EXPERIMENTAL STUDIES ON YELLOW FEVER IN NORTHERN PERU.

BY HIDEYO NOGUCHI, M.D., AND I. J. KLIGLER, Ph.D.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

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*Leptospira icteroides*, first isolated from certain cases of yellow fever in Guayaquil,¹ and later from a case of this disease in Merida,² had assumed such significance in the study of the etiology of yellow fever as to make further investigations advisable. Peru, which has had many visitations of yellow fever, was again invaded in June, 1919, in the province of Piura, the northernmost region, bordering on Ecuador. From this invasion a small epidemic arose which had not entirely disappeared in May, 1920. During the outbreak the following towns were affected: Sechura, Morropon, Tambogrande, Chulucanas, Piura (500 cases among the 10,000 inhabitants) in 1919, and Payta (108 cases among 3,000 inhabitants) in 1920. The mortality was estimated to have been about 10 per cent, which is considerably lower than was the case with yellow fever in Guayaquil and Merida, where it was about 50 per cent. An expedition to Peru was therefore undertaken.³ The present report deals with the results of bacteriological studies at Payta, Piura, and Morropon extending over a period of 3 months, March, April, and May, 1920.

When one of us reached Peru (March 1, 1920) Payta was the only town where the epidemic of yellow fever was still in progress; the last case occurred there on April 16. The first experiments were

³ This expedition was undertaken under the auspices of the International Health Board of The Rockefeller Foundation, and The Rockefeller Institute for Medical Research. We wish to thank the federal and local authorities in Peru for their courtesy and cooperation in this work.
carried out in Payta. In April an epidemic was reported at an inland town, Morropon, and a trip was made to that place to secure material for further studies to be carried out in Piura, where better laboratory facilities were available.

Studies in Payta.

Payta, a town of about 3,000 inhabitants, is the principal port in northern Peru. It consists of a cluster of bamboo huts on a strip of sandy shore. Rain is rare, and water is very scarce. The water supply comes from a river about 7 kilometers from the town, but the amount is hardly sufficient for ordinary daily needs.

A provisional laboratory was set up in Payta in a small bungalow consisting of three rooms. One room served as a laboratory, another as an animal room, and the third as a sleeping room. The laboratory supplies brought from The Rockefeller Institute had been resterilized in the laboratory of the Municipal Institute of Hygiene in Lima, as there were no facilities for steam or hot air sterilization at Payta. The rabbit serum used in the culture media had been brought from New York and as a result of the long voyage in a tropical climate a precipitate had appeared in it. The guinea pigs, also brought from New York, had suffered severely, and about two-thirds of them had succumbed within 2 weeks of their arrival in Payta. The feed for the guinea pigs was scarce, so that only the larger and hardier animals survived. This was unfortunate, as the larger animals are less suitable for initial inoculation; however, a certain number of native guinea pigs was procured. A very serious circumstance was the fact that, owing to the lack of electric current, the dark-field microscope could not be used. Moreover, the effort to obtain Giemsa preparations of the blood were unsuccessful because of the quality of the water. Since most yellow fever patients were treated by their physicians at their own homes it was not always easy to secure consent to obtain blood for inoculation, and it was practically impossible to obtain blood twice from the same patient. Finally, the cultures of *Leptospira icteroides* brought to Payta from Merida did not survive the journey.

Under the adverse conditions and the lack of laboratory facilities, the bacteriological work was confined to cultivation and animal
transmission with such samples of blood as could be obtained, the
object being to infect guinea pigs and to produce the characteristic
symptoms and lesions in these animals. In all, nine cases of yellow
fever were studied. Guinea pigs brought from New York were
inoculated from seven and native guinea pigs from two of the cases.

per 1,000 cc. Mar. 16. Delirious; black vomit; anuria. Mar. 17. Died.
Mar. 12 (3rd day of illness). Blood taken and cultures made (nine tubes).
Mar. 16. 1 cc. of citrated blood (kept in the ice box for 4 days) was inoculated
intraperitoneally into Guinea Pig 1 and 2 cc. into Guinea Pig 2. The culture
tubes, which had stood at room temperature for 4 days, appeared free from con-
tamination, and three guinea pigs were inoculated with material from Tubes 1 to
4 and three with material from Tubes 5 to 9. Some of the culture material was
left standing until Mar. 23, when it was inoculated into three guinea pigs.
The two guinea pigs inoculated with blood 4 days old showed no fever. A few
old hemorrhagic areas were found in the lungs of Guinea Pig 2 when it was killed
on the 15th day. Of six guinea pigs inoculated with culture material 4 days old,
three had fever on the 6th day and showed petechial lung hemorrhages when
killed on the 15th day. The three remaining animals either suffered from an
intercurrent infection (pneumonia) or escaped any obvious infection.
Of the three guinea pigs inoculated with the 11 day culture material, one
(Guinea Pig 43) showed petechial hemorrhages in the lungs when killed on the
15th day. The other two showed on autopsy no leptospira lesions but enlarged
spleen and pulmonary congestion, which were taken as evidence of secondary
infection.

The findings described show that in no instance was a fatal infection
by Leptospira icteroides induced, but they raise the question whether
the characteristic hemorrhagic areas in the lungs in Guinea Pigs
2, 3, 5, 8, and 43 did not indicate a mild infection with this organism.

Case 2 (Moderate; Recovered).—C. Onset Mar. 13, 1920. Moderate case;
Mar. 14 (2nd-day of illness). Blood taken and used wholly for making cul-
tures. Three guinea pigs were injected with a 3 day culture, three with a 4 day
culture, and three with a 9 day culture.
Of this series almost all, except Guinea Pig 14, which received a 3 day culture,
and No. 19, which received a 4 day culture, died of intercurrent infections (pneu-
monia, paratyphoid, cocci), while two remained well. Guinea Pig 14 showed
on the 5th day a temperature of 39.7°C., and on the 6th 39.6°C., while at
autopsy the lungs showed several hemorrhagic areas. Guinea Pig 19 showed on.
the 7th day a temperature of 39.5° and on the 8th and 9th days 39.8°. It was killed on the 10th day for examination and transfers. Few small petechiae in the lungs and minute points of hemorrhage in the right kidney. The blood and organ emulsions from this animal were inoculated on Mar. 28 into two guinea pigs, both of which soon returned to normal.

The experiments on Case 2 are suggestive and lead to the tentative conclusion that in two at least of the nine guinea pigs inoculated with culture materials prepared with blood drawn on the 2nd day a mild infection with *Leptospira icteroides* was induced. It is possible that Guinea Pig 19, if allowed to live longer, might have developed a typical form of the *icteroides* infection, as, when killed on the 10th day, definite lesions were present in the lungs and kidney. The failure to transfer the infection from this animal into two others is not conclusive, as in the early transfers a larger number of guinea pigs should be employed, because of the resistance to infection which certain guinea pigs usually exhibit.

Case 3 (recovered) gave similar results with blood drawn on the 3rd day of illness.

Two guinea pigs were inoculated with 2 cc. of blood from Case 4 (fatal; blood drawn on 2nd day of illness) soon after it was drawn, and two with the same amount of blood from Case 5 (recovered; blood drawn on 3rd day of illness). Cultures made with blood from each of these patients were left at room temperature for 4, 7, and 10 days and then inoculated into eight guinea pigs (Mar. 19, 22, and 26). Some of the animals inoculated with material from Cases 4 and 5 had definite febrile reactions and showed at autopsy lung lesions suggestive of a mild leptospira infection, but there was no fatal infection with typical jaundice.

With Case 6 (recovered; blood drawn on Mar. 19, 2nd day of disease) and Case 7 (recovered; blood drawn on Mar. 20, 3rd day of disease), the blood was drawn into citrate serum agar mixed in equal parts, and 3 cc. of the mixture were inoculated into each of two guinea pigs. Cultures made in the usual way were allowed to stand at room temperature for 3 to 7 days, and two sets of six guinea pigs were inoculated with this material. The results with Cases 6 and 7, both with blood and cultures, were unsatisfactory. The majority of the guinea pigs showed irregular febrile reactions, and from these animals, owing to the scarcity of guinea pigs, no transfers were made. Some of them, when killed later, were found to have hemorrhagic areas in the lungs, some showed indications of secondary infection (enlarged spleen), while others showed no lesions. In no instance was there a typical fatal leptospira infection.

Two more cases were studied before the epidemic in Payta subsided, Case 8 (recovered; blood drawn on 3rd day of illness, Mar. 29) and Case 9 (recovered;
blood drawn on Apr. 1, 2nd day of illness). In view of the failure to secure a definite transmission with fatal outcome with the larger guinea pigs still alive from the lot brought from New York, we decided to test native guinea pigs. For this purpose six native and two American guinea pigs, weighing 600 gm., were inoculated with a 7 day culture of the blood of Case 8 and five native guinea pigs with the 10 day culture from Case 9. None of the animals developed a typical fatal leptospira infection, although some undoubtedly had a mild infection, since lung lesions were found at autopsy and in two instances there was a suggestion of jaundice. It is interesting to note here that later experiments demonstrated that the native guinea pigs possess a greater resistance to the icteroides infection than the domesticated variety brought from the United States.

It is obvious that in the transmission experiments just described as having been carried out at Payta, no typical instance of fatal infection with Leptospira icteroides was obtained, and in no instance was the leptospira observed under the microscope. As the dark-field microscope was not available and no proper Giemsa staining could be secured, the latter circumstance is without value.

On the other hand, certain positive results were obtained in inoculated guinea pigs which led to the belief that a mild form of Leptospira icteroides infection had in some instances been induced; i.e., rise of temperature after the period of incubation common in this infection (3 to 5 days) and at autopsy definite hemorrhagic areas in the lungs and in one instance in both lungs and kidney, with occasionally a suggestion of jaundice. The failures to obtain more pronounced results are not difficult to account for. As stated above, almost all the guinea pigs of the most favorable age and weight shipped from New York succumbed en route or soon after arriving at Payta. Those remaining were so few in number that they were used sparingly; hence fewer were injected with given samples of blood or cultures than would ordinarily have been employed. The rabbit serum which is essential to successful cultivation of the leptospira had undergone changes with the formation of a precipitate, and the reaction became so alkaline as to prevent a growth of Leptospira icteroides to any extent. And yet a degree of success, which was confirmed by subsequent results, was, we believe, achieved.
Studies in Piura.

The epidemic having subsided in Payta, the laboratory was removed to Piura, where sterilizing facilities and adequate water were available. A laboratory was set up, through the cooperation of the government, in the Belan Hospital. A detached building was used for animal quarters and feed was also more plentiful.

By the time the small laboratory had been started cases of yellow fever were reported in Morropon, a small town of 2,000 inhabitants in the foothills of the Andes. The distance from Piura was about 65 miles, a desert separating the two places. Arrangements were made at once to investigate the cases. As the journey was made on horseback it was obviously out of the question to transport experimental animals, etc., by this means through a tropical region; hence it was decided to rely entirely on cultures. Fresh rabbit serum was obtained from local rabbits, and in order to guard against adverse changes in the culture media the component parts, consisting of serum, and 0.3 per cent semisolid agar, were carried separately.

The cultures were made by drawing the blood from an arm vein of the patients directly into the tube of semisolid agar, the rabbit serum then being added in a proportion of 1:5. The whole was thoroughly mixed and covered with a layer of liquid paraffin, and the tubes were carefully capped with tin-foil and carried back to Piura.

On arrival at Morropon it was ascertained that cases of yellow fever had been occurring for some time, and the epidemic was regarded as declining; however, by making house to house visits several cases diagnosed as yellow fever by Dr. Caballero were found on April 23. Between April 24 and 27 cultures with the blood were made from six cases, one of which proved later not to have been yellow fever. The remaining five cases pursued a clinical course which left no doubt as to their yellow fever nature. Because of the illness of one member of the party, the work at Morropon was suspended on April 28.

The journey on horseback from Piura to Morropon usually takes 1½ days. Our party consisted of Dr. Enrico Caballero, the government expert stationed at Catacaos, who showed us every courtesy, Mr. John Mitchell, a sanitary engineer, and Dr. Kligler. The expedition started from Piura on April 21 and arrived at its destination on April 22.
The party returned with the cultures to Piura, reaching there on May 3. At the same time the stock of guinea pigs had been renewed, 300 young, healthy animals having been brought from The Rockefeller Institute. Moreover, because of the lack of electric lighting facilities which had made it impossible to use the dark-field microscope, a storage battery suited to that instrument had also been brought from New York.

Very few of the tubes showed contamination, the blood still appearing bright red in the upper zone of the media. The cultures were inoculated into guinea pigs on May 6, or 9 to 12 days after they had been set up in Morropon. The inoculation procedure was identical with that employed in Merida, the upper portions of several selected tubes of culture from a case being pooled and the mixture inoculated intraperitoneally into six young normal guinea pigs.

Dark-field examination of the culture tubes undertaken the next day (May 7, 10 to 13 days after they were made) revealed the presence of active leptospiras in the cultures from three of the five cases. They were few in number and required careful search in some instances. In some tubes no leptospira was detected. As the details of the experiments show, the inoculation of cultures from four of the five cases induced typical fatal infections in some animals, other animals showing only a mild infection or escaping infection altogether.

Case 10 (Severe; Recovered).—C. M., male, age 16 years; born in Salitrá; resident of Morropon. Onset Apr. 22, 1920, 7 p.m. Headache; backache; pains in muscles; nausea; no vomiting. Apr. 23. Temperature 39.6°C.; pulse 106. Apr. 24. Temperature 39.9°C.; pulse 100; albumin +. Apr. 25. Temperature 39°; pulse 90; albumin + +; nausea. Apr. 26. Temperature 37.4°; pulse 90; albumin + +; black vomit. Apr. 27. Temperature 37.6°; pulse 78; albumin + +; epigastric pain; epistaxis; urine increasing towards normal amount; jaundice. Apr. 28. Temperature 37°; pulse 76; recovering.

Blood was taken on the 2nd day of illness at 11 a.m. Cultures examined after 12 days contained living leptospiras. Six guinea pigs (Nos. 13 to 18) were inoculated with material from Tubes 1 and 2 on May 6, with positive transmission in all.

Guinea Pig 13.—Temperature 39.2° on the 5th day. Died on the 6th day. Autopsy.—Epistaxis; subcutaneous petechiae; marked hemorrhages in lungs and gastric mucosa; jaundice slight.

The emulsions of kidney and liver were inoculated into three guinea pigs (Nos. 39 (Chart 1), 40, and 41), all of which developed typical fatal infection, dying
Guinea Pig 14.—Killed, when moribund, to obtain infective material for therapeutic experiments to be described in the following paper.\(^6\)

Autopsy.—Numerous petechiae in lungs; hemorrhage and blood in stomach; liver slightly degenerated; kidneys congested; spleen normal.

Guinea Pig 15.—Killed for transfer 4 days after inoculation, at first rise of temperature to 39.4\(^\circ\)C.

Autopsy.—No lesions were noted, but all three of the guinea pigs (Nos. 36, 37, and 38) inoculated with blood and emulsions of liver and kidneys succumbed with typical infection.

Guinea Pigs 16 and 18.—Developed characteristic infection. When jaundice appeared they were utilized for testing the curative effect of the anti-icteroides immune serum brought from The Rockefeller Institute, as will be described in the following paper.\(^5\)

With this case leptospiras were found in the initial culture, with which a typical infection was induced in guinea pigs, and further transfer from animal to animal was accomplished. Pure cultures of the leptospira were in turn recovered from the infected guinea pigs.

Case I1 (Mild; Recovered).—O. V., male, age 18 years; native of Morropon. Onset in afternoon of Apr. 21, 1920. Chills; headache; backache; fever. First seen morning of Apr. 24. Temperature 40\(^\circ\)C.; pulse 100; albumin +. Apr. 25. Temperature 38.5\(^\circ\); pulse 70; albumin ++; nausea, but no vomiting. Apr. 26. Temperature 38.5\(^\circ\); pulse 66; albumin +++. Apr. 27. Temperature 37.2\(^\circ\); pulse 58; albumin ++; abundant urine. Apr. 29. Temperature 36.6\(^\circ\); pulse 50; mild jaundice; recovering.

Apr. 24 (3rd day of illness). Blood was drawn. The dark-field examination of cultures 13 days after they were made failed to reveal any leptospira, owing to accidental contamination of the tubes while they were being handled the previous day for animal inoculation.

May 6. Six guinea pigs (Nos. 25 to 30) inoculated with culture, then 12 days old. Three of these (Nos. 25, 26 (Chart 2), and 29) developed typical severe infections, while the remaining three showed no perceptible symptoms. When killed for examination, however, all showed some hemorrhagic areas in the lungs, indications of a mild infection. It is interesting to note the different results with the same culture material, due to variations in individual susceptibility of the guinea pig to \textit{Leptospira icteroides}. The symptoms and lesions in fatally infected animals were altogether typical and hence will not be described in detail except in unusual instances. Leptospiras were found in the organ emulsions and a culture was obtained from the blood.

Transfer from one of these animals was made into three guinea pigs, all of which succumbed in due time to typical fatal infection.

One of the guinea pigs (No. 25) was used, when near collapse, for testing the efficacy of the anti-icteroides serum. The animal recovered, having received 1 cc. of the serum.

Case 12 (Fatal).—P. C., male, age 28 years; native of mountainous region. Onset, Apr. 23, 1920, typical. Apr. 25. Seen for the first time; temperature 39.4°C.; pulse 102; albumin ++; epigastric pain; no vomit. Apr. 26. Temperature 34.8°; patient in state of collapse; bleeding from nose and gums; black vomit; jaundice. Apr. 27, 6 a.m. Died.

Blood was taken in the morning of the 3rd day of illness. Cultures contained living leptospiras when examined on May 7 (12 days old).

May 6. Six guinea pigs (Nos. 1 to 6) were inoculated with culture material. Of these, three (Nos. 2, 3, and 4) developed severe infections, one dying on the 7th, and one on the 8th day (Chart 3), and the third being killed for transfer on the 6th day, when it was intensely icteric. Three guinea pigs inoculated with blood and liver and kidney emulsions from this animal died with typical symptoms. Three of the six original guinea pigs showed no sign of infection (Nos. 1, 5, and 6), but examination after 12 days revealed hemorrhagic areas in the lungs and also, in one instance, in the suprarenal. Leptospiras were found in the blood and organs in some of the guinea pigs, and cultures were obtained from the blood. The outstanding feature of this strain was the predominance of jaundice which it produced in the animals.

Case 13 (Moderate; Recovered).—J. C., male, age 14 years; native of Morropon. Onset Apr. 23, 1920. Apr. 26. Seen for the first time; epistaxis; black vomit; melena; temperature 37°C.; pulse 80; albumin +++. Apr. 27. Temperature 36.6°; pulse 66; albumin +++. Apr. 28. Temperature 37°; pulse 80; albumin +++. Recovery.

Apr. 26 (4th day). Blood taken for cultures. Dark-field examination of cultures 11 days after they were made failed to reveal any leptospira. Six guinea pigs inoculated with the 10 day culture material from this case also yielded negative results.

Case 14 (Severe; Recovered).—F. N., female, age 25 years; native of Morropon. Apr. 25, 1920, 11 a.m. Onset. Apr. 26. Temperature 38.5°C.; pulse 114; albumin trace; headache; backache; muscular pain; face flushed; conjunctive congested. Apr. 27. Temperature in morning 39.2°; pulse 106; albumin +. 4 p.m. Temperature 39.5°; pulse 120; severe pain and weakness; nausea, but no vomiting. At request of patient 20 cc. of anti-icteroides serum brought from The Rockefeller Institute were injected intravenously. 7 p.m. Temperature 38.9°; pulse 100; stronger; pains relieved. Apr. 28. Afternoon temperature 38.2°; pulse 100; albumin +++; nausea; bilious vomit. Apr. 29. Temperature 38.2°; pulse 90; no nausea; albumin ++. Apr. 30. Temperature 37.9°; pulse 86; mild pharyngitis. May 1. Temperature 36.8°; pulse 80; albumin +; recovering.
Charts 1 to 4. Temperature curves of guinea pigs inoculated with material from yellow fever cases in Peru.
Apr. 27 (48 hours after onset). Blood taken in the morning. The cultures were examined on May 8 when 11 days old, and living leptospiras were found in one tube, apparently dead ones in another.

May 6. Six guinea pigs (Nos. 7 to 12) were inoculated with the 9 day culture material. A typical infection was induced in three, while the other three remained apparently well. One of the latter showed hemorrhagic areas in the lungs when examined after 12 days. The other two had no macroscopic lesions, indicating that they were both completely refractory to this strain.

Transfer was made from one of the positive animals into three guinea pigs, all of which died with typical infections. Leptospiras were demonstrated in varying number in the blood as well as in the emulsions of liver and kidneys, and pure cultures were obtained from the blood of these animals. Chart 4 shows the temperature curve of one of the guinea pigs (No. 45) infected with Strain 4.

In Case 14, as in Cases 10, 11, and 12, positive transmission to guinea pigs was obtained by means of culture material. The initial cultures usually contained living leptospiras. In the blood and liver or kidneys of the infected animals the leptospiras were demonstrated, and a pure culture of *Leptospira icteroides* was recovered from the blood.

*Identification of the Morropon Strains.*

Upon our return to The Rockefeller Institute, we proceeded with the identification of the strains of leptospira isolated from yellow fever cases in Morropon along the lines previously followed. The Pfeiffer phenomenon was determined, as well as the effects of immune serums upon the organism *in vitro*.

Rich cultures of leptospira strains (Nos. 1 and 2) were employed in these experiments. The serums used were monovalent immune serums prepared in rabbits with Guayaquil Strain 1 of *Leptospira icteroides*. For purposes of control American Strain 2 of *Leptospira icterohaemorrhagiae* was tested simultaneously. Moreover, a polyvalent immune serum prepared in a horse with several Guayaquil strains of *Leptospira icteroides* was also tested. The results obtained are given in Table I.

As Table I shows, the leptospira strains from Morropon gave positive Pfeiffer reactions with the immune serums prepared with the Guayaquil strains, but negative reactions with the *anti-icterohaemorrhagiae* serum. Likewise, with respect to their behavior towards
these serums \textit{in vitro} an indubitable specificity for the anti-\textit{icteroides} serum is evident. The slight reaction with the anti-\textit{icterohemorrhagiae} serum may be regarded as a group reaction among closely allied species. It is concluded, therefore, that the leptospiras isolated from Morropon and Guayaquil cases of yellow fever are of the same species.

\textbf{TABLE I.}

\textit{Identification of the Morropon Strains.}

<table>
<thead>
<tr>
<th>Mode of test</th>
<th>Strain No.</th>
<th>Anti-\textit{icteroides} serum (monovalent, rabbit)</th>
<th>Anti-\textit{icteroides} serum (polyvalent, horse)</th>
<th>Anti-\textit{icterohemorrhagiae} serum (monovalent, rabbit)</th>
<th>Controls without immune serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{In vitro; allowed to stand for 18 hrs.}</td>
<td>1</td>
<td>Complete disintegration (positive).</td>
<td>Complete disintegration (positive).</td>
<td>Complete agglutination and immobilization.</td>
<td>Very active (negative).</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Complete agglutination; some motile leptospiras.</td>
<td>Complete agglutination; some motile leptospiras.</td>
<td>Complete agglutination; few motile leptospiras.</td>
<td>Very active; no agglutination.</td>
</tr>
</tbody>
</table>

\textbf{DISCUSSION AND SUMMARY.}

Fourteen typical cases of yellow fever were studied in northern Peru during an epidemic occurring in 1920, nine in Payta in March and April, and five in Morropon and Piura in April and May. The method of investigation was similar to that previously employed, but as the laboratory facilities were very meager certain changes were required. Although in Payta the work was handicapped by the lack of electric light, the scarcity of water and animal food, the unsuitability of the guinea pigs for inoculation, and the changes in culture media due to age, the results obtained under these adverse conditions were by no means negative. While in no instance was there a typical
infection produced in animals, either by direct inoculation of blood or with culture materials, yet certain guinea pigs in each series showed temporary febrile reactions or definite hemorrhagic lesions of the lungs indicative of a mild leptospira infection. Direct search for *Leptospira icteroides* in the blood of patients or in culture materials was not made because the dark-field microscope could not be used.

Subsequently, at Piura, the laboratory facilities were vastly improved, the use of the dark-field microscope was made possible by means of a storage battery, and a fresh stock of young healthy guinea pigs was received from New York, and fresh rabbit serum obtained in Piura. In the study of the materials obtained from five cases of yellow fever in Morropon all these added facilities were taken advantage of, with the result that the outcome was positive and convincing. Cultures from the five cases were examined after 11, 12, and 13 days, and in those from three cases living leptospiras were found.

By inoculation into suitable guinea pigs of culture material from these five cases, irrespective of whether or not leptospiras were detected under the dark-field microscope, a typical *Leptospira icteroides* infection was produced from four of the five cases. In one of these no leptospira had been detected in the culture tubes. Thus one case only yielded negative results, in that no leptospiras were found under the dark-field microscope and the animal inoculation was negative.

The leptospira was demonstrated in the blood or organ emulsions of the infected guinea pigs, and further transmission of each strain to other guinea pigs was obtained and pure cultures were secured.

A few points of practical significance appeared in the course of the present investigation. One is the importance of using fresh rabbit serum for culture media. Old rabbit serum, whether in pure form or incorporated with agar, etc., which had been kept for several months in a tropical climate, proved to be unsatisfactory for obtaining growth of *Leptospira icteroides*. A second point of interest is the variation in susceptibility of guinea pigs to infection with *Leptospira icteroides*. In two of four series of positive animal inoculations with the Morropon culture materials only one-half of the guinea pigs inoculated with given materials developed typical symptoms. The other half either suffered from a transient mild infection, as evidenced by a few hemorrhagic foci in the lungs, or escaped infection altogether.
From these facts it is highly probable that the lung lesions and febrile reactions observed in certain guinea pigs inoculated with the Payta materials were due to a mild leptospira infection. In a comparative experiment the native guinea pigs procured in Payta were found to be more resistant to the leptospira infection than those recently brought from New York. In fact, only a small portion of the former succumbed to typical infection even when inoculated with a virulent strain of *Leptospira icteroides* obtained from the Morropon epidemic.

In conclusion it may be stated that of fourteen cases of yellow fever studied in Peru, a typical leptospira infection, together with the demonstration of the organism in experimentally infected guinea pigs, was obtained in four, while in the majority of instances indications of a mild, non-fatal leptospira infection were observed. In a few cases only were the results entirely negative.

The leptospira isolated from Morropon cases of yellow fever, which is morphologically and culturally identical with the Guayaquil and Merida strains of *Leptospira icteroides*, was also shown by immunity test to be indistinguishable from the Guayaquil organism.