growth to cystine agar slants, plain agar growth to plain agar slants plus a piece of fresh sterile rabbit spleen, serum glucose agar growth to serum glucose agar slants, and all seven to control tubes of plain agar slants and glucose fermentation tubes.

The controls are all negative at the end of ten days, but all other tubes showed good growth at the end of 24 hours, which has been subcultured daily, each to its own kind of medium. These subcultures in the fifth generation were rubbed on the shaved abraded skin of the abdomen of guinea pigs on April 25, causing acute death with typical lesions of tularæmia.

(2) *Growth from guinea pig's heart's blood.*—Growth appeared on the sixth day on the cystine agar slants planted with the guinea pig's heart's blood, and by the eighth day growth was abundant. Daily subcultivation on cystine agar and plain agar give good growth on the former but no growth on the latter. The fermentation tubes planted with the guinea pig's heart's blood are sterile on the thirteenth day.

## CONCLUSION.

Cultures of *Bacterium tularense* of human and ground-squirrel origin which have been carried one year on artificial mediums other than coagulated egg yolk grow well on (1) cystine agar, (2) plain agar plus a piece of fresh sterile rabbit spleen, and (3) Loeffler's blood serum coagulated at 70° C. The same cultures fail to show growth on plain agar and in fermentation tubes containing beef infusion broth.

Cultures in the fifth generation on these special mediums caused acute death with typical lesions of tularæmia in guinea pigs from which *Bacterium tularense* was cultured on the same mediums; these latter cultures in the fifth generation caused acute death in guinea pigs with typical lesions of tularæmia.

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NOTE.—Old cultures of gonococcus and *B. diphtheriæ* grow abundantly on cystine agar.

## HYGIENIC LABORATORY BULLETINS OF THE PUBLIC HEALTH SERVICE.

The Hygienic Laboratory was established in New York, at the Marine Hospital on Staten Island, August, 1887. It was transferred to Washington, with quarters in the Butler Building, June 11, 1891, and a new laboratory building, located in Washington, was authorized by act of Congress March 3, 1901.

Of the bulletins published by the laboratory since its establishment, copies of the following are available for distribution and may be obtained without cost by applying to the Surgeon General, United States Public Health Service, Washington, D. C.

No. 2.—Formalin disinfection of baggage without apparatus. By M. J. Rosenau.

No. 43.—The standardization of tetanus antitoxin (an American unit established under authority of the act of July 1, 1902). By M. J. Rosenau and John F. Anderson.

No. 55.—Quantitative pharmacological studies; adrenalin and adrenalin-like bodies. By W. H. Schultz.

No. 59.—The oxidases and other oxygen catalysts concerned in biological oxidations. By Joseph Hoeing Kastle.

No. 65.—Facts and problems of rabies. By A. M. Stimson.

No. 73.—The effect of a number of derivatives of choline and analogous compounds on the blood pressure. By Reid Hunt and R. de M. Taveau.

No. 78.—Report No. 4 on the origin and prevalence of typhoid fever in the District of Columbia (1909). By L. L. Lumsden and John F. Anderson. (Including articles contributed by Thomas B. McClintic and Wade H. Frost.)

No. 81.—Tissue proliferation in plasma medium. By John Sundwall.

No. 89.—Sewage pollution of interstate and international waters with special reference to the spread of typhoid fever. VI. The Missouri River from Sioux City to its mouth. By Allan J. McLaughlin.

No. 95.—Laboratory studies on tetanus. By Edward Francis.

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C. Evans. III. Effect of freezing and thawing upon the antibody content of antimeningococcus serum. By C. T. Butterfield. IV. The fermentation reactions and pigment production of certain meningococci. By Clara E. Taft. V. Studies on the lethal action of some meningococci on mice with special reference to the protective properties of antimeningococcus serum. By M. H. Neill and Clara E. Taft.

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No. 128.—Quantitative pathological studies with arsenic compounds. By Charles W. Hooper, Alfred C. Kolls, and K. Dorothy Wright.

No. 129.—Digest of comments on the Pharmacopœia of the United States of America and on the National Formulary for the calendar year ending December 31, 1919. By A. G. DuMez.

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