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EXPERIMENTAL STUDIES ON YELLOW FEVER OCCURRING IN MERIDA, YUCATAN.

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INTRODUCTION.

In 1918 a leptospira was detected in the blood and liver from certain cases of yellow fever in Guayaquil.¹ The organism was demonstrated also in the blood and in organ emulsions from guinea pigs which had been inoculated with blood or liver emulsions from yellow fever patients and had shown typical symptoms of the disease, fever, hemorrhages, jaundice, and nephritis. A pure culture of the same organism was made directly from the blood of yellow fever cases and the blood of experimentally infected guinea pigs, and the pathogenicity of the culture was demonstrated in guinea pigs, marmosets, and pups. The organism, which has been designated *Leptospira icteroides*, was found to be a filter passer, easily destroyed by a temperature of 55°C. within 5 minutes, an obligatory aerobe, and requiring for growth a certain amount of unmodified blood serum from man or suitable animals. Its growth is usually suppressed by secondary bacterial contamination, but when successfully obtained it remains almost invisible to the naked eye, forming neither a discrete colony nor a striking change of the culture medium, and hence the culture may be overlooked under ordinary circumstances as sterile. An ordinary microscope does not render it visible in the fresh state, and staining with ordinary dyes fails to bring out its presence. Through a dark-field microscope with a powerful illumination, however, it can readily be recognized. In the blood of yellow fever patients the organism may be present in such small numbers that an average dark-field search usually misses it. From the comparatively small percentage of positive transmissions from yellow fever patients to guinea pigs, it seems probable that many strains of the organism fail to infect this animal fatally or even with moderate severity. Whenever a secondary bacterial infection occurs after an attempt at transmission, either the animal dies before the infection with *Leptospira icteroides* fully develops, or the course of infection is atypically modified and the strain thereby lost. The various factors just enumerated no doubt constitute some of the reasons why animal inoculation or the detection of the organism has not been successful in the hands of different investigators at an earlier period.

¹ Noguchi, H., *J. Exp. Med.*, 1919, xxix, 547, 565, 585.

It has been shown also² that the majority of the serums from persons recently recovered from an attack of yellow fever possess a specific dissolving power upon the organism when tested by the Pfeiffer reaction. This phenomenon has been regarded as significant in establishing a possible relation between yellow fever and *Leptospira icteroides*. Another important point with respect to the etiological relation was furnished by the experimental transmission of the leptospira infection from yellow fever patients to normal guinea pigs, or from infected guinea pigs to normal guinea pigs, by the bite of infected stegomyia females,³ or with an emulsion of such mosquitoes, 8 days or longer after they had fed on infected individuals or animals.

By analogy *Leptospira icteroides* presented the principal characteristics of the virus of yellow fever as experimentally determined by Reed, Carroll, Agramonte, and Lazear⁴ and by later investigators (Marchoux, Salimbeni, and Simond,⁵ and Parker, Beyer, and Pothier⁶). Both are filter passers, readily killed at 55°C., transmitted by *Stegomyia calopus* after a period of incubation, not amenable to cultivation in ordinary media, invisible under the ordinary microscope, not resisting bacterial putrefaction, but capable of preservation in citrated blood under a layer of liquid paraffin for several days at room temperature,⁵ inoculable by hypodermic inoculation (in the case of the yellow fever virus inoculation was made into volunteer human beings,^{4, 5} in the case of *Leptospira icteroides* into susceptible animals), and producing similar symptoms. From these circumstances it seemed certain that *Leptospira icteroides* was responsible for the infection in the cases from which the organism was isolated or in which a positive Pfeiffer reaction was obtained, but the question as to whether or not *Leptospira icteroides* is also responsible for the disease known as yellow fever elsewhere than in Guayaquil was left open to further experimental determination.

The present paper gives the results obtained by us during a recent expedition to Merida, Mexico.⁷ Merida, a city of 100,000 inhabitants, and the capital of Yucatan, has a small proportion of non-

² Noguchi, H., *J. Exp. Med.*, 1919, xxx, 9.

³ Noguchi, H., *J. Exp. Med.*, 1919, xxx, 401.

⁴ Reed, W., Carroll, J., Agramonte, A., and Lazear, J. W., *Senate Doc. No. 822, 61st Cong., 3rd Sess.*, 1911, 56.

⁵ Marchoux, Salimbeni, and Simond, *Ann. Inst. Pasteur*, 1903, xvii, 665.

⁶ Parker, H. B., Beyer, G. E., and Pothier, O. L., *Bull. Hyg. Lab., U. S. P. H.*, No. 13, 1903.

⁷ This expedition was undertaken under the auspices of the International Health Board of The Rockefeller Foundation and The Rockefeller Institute for Medical Research during the months of Dec., 1919, and Jan., 1920. The Commission received hearty and efficient cooperation from the hospital and sanitary officials in Merida, to whom we wish to express our appreciation and thanks.

immune foreign population, but it owes its previous epidemics of yellow fever to the occasional influx of foreigners or of troops sent thither from other and mountainous states where there is no yellow fever. It is reported that a systematic anti-stegomyia campaign had kept the city free from yellow fever during the 4 years preceding 1919, and that it was owing to an enormous crop of stegomyias in 1919 that an epidemic broke out in July and lasted until November, claiming about 100 patients among the newly arrived troops. The death rate was about 50 per cent.

At the time when one of us arrived in Merida,⁸ there had been no new case of yellow fever for 13 days. On December 15 a soldier, J. M., was brought to the yellow fever hospital in a moribund condition and died the following morning. Postmortem examination, performed 4 hours after death by Dr. A. Lara, Director of the Hospital, and Dr. Diego Hernandez, a representative of the Department of Public Health and Charities of Mexico, established the diagnosis of yellow fever. Blood from the heart and pieces of liver and kidney were obtained for experimental purposes. The details of the experiments will be given later under the heading Results obtained with Case 1.

As soon as the diagnosis of yellow fever had been established in Case 1, Dr. Hernandez ordered the soldiers (eleven in all) who had been quartered with this patient to the yellow fever hospital, there to be placed under quarantine. Among these eleven was a young soldier, A., who was apparently convalescing from a recent attack of yellow fever. The urine and serum of this individual were studied later and will be referred to in the report as Case 3.

On December 18 one of the exposed soldiers, M., began to complain of an illness which suggested strongly the beginning of yellow fever. A systematic study of this case was started at once, the blood being withdrawn from the median basilic vein at first daily and then every other day (December 18, 19, 20, 22, and 24) and used on the one hand for cultivation and on the other, if possible, for inoculation into guinea pigs. As Chart 7 shows, the fever became typical within 3 days, with the development gradually of all other symptoms, congested conjunctivæ, flushed face, muscular pains in the limbs and loins,

⁸ Dr. Kligler arrived Dec. 12, 1919, Dr. Noguchi Dec. 23.

severe headache, swollen gums, nausea, epigastralgia, albuminuria, and cylindruria. On December 24 there occurred the characteristic "coffee-grounds" vomit, also definite jaundice, which became much deeper within the next several days. The albumin and casts gradually increased in the urine, and there was an abundance of bile pigment, the amount of urine being also diminished. The patient was discharged at the end of 3 weeks. Before discharge the serum and urine were collected for further study. This case will be referred to as Case 2.

RESULTS OBTAINED WITH CASE 1.

Case 1.—J. M., soldier; brought to the Casa de Salud (the yellow fever hospital) from the barracks in the night of Dec. 15, 1919, in a critical condition, with characteristic black vomit and jaundice. Death occurred at 6 a.m., and autopsy was made at 10 a.m., 4 hours post mortem.

Autopsy.—There was general jaundice, which was particularly marked in the scleras. The face was covered with black vomit. The liver was dotted with small yellowish areas. There were petechial hemorrhages in the stomach wall; the kidneys were hemorrhagic; the pericardium contained yellowish fluid; the serum was icteric.

Blood was drawn from the heart, and a portion of the liver and kidney was removed at autopsy for the experiments to be described. For the purpose of transmission guinea pigs were used throughout the present work.

Transmission Experiments with the Blood, Liver, and Kidney of Case 1.

Blood.

On December 16, 1919, intraperitoneal inoculation of 1 cc. of the heart's blood⁹ was made into two guinea pigs (Nos. 1 and 2). Both had a temporary rise of temperature after 7 days but returned to normal within a few days. No jaundice developed at any period. On examination after 18 days there were some suspicious lesions in the lungs, otherwise the findings were negative.

⁹ The same specimen of blood kept at 8°C. for 8 days was inoculated into Guinea Pigs 18 and 35 intracardially, but neither symptoms nor high temperature were seen to develop within 2 weeks (negative).

Kidney.

An emulsion of the kidney in sterile saline solution was injected on December 16 into two guinea pigs, one of which showed a very suggestive reaction (Guinea Pig 3).

Guinea Pig 3 (Chart 1).—Dec. 16, 1919. Intraperitoneal inoculation of 1 cc. of the kidney emulsion. Temperature rose to 103° F. in the afternoon of Dec. 19 and was 103° a.m. and 104.6° p.m. on Dec. 20. The animal was killed for transfers to three new guinea pigs on Dec. 21.

Transfers from Guinea Pig 3 (Second Generation).—December 21. 1 cc. of an emulsion prepared from the liver and kidney from Guinea Pig 3 was inoculated intraperitoneally into each of two normal guinea pigs, and 1 cc. of the heart's blood into a third. One died early of a secondary infection, and another remained well after having shown a slight elevation of temperature, while the third animal (Guinea Pig 3c) had definite signs of the leptospira infection.

Guinea Pig 3c (Chart 2).—Inoculation of 1 cc. of the blood of Guinea Pig 3. This animal began to show fever (103.2°) 5 days after the inoculation. The following morning the temperature remained at 103.2° and in the afternoon reached 105.6°. The animal was killed for examination and transfer.

Autopsy.—There was a light yellowish color throughout the subcutaneous tissues. The lungs showed numerous hemorrhagic foci, the gastric mucosa was highly congested, but there were no macroscopic hemorrhages in the latter. The intestines were icteric and congested, the liver was slightly icteric, the kidneys and adrenals were moderately congested, and the spleen was normal. The urine was slightly turbid and contained a considerable amount of albumin (++) and a few casts.

The blood from the heart of Guinea Pig 3c, as well as emulsions of the liver and kidney, were used to inoculate nine new guinea pigs. Two of the three inoculated with the blood and three of the six inoculated with the mixture of emulsions of the liver and kidney succumbed to a secondary infection with the paratyphoid bacillus. Two, however, of those escaping the secondary infection showed temperature curves and lesions quite typical of *Leptospira icteroides* infection, as described below.

Transfers from Guinea Pig 3c (Third Generation).—

Guinea Pig 54 (Chart 3).—Dec. 27, 1919. Received 1 cc. of the heart's blood from Guinea Pig 3c intraperitoneally. The temperature rose on the 7th day, and the animal remained febrile for 48 hours. It was killed for transfer and examination 10 days after the inoculation.

Autopsy.—The lungs showed numerous hemorrhagic foci. The gastrointestinal mucosa was highly congested. The liver was pale, perhaps slightly yellowish, with granular surface and several hemorrhagic spots. The kidneys were apparently swollen and degenerated, the adrenals were small and hemorrhagic, and the spleen was swollen.

Guinea Pig 59 (Chart 4).—Dec. 27, 1919. Received 1 cc. of a mixture of the liver and kidney emulsion from Guinea Pig 3c intraperitoneally. The temperature rose to 104° F. on the 6th day, and was 103° a.m. and 105° p.m. the following day. The animal was killed for transfer and examination on that day.

Autopsy.—There was a slight yellowness throughout the body, especially on the abdominal wall; no subcutaneous or intramuscular hemorrhages. The lungs showed several petechial hemorrhages on the posterior surface; there was a general congestion of the lobes. The liver was slightly icteric. The gastrointestinal tract showed scattered hemorrhagic areas, especially in the mucosa of the large intestine. The kidneys were highly congested, the spleen was normal, and the urine icteric.

Guinea Pigs 54 and 59 undoubtedly represented a positive transmission. The heart's blood and emulsions of the kidney and liver of Guinea Pig 59 were inoculated intraperitoneally into six normal guinea pigs, but the animals were lost through secondary bacillary infection.

Liver.

A small piece of the liver from Case 1 was emulsified in a sterile mortar with saline solution, and 1 cc. of the emulsion was inoculated into each of two guinea pigs (Nos. 5 and 6) intraperitoneally on December 16, 1919. One of them (Guinea Pig 6) presented a definite picture of the leptospira infection, while the other gave a suggestive reaction only. The protocol of Guinea Pig 6 and details regarding further transfers are given below.

Guinea Pig 6 (Chart 5).—Dec. 16, 1919. Received 1 cc. of the liver emulsion intraperitoneally. As the chart shows the temperature rose sharply (103° F.) in the afternoon of the 7th day and still more the next day (104° a.m. and 104.9° p.m.). On the 9th day it fell to 100.5° with the simultaneous appearance of a

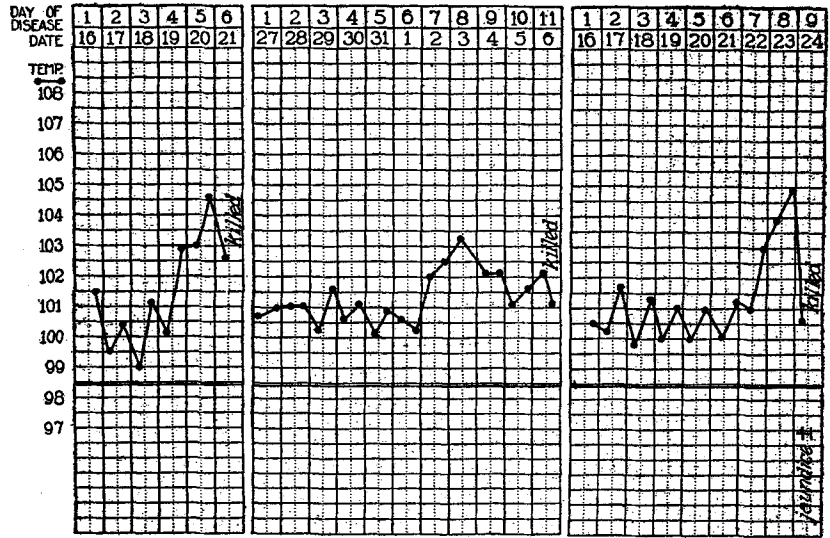


Chart 1 (GP#3). Inoc. with human kidney (Case 1).
 Chart 3 (GP#54). Transfer from GP#3c.
 Chart 5 (GP#6). Inoc. with human liver (Case 1).

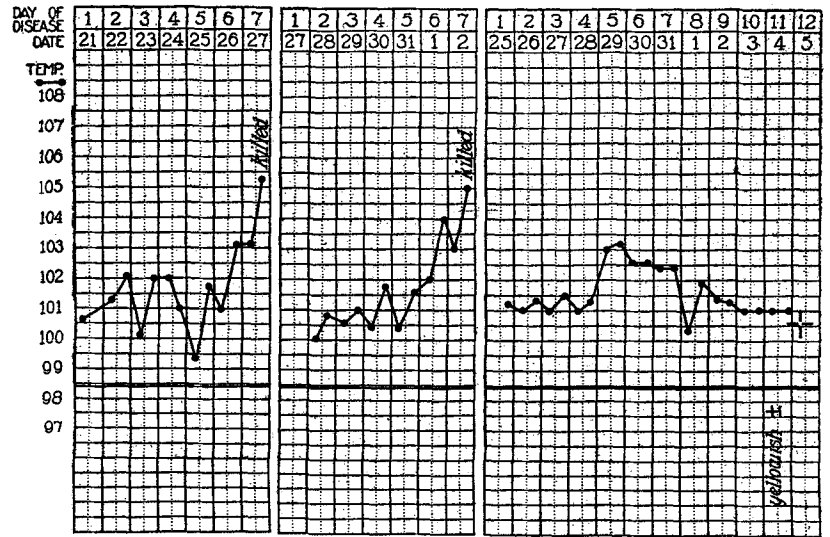


Chart 2 (GP#3c). Transfer from GP#3.
 Chart 4 (GP#59). Transfer from GP#3c.
 Chart 6 (GP#33). Inoculated with culture from Case 1.

CHARTS 1 TO 6. Transmission experiments with autopsy material from Case 1.

trace of jaundice in the scleras and abdominal wall. The animal was killed for transfer and examination.

Autopsy.—Faint jaundice throughout the body. Lungs showed a few ecchymoses and infarctions. Liver congested, with numerous subcapsular hemorrhagic mottles, perhaps more brownish than normally. Stomach and intestines highly congested. Kidneys and adrenals highly congested, the former showing a parenchymatous degeneration. Spleen normal. Urine turbid, brownish, containing casts and albumin.

Guinea Pig 6 represented a positive transmission, and from this animal transfer was made to three normal guinea pigs (Nos. 19, 27, and 28). The results obtained in these three animals were, first, a definite febrile reaction on the 4th day, followed by an irregular fluctuation of temperature during the several successive days (indicative of a secondary infection) without jaundice at any time. These animals were killed on the 13th day for examination. The lungs showed more or less numerous hemorrhagic foci; the other organs appeared normal. No further passage was made with this material.

*Transmission Experiments by Means of Cultures from the Blood
of Case 1.*

Attempts were made to obtain a culture of *Leptospira icteroides* with the blood obtained from Case 1. Into each of a series of culture tubes containing the medium previously found suitable¹⁰ for the growth of *Leptospira icteroides* 0.1 to 0.5 cc. of the blood was introduced, and the tubes were placed in a thermostat, the temperature being about 26°C. A portion of blood was also mixed with sodium citrate saline solution (equal parts) and after being kept at room temperature (26°C.) for 24 hours was inoculated in amounts of 0.1 to 0.5 cc. into three tubes on December 17, 1919.

Nearly all these tubes were found to be contaminated with a *coli*-like bacillus, owing undoubtedly to a terminal or postmortem invasion by this organism of the blood circulation. On December 25 two culture tubes, inoculated on December 17, appeared to be free from any secondary bacterial contamination. Under the dark-field microscope no leptospira could be found. From each of these two tubes about 1.5 cc. of the uppermost layer of the medium, just beneath

¹⁰ Noguchi, H., *J. Exp. Med.*, 1919, xxx, 13.

the paraffin oil, were taken out by means of small sterile pipettes and the mixture of the two cultures was used for inoculating three guinea pigs. Two of the animals died of a secondary infection within 2 days, and the third (Guinea Pig 33) was found dead on the 12th day after having had slight fever from the 5th to 7th day. The protocol of this animal is given below.

Guinea Pig 33 (Chart 6).—Dec. 25, 1919. Inoculated intraperitoneally with 1 cc. of the mixture of Culture Tubes 1 and 2 which had been kept 8 days at room temperature after inoculation with citrated blood from the heart of Case 1 at autopsy. The temperature rose to 103° F. a.m. and 103.2° p.m. on the 5th day, and was 102.6° and 102.4° respectively for the 2 following days. It returned to normal on the 10th day, and death occurred during the night of Jan. 4, 1920, 11 days from the time of inoculation.

Autopsy.—The cadaver had undergone considerable postmortem decomposition, owing to the warm temperature of the laboratory, when found lying dead in the cage on Jan. 5. Notwithstanding postmortem discoloration, there was a slight but distinct yellowness in the skin and scleras. On opening the body also the postmortem changes greatly interfered with the efforts to determine any characteristic lesions. The lungs were congested and showed a few old hemorrhagic areas. There was a small localized abscess on the abdominal wall, and the peritoneal cavity contained some turbid fluid.

It is difficult to determine whether or not the temperature curve and lesions in Guinea Pig 33 were due wholly to the abscess or in part to a mild leptospira infection.

RESULTS OBTAINED WITH CASE 2.

Case 2 (Chart 7).—P. M., soldier; exposed to infection through the preceding case. Dec. 17, 1919, 5 p.m. Complained of headache. Dec. 18. Increase in general malaise; headache; rachialgia; pains in the limbs. Blood was drawn at 4 p.m. Temperature 100.9°; pulse 96. No albumin in urine. Dec. 19. Temperature 101.5°; pulse 92. Blood drawn in the morning; citrated and inoculated into two guinea pigs. Trace of albumin. Dec. 20. Temperature 103.5°. Blood drawn and inoculated into three guinea pigs. Albumin 0.05 gm. Dec. 21. Trace of albumin. Dec. 22, 3 p.m. Temperature 99.3°; pulse 78. Conjunctiva injected; scleras slightly icteric. Vomited. Stool chocolate color. Urine turbid; albumin 0.18 gm. Blood drawn for culture. Dec. 23. Albumin 1.4 gm. Pulse 72. Dec. 24. Albumin 1.8 gm.; casts and renal epithelium. Jaundice marked. Blood drawn for culture and animal inoculations. Pulse 72. Dec. 25. Albumin 2.2 gm. Dec. 26. Albumin 5.5 gm. Dec. 27. Albumin 0.8 gm.

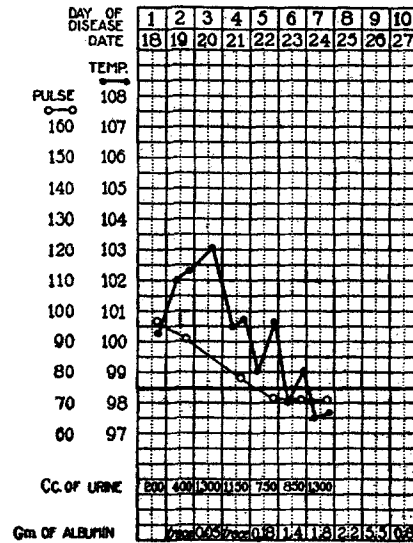


Chart 7 (patient).
Temperature curve, Case 2.

Transmission Experiments with Blood of Case 2.

First Specimen of Blood, Drawn on the 2nd Day of the Disease, December 19, 1919.

1 and 2 cc. respectively of the citrated blood were inoculated intraperitoneally into two guinea pigs (Nos. 7 and 8).

Guinea Pig 7.—The animal had a temperature of 103° F. on the 5th day; during the following 48 hours it was normal but rose again to 102.8–102.5° for 3 succeeding days. On the 12th day it was normal. On the skin of the abdominal wall were several ecchymotic areas. The animal was killed for examination.

Autopsy.—With the exception of one small hemorrhagic spot in the lungs nothing abnormal was found.

Notwithstanding this apparently negative finding, however, the blood, as well as emulsions of the liver and kidney of this animal, were inoculated into six normal guinea pigs, the blood into one and the mixed emulsions of the liver and kidney into five. The results were of doubtful nature with respect to positive transmission, although two of these animals showed some punctiform hemorrhages in the lungs when examined after 14 days.

Guinea Pig 8 (Chart 8).—This experiment was a duplicate of the experiment with Guinea Pig 7; that is, the animal was inoculated with the blood taken on

Dec. 19, 1919. As Chart 8 shows, the temperature rose to 104° F. in the afternoon of the 4th day, remaining high for the following 24 hours. It fell to 100.5° on the 6th day, when it seemed advisable to make a transfer.

Autopsy.—The examination showed a few hemorrhagic foci in the lungs, highly congested liver and kidneys, and a few hemorrhagic areas in the mucosa of the intestines.

Transfers with the blood and organ emulsions (liver and kidneys) of Guinea Pig 8 were made to several normal guinea pigs.

Transfers from Guinea Pig 8 (Second Generation).—

Guinea Pig 20 (Chart 9).—Dec. 24, 1919. Inoculated with the citrated blood from Guinea Pig 8, 1 cc. intraperitoneally. The temperature rose suddenly to 105° on the 5th day and remained at that point during the day. It fell to 103° the next day and to 101° on the 8th day. At this period the scleras seemed slightly icteric. The urine diminished to less than 2 cc. and contained albumin. The condition remained unchanged for the 3 succeeding days, the urine still containing albumin. Casts appeared on the 10th day. The yellowness in the conjunctiva was definite on the 11th day, and the animal died in the afternoon after subnormal temperature.

Autopsy.—Small hemorrhagic and congested areas in the lungs; liver smaller in size (?) and reddish brown with yellow mottles; kidneys unusually pale, and the demarcation between cortex and medulla indistinct. The spleen was somewhat enlarged, perhaps owing to secondary infection, and granular. In the liver and kidney stained by Levaditi's method fairly numerous leptospiras were demonstrated.

Guinea Pig 21.—Dec. 24, 1919. Inoculated intraperitoneally with 0.5 cc. of the mixture of the liver and kidney emulsions of Guinea Pig 8. Died of a secondary infection, but showed the presence of a mild leptospira infection, with albumin and casts in the urine.

Guinea Pig 22.—This experiment was a duplicate of the foregoing experiment. Temperature 103° F. on the 4th day, and fever continued for several days. Jan. 5, 1920. The animal was killed for examination.

Autopsy.—The lungs showed old hemorrhagic areas, but the other organs seemed normal.

The remainder of the citrated blood of December 19 was preserved in the ice box (about 10°C.) and after 5 days was inoculated intracardially into two normal guinea pigs, with negative results.

*Second Specimen of Blood, Drawn on the 3rd Day of the Disease,
December 20, 1919.*

1 cc. of the citrated blood drawn on December 20 was inoculated intraperitoneally into three guinea pigs (Nos. 9, 10, and 11) on the same day.

Guinea Pigs 9 and 11.—These two animals had suggestive and almost identical temperature curves.

Autopsy.—When killed for examination on the 15th day, there were several foci of recent hemorrhage in the lungs, and the kidneys were congested; the other organs appeared normal.

Guinea Pig 10 (Chart 10).—This animal presented a more definite febrile reaction. On the 14th day it had a normal temperature and appeared well. It was killed for examination on that day.

Autopsy.—There were numerous hemorrhagic foci in the lungs; the abdominal wall and subcutaneous tissues were icteric and showed several large hemorrhagic areas. The adrenals were hemorrhagic, the kidneys highly congested, and the liver was apparently normal. There were pin-point hemorrhages in the gastric mucosa, and hemorrhages in the intestinal mucosa. The spleen appeared normal.

The remainder of the citrated blood of December 20 was preserved in the ice box (about 10°C.) for 4 days and 1 cc. was inoculated intracardially into each of two normal guinea pigs (Nos. 14 and 15).

Guinea Pig 14.—Sudden rise of temperature to 104° F. in the afternoon of the 4th day, but it dropped to normal (102°) on the next day. On the 6th day there was another rise to 103.5°, from which the temperature gradually fell to sub-normal on the 8th day. The urine gradually diminished in quantity after the 4th day, and albumin and casts were present for several days. Ecchymotic hemorrhages were observed on the abdominal wall on the 8th day. There was a suspicion of jaundice. The animal rapidly recovered and was normal on the 13th day.

Guinea Pig 15.—Lost through secondary infection.

*Third Specimen of Blood, Drawn on the 5th Day of the Disease,
December 22, 1919.*

The citrated blood from Case 2 drawn on December 22 was kept on ice for 48 hours (about 10°C.) before it was inoculated into two guinea pigs (Nos. 16 and 17) on December 24.

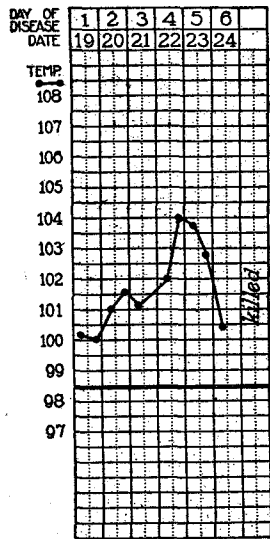


Chart 8 (GP#8).
Inoc. with 2nd day blood.

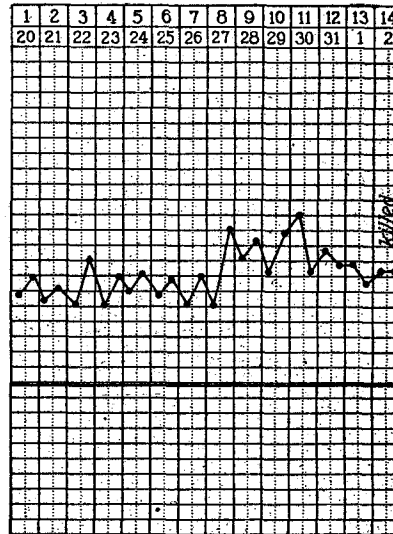


Chart 10 (GP#10).
Inoculated with 3rd day blood.

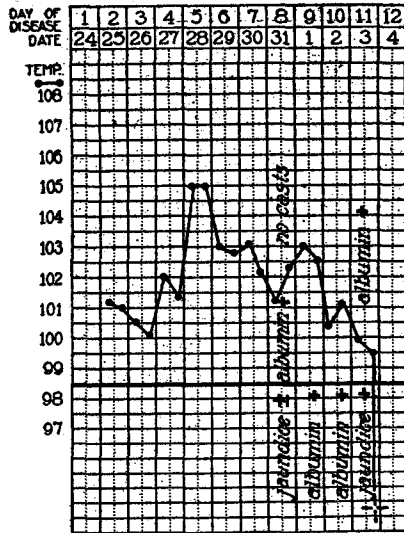


Chart 9 (GP#20)
Transfer from GP#8

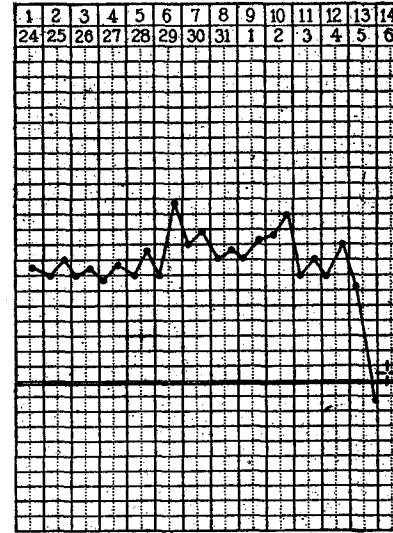


Chart 11 (GP#25)
Inoculated with 7th day blood.

CHARTS 8 to 11. Direct transmission experiments with blood from Case 2.

Guinea Pig 16.—Showed a rise of temperature to 103° F. on the 4th day, a decline to 102.5° on the 5th day, and a rise again to 103.5° on the 6th. At this time the animal had congested conjunctivæ. On the 9th day the temperature became somewhat subnormal but soon returned to normal during the succeeding days. The animal was killed on the 13th day.

Autopsy.—A number of hemorrhagic foci were found in the lungs, the liver appeared very pale and brownish, the kidneys were somewhat congested, the spleen normal. The animal was probably recovering from an abortive infection.

Guinea Pig 17.—This animal had practically the same febrile reaction as Guinea Pig 16.

Autopsy.—Performed on the 13th day. The organs appeared normal, with the exception of a few hemorrhagic foci in the lungs.

*Fourth Specimen of Blood, Drawn on the 7th Day of the Disease,
December 24, 1919.*

The citrated blood was intracardially inoculated into three guinea pigs (Nos. 24, 25, and 26) within 3 hours from the time of withdrawal, each animal receiving 0.2 to 0.5 cc.

Guinea Pigs 24 and 25 (Chart 11).—Both animals had a suggestive temperature curve but had no jaundice at any period.

Autopsy.—The lungs showed some suspicious recent hemorrhagic areas; the kidneys were rather congested. The other organs were normal. Results inconclusive.

Guinea Pig 26.—Perhaps died of secondary infection.

*Transmission Experiments by Means of Cultures from the Blood of
Case 2.*

Cultures were made with the citrated blood of Case 2 by introducing into a series of six tubes containing the necessary culture media¹¹ quantities of the blood varying from 0.1 to 0.5 cc. and adding to each a layer of paraffin oil. The tubes were kept for several days at a temperature of 26–28°C. Then, since the leptospira grows only in the presence of oxygen, the upper layer of the semisolid medium was removed from each tube by means of a sterile pipette and the contents of two or more tubes were mixed in a Petri dish. Several guinea

¹¹ This consisted of a rabbit serum diluted three times with 0.9 per cent sodium chloride solution and rendered semisolid by the addition of 0.1 to 0.3 per cent nutrient agar (melted). Each tube contained about 6 cc. of the mixture.

pigs were inoculated with the same material. For every series of cultures made from any given specimen of blood at least two sets of guinea pigs were inoculated, and three sets if there was a larger number of tubes.

The cultures set up with the specimens of blood taken on five occasions (December 18, 19, 20, 22, and 24) remained for the most part free from any secondary contamination during the first 5 days. Shortly afterwards, however, darkening of the hemoglobin in several tubes indicated the presence of some sort of contamination, and microscopically a fungus growth was found. Unless a surface growth of fungus was already evident, however, the contents of these tubes were also used for inoculation, though tubes showing bacterial contamination were discarded. Such contamination was rarely met with in experiments with the blood of Case 2, and that occasionally occurring was probably due to technical error. Following are the results of animal inoculations with the various culture series.

Culture, First Series. Blood Drawn on the 1st Day of the Disease, December 18, 1919.

Dark-field examination of Tubes 1 and 2, 7 days after the cultures had been set up, revealed no organism. The contents of these two tubes (2 cc. of the uppermost layer of medium from each) were mixed together in a Petri dish, and 1 cc. of the mixture was inoculated intraperitoneally into each of three normal guinea pigs (Nos. 36, 37, and 38). No definite reaction followed the inoculations in Guinea Pigs 36 and 38, which, on examination 15 days after inoculation, presented nothing remarkable in any organ except for a congestion of the lungs.

Guinea Pig 37.—Slight rise in temperature on the 7th day (102.5° F. a.m., 103° p.m.), with return to normal the next day. On the 14th day the temperature rose suddenly to 105° a.m. and 103° p.m.; on the 15th day it was 102.6° a.m. The animal was killed the same day for examination.

Autopsy.—The lungs were typical, showing numerous hemorrhagic foci, but there was no other change.

Dark-field examination of Tubes 3 and 4 of the same series demonstrated the presence of a few fungi on December 25, 7 days after

inoculation. The uppermost layers of media from the two tubes were mixed and 1 cc. of the mixture was inoculated intraperitoneally into two guinea pigs (Nos. 39 and 40) on that day (December 25), with negative results.

Culture, Second Series. Blood Drawn on the 2nd Day of the Disease, December 19, 1919.

Five culture tubes were available for these experiments. These were divided into two groups, Tubes 1 and 2, and Tubes 3 to 5. Dark-field examination of these tubes was made 6 days after the inoculation. Tubes 1, 2, 3, and 5 showed no organism; Tube 4 showed a few immotile leptospiras, some apparently degenerating. Occasional coarse, short filaments (fungus) were also found. The number of leptospiras was so small that a repeated, thorough search with a powerful illumination was necessary to find one. The results of animal inoculation follow.

The contents (uppermost layers) of Tubes 1 and 2 of the second culture series were taken out and mixed. 1 cc. of the mixture was inoculated intraperitoneally into two guinea pigs (Nos. 41 and 42) on December 25 and into two more on December 26. Two died of secondary infection; the others remained well.

The uppermost layer was taken out of Tubes 3, 4, and 5 of the second culture series, and 1 cc. of the mixture of these, which had been found by dark-field examination to contain a few leptospiras, was inoculated intraperitoneally into each of three guinea pigs (Nos. 43, 44, and 45) 6 days after the cultures had been made. The results of these inoculations were all positive, as the protocols show.

Guinea Pig 43 (Chart 12).—The temperature rose to 103° in the morning of the 5th day. During the next 36 hours it fluctuated between 102° and 102.5°. On the 7th day it rose to 105.2° a.m. and 103.5°³p.m. The amount of urine was still undiminished. On the 8th and 9th days the morning temperature was 102° and the evening 104°; on the 10th it was 102.5° in the morning and 103.4° in the evening; on the 11th it fell rapidly to normal. The animal was decidedly jaundiced. The urine diminished daily after the 9th day, and only 3 cc. were secreted during the 24 hours from Jan. 3 to Jan. 4, 1920. Albumin and casts were present. The animal was killed for examination and transfer on the 11th day.

Autopsy.—Findings typical. Intense jaundice; extensive subcutaneous ecchymoses; lungs distinctly hemorrhagic; gastrointestinal mucosa congested with

hemorrhagic areas; liver yellowish; kidneys hemorrhagic and yellowish; spleen somewhat enlarged.¹² Dark-field examination revealed the presence of leptospiras in the blood as well as in emulsions of the liver and kidney.

Transfers from Guinea Pig 43.—Blood from the heart and a mixture of emulsions of the liver and kidney were intraperitoneally inoculated into six normal guinea pigs, the blood into Nos. 106 and 107, and the emulsion into Nos. 108, 109, 110, and 111. The protocols follow.

Guinea Pig 106.—Jan. 4, 1920. Inoculated with 1 cc. of the citrated blood from Guinea Pig 43. Temperature 104° F. on the 3rd day, fell to normal on the 4th, rose to 104° a.m., 104.5° p.m. on the 5th, remaining high for another 24 hours. On the 7th day it was 103.5° a.m. and 102.2° p.m. Jaundice and albuminuria were both distinct. On the 8th day died after subnormal temperature.

Autopsy.—All the characteristic lesions, together with signs of a secondary infection with the paratyphoid bacillus (enlarged spleen and fibrinous exudate in the peritoneal cavity).

Guinea Pig 107 (Chart 13).—This experiment was a duplicate of the foregoing experiment. As shown in Chart 13 the course of the infection was typical, the temperature rising to 105.2° a.m. and 104.6° p.m. on the 5th day. On the 7th day the animal was intensely yellow, with albumin and casts in the urine. It was killed for examination on the 7th day.

Autopsy.—Findings typical. The blood and organ emulsions showed a few leptospiras.

Guinea Pig 108.—Jan. 4, 1920. Inoculated intraperitoneally with 0.5 cc. of the mixture of the liver and kidney emulsions from Guinea Pig 43. This animal suffered from a secondary paratyphoid infection and showed a temperature of 104.6° in the afternoon of the 3rd day. This early fever endured for 3 days longer. On the 7th day it fell to 103.5°. On the 8th day it reached normal, and the animal died after subnormal temperature on the 9th day. No jaundice developed.

Autopsy.—Several hemorrhagic foci in the lungs. No leptospira was found.

Guinea Pig 109.—This animal must have been suffering from an intercurrent infection before the inoculation, as the temperature was 103.4° in the afternoon of Jan. 3, 1920, a day before the inoculation. After inoculation the temperature fluctuated between 102° a.m. and 102.5° p.m. during the 2 days that followed. On the 4th day it was normal. On the 5th it rose to 103.5° a.m. and 103.2° p.m., on the 6th it was 102.5°, on the 7th 101.5° a.m. and 96° p.m. Jaundice developed on the 6th day.

Autopsy.—Performed on the 7th day. The lesions were typical, hemorrhages in the lungs, stomach, and intestines, kidneys highly hyperemic with minute hemorrhages, liver yellowish. The spleen was enlarged, showing the effect of a

¹² Blood culture showed the presence of the paratyphoid bacillus.

secondary infection with the paratyphoid bacillus. The blood and organ emulsions contained leptospiras. A blood culture from the heart showed the presence of the paratyphoid bacillus.

Guinea Pig 110 (Chart 14).—Duplicate experiment. This animal escaped secondary infection with the paratyphoid bacillus and ran a typical course of the leptospira infection. The temperature rose to 103° in the afternoon of the 3rd day, was 102.2° a.m. and 104° p.m. on the 4th, 103.8° a.m. and 104.6° p.m. on the 5th. From the 6th day the temperature rapidly fell and was 98° when death occurred in the morning of the 7th day. Jaundice was definite on the 6th day.

Autopsy.—Findings typical. The leptospira was demonstrated in the organ emulsions. The animal had epistaxis at the time of death.

Guinea Pig 111 (Chart 15).—Duplicate experiment. This animal also escaped secondary paratyphoid infection and ran a course of fever similar to that of Guinea Pig 110, except that the highest temperature never exceeded 104° (4th day). On the 5th the temperature was still 103.8°. The examination of the blood showed no leptospiras. The animal was killed for examination and culture in the afternoon of the 5th day. The citrated blood from the heart was examined carefully for the leptospira, and one was found after long search. This blood was used for cultivation, which in 7 days was successfully accomplished.

Autopsy.—Examination of the organs showed that jaundice had not yet developed. The lungs, stomach, and intestines were dotted with hemorrhagic foci. The liver was congested, with yellowish areas. The kidneys were congested. Emulsions of the liver and kidney contained a small number of leptospiras.

Guinea Pig 44 (Chart 16).—This is one of the three guinea pigs inoculated with 1 cc. of the mixture of Culture Tubes 3, 4, and 5 of the second culture series on Dec. 25, 1919. This animal, in striking contrast with the foregoing ones (Guinea Pig 43 and its transfers) showed a rather slowly developing infection without much febrile reaction. The temperature remained entirely within the normal fluctuation until the 7th day, and on the 8th day the morning temperature was slightly above normal (102.6° F.), rising to 103.6° in the afternoon. On the 9th day the temperature was normal in the morning and somewhat lower in the afternoon, and the animal was distinctly yellowish. On the 10th day the temperature registered 101.3° a.m. and 101.2° p.m., perhaps somewhat below the normal, and jaundice was intense. The animal died during the night.

Autopsy.—Performed in the morning, Jan. 4, 1920. Intense jaundice. There were extensive subcutaneous hemorrhagic foci on the abdominal wall and in adjacent regions. The lungs were yellowish, with numerous hemorrhagic areas, and the heart was dilated and contained dark clot and blood. The liver was yellowish brown, mottled with congested areas, the gall bladder empty, the kidneys were swollen, hemorrhagic, and degenerated, deeply bile-stained, and the adrenals hyperemic. The stomach contents were bloody, and there were ecchymoses in the mucosa. The intestines contained bloody stools; the mucosa was congested and showed many hemorrhagic spots. The spleen was normal. The

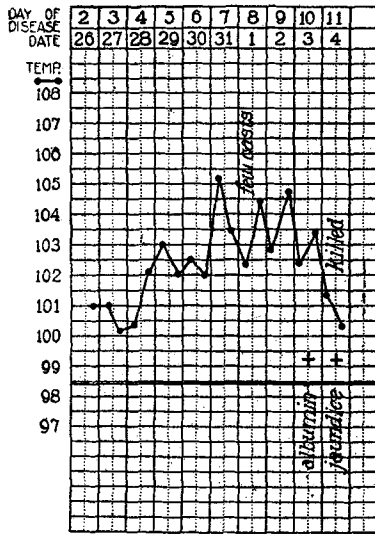


Chart 12 (GP#43).
Inoc. with culture of 2nd day blood.

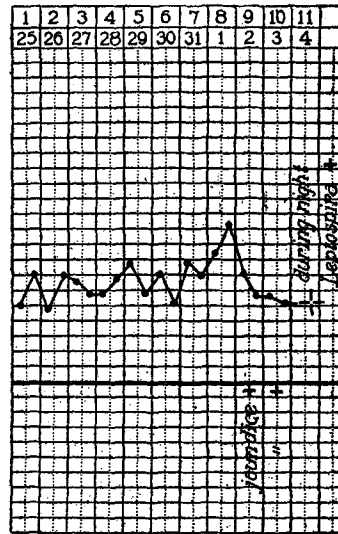


Chart 16 (GP#44).
Inoc. with culture of 2nd day blood.

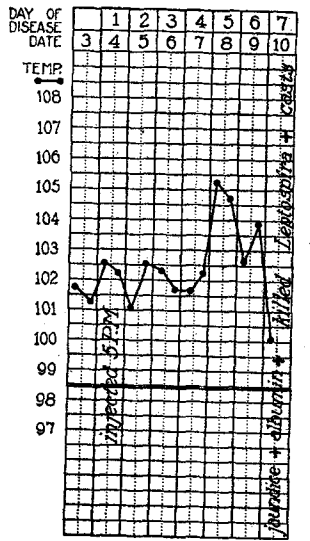


Chart 13 (GP#107).
Transfer from GP#43.

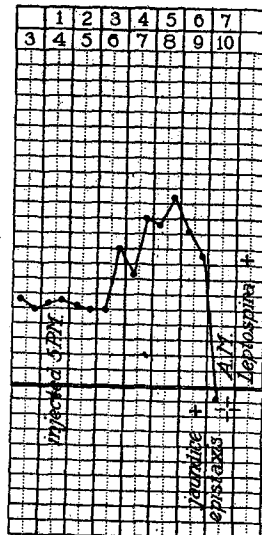


Chart 14 (GP#110).
Transfer from GP#43

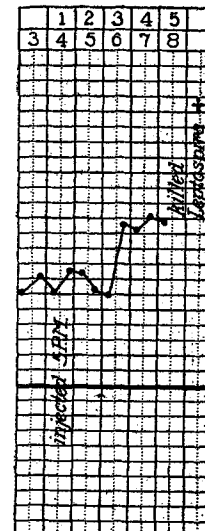


Chart 15 (GP#111).
Transfer from GP#43

CHARTS 12 TO 16. Transmission experiments with cultures from Case 2.

bladder was almost empty (anuria); ovaries and uterus congested. In the scanty urine removed from the bladder there were albumin and numerous casts with erythrocytes. The leptospira was found in the blood.

Transfer with a mixture of the liver and kidney emulsions from Guinea Pig 44 was made into Guinea Pig 105 on January 4, 1920. The result was positive in every respect. The animal succumbed to a typical leptospira infection on the 7th day, and the leptospira was detected in the blood and organs.

Guinea Pig 45.—This is the last of the three guinea pigs (Nos. 43, 44, and 45) inoculated with the same material (culture made with the blood drawn on the 2nd day) on Dec. 25, 1919. The result of the inoculation was likewise positive, although the temperature curve deviated considerably from the curves of the other two animals, owing probably to a secondary infection with another organism. On the 11th day the animal had been running a temperature of 103–104° F. for 3 days. It was killed for examination on the 11th day.

Autopsy.—The lesions were typical except for the absence of distinct jaundice. The liver was, however, somewhat yellowish. The leptospira was not found by routine dark-field examination.

Culture, Third Series. Blood Drawn on the 3rd Day of the Disease, December 20, 1919.

The citrated blood of Case 2 drawn on December 20 was introduced into five culture tubes and incubated for 5 days at 26°C. Tubes 1, 2, and 5 were free from any contamination, while Nos. 3 and 4 showed a fungus growth. A search for the leptospira by dark-field examination showed occasional immotile leptospiras in the contents from Tube 4, but was unsuccessful in the case of the other tubes.

The uppermost layer of culture medium from Tubes 1 and 2 was taken out and the two were mixed, as was done also with Tubes 3, 4, and 5. The two mixtures were inoculated into corresponding groups of guinea pigs.

Guinea Pigs 46 and 47.—Inoculated with 1 cc. of the mixture of Culture Tubes 1 and 2. Both animals had a suggestive temperature curve. On the 13th day both guinea pigs were killed for examination.

Autopsy.—Findings similar, both animals having old hemorrhagic foci in the lungs, congested stomach and kidneys, and normal spleen. The liver was slightly yellowish in Guinea Pig 46.

There was perhaps an abortive infection in both these animals.

Three guinea pigs (Nos. 48, 49, and 50) were inoculated each with 1 cc. of the mixture of Culture Tubes 3, 4, and 5. The results were highly suggestive of an abortive leptospira infection with Guinea Pigs 48 and 49, and quite definite with Guinea Pig 50, in which jaundice was also present. From Guinea Pig 48 a positive transfer to another guinea pig was also accomplished.

Guinea Pig 48.—Typical temperature rise on the 5th day, reaching 103.8° F. a.m. and 103.4° p.m. The temperature remained normal on the 6th day and continued so until the 9th day, when the animal was killed for examination and transfer.

Autopsy.—The lesions found were small, discrete hemorrhagic foci in the lungs, swelling and congestion of the kidneys, and congestion of the stomach mucosa. The other organs appeared to be normal.

Transfers were made with the citrated blood from the heart of Guinea Pig 48 into two guinea pigs and with a mixture of the liver and kidney emulsions into four guinea pigs. One of the animals inoculated with the blood showed a temperature of 102.8°F. in the afternoon of the 8th day and 103° in the morning of the 9th day, but soon returned to normal. When it was killed for examination on the 12th day there were a few hemorrhagic patches in the lungs, the liver was somewhat yellowish, and the kidneys were congested.

Transfer with the organ emulsions from Guinea Pig 48 gave a more decided picture of the leptospira infection in some but no reaction in others.

Guinea Pig 98.—Temperature of 102.8° on the 5th day, 102.5° a.m. and 103.2° p.m. on the 6th, 103° a.m. and 102° p.m. on the 7th. On the 9th the temperature became normal. The animal was killed on the 12th day for examination.

Autopsy.—The lungs showed many recent hemorrhagic patches, the mucosa of the stomach several old hemorrhagic foci. The kidneys were pale; the spleen was normal.

Culture, Fourth Series. Blood Drawn on the 5th Day of the Disease, December 22, 1919.

Only four tubes were made with the citrated blood drawn on December 22 from Case 2, and after 9 days only two tubes appeared to be free from bacterial contamination. These tubes also contained a slight fungus growth which did not change the appearance of the

medium to any marked extent. Under the dark-field microscope two undoubted immotile leptospiras were found in the mixture of the contents of the two tubes. 1 cc. of the mixture was also inoculated into each of four guinea pigs.

The results of this series of inoculations were unsatisfactory, owing to a secondary infection in all the guinea pigs.

Culture, Fifth Series. Blood Drawn on the 7th Day of the Disease, December 24, 1919.

Six culture tubes were inoculated with the citrated blood of this date. Unfortunately the tubes were covered with an accidentally contaminated paraffin oil, and within a few days incubation at 28°C. all of them contained a profuse growth of fungus.

SUMMARY.

Injections into guinea pigs of the blood and the emulsions of liver and kidney obtained at autopsy from a fatal case of yellow fever in Merida induced in some of these animals, after a period of several days incubation, a rise of temperature which lasted 1, 2, or more days. When killed for examination at this febrile stage the animals invariably showed hemorrhagic areas of various size, sometimes few and sometimes numerous, in the lungs, and also, though less constantly, in the gastrointestinal mucosa, together with general hyperemia of the liver and kidneys. In a guinea pig (No. 6) inoculated with the liver emulsion of Case 1 there was a trace of jaundice on the 9th day. Injections of the blood or liver and kidney emulsions from such animals into normal guinea pigs reproduced the febrile reactions and the visceral lesions. The majority of the animals which were allowed to live and complete the course of the infection rapidly returned to normal (within several days). Examinations of these surviving guinea pigs after 2 weeks revealed the presence of rather old hemorrhagic foci in the lungs.

In the course of further attempts to transfer the passage strain, a secondary infection by a bacillus of the paratyphoid group caused many deaths among the guinea pigs and resulted finally in the loss of the strain from Case 1.

Most of the cultures made with the heart's blood taken at autopsy from Case 1 proved to be contaminated with a bacillus of the *coli* group. The contents of the apparently uncontaminated tubes were inoculated into guinea pigs, but the results were for the most part negative or vitiated by a secondary infection.

Dark-field search for the leptospira with the autopsy materials was negative, although prolonged and thorough examination was not practicable at the time of these experiments. Our efforts were concentrated on obtaining positive animal transmission rather than on the time-consuming demonstration of the leptospira, which when unsuccessful does not necessarily exclude the presence of the organism in small numbers. Likewise, the dark-field work with the material from guinea pigs was confined to a brief examination and was omitted in many instances. Under these circumstances no leptospira was encountered in any of the material from Case 1.

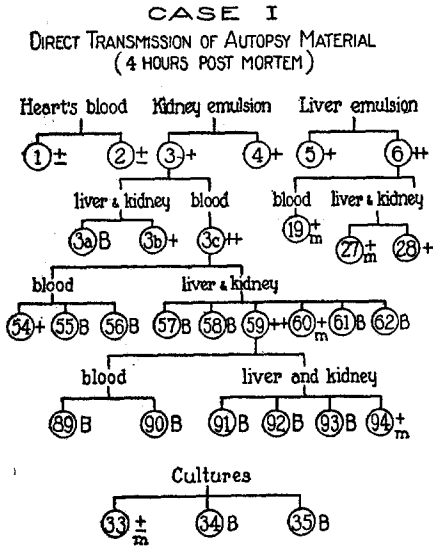
On the other hand, the results obtained with the specimens of blood from Case 2 were definitely positive, not only in the transmission of the disease directly, or indirectly by means of cultures, into guinea pigs, but also in the demonstration of the leptospira in the primary cultures and in the blood and organ emulsions of guinea pigs experimentally infected with such cultures.

Definite positive direct transmissions were obtained with the specimens of blood drawn on the 2nd and 3rd days. No blood was taken on the 4th or 6th days. There were indications of abortive or mild leptospira infection in the guinea pigs inoculated with the blood taken on the 5th day.

Regarding the inoculation of cultures from Case 2, it may be stated that only the cultures (leptospira +) made with the blood drawn on the 2nd day caused a definite fatal infection in guinea pigs. From this series a continuous passage in the guinea pig has been successfully accomplished. One of the guinea pigs (No. 48) inoculated with the culture 5 days old (leptospira +) made from the blood taken on the 3rd day presented typical symptoms, and a positive transfer from this to another animal (No. 98) was also made. Cultures of the blood drawn on the 5th and 7th days gave unsatisfactory results, owing to a secondary contamination. Leptospiras were detected in some of the culture tubes containing 2nd and 3rd day

specimens of blood from Case 2; they were few in number and for the most part immotile, owing perhaps to some unfavorable cultural condition such as a fungus contamination.

Charts 17, 18, and 19 give a summary of the experiments.



- + Temperature & lesions typical.
- ++ All symptoms, including jaundice & lesions.
- +_m Mixed infection.
- ± Doubtful leptospira infection.
- Negative result in regard to leptospira infection.
- ++_m Severe leptospira infection with all symptoms, but concomitant secondary infection.
- B Bacterial infection.
- L Leptospira.

Chart 17.

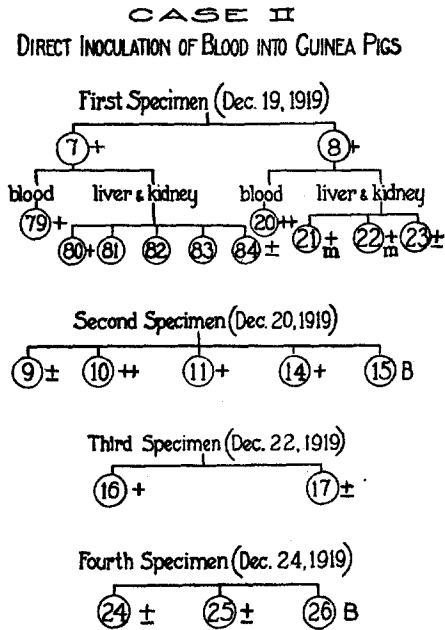
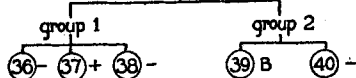


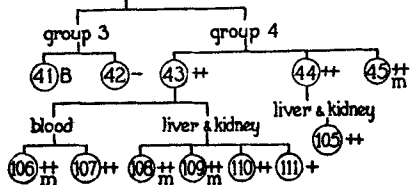
Chart 18.

CASE II
TRANSMISSION BY MEANS OF CULTURES FROM BLOOD

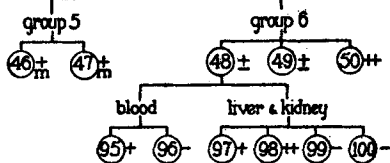
First Series (Dec. 18-25 = 7 days old) L?



Second Series (Dec. 19-25 = 6 days old) L +



Third Series (Dec. 20-25 = 5 days old) L +



Fourth Series (Dec. 22-31 = 9 days old) L + ? B



Chart 19.