ETIOLOGY OF YELLOW FEVER.

III. Symptomatology and Pathological Findings in Animals Experimentally Infected.

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Plates 36 to 38.

(Received for publication, March 26, 1919.)

Mention has already been made[1] of successfully inducing in guinea pigs symptoms and pathological conditions simulating those observed in yellow fever patients in Guayaquil by inoculating these animals with the blood or organ emulsions from yellow fever patients. It has also been stated[1] that in the blood and organ emulsions of the infected guinea pigs an organism belonging to the genus *Leptospira* has been demonstrated and that the organism, after having been obtained in culture, is capable of inducing the same symptoms and pathological changes in these animals as does the original blood of yellow fever patients. In this paper the mode and course of this infection as observed in guinea pigs, dogs, and monkeys will be described.

Mode of Experimental Infection.

Infection with this organism may be induced either by injection into the peritoneal cavity, the blood circulation, or the subcutaneous tissues, or by application to the scarified, depilated surface of the skin or to the mucous membranes, or by feeding the animal with infected tissue or culture.

Experimental Infection in Guinea Pigs (Text-Fig. 1, a to f).

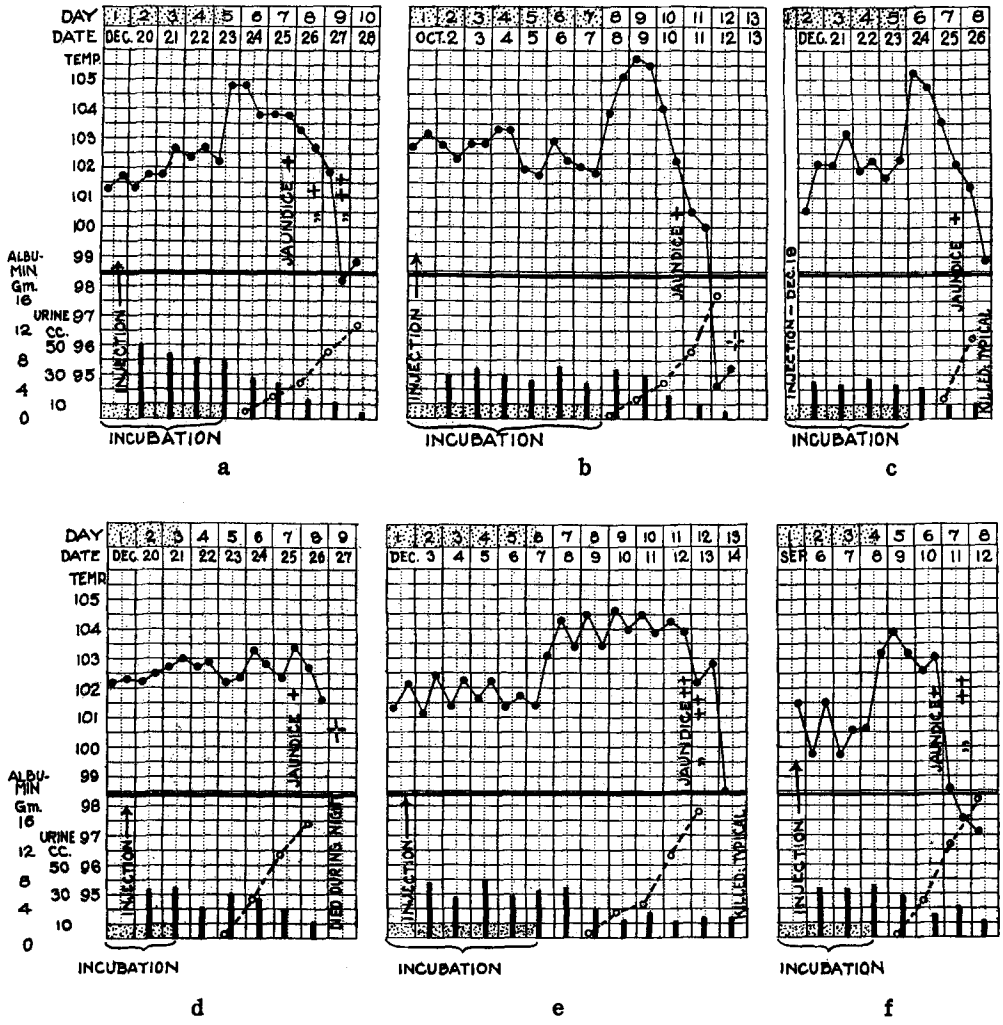
Incubation Period.—The incubation period varies according to the mode of infection and the quantity of virus introduced. When a large

[1] Noguchi, H., Etiology of yellow fever. II. Transmission experiments on yellow fever, *J. Exp. Med.*, 1919, xxix, 565.

amount is inoculated intraperitoneally or into the circulation the first symptoms—inactivity, anorexia, hyperleucocytosis—make their appearance after 48 hours, followed by a rise in temperature and slight albuminuria within the next 24 hours. With subcutaneous inoculation the symptoms do not appear until the 4th (72 hours) or 5th (96 hours) day, and with a very small amount of the virus a few days later. The percutaneous and *per os* modes of infection require a period of about 6 and sometimes as many as 12 days of incubation.

Onset and Course.—The onset is indicated by loss of appetite, inactivity, injection of the bulbar conjunctivæ, and a sudden rise in temperature ranging from 39.8–40.5°C. and rarely 41°C. The animal offers little resistance to handling, plaintive cries indicating intense muscular pains. The urine diminishes in quantity from an average daily output of about 25 cc. to almost half that, and its color changes from a pale straw tinge to dark brownish yellow. It now contains a moderate amount of albumin, with some epithelial cells and granular casts. At this stage no bile pigment can be demonstrated in the blood serum and no jaundice in the scleras or other parts of the body. The leucocytes are increased during the 1st day, but leucopenia follows within a few days, the differential count showing a marked increase of polymorphonuclear leucocytes.[2] Within the next 24 hours the temperature shows a slight drop, only to return to 39.8–40°C. during the following day. The fever then subsides gradually until the temperature drops below the normal in 3 or 4 days more. In fatal cases it sometimes drops to 34°C. just before death. Although the temperature begins to fall on the 2nd day all the other symptoms are aggravated. The urine is reduced still further in quantity and contains an enormous amount of albumin, renal epithelia, granular and hyaline casts, and the bile pigment first appears. At the same time the scleras become deeper yellow and are intensely suffused. At this stage there is a trace of bile pigment in the serum. On the 3rd day icterus becomes quite distinct

[2] I am greatly indebted to Dr. Jorge Larrea, Director of the Laboratories of the Guayaquil Yellow Fever Hospital, for total leucocyte counts on some of the experimental animals. My thanks are also due to Dr. F. Rojas of the General Hospital in Guayaquil.

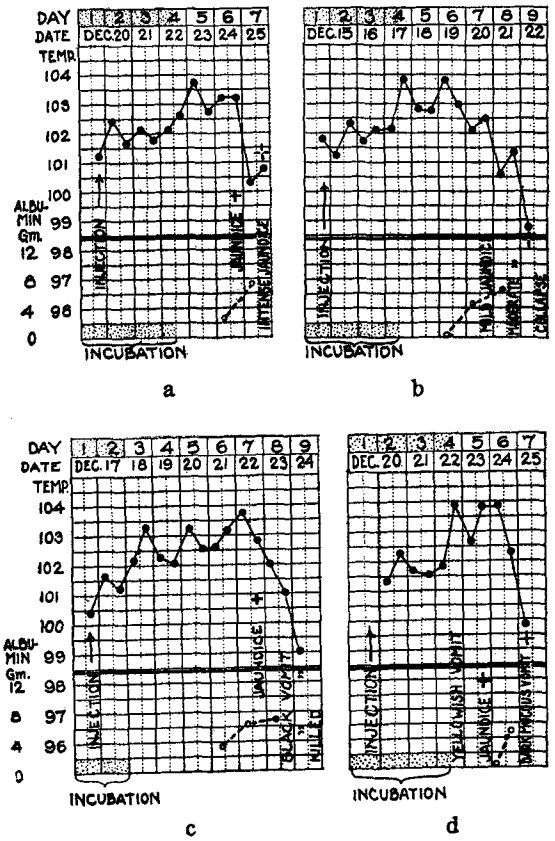


TEXT-FIG. 1, *a* to *f*. Observations on guinea pigs inoculated with the cultures of the different strains of *Leptospira icteroides*. (*a*) Guinea pig. Strain, Case 5. (*b*) Guinea pig. Strain, Case 5. (*c*) Guinea pig. Strain, Case 6. (*d*) Guinea pig. Strain, Case 5. (*e*) Guinea pig. Strain, Case 6. (*f*) Guinea pig. Strain, Case 1.

in the ears, scleras, palms, and soles. There may be ecchymoses in the scleras. Epistaxis and hematuria are frequent symptoms. The urine is now a deep grayish brown hue, and only a few cubic centimeters may be excreted. The animal still keeps on its feet but is quiet. On the 4th day the jaundice becomes general, and the serum taken is deep brownish yellow, as is also the urine. Erythrocytes, renal cells, albumin, and bile pigment are very abundant in the scanty urine which may still be passed, or there may be total anuria. Melena or bleeding from the rectum has been observed, and epistaxis is a frequent phenomenon in a dying animal (Fig. 13). Death occurs between the 5th and 7th days after onset of the disease and is accompanied either by clonic convulsions or by gradual asphyxiation, with air-hunger. The blood pressure becomes so low that about 2 days before death very little or almost no blood flows from the largest ear veins, even when they are completely cut across.

Non-Fatal and Abortive Infections.—Experimental yellow fever in the guinea pig is not always fatal. A certain proportion of the animals inoculated with a virulent strain of the virus show a temporary febrile reaction and albuminuria with few casts. Jaundice is slightly noticeable in the ears (if the animal is white or light in color) and scleras, but it is sometimes apparently absent. The fever and other symptoms set in as in fatal cases but completely pass away within a few days. In a week the animal has regained its previous health. That these reactions are mild or atypical symptoms of the same infection is evident from the fact that such animals cannot be reinfected, even with a quantity of the virus sufficient to kill control guinea pigs with the typical symptoms and lesions. Furthermore, these mild infections occur among guinea pigs inoculated with the same quantity of virus which produces a fatal infection among others in the same group, and especially do they occur frequently when the amount of the virus injected is rather small or the strain attenuated in virulence for the guinea pig. As in yellow fever in man, therefore, so in the experimental condition in guinea pigs, there apparently exist varying grades of severity of infection according to the individual resistance to the same virus. Many instances have been encountered, among many hundreds of guinea pigs purchased in Guayaquil, in which the animals showed a complete immunity to the

inoculation of the virus. It is possible that as some of them had been kept in houses or markets for varying periods of time before purchase they were rendered immune through a previous mild infection.



TEXT-FIG. 2, *a* to *d*. Observations on dogs inoculated with the cultures of the different strains of *Leptospira icteroides*. (*a*) Dog (pup). Strain, Case 6. (*b*) Dog (pup). Strain, Case 1. (*c*) Dog (pup). Strain, Case 5. (*d*) Dog (pup). Strain, Case 3.

Experimental Infection in Dogs (Text-Fig. 2, a to d).

Incubation Period.—This is practically the same as in guinea pigs.

Onset and Course.—The onset and course are also similar to those observed in guinea pigs, some dogs succumbing to the infection and

others recovering from a mild attack. In a fatal infection the temperature may reach 40°C., but usually it is not over 39.5°C., and lasts for 3 or 4 days, when it gradually drops by lysis. The skin then becomes yellowish and the conjunctivæ injected, and the animal refuses to eat. It often vomits bilious and sometimes blackish, mucous, frothy contents from the stomach. The amount of urine diminishes, with increasing albumin, casts, and bile pigments. Death occurs in coma or in clonic convulsions, the temperature falling below normal.

Experimental Infection in Monkeys (Text-Fig. 3, a to e).

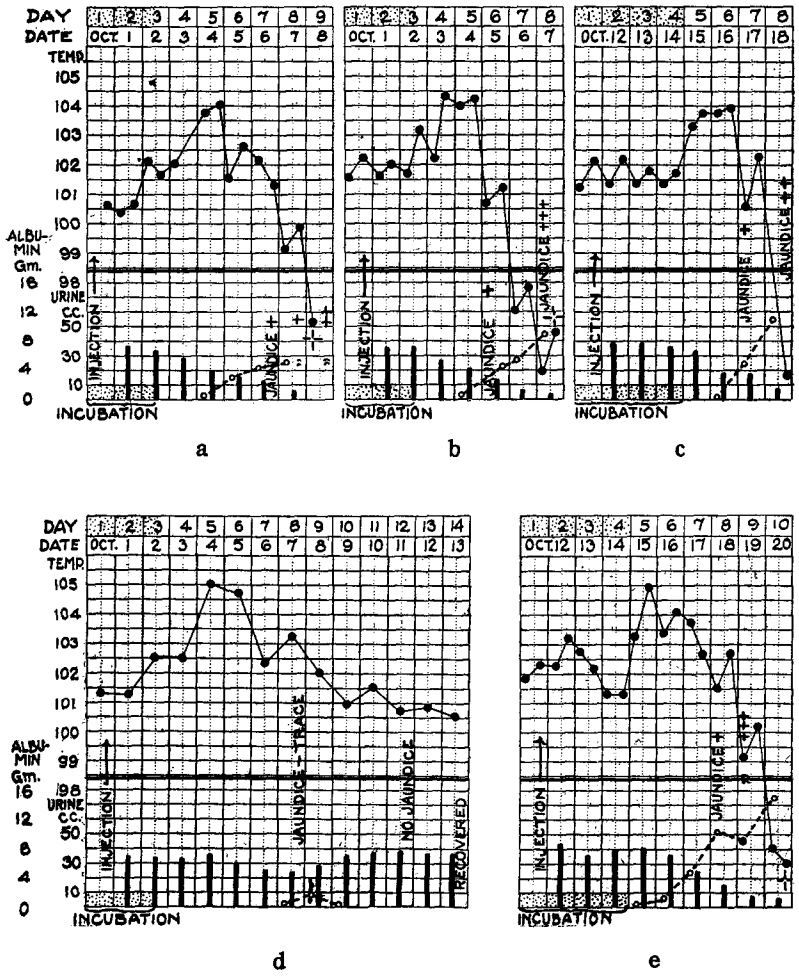
As stated elsewhere, the South American ringtail monkeys were found refractory to infection with the blood of yellow fever patients or the culture of the yellow fever leptospira.[1] With both a temporary febrile reaction was observed, but the animal invariably recovered. Five marmosets,[3] however, proved to be susceptible to the organism, four being fatally and one mildly infected.

The incubation period varied from 3 to 4 days, during which time the animals appeared well. The disease became noticeable by their inactivity, loss of appetite, non-resistance to handling, and slight greenish pigmentation of the urine. Albumin, casts, and bile pigments began to appear in the urine when the fever reached its height. Mild jaundice in the scleras, abdominal skin, and oral mucous membrane became noticeable a day or two after the fever began to subside. On the day of death the animals lay in a helpless position, offering only feeble resistance to handling. Death occurred during a condition of general weakness, coma, and sometimes convulsions. The temperature was usually subnormal, and the body of the animal more distinctly and generally yellowish.

Autopsy Findings in the Experimental Infection (Fig. 14).

The guinea pigs, dogs, and marmosets dying of the experimental infection invariably show a pronounced jaundice ranging from a light yellow to a deep bright yellow. Blood clots or stains are often

[3] Species *Midas œdipus* and *Midas geoffroyi.*



TEXT-FIG. 3, *a* to *e*. Observations on monkeys inoculated with the different strains of *Leptospira icteroides*. (*a*) Monkey 1. Strain, Case 1. Culture of second generation, 2 cc. intraperitoneally. (*b*) Monkey 2. Strain, Case 3. Culture of first generation, 4 cc. intraperitoneally. (*c*) Monkey 5. Strain, Case 6. Guinea pig kidney emulsion, 6 cc. intraperitoneally. (*d*) Monkey 3. Strain, Case 1. Culture of second generation, 4 cc. intraperitoneally. (*e*) Monkey 4. Strain, Case 5. Guinea pig liver emulsion, 6 cc. intraperitoneally.

present in the nostrils, mouth, anus, or vagina. Subcutaneous ecchymoses are frequently present in the guinea pig but seldom in the dog or marmoset. Postmortem rigor and lividity are marked. In guinea pigs the subcutaneous tissues are intensely jaundiced, and ecchymoses are seen in the axillary and inguinal regions. The abdominal muscles are often spotted with minute ecchymoses but sometimes these are absent. In the dog and marmoset the muscles show very slight or no hemorrhage.

The lungs of the guinea pig almost always show ecchymoses varying in extent from few and minute to numerous and large ones, roughly round, oval, or irregularly oblong or square, and with or without a sharp outline, vivid red, dark, or bluish red in color, sometimes light red with a darker center. Undoubtedly the color of the spots becomes darker and more bluish as they become older, since the longer the animal lives the darker are these ecchymoses. Postmortem hypostasis is marked. There may be some ecchymoses in the pleuræ. In the dog and marmoset the lungs are much less affected, only a few spots of hemorrhage being found, and the pleuræ are usually free.

There may be some dilation of the right heart. The pericardium is often studded with minute hemorrhagic spots. The fluid is clear and icteric. The muscle is friable and brownish yellow, and there are few or sometimes many ecchymoses on the surface. The endocardium and papillary muscles seem normal except for occasional punctiform ecchymoses. The valves are not altered but are yellowish in color, the aorta usually being deeply jaundiced. These changes apply equally to the guinea pig, dog, and marmoset.

The liver is usually slightly enlarged and varies in color from a yellowish brown-red to a bright yellowish brown. In instances in which death occurred within the first 3 days the degeneration was less advanced and the yellowish color not so pronounced. The surface is often mottled or striped with yellowish brown and brownish red, and the markings are very distinct.

The gall bladder is usually filled with a dark green or greenish yellow bile, and the wall is often spotted with minute ecchymoses.

The stomach usually contains some undigested food which is mixed with blood from the adjacent ecchymotic area of the mucosa. The contents are sometimes semifluid containing blackish blood re-

sembling coffee-grounds in appearance. In dogs and marmosets the greater quantity of mucus renders the appearance of the stomach contents indistinguishable from those found in human autopsies. The mucosa is somewhat hyperemic, and numerous ecchymoses are found, especially near the cardia. The serosa of dogs and marmosets is free of hemorrhages.

The small intestine and colon, including the rectum, are intensely injected, and numerous hemorrhages are found in the mucosa. The serosa is sometimes affected. The contents are blackish in color and may contain freshly escaped blood (melena). In the dog the character of the intestinal contents is a closer reproduction of what is observed in man, the serosa in this animal not showing the ecchymoses which are present in the guinea pig or any appreciable fluid in the peritoneal cavity. The nature of the findings in marmosets is about midway between that of guinea pigs and that of dogs.

In guinea pigs the kidneys are extensively involved. Hyperemia and punctiform hemorrhages in the parenchyma are almost constant. The ecchymoses, however, vary from a few to almost countless numbers in extreme instances. In dogs and marmosets there may be only a few minute spots. On section the cortex is broader than normally and highly hyperemic, with cloudy swelling. The medullary portion is succulent and hyperemic near the border. Bloody fluid or clot may be found in the pelvis, and sometimes numerous punctiform ecchymoses.

In the guinea pig hyperemia and hemorrhages were found in the suprarenal glands, but in dogs and marmosets only a comparatively slight degree of hyperemia. There were no changes in the pancreas or spleen, except a slight enlargement of the latter in rare instances.

The lymphatic glands show, in guinea pigs, general adenopathy, with occasional hemorrhages. In dogs and marmosets some glands only are enlarged and congested.

The bladder frequently contains bile-stained urine full of albumin, casts, and cells.

The testicles are apparently not affected, although in the guinea pig hemorrhages are frequent in the adipose tissue around them.

The ovaries are usually congested, and the uterus very much so, and in the pregnant state there were hemorrhages into the amnionic

fluid. The endometrium is congested, sometimes with a clot in the cavity.

No gross changes are observed in the nervous system. The membranes are hyperemic and the fluid is icteric.

Histological Examination (Figs. 1 to 12).

Lungs (Figs. 3 and 6).—The areas of hemorrhage are most abundant and extensive in the guinea pig. The alveoli in the hemorrhagic areas are completely filled with blood corpuscles, and in the adjacent zone a marked degree of edema is evident. There are also small accumulations of polymorphonuclear leucocytes and endothelial cells. In marmosets and dogs these changes are far less extensive. The leptospiras were demonstrated in the tissues by the Levaditi method.

Liver (Figs. 1 and 4).—The degree of degeneration of the liver cells is variable in different animals. In well marked instances the majority of the cells are swollen and necrotic. Vacuoles are found in some cells. The nuclei are swollen and degenerated. In some areas the liver cells are dissociated, swollen, and appear to have lost their sharp hexagonal outlines. The liver cells near the blood vessels seem to be less affected than those around the portal canal. Hemorrhagic foci of varying dimension are distributed irregularly. The endothelial cells of the bile ducts are increased in size, and there are some lymphoid cells around the portal canal zone. Mitotic figures are found. The organisms were found in the tissues stained by Noguchi's method (Figs. 7 and 10).

Kidneys (Figs. 2 and 5).—The epithelium of the convoluted tubules shows granular, swollen, and sometimes vacuolated cytoplasm. The cells may be detached from the membrane and fill up the lumen, which is distended with granular and hyaline casts. The glomeruli are considerably injected, and numerous hemorrhages are found throughout the cortex and medulla (Figs. 8, 9, 11 and 12).

Stomach.—There are superficial congestion of the mucosa and some hemorrhagic foci. In certain areas there is an accumulation of lymphoid and plasma cells.

Large and Small Intestines.—The intestines show injection and occasional hemorrhages.

Heart.—Certain fibers are swollen and contain vesicular nuclei.

Spleen.—There are hemorrhages, and the pulp is rich in blood.

Lymph Nodes.—These show phagocytosis and central degeneration of the follicles.

Adrenal.—In guinea pigs there are parenchymatous degeneration, congestion, and hemorrhages in some instances. These changes are less frequent in dogs and marmosets.

Pancreas.—Little change.

Nervous System.—Little change.

SUMMARY.

Studies are reported on the type of disease induced in guinea pigs, dogs, and monkeys by inoculating them (1) with the blood or organ emulsions of guinea pigs or other susceptible animals experimentally infected with *Leptospira icteroides*, and (2) with a pure culture of the organism. Particular attention has been given in these experiments to the clinical features of the experimental infection in the various animals and to the pathological changes resulting from the infection.

The symptoms and pathological lesions induced in guinea pigs are much more pronounced than those observed in dogs or marmosets. The period of incubation is nearly the same in all three species, 72 to 96 hours with intraperitoneal or subcutaneous inoculation, and a day or more longer when the infection is induced percutaneously or *per os*. The febrile reaction in the guinea pig and marmoset is about the same; in the dog there is less fever. The amount of albumin, casts, and bile pigments in the urine is more abundant in the guinea pig and marmoset than in the dog, and these animals also appear on the whole to become more intensely icteric. The black or bilious vomit, however, though occurring frequently in dogs during life, is observed in the guinea pig and marmoset at autopsy. The hemorrhagic diathesis is most pronounced in guinea pigs, less so in marmosets, and least in dogs. In dogs, for example, subcutaneous hemorrhages almost never occur, and the lungs usually show only a few minute ecchymoses. The pleuræ, pericardium, and other serous surfaces of the thorax and abdomen remain free from ecchymoses,

which, however, with hyperemia, are very marked along the gastro-intestinal tract.

The symptoms and lesions observed in animals experimentally infected with *Leptospira icteroides* closely parallel those of human yellow fever.

The pathological changes occurring in human cases of yellow fever are similar to those induced by inoculation in guinea pigs and marmosets and in respect to their intensity stand intermediate between those arising in the two animals mentioned.

EXPLANATION OF PLATES.

PLATE 36.

Sections of tissues from yellow fever cases, fixed in Zenker's fluid and stained with eosin and methylene blue. × 150.

FIG. 1. Section of the liver of a marmoset inoculated with the organism isolated from Patient E. Ch., Case 5, showing hemorrhage and necrosis, vacuolization, and dissociation of the liver cells.

FIG. 2. Section of the kidney from the same marmoset, showing necrosis, detachment, and some vacuolization of the renal epithelia. Some of the lumina are seen to contain granular casts. The glomeruli seem to be highly congested; hemorrhage in some portions of the tissue.

FIG. 3. Section of the lung from the same marmoset, showing hemorrhage in the tissue.

FIG. 4. Section of the liver from a guinea pig inoculated with the organism isolated from Patient M. G., Case 3. The liver cells are seen to have been largely disintegrated and replaced by red corpuscles and debris.

FIG. 5. Section from the kidney of same guinea pig, showing advanced degeneration of renal epithelia, with granular and hyaline casts in the lumen.

FIG. 6. Section of the lung from a guinea pig inoculated with the strain isolated from Patient A. Ce., Case 7. There is a considerable degree of hemorrhage and edema. Some leucocytic infiltration seems to be evident.

PLATE 37.

Sections from the liver and kidney of marmosets and guinea pigs inoculated with the strains of *Leptospira icteroides* derived from Cases 1, 3, and 5. Figs. 10 to 12 are stained by the method of Levaditi, Figs. 7 to 9 by the writer's modification of that method. × 1,000.

FIG. 7. Liver of marmoset, showing the organism between the hepatic cells; strain from Case 5.

FIG. 8. The same strain. There are two minute, delicate organisms in the lumen of a kidney tubule.

FIG. 9. The same.

FIG. 10. The organism in the guinea pig liver, in which it appears somewhat coarse.

FIGS. 11 and 12. The organism in the kidney; coarse and quite numerous.

PLATE 38.

FIG. 13. Epistaxis in a guinea pig inoculated with a culture of *Leptospira icteroides* isolated from Patient A. Ce., Case 6. The picture was taken at the moment of death.

FIG. 14. Autopsy of a guinea pig killed on the 6th day after inoculation with a culture of *Leptospira icteroides* isolated from Patient A. Ce., Case 6. The animal showed typical symptoms and was killed at the time of collapse. The picture shows the general jaundice of the skin and subcutaneous tissues and the yellowish liver. Hemorrhages are evident in the lungs, and can be seen to have taken place in the stomach, as shown by its dark color. The kidney is congested, and sometimes a few ecchymotic spots can be seen. The spleen appears to be normal. The intestines and also the muscles along the thorax, abdomen, and postperitoneal region are comparatively free from hemorrhage, presenting a striking contrast to the highly congested condition of these muscles in guinea pigs inoculated with the organism of infectious jaundice. The strikingly yellow color of the liver is far more intense than is usually observed in experimental infectious jaundice in the guinea pig.

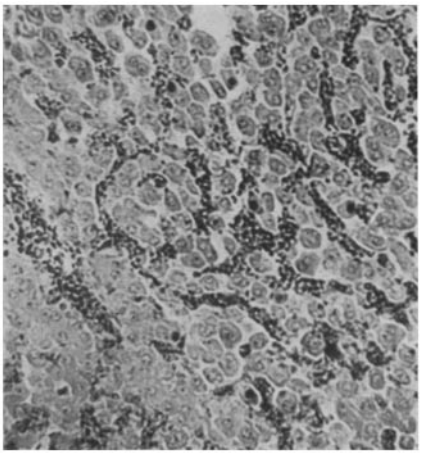
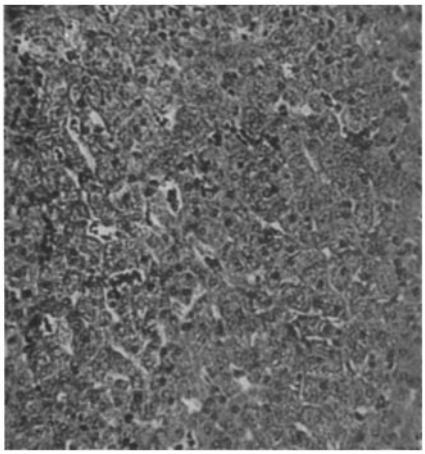


FIG. 1.



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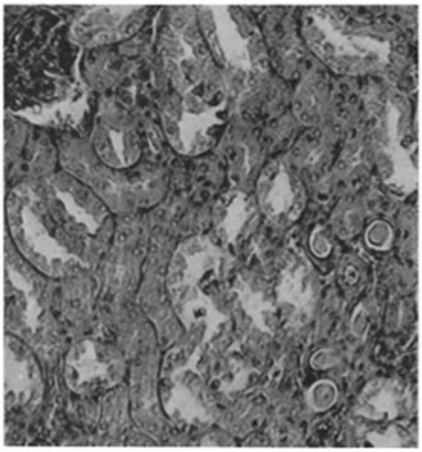

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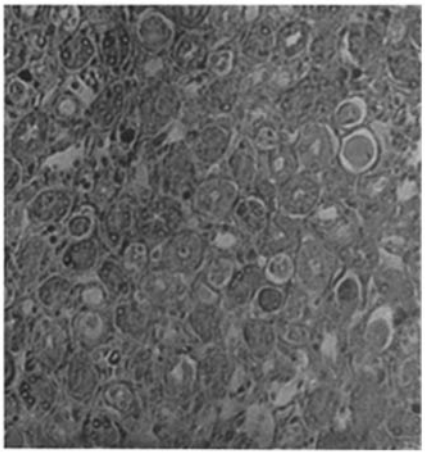

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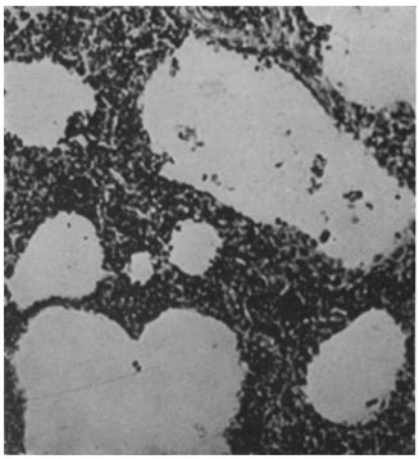

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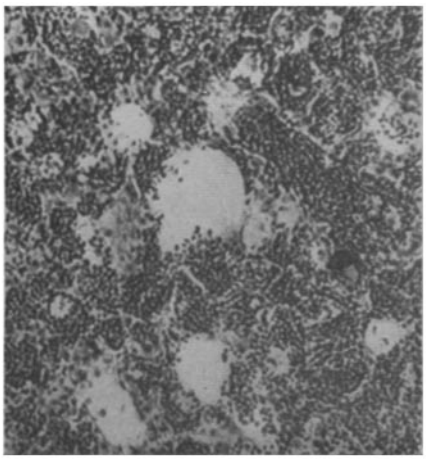
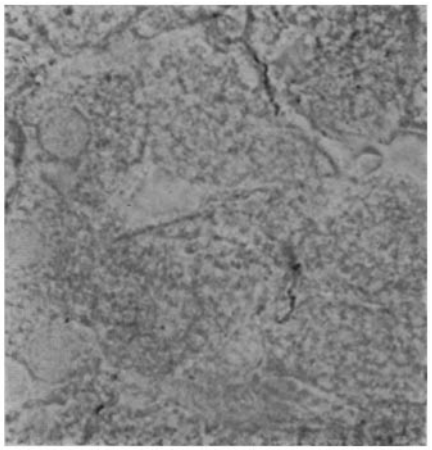


FIG. 6.

(Noguchi: Etiology of yellow fever. III.)



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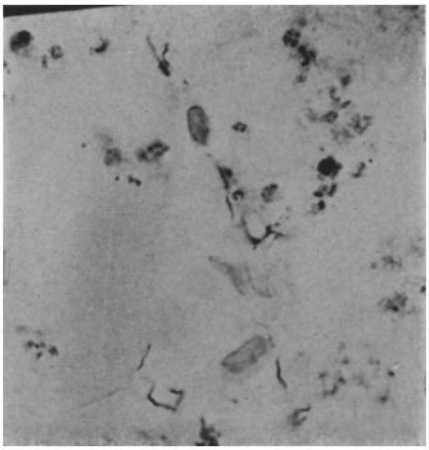
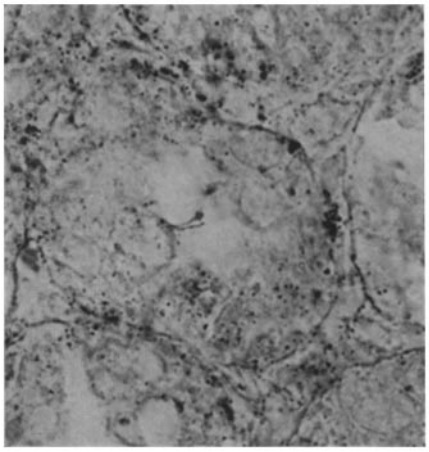


FIG. 10.



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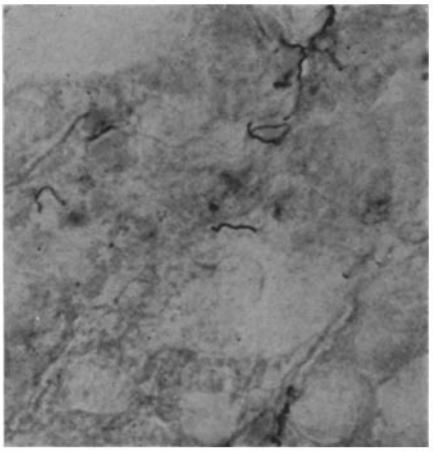

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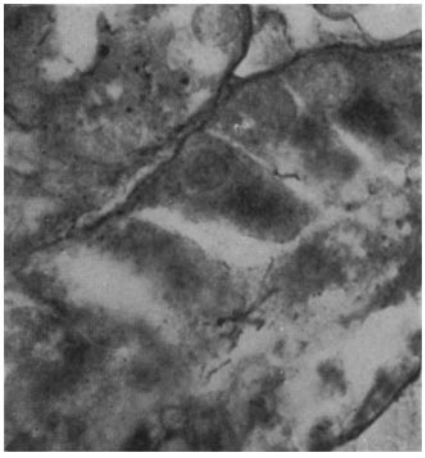

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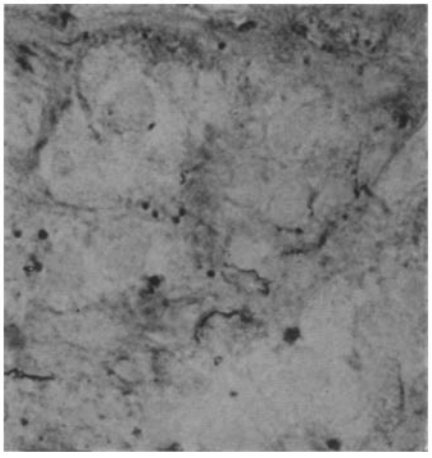

FIG. 12.

(Noguchi: Etiology of yellow fever. III.)

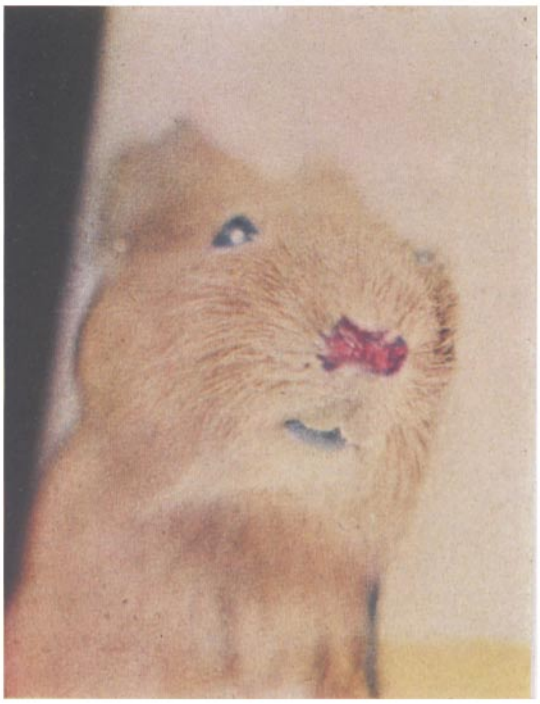

FIG. 13.

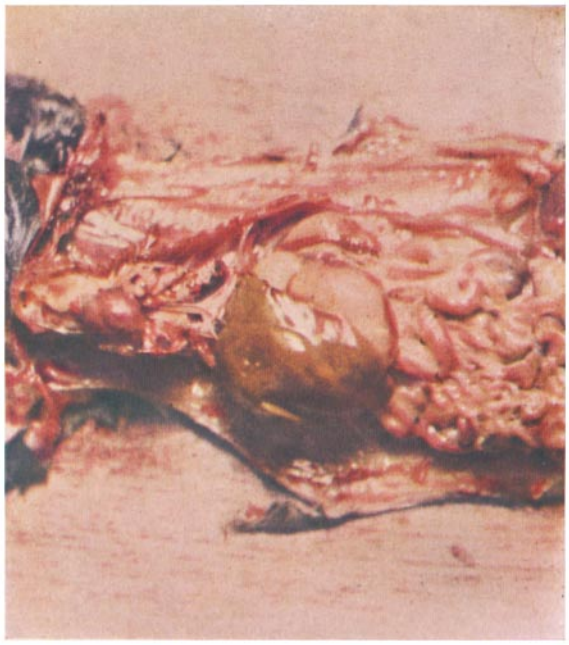

FIG. 14.

(Noguchi: Etiology of yellow fever. III.)