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Recent studies in leptospirosis

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FOREWORD

This thesis is primarily concerned with a review of the literature that has been compiled by the many investigators of leptospiral disease. The classical strain of leptospira, Leptospira icterohaemorrhagiae the cause of Weil's disease, seems to be the focal point in any study of leptospirosis for several reasons. It was the first leptospiral disease from which the etiological agent was isolated, and it is the most frequent agent that affects man in the United States of America and Western Europe. The proximity of heavily populated areas and rats (Rattus species) has aided the incidence and spread of the disease in such groups.

The causative agents of leptospirosis will be discussed from the aspect of their description, similarities and differences, nutritive requirements, pathogenicity, diagnosis, epidemiology and prophylaxis.
INTRODUCTION

Through the ages, a disease that has usually been associated with epidemics, and frequently described by such names as "typhus icterode", "yellow fever" and "acute atrophy of the liver" would now be called Weil's disease, or leptospirosis icterohaemorrhagiae.

The occurrence of the disease has been noted to be most frequent among young adults, particularly soldiers, butchers and sewer men. Buchanan (1927) refers to cases of jaundice with hyperpyrexia which were reported in 1886 by Mathieu, who considered that "infectious jaundice" was justified as a name for this disease in view of the fever, general body symptoms, splenomegaly and albuminuria. Sometime later in 1886, Weil published an account of four cases of infections with severe jaundice, in two of which febrile relapses were described. The disease (infectious jaundice) has been known since that time (1886) as Weil's disease.

Although Stimson (1907) noted a spirochaete, which he named *Spirochaeta interrogens*, in the autopsy kidney section of a human case that had supposedly succumbed to yellow fever, the credit for the first critical studies of the aetiology of Weil's disease goes to the Japanese workers. Inada, et al., published their monograph on Weil's disease in 1916, in which the detection of a spirochaete in the liver of a guinea-pig that had been injected with the blood of a patient suffering from Weil's disease was disclosed. The organism, which they named *Spirochaeta icterohaemorrhagiae*, reproduced the disease through fifty-one passages of guinea-pigs. Ido, et al., (1917) demonstrated that the
wild rat (*Mus decumanus*) was an asymptomatic host of the causative organism.

Noguchi (1918) renamed the causative agent of Weil's disease, *Leptospira icterohaemorrhagiae*, after careful morphological studies of the etiological agent and the spirochaete group as a whole.

The work in Japan had laid the groundwork for future studies of leptospirosis. Studies on leptospirosis during the next few decades demonstrated that infection with leptospirae took many and varied clinical forms as was shown by ensuing immunological and epidemiological studies.
THE LEPTOSPIRAE

A. Morphology:

The biological nomenclature of the leptospirae places them in the order Spirochaetales because of their motility, slender, flexuous body, and the presence of spirals. The family Treponemataceae claims this microorganism because of its lack of a chambered structure in its body, crista (a membranous ridge running longitudinally along its body), and its generally specialized habitat in the blood and tissues of vertebrate hosts. The species of Leptospira are placed in a genus of their own because of persistence of elementary spirals, lack of an axial filament, and resistance to 10% Saponin. The genera Borrelia and Treponema both possess an axial filament and are dissolved by the action of 10% Saponin.

The type species of the genus is Leptospira icterohaemorrhagiae which was described by Noguchi (1917) after isolating the organism from a wild rat (Rattus norvegicus) in New York City. The organism was so-named by Noguchi (1917) because of its resemblance to a fine coil; hence, the Greek name, Leptospira. Noguchi's organism has been proven to be immunologically, epidemiologically, and clinically identical to that isolated by Inada et al., (1916) in Japan, and Stokes, et al., (1917) in Flanders.

Leptospira icterohaemorrhagiae is a strikingly flexible, motile microorganism measuring seven to fourteen microns in length with exceptionally long forms measuring thirty to forty microns due to delayed transverse fission. The organism is finely coiled with regular spirals measuring 0.15 to 0.5 micron in amplitude, and 0.3 micron
in depth. There are one or more undulations throughout the entire length and the body culminates in finely-pointed ends. The lack of an axial filament, flagella, chambered structures, crista, and a membrane as shown by electron microphotography (Morton, 1945) justifies the generic classification of the organism.

A darkfield examination of the leptospira from a liquid culture medium exhibits the appearance of a fine tapering filament with close coils that resembles a rope. Their bodies may assume the forms of semicircles, figures of eight, and S-shapes. The reflection of light from each spiral of its body gives an appearance of a row of brightly shining dots, due to the refractive distances of each portion of its body. In semi-solid media the organisms appear serpentine, undulating or bent.

Ito et al., (1916) showed that the leptospira does not take the ordinary aniline dye stains, but can be demonstrated with Giemsa's stain by assuming a pinkish or pink-purple color. The appearance of the Giemsa stained organism differs from the living leptospira by obscuring the spirals somewhat so that the middle portion is heavier, and the ends taper off into fine points.

Fletcher (1927) states that the silver impregnation method of staining of Fontana makes the leptospira much easier to demonstrate than by darkfield examination. The former found this staining method most useful in kidney or liver emulsions where the leptospiroae are in marked contrast to the tissue parenchyma due to the deep silver stain on their bodies.

To date (1949) there have been four distinct species of
leptospirae classified. Bergey (1948, 6th Edition) classifies Leptospira icterohaemorrhagiae, Leptospira hebdomadis, Leptospira biflexa and Lepto-
spira canicola as separate species. However, the ability to distin-
guish one species from the other on morphological grounds alone is not possible. Resort must be made to more careful studies in the realms of immunology, epidemiology and pathogenesis.
1. Cultural Characteristics:

The leptospirae, like many of the other varieties of blood spirochaetes, require one or more of the constituents of the blood of suitable animals or humans for growth. Noguchi (1918) reported the first critical analysis of the conditions required for uniform success in the *in vitro* culturing of various strains of *Leptospira icterohaemorrhagiae* from Asiatic, European, and American sources. The basis nutrient is diluted animal serum in an isotonic salt solution, such as Ringer's solution, buffered to a pH range of 7.3 to 7.5.

It is of biological interest to note that the best growth is obtained from the sera of susceptible animals, i.e., guinea-pig and horse sera. The poorest growth is obtained from rat serum. The latter animal not only tolerates infection of pathogenic leptospirae, but becomes a healthy carrier of the disease.

Schuffner (1934) and Lewis (1942) state that guinea-pig serum is the nutrient of choice. Fletcher (1927) used rabbit serum with success. Chang (1947) used horse, rabbit, and guinea-pig sera for the cultivation of *Leptospira icterohaemorrhagiae* with equal success.

It has been repeatedly shown by the workers in the field that the growth factor or factors in the serum is contained in the thermolabile portions, since growth is greatly reduced when the serum is preheated at sixty degrees Centigrade for thirty minutes before inoculation with viable leptospirae. Preheating of the serum at one hundred degrees Centigrade for fifteen minutes destroys the nutrient value of the serum. Controls of unheated serum show rich growth of leptospirae.
That the leptospira is an obligatory aerobe was first shown by Noguchi (1918) when he found that any hindrance to the access of oxygen was unfavorable in obtaining a culture of the organism. Chang (1947) noted that growth of Leptospira icterohaemorrhagiae in tubes of his semi-solid medium was best shown in the zone of optimal oxygen availability.

Noguchi (1918) and Chang (1947) have both shown that the leptospirae (Leptospira icterohaemorrhagiae, Leptospira canicola and Leptospira biflexa) apparently do not possess enzyme systems that utilize carbohydrates. The reaction of cultures containing glucose, lactose, maltose, levulose, galactose, sucrose, dextrin, imulin, mannitol, dulcitol, isodulcitol, arabinose, raffinose, and salicin failed to indicate any difference in pH as compared to uninoculated controls of these same carbohydrates.

The temperature range of the pathogenic leptospirae is from ten degrees to thirty-seven degrees Centigrade. The optimum range from in vitro cultures is from thirty degrees to thirty-seven degrees Centigrade. Noguchi (1918) failed to obtain growth in cultures at forty-two degrees Centigrade.

The growth rate of pathogenic leptospirae in culture is determined by the density of the leptospiral population and the temperature. It can be shown that the survival time of leptospirae in culture is inversely proportional to the temperature of incubation. At higher temperatures the leptospirae increase their numbers rapidly, hence, use up the available nutrient materials rapidly and die off either from starvation or from the toxic action of metabolites formed in the culture.
The temperature choice for maintaining viable cultures in the laboratory is twenty-five degrees Centigrade. Chang (1947) has found that pathogenic leptospiroa can be maintained in culture for seven weeks or more at this temperature.

The growth curve of pathogenic leptospiroa is essentially the same as is seen in other pathogenic microorganisms. The pattern remains the same but the time in each phase is prolonged, (Chang, 1947). The initial phase or lag phase, wherein the organism is adapting itself to an adequate nutrient medium, requires an hour or so for an organism like Escherichia coli; whereas, this phase is prolonged to twenty-four hours in the case of the pathogenic leptospiroa. The accelerated growth phase, wherein the Escherichia coli reproduces itself to a peak in twenty-four hours, requires seven days for the leptospiroa. The stationary growth phase, wherein an organism like Escherichia coli has utilized its available nutrients and growth is at a standstill, requires only a number of hours, whereas, the leptospiroa require seven days. The phase of declining growth is prolonged to eight weeks in the leptospiroa. This knowledge of the growth phase in the case of pathogenic leptospiroa is useful in that these organisms may be maintained in culture for seven weeks or more. The low temperatures at which the leptospiroa remain viable and its prolonged growth phase is important from the epidemiological point of view in that pathogenic leptospiroae may be deposited by infected hosts and remain viable until an opportunity is afforded for the organisms to invade a new host.

The first requisite to the successful cultivation of pathogenic leptospiroa was shown by Noguchi (1918) to be the optimum reaction of the culture medium. A slight shift in the pH to the alkaline or to acid
range renders the medium unsuitable for growth. The fact that the sera of many animals is unsuitable for culture is easily demonstrated by titrating a given serum with 0.1 normal hydrochloric acid. Noguchi (1918) showed that two ml. of the sera of sheep, ox, pig and donkey, when mixed with three ml. of distilled water, required 0.4 ml. of 0.1 normal hydrochloric acid to reach a pH of 7.0, and 0.6 ml. to lower the pH to the point of precipitation of the proteins. Rabbit serum was shown to be less alkaline, i.e., only 0.2 ml. of 0.1 normal hydrochloric acid was required to bring the pH to 7.0, and 0.35 ml. to the acid range. The natural pH of horse serum was found to lay between that of rabbit serum and the sera of the sheep, ox, pig and donkey group. Hence, it seemed that cultivation of the leptospiroae could be successful only when it was possible to obtain a culture medium that could maintain the hydrogen ion concentration near a neutral reaction or slightly alkaline, i.e., pH 6.8 to 7.2.

Schuffner (1934) found the answer to the problem of maintenance of pH by utilizing his knowledge of the action of buffers by incorporating Sorensen's double phosphate buffer solution in his culture media. A buffer has the ability to absorb or offset any changes that may take place in the hydrogen ion concentration of liquids used in a culture medium.

The culture medium in use today is essentially the same as Noguchi's original medium with Schuffner's addition of a buffer. The media described and used by Ito et al. (1916), Fletcher (1927), and Molner et al. (1941) are all basically similar in that they all contain blood and isotonic salt solutions. Chang (1947) states that he obtained
a three or four times greater growth, at the growth peak, in his semi-solid medium (0.2 percent agar added) than with Noguchi's medium by adding 0.05 percent pulverized liver extract and 0.08 percent Bacto-Tryptose.

When growth is obtained in any of these liquid or semi-solid media the macroscopic appearance is that of a light haze in a narrow zone just below the surface of the medium. Loopfuls of this material when taken from the hazy layer and viewed under the darkfield microscope demonstrate the classical morphological picture of leptospiroae.

The cultural methods required for Leptospira biflexa, the non-pathogenic species of the genus, are far more simple than for their pathogenic cousins. This organism can be grown in distilled water with 0.1 percent potassium nitrate added. The best medium is ten percent rabbit serum in distilled water (Chang, 1947).

Metabolism:

The study of the metabolic activities of microorganisms is useful in that it often leads the way to methods of culture which allow for richer growths of organisms than are obtained by the addition of so-called nutrient materials without knowing just what factor (or factors) is actually needed. The metabolic requirements of microorganisms can be used by the epidemiologist in his correlating of the facts that lead to the institution of prophylactic methods. This is especially important in the treatment of soil or water where leptospiroae may be deposited by infected rats.

Noguchi (1918) has shown that the leptospiroae are unable to utilize at least fourteen of the usual carbohydrates that are attacked by many of the common pathogens. Chang (1947) failed to show any evi-
ence that either *Leptospira biflexa*, *Leptospira icterohaemorrhagiae*, or *Leptospira canicola* utilize any of the simple sugars.

Many of the workers have used haemoglobin in some form in their culture media, i.e., Inado (1915), Ito (1916), Fletcher (1927). However, Chang (1947) showed that a small amount of haemoglobin seemed to improve growth in the culturing of leptospiroae, the growth was greatly diminished when used without serum, and cannot be used to support growth indefinitely.

Rosenfield and Greene (1941), working on the assumption that the leptospiroae require a growth factor or factors that they are unable to synthesize themselves, tested a group of substances that were known to exhibit definite effects upon the development of other organisms. These substances were tested with known numbers of *Leptospira canicola* (the leptospiral pathogen of dogs). Nicotinic acid, thiamin hydrochloride, nicotinic acidamide, riboflavin, folic acid, and ascorbic acid were included in Schuffner's medium both with and without the presence of serum. Their results showed that no factor or combination of factors gave growth without the presence of animal serum. Both nicotinic acid and nicotinic acidamide gave marked stimulation to growth at a concentration of one gamma/ml., but growth was markedly decreased with greater concentrations. Folic acid showed no effect on the proliferation of the organisms. Riboflavin, in Schuffner's medium, caused death of all the organisms except in very low concentrations. The adverse effect of higher concentrations of these substances was perhaps due to the mechanism of hydrogen peroxide production whose breakdown by catalase is not provided for in the enzyme systems of the leptospira. Chang (1948) found that the various vitamins of the B-complex
and ascorbic acid failed to replace the animal serum in supporting growth of Leptospira canicola, Leptospira biflexa or Leptospira icterohaemorrhagiae.

Chang (1948) did a series of studies on the metabolism of virulent cultures of Leptospira icterohaemorrhagiae and found that the thermolabile factors of animal serum (guinea-pig, rabbit or horse serum) are required for growth and cannot be entirely replaced by glycerol, cytochrome-c or serum albumin. It was shown that iodoacetic acid (eighty micrograms per milliliter) completely inhibited growth, hence, concluded that leptospirae live chiefly, if not exclusively, on proteins. Iodoacetic acid apparently poisons the proteolytic enzyme systems of the leptospirae.

Thus, it would seem that the leptospirae possess enzyme systems that are only able to attack proteins. Haemoglobin can apparently be used as a temporary substitute, but the thermolabile fractions of animal serum are needed for continued growth. The common vitamins, especially nicotinic acid and nicotinic acid amide can act as accessory growth factors when used in small amounts. Greater concentrations of these substances are detrimental to growth because of harmful metabolites that are formed, i.e., hydrogen peroxide.
Antigenicity

The most enlightening studies of leptospirosis have been made with the aid of immunological tools, i.e., tests involving the phenomena of agglutination, precipitation, alexin fixation, and lysis.

The antibody-antigen reaction can be manifested in the form of agglutination, by adding a suspension of leptospirae (antigen) to dilutions of serum, containing specific antibodies, in 0.85 per cent saline solution. If the specific antibody or agglutinin is contained in the serum, an aggregation and precipitation of particulate antigen will become visible in the tubes.

Precipitation may be manifested by treating the antigen so that it becomes soluble (Schuffner, 1934), and combining this soluble antigen with its specific antibody will result in a visible precipitation.

Lysis may be demonstrated by first sensitizing a specific antibody with alexin (complement) and then adding the antigen to this mixture. The antigen-antibody reaction here results in dissolution of the bacterial cell.

By studying the antigenicity of the leptospirae, one is able to obtain much valuable information in regard to the differentiation of the species or strains within the genus. In general, it seems possible to separate the leptospirae into two distinct serological groups. The first group of serological strains or species may arbitrarily be called the cosmopolitan group since these organisms have been isolated from hosts all over the world. This group includes Leptospira icterohaemorrhagiae, Leptospira canicola, and Leptospira grippotyphosa.

The second group of serological strains may be called the Far East group.
since these organisms have been found to be present in that sector of the world. This group includes Leptospira hebdomadis, Leptospira autumnalis and Leptospira febrilis.

Schuffner (1934), Walch-Sorgdrager (1939), Kolachine (1945) all consider that Leptospira icterohaemorrhagiae and Leptospira canicola are distinctly different organisms, although there is a similarity of antigenic structure. This similarity of structure can be shown by agglutination tests. An agglutination of Leptospira icterohaemorrhagiae (antigen) with Leptospira canicola antiserum (antibody) can be shown to reach a titer of 1:3,000, and a titer of 1:30,000 can be reached when homologous antigens and antibodies of these organisms and reacted with one another. Hence, the distinction is based on the degree of agglutination. Leptospira grippotyphosa also shows the greatest agglutinating titer with its homologous antiserum and lesser agglutinating titers with the other two members of this group.

In the Far East group of pathogenic leptospirae Ido (1918) differentiated Leptospira hebdomadis from Leptospira icterohaemorrhagiae by cross-protection tests, and by in vivo lysis tests (Pfeiffer phenomenon). Walch-Sorgdrager (1939) reported that the antiserum of a patient with canine leptospirosis (Leptospira canicola) gave agglutinating titers of 1:10,000 when treated with Leptospira canicola, 1:3,000 when treated with Leptospira hebdomadis, 1:10 when treated with Leptospira autumnalis, and 1:30 when treated with Leptospira icterohaemorrhagiae. Hence, the serological differentiation here seems to be based on the degree of agglutinating titers as well as in the first group. Fletcher (1927) regarded Leptospira febrilis as a distant strain, but Schuffner (1934) considered this organism a weakly antigenic
strain of Leptospira icterohaemorrhagiae. The latter reached this conclusion in view of the clinical pictures and agglutinating titers.

Thus it seems that the many strains of leptospiroae which have been isolated can be placed in six serological groups, the differentiation being made on higher serological titers of these strains with their homologous antisera.
The Pathogenicity of Leptospirosis and its Manifestations

The ability of the leptospirobes to produce disease has been shown to be dependent not only upon the species of the organisms within the genus, but also upon the hosts which the leptospirobes have successfully invaded. However, it must be pointed out that virulence varies greatly in different strains of the same species. Likewise our estimate of severity of the disease in man depends upon the selection of cases, since only the severe cases in one locality may come to the attention of the physician, while in another locality both mild and severe cases may apply for medical treatment. Mason (1938) and Havens, et al. (1941) have both shown that subclinical infections existed in persons whose occupations were such that they were in proximity to rat infested areas. The incidence of subclinical infections has been proven by the selection of eleven rat catchers from whom sera were taken and treated with Leptospira icterohaemorrhagiae antigen. Six out of the eleven subjects from this group agglutinated the antigen totiters of 1:30 - 1:300 (Mason 1938). Yet these six subjects had no history of the disease. Syverton, et al. (1938) have shown that the blood taken from patients suspected of having Weil's disease failed to infect guinea-pigs. Usually, the diagnostic laboratory would consider the results of this test negative for Leptospira icterohaemorrhagiae infection since the guinea-pig is a highly susceptible host. These workers showed that a highly virulent strain of Leptospira icterohaemorrhagiae could be developed when the liver and kidneys of apparently negative guinea-pigs were emulsified and injected into healthy guinea-pigs for three successive passages.
1. Leptospirosis in man.
   a. Leptospira icterohaemorrhagiae.

   Weil’s disease in man is characterized by a sudden onset with alarming symptoms, i.e., fever, headache, muscular pains and sometimes jaundice. The disease may be divided into three stages: (1) septicemic, (2) icteric or toxic, and (3) convalescent.

   **Incubation period.**

   The average period of incubation in Holland for 56 cases was 9.6 days with a range of four to nineteen days (Walch - Sorgdrager, 1939), and 10.4 days with a similar range in 34 cases (Schuffner, 1934). Inada, et. al., 1916, reported an incubation of five to seven days in Japan which suggests a more virulent strain in that area.

   **Onset.**

   The onset is usually sudden, enabling the patient to tell the exact hour that he became ill. There is hyperpyrexia, severe headache of the frontal type, often chilly sensations and occasionally a severe shaking chill, and general malaise with prostration.

   **Septicemic Stage.**

   The septicemic stage is that period from the onset to the icteric stage, or in the non-icteric cases as long as the leptospirae are circulating in the blood. It covers a period of two to nine days, usually averaging five days. The temperature ranges from 102 to 106 degrees Fahrenheit with a corresponding full fast pulse. The conjunctivae are injected due to the flushing of the episcleral capillaries. There is a leukocytosis of 14,000 to 20,000 with a Schilling shift to the left suggesting that the leptospira is a pyogenic organism. There
is oliguria with albumin and the blood nitrogen is elevated from fifty to one hundred milligrams per cent.

Larson (1941) in thirty-three patients found the following: headache 54%, liver enlargement 21%, conjunctivitis 21%, haemorrhages 51%, oliguria 30%, hyperpyrexia 100% and jaundice 100%. Buchanan (1927), in twenty-two cases found the following: jaundice 100%, haemorrhages 60%, and liver enlargement 10%. Hence, in these two groups haemorrhage and jaundice seem to be a fairly constant occurrence.

**Toxic Stage.**

This stage lasts from two to nine days in which the fever continues but is markedly lower. The patient is extremely toxic and semi-comatous. There is a reduction in the erythrocytes with anemia, and a thrombocytopenia. The leukocyte count usually runs between ten to fourteen thousand but may surpass thirty thousand. There is a Schilling differential shift to the left. In fatal cases death usually occurs between the eighth and sixteenth day, as the result of renal failure, heart failure, haemorrhage, severe toxemia or pneumonia. Inada (1916) reported a death rate of thirty to forty-eight per cent for *Leptospira icterohaemorrhagiae* in Japan. Buchanan (1927) reported a death rate of twenty-five per cent in Scotland. In the non-fatal cases the patient becomes more rational, and recovers from his toxicity and jaundice by the end of the second week.

The pathogenesis of the icterus is yet obscure (Stavitsky, 1945). But it may be due to hepatitis (Basile, 1921) or hemolysis (Walch - Sorgdrager, 1939). The Van den Bergh test is positive both direct and indirect suggesting that the icterus may be due to both hepatitis and
hemolysis.

Renal failure becomes pronounced in this stage. Leptospirae appear in the urine between the ninth and sixteenth day, and persist for a few weeks. Ashe, et. al. (1941) report that the kidney damage varies from a slight albuminuria to a severe haemorrhagic nephritis with a great number of renal casts. The renal damage due to the leptospirae appears to be of toxic origin. Packchanian (1941) states that the nephritis is more severe in the icteric cases with biliuria.

Convalescent Stage.

The convalescent stage usually sets in after the fourteenth day of illness but may be delayed until the twenty-first day. In some cases jaundice is the last symptom to disappear. Relapses often occur in the third week of illness in from twenty-eight to seventy-five per cent of the patients in different parts of the world. They last from one to seven days and are never serious (Walch - Sorgdrager, 1939).

Complications.

The most important complication is that of leptospiral meningitis. Walch - Sorgdrager (1939) state that it occurs usually in the second stage of the disease at about the fifteenth day, and is most common in the non-icteric cases, i.e., of 129 cases in the Netherlands, 15.5 per cent developed meningitis. The spinal fluid shows a leukocyte count of two hundred to three hundred with thirty to ninety per cent polymorphonuclear leukocytes, averaging fifty per cent, an increase in sugar, reduced chlorides, and occasionally a cloudy appearance.

b. Leptospira canicola.

Although Okell, et. al., (Walch - Sorgdrager, 1939) first
noted an infectious jaundice in dogs that was due to a leptospira, in 1925, this species was not proven to be distinct until Schuffner (1934) showed that it was immunologically separate from the classical strain of leptospira.

This type of leptospirosis is rare in man, and there is a paucity of written material concerning this disease. This is perhaps due to its comparative mildness in man. Walch - Sorgdrager (1939) reported that in a total of twelve cases in the Netherlands, the disease hardly ever produced jaundice and was not fatal. A weak positive direct Van den Bergh test was reported in two of these cases where a slight icterus of the sclera was noted. There was a febrile albuminuria, and erythrocytes, leukocytes and hard renal casts were present in the urine for a few days. Meningeal symptoms were present in four cases, and marked debilitation was noted in all these cases during the convalescent stage.

The first case of Leptospira canicola infection in man was reported by Stewart, et al., (1938) which was proven immunologically. Rosenbaum (1946) reported a case of canicola fever with the same symptoms as is presented in Leptospira icterohaemorrhagiae infection. The icteric index was ninety-four units, the N.P.N. was thirty-five mg. per cent, the stools were pale, the cephalin flocculation test was two-plus denoting liver damage, and there was biliuria. There was no nephritis, meningitis nor haemorrhagic effects.

In general, canicola fever in man shows a variable degree of jaundice. There was no jaundice in the twelve cases in Holland. Jaundice occurs in about fifty per cent of the cases of the United States.
Nephritis is mild, the fever is variable, and the haemorrhagic tendencies are mostly manifested by injected conjunctivae and epistaxis.

c. Leptospira grippotyphosa.

This leptospirosis has been noted in man since the end of the 19th century, especially in Western and Central Europe, Russia, Italy, and in the Andaman Islands. It has been known under such synonyms as summer influenza, mud fever, swamp fever, and Schlamm fieber.

The cause of this disease in man is much milder than is seen in Weil's disease. The liver is not enlarged and jaundice is present in about two per cent of the cases.

*Leptospira andaman* B of the Andaman Islands has been shown to be serologically identical with *Leptospira grippotyphosa* (Walch-Sorgdrager, 1939).

d. Leptospira hebdomadis.

This leptospirosis has been endemic in Japan and Java for many years. The causative organism was isolated from patients in Japan by Ido, et al. in 1918. The disease is synonymous with *Leptospira akiyami* B, Japanese seven day fever, Autumn fever, Nanukayami and Sakushyn fever.

The onset of the disease is abrupt with hyperpyrexia, general malaise, headache, and injected conjunctivae. There is usually lymphadenopathy, albuminuria, and marked leukocytosis. The disease lasts about one week. Jaundice is never present. There have been no fatalities reported.

e. Leptospira autumnalis.

Hasamiyami or Akiyami fever, harvest sickness, Leptospira
akiyami A, Spirochaeta autumnalis A Rachmat group is endemic in Japan, Sumatra, and the Federated Malay States (Fletcher, 1927).

The course of the disease in man is similar to Weil's disease although the course is shortened from six to twelve days. Jaundice and haemorrhages are very frequent.

f. Leptospira febrilis.

This leptospirosis has been described by Fletcher (1927) as the causative organism of a febrile disease in Malaya. It is also known as Leptospira pyrogenes.

The pathogenicity of this disease is similar to Leptospira autumnalis. However, statistically, it is more severe and fatal in man. Schuffner (1934) considered that this organism was a strain of Leptospira icterohaemorrhagiae.

g. Miscellaneous Leptospirosis.

Walch (1926), Fletcher (1927), and Walch - Sorgdrager (1939) have discussed a group of leptospiroa in man which are serologically distinct from the other leptospiroae after agglutinin adsorption tests. Among this group are Leptospira australis A and B, Leptospira pomona, and Leptospira mitis. The pathogenicity of these leptospiroae is relatively mild for man and no cases exhibit jaundice.
2. Leptospirosis in Guinea-pigs.

   a. Leptospira icterohaemorrhagiae.

   The guinea-pig has been the favorite animal of study for Weil's disease since the causative organism was first isolated by Inada, et. al. in 1914. This little rodent is highly susceptible to the disease. It usually succumbs in five to eight days after introduction of the etiological agent, intraperitoneally or orally. The symptoms of the disease in the guinea-pig are similar to those seen in man.

   The pathogenicity of the leptospira in the guinea-pig is manifested by the haemorrhages and jaundice. (Basile, 1921). Inada, et. al., (1916) reported that jaundice occurred seven to eight days after injection in all his animals. Basile (1921) reported that jaundice varied in the guinea-pigs that he had inoculated with Leptospira icterohaemorrhagiae. The haemorrhages may be diffuse involving the skin, muscles, and internal organs. The haemorrhages of the lungs are particularly characteristic, and are described by Inada, et. al. (1916) as having the appearance of the wings of a butterfly of the genus Vanessa.

   Basile (1921) tried to explain the pathogenicity of the leptospiroa on the basis of an endotoxin that is released by the infective agent which has adverse effects on the endothelial cells of the capillaries. The jaundice which follows the haemorrhagic stage is brought about by the toxic effect of the leptospiroa on the erythrocytes; hence, haemoglobin is released and converted to bilirubin without the assistance of the liver.
Wylie (1946) pointed out that too much emphasis has been placed upon the importance of jaundice intensity by the workers in the past. Attention was turned to the kidneys which have long been recognized to be damaged in leptospirosis. Evidence of this is seen in albuminuria, renal casts, and increased non-protein nitrogen blood levels. This worker compared the blood urea levels of two groups of guinea-pigs, with twenty animals in each group. One group was injected with the kidney emulsions of two guinea-pigs that has been infected with a stable strain of virulent *Leptospira icterohaemorrhagiae*. The dose given each animal was one milliliter via the intraperitoneal route. Early in the eighth day, when all the inoculated animals were in extremis, blood was withdrawn by cardiac puncture and the blood urea was estimated. The average blood urea in the twenty normal animals was 16.25 mg. per cent in contrast to a level of 231.3 mg. per cent in the infected group of animals. Wylie (1946) then compared the degree of liver damage in animals that had been given liver protective substances (methionine, 1.0 mg./kilogram of body weight) with an equal number of animals that had not been given methionine. Both groups were then inoculated intraperitoneally with 1.0 ml. of kidney emulsion containing *Leptospira icterohaemorrhagiae*. All animals died at the end of eight days. The liver sections of the animals from both these groups were studied. Only five per cent of the methionine treated and sixty per cent of the untreated guinea-pigs exhibited liver damage. Yet the urea blood levels were 216 mg. per cent for the treated group and 212 mg. per cent for the untreated group. Wylie (1946) concluded that in view of the results of his experiments, the pathogenicity of Weil's disease in guinea-pigs is
due to renal failure regardless of liver damage.

Stavitsky (1945) concluded, from his study of the pathogenicity of *Leptospira icterohaemorrhagiae* in guinea-pigs, that the mode in which the organisms cause jaundice and haemorrhage is difficult to demonstrate. He suggested that the organisms spread throughout the organs of the susceptible host by their rapid motility, since attempts to demonstrate a "spreading factor" in filtrates and autolysates of leptospiral cultures were unsuccessful. The question is raised as to the possibility of the toxin or toxic antigen of the organism being extremely labile and easily altered by *in vitro* testing of spirochaetal extracts.
b. **Leptospira canicola.**

This organism has slight pathogenicity for guinea-pigs. It is not fatal and causes no jaundice (Schuffner, 1934). However, Meyer, et al. (1938) was able to show icterus after two or three passages in guinea-pigs from twenty-two strains of *Leptospira canicola* isolated in California. Walch – Sorgdrager (1939) showed that the infective material from an icteric "pig" may or may not produce jaundice in another "pig".

Schuffner (1934) showed that the virulence of the organism can be raised by serial passages to produce death and numerous leptospiroae in the blood. Guinea-pigs became carriers when they recover from the infection.

Jaundice may be produced through serial passages of the aetiological agent. These animals show congested viscera and lymph nodes as well as distended gallbladders. Syverton found an icteric incidence of one per cent in a series of eight hundred guinea-pigs (Meyer, et al., 1939).

In general, it can be said that *Leptospira canicola* infection in guinea-pigs is a relatively mild disease. The usual symptoms are hyperpyrexia without jaundice. The animals rarely succumb to infection.

c. **Leptospira grippotyphosa.**

Guinea-pigs are only mildly affected by this leptospiral infection. There is hyperpyrexia, cachexia, and no icterus.

d. **Leptospira hebdomadis.**

Walch – Sorgdrager (1939) report that *Leptospira hebdomadis* is moderately virulent for guinea-pigs. Young animals show a mortality of sixty per cent with lymphadenopathy. There is a four per cent in-
cidence of nasal hemorrhages in this group. Fletcher (1927) noted that hyperpyrexia and lymphadenopathy were the marked symptoms of his test animals in Malaya.

e. **Leptospira autumnalis.**

Most of the work (in English) on this disease has been reported by Fletcher (1927), and Walch - Sorgdrager (1939). Guinea-pigs are readily infected with *Leptospira autumnalis* demonstrating hyperpyrexia and jaundice in more than fifty per cent of the infected animals. Death occurs in four to five days in the icteric animals with mucosal haemorrhages.

f. **Leptospira fibrilis.**

The pathogenicity for guinea-pigs with this type of leptospirosis is similar to *Leptospira autumnalis* (Fletcher, 1927).

g. **Miscellaneous Leptospirosis.**

This group of leptospirosis has low pathogenicity for guinea-pigs.

3. Leptospirosis in Dogs.

The leptospiroae that infect dogs are *Leptospira canicola*, *Leptospira icterohaemorrhagiae* and *Leptospira hebdomadis*. The pathogenicity in these animals ranges from slight to severe depending upon the organism involved and the age of the dog.

a. **Leptospira icterohaemorrhagiae.**

The pathogenicity is much greater for young dogs. Klarenbeek and Schuffner (1933) report that dogs affected with this disease showed jaundice in older dogs, and severe haemorrhages with icterus and death.
in puppies. Of ninety-four dogs infected, three per cent were jaundiced and forty-one per cent fatal.

b. Leptospira canicola.

Canicola fever is a prime disease of dogs. Meyer, et al. (1939) reported that in a study of eighty-eight dogs infected with this leptospiral disease, in California, forty-one were icteric, forty were haemorrhagic, and seven were both icteric and haemorrhagic.

In the icteric dogs there was pronounced jaundice in the subcutaneous, facial, and visceral tissues. The livers were clay colored, slightly enlarged and congested; the bile ducts were patent. Haemorrhages were most prevalent in the portal lymph nodes, and occasionally in the renal cortex and lungs. There was catarrhal inflammation of the intestinal mucosa.

The haemorrhagic group of dogs demonstrated necrotic ulcerations of the buccal mucosa. The lymph nodes were edematous and haemorrhagic. The liver was mottled, hyperemic and friable with fatty degeneration. The lungs, pleura, myocardium and pericardium showed petechiae. The gastrointestinal tract was infiltrated with the results of erythrocytic degeneration, i.e., a dark purplish or brown color.

Walch - Sorgdrager (1939) report that the leptospirae were chiefly found in the lumina of the kidney tubules suggesting that the organisms may cause kidney damage by their toxic action on the epithelium. In the icteric form the organisms were situated outside the lumen in the interstitial tissue, and only occasionally in the epithelium of the tubules.
c. **Leptospira hebdomadis.**

Fletcher (1927) reported that a number of young dogs were dying from a "natural" leptospirosis in Malaya. Some of these showed icterus while others demonstrated haemorrhage with jaundice. The Dogs became seedy, refused food, vomited and died in four to five days. Four of these dogs showed pathogenic changes in the lung similar to those seen in guinea-pigs with *Leptospira icterohaemorrhagiae* infection. A full grown dog and several puppies were inoculated with material, obtained from a guinea-pig that had succumbed to an infection produced by inoculation of visceral emulsion from an icteric dog. One of the puppies died, the others became icteric and haemorrhagic but recovered from the infection. The adult dog seems relatively immune. This dog strain of leptospirosis was proven to be serologically identical to *Leptospira hebdomadis.*
4. Leptospirosis in Rats.

a. Leptospira icterohaemorrhagiae.

It has been reported on many occasions that the rat is a healthy carrier of this organism. Walch - Sorgdrager (1939) reported that the young sewer rat (Rattus norvegicus) is easily infected and may develop icterus. Schuffner (1934) inferred, by examining rats from Amsterdam sewers, that young rats are far less frequently affected by leptospirosis, i.e., in a group 124 adult rats forty-five per cent were infected while only 2.5 per cent of a group of eighty-three young rats were infected. Rats are generally healthy carriers of leptospirosis. Buchanan (1927) described the kidney and liver as showing the most constant lesions in rats trapped in Scotland. The kidney showed marked degenerative changes and areas of necrosis. The liver was not so profoundly changed as the kidney. Stuart (1938) demonstrated masses of leptospirae in the lumen of the convoluted kidney tubules of rats in Levaditi-stained kidney sections. These rats were serologically positive for leptospiral infection. Ido, et al. (1917) were unable to demonstrate leptospirae in the blood of sixty-four rats who were infected. Inada, et al. (1916) have never been able to infect white rats; whereas Ido, et al. (1917) succeeded in infecting one of four white rats. This albino rat became icteric and died, but leptospirae were isolated only in the kidney.

Kalfayan (1947) studied the kidneys of rats (Rattus norvegicus) who were carriers of Leptospira icterohaemorrhagiae in Beirut, Syria. Sections of the rats' kidneys showed numerous leptospirae on the surface of the epithelial cells of the tubules, and one or more leptospirae in the connective tissue around the tubules. It is interesting to note
that fourteen out of a total of seventy rats showed histological changes in the kidney that were similar to acute or subacute glomerular nephritis in man. Six of these fourteen rats were positive for leptospiroae.

In general, leptospiroae in infected rats appear only in the kidneys. Ido (1917) noted that man presents this picture in the convalescent stage of Weil's disease, and in guinea-pigs after immune serum has been administered. When antibodies have fully developed, the leptospiroae are present only in the kidneys. Perhaps there is some evidence here that rats are immune to leptospirosis.

Immunity Studies

Immunity to leptospirosis is manifested by the presence of antibodies in the serum of the individual person or animal examined. Much valuable information may be gained by finding agglutinins for leptospiroae present in the plasma of an individual since it is indicative of past or present infection.

The presence of immune bodies was first demonstrated in Japan by Inada, et al. (1918). These workers were able to demonstrate agglutinating titers for leptospiroae in convalescent and immune sera of persons known to have had Weil's disease.

Man has no natural immunity against leptospirosis; however, solid immunity may be produced by an attack. In one case, Packchanian and Tom (1943) were able to demonstrate leptospiroa antibodies in the blood, in titers as high as 1:10,000, four years after an attack of leptospirosis. In another case, they were able to demonstrate agglutinins to a titer of 1:300 after a period of twenty-two years from the initial illness.
Schuffner (1934) was able to diagnose many cases of *Leptospira icterohaemorrhagiae*, that were not recognized clinically, by means of agglutination tests. Antibodies develop seven to fifteen days after the onset of illness, and the agglutinin titer may run as high as 1:30,000 (Schuffner, 1934).

The administration of immune serum may affect the immunity of the individual since the antiserum may destroy the circulating leptospirae thus reducing their antigenicity.

Schuffner (1934) noted that the administration of immune serum to persons not affected with leptospirosis failed to cause agglutination when their sera was tested.

Raven (1941) was able to demonstrate retrospect infection in dogs by testing for the presence of immune bodies. She found that antibodies for leptospirae in infected dogs appear about the tenth day of the disease and reach a high level shortly thereafter (thirty days) which is maintained for many months. This high level of agglutinins (1:30,000) declines to a lower level (1:300) after a period of two to three years.

The immunity studies in guinea-pigs parallels that of man.
Diagnosis

The methods of the diagnosis of leptospirosis depends upon the animal studied and the phase of the disease. One is able to detect past infection by serological studies.

a. Man.

1. Darkfield.

Darkfield examination may be made of the blood up to the fourth or fifth day following the onset since the organisms are present up to this time, although they have been found as late as the eighth day. In no instance have they been found beyond the twelfth day. The leptospirae appear in the urine after the tenth day of illness. But usually it is difficult to demonstrate the organisms in this manner because they are so few in number. After autopsy, the organisms are much easier to demonstrate by the darkfield examination of liver and kidney emulsions.

2. Stained Preparations.

Blood films and thick drop smears may be stained by any of the Romanovsky stains, i.e., Giemsa's, Wright's and Leishman's stains, in order to demonstrate the leptospirae. But this method also has its limitations because of the low concentration of organisms in the blood and urine of man. Autopsy organs (liver and kidney), when emulsified and stained with Giemsa's stain or Fontana's silver stain, readily demonstrate the leptospirae in moderate numbers. Tissue sections of the kidney and liver stained by Levediti's silver impregnation method readily demonstrate the organisms.
3. Animal Inoculation.

(a) Guinea-pig.

Since the time of Inada (1916), the guinea-pig has been the animal of choice of many workers. When no leptospiroa are found in the blood or urine by darkfield or stained preparations, guinea-pig inoculation is resorted to.

The blood of a patient is withdrawn in amounts of 3 - 5 ml. during the first few days of the disease and injected intraperitoneally into young guinea-pigs (175 gms.). Fresh urine is collected between the tenth and twentieth day, centrifuged in volumes of 100 ml., and the sediment is injected intraperitoneally into guinea-pigs. These test animals demonstrate the symptoms of the disease and usually succumb in about eight days.

(b) Mice.

Packchanian (1940) suggested the use of the albino deer mouse (Peromyscus maniculatus gambelii) as a suitable test animal for the diagnosis of Leptospira icterohaemorrhagiae. Leptospiroa are found in the blood one to five days after intraperitoneal injection of infective material. The infected animals show almost constant jaundice and haemorrhage into the peritoneal cavity. Serial passages were shown to increase the pathogenicity of the leptospiroa so that death occurred much sooner in these animals (three to fifteen days). Autopsy examination demonstrated necrotic areas in the liver, jaundice, and haemorrhage in all organs with massive numbers of leptospiroa. Any strain that was infective for guinea-pigs produced a fatal infection in the deer mice.
Larson (1941) found that susceptibility of white mice (Mus musculus) was dependent upon age. He succeeded in fatally infecting all mice (with Leptospira icterohaemorrhagiae) who were three to four weeks old. At the age of five weeks there was a thirty per cent survival. Forty-three per cent survived at the age of six weeks, and seventy per cent survived at the age of seven weeks. Leptospirae were found in the liver, kidneys, spleen, lungs and brain upon autopsy. After an incubation period of three to seven days the mice became seedy and inactive. Jaundice occurred one to two days before death. At autopsy the lungs were haemorrhagic, the lymph nodes congested and the animal had generalized icterus.

(c) Hamsters.

Larson (1944) compared the use of hamsters (Cricetus auratus) and guinea-pigs as test animals for the diagnosis of Leptospira icterohaemorrhagiae and Leptospira canicola. Inocula were obtained from organisms grown on Verwoort's medium at thirty-two degrees Centigrade, or of ten per cent emulsions of liver and kidneys from animals infected with leptospirae. All injections were intraperitoneal in doses of 0.3 ml. The strains of Leptospira canicola and Leptospira icterohaemorrhagiae used were able to produce virulent manifestations in guinea-pigs and young mice.

Young (150 gms.) guinea-pigs survived after being inoculated with Leptospira canicola. The same inoculum caused death of young guinea-pigs when Leptospira icterohaemorrhagiae was used. Injections of both Leptospira icterohaemorrhagiae and Leptospira canicola produced death in four weeks old hamsters at the end of five to six days.
Autopsy examination showed lung haemorrhages and leptospiroa in the peritoneal fluid of the hamsters that had succumbed to *Leptospira canicola*. The hamsters that were fatally infected with *Leptospira icterohaemorrhagiae* demonstrated the same pathologic changes as is seen in fatally infected guinea-pigs.

Randall and Cooper (1944) suggested that the hamster and guinea-pig may be used for the differential diagnosis of *Leptospira icterohaemorrhagiae* and *Leptospira canicola*. This conclusion was reached after catheterized urine sediment, from an acutely ill dog whose serum had a positive titer of 1:2,000 for *Leptospira canicola*, was injected (in a sterile saline suspension) intraperitoneally into four young hamsters and four young guinea-pigs. Within nine to ten days the hamsters died of leptospirosis. *Leptospira canicola* was demonstrated by the darkfield examination of portions of the hamsters' kidneys and livers. The inoculated guinea-pigs remained normal in appearance. Both guinea-pigs and hamsters were fatally infected with injections of *Leptospira icterohaemorrhagiae*. Hence, if an unknown species of leptospiroa are injected into both young guinea-pigs and hamsters, fatal infections in both groups would indicate an infection with *Leptospira icterohaemorrhagiae*; whereas, death in hamsters alone would point to an infection with *Leptospira canicola*.

However, it seems that variability in pathogenicity should be taken into account since Syvertton, et al. (1938) showed that some strains of *Leptospira icterohaemorrhagiae* had to be passed serially through three guinea-pigs before infection could be noted.
Stavitsky (1945) has shown that the hamster is as good a test animal as the guinea-pig for the diagnosis of Weil's disease. He found that both guinea-pigs and hamsters react similarly to infection with Leptospira icterohaemorrhagiae.

(d) Gophers.

Syverton, et al. (1938) found that the gopher, Citellus richardsoni, was susceptible to experimental infection with Leptospira icterohaemorrhagiae. However, these animals are not easily obtained for laboratory use, and its use affords no advantage over other test animals.

(e) Chick Embryo.

Davis (1939) has shown that twelve day old chick embryos could be used to proliferate Leptospira icterohaemorrhagiae. Loopfuls of culture material, proven to contain viable leptospirae via darkfield, were inoculated on the chorioallantoic membrane through a slit in the cell membrane. After incubation at thirty-four degrees Centigrade for eight to ten days, the eggs were opened and examined grossly and microscopically via histological sections. Leptospirae appeared on the chorioallantoic membrane as thickened white patches. There was edema of the mesenchyme, and mononuclear infiltration and hyperplasia of the epithelium. The heart blood, liver, lungs and kidneys showed fatty necrosis.

It seems that the chick embryo offers no particular advantage over animal inoculation. Although the pathogenicity of organs parallels that of the guinea-pig, it must be shown by time-consuming histological techniques. The great disadvantage is that
the inoculum must be sterile, since chance contaminants, which are destroyed by the immunological defenses of animals, will cause death of the chick embryo.


The diagnosis of leptospirosis may be made by obtaining inoculum from the blood, urine, or emulsified organs (liver and kidney) of a susceptible host. The advantage of cultivation over direct examination of organisms as a means of diagnosis has been shown by Lewis (1942). He found that he was able to demonstrate an incidence of 18.1 per cent when he examined the urine of 110 rats, (Rattus norvegicus) that he had trapped in Philadelphia, by darkfield, whereas he showed a 36.6 per cent incidence of leptospirosis by culture. The kidney emulsions, that he obtained by aseptically removing them from rats and macerating them in a sterile mortar, showed an 18.1 per cent incidence by darkfield examination. Culture of the kidney emulsions in Schuffner’s medium showed a 90.1 per cent incidence of *Leptospira icterohaemorrhagiae*.

Schuffner (1934) cultivated *Leptospira icterohaemorrhagiae* from fifty-one cases of which eighteen were without jaundice.

Hence, it seems that one is able to demonstrate leptospiroa more readily when they are cultured since this means permits the organisms to proliferate in great enough numbers to be detected by darkfield examination. This is especially true in human leptospirosis where there is a paucity of organisms in the blood during the disease. Cultivation of the leptospiroa is especially adaptable to laboratories that do not have the facilities for animal studies.
The leptospiroa must be cultivated when agglutination testing is to be done, since the culture is the antigen source for this test.

In human leptospirosis, it is, of course, needless to say that cultivation of the organisms must be done before the seventh day of the disease since the organisms are not usually present in the blood after that time. Better results are obtained if guinea-pigs are injected and cultures made from the infected animals. In old blood, Schuffner (1934) suggested that it is better to inject the triturated coagulum, rather than the serum, into guinea-pigs. This is obvious since the leptospiroa may bore their way into the coagulum leaving the serum a poor inoculum.

The organisms have been cultured from the urine of humans as late as the twentieth day of illness, but here too, it is best to inoculate a susceptible test animal with the sediment of the urine. The organisms may then be demonstrated and cultured from the blood or organs of the infected animal.

Since the isolation of the aetiological agent by Inada, et al., (1916), there have been many methods of cultivation suggested and used. Some of those more generally used today are Schuffner's liquid medium (1934), and its many modifications, Noguchi's semi-solid medium and its modifications, and Verwoort's liquid medium.

The conditions for the cultivation of leptospiroa have been discussed in the section on the physiology of the leptospiroa.
5. Serological Tests.

The different types of serological tests have proven to be a valuable aid in the diagnosis of leptospirosis. The diagnosis of cases not recognized clinically has been made possible by the agglutination test. The agglutination test is of no value before the ninth or tenth day, in human leptospirosis as agglutinins are not elicited in the blood before the sixth to eighth day of illness. A rise in the agglutinating titer is of diagnostic value between the tenth and fourteenth day since it confirms a course of leptospiral infection. A negative reaction after the thirtieth day of illness rules out leptospirosis. A titer of 1:1000 is indicative of present or recent infection. The diagnostic titer is 1:300. Agglutination in low titers may represent an old infection and not the results of the present acute infection.

(a) Agglutination Tests.

This test is manifested by the reaction of the immune serum with the leptospiroae (antigen). The antigens may be either viable leptospiroae, or organisms killed by heat or formalin. The live antigens are more satisfactory for agglutination testing since the killed antigens tend to clump spontaneously. Raven (1941) found that motile leptospiroae (Leptospira icterohaemorrhagiae and Leptospira canicola) were most useful for agglutination testing since the decrease in the motility of the organisms could be noted by darkfield, and used as a check on the gross appearance of agglutination in tubes. The serum being tested should be inactivated in order to prevent lysis of the test organisms.
In general, the test is performed by diluting the test serum in Sorensen's buffer solution (pH 7.2) in serial titers of 1:30 to 1:30,000. A constant concentration of leptospira antigen (0.15 ml.) is added to each serum titer. The antigen may be prepared by using leptospirae cultured in Schuffner's liquid medium for a period of four to seven days. A good concentration of organisms is a suspension of twenty-five to fifty leptospirae per "high dry" darkfield. If the organisms from the culture are more numerous, they may be diluted in Sorensen's buffer solution until the desired concentration is obtained. The mixtures of serially titered, buffered serum and leptospirae are incubated at thirty-seven degrees Centigrade for two hours to allow antigen-antibody reaction to take place. The results of the tests are read by examining a loopful of the material from each tube under a darkfield microscope. Positive and negative controls are, as always, read along with the test serum. The test is reported positive in the highest serum titer in which agglutination is noted, i.e., if agglutination is noted in a titer of 1:3000, but not in 1:6,000 the test is reported as positive in 1:3,000.

Starbuck and Ward (1942) reported a modification of the agglutination test which may be performed on spot plates and read macroscopically. The antigen is prepared by concentrating formalized leptospiroae by high speed centrifuge and adding a small amount of gentian violet to this concentrate. One drop of the colored antigen is added to one drop of serially diluted serum on a clean glass slide plate ruled off in squares. The plate is rocked gently for
ten minutes to allow the antigen-antibody reaction, to take place. The reading of the test is done against a white background of diffuse transmitted light. A positive reaction is indicated by the appearance of light blue aggregates in the drop of antigen-antibody mixture.

Gardner (1947) has shown that it is possible to detect homologous antigen-antibody reactions, from antigenically related antibodies of other leptospires, by darkfield examination of material from agglutination test material. The former reactants form aggregates of leptospires, which are irreversible. The latter reactants demonstrate loosely clumped aggregates, which are partially reversible due to the cells being only weakly sensitized by the heterologous antibody.

(b) Alexin-Fixation.

The alexin-fixation test seems to be less reliable than Schuffner's agglutination test and its modifications. It is more complex and less adapted to the average laboratory or survey work where large groups are tested.

The test requires a rich culture growth of leptospira antigen that has been refrigerated for eighteen hours previous to testing and sensitized sheep erythrocytes whose hemolytic dose has been previously determined. The test serum is diluted serially in geometrical progression from 1:20.

Molner and Kaspar (1941) used formalized as well as viable suspensions of leptospires. The former detects antibodies in low titered sera and the latter in high titered sera by lysis.
(c) Lysis.

Davidson and Smith (1936) used this test as a means of detecting antibodies in the blood and urine of fish workers who were suspected of infection with Weil's disease.

The urine was collected and passed through a 15 Chamberland Candle filter to render it bacteria free. The filtrate was titered in dilutions of 1:2 and 1:6. When viable leptospiroae were added to the serially diluted urine, lysis of the organisms was demonstrated after incubation at thirty-seven degrees Centigrade for four hours. Thus, it was shown that lysins are present in the urine of patients with latent Weil's disease.

Lysis is demonstrable via leptospiral antibodies in the blood in the presence of alexin. The technique of testing is the same as is used in the agglutination tests. The tests are read by darkfield examination. The highest dilution in which lysis is present is the reading that is reported as positive.

(d) Precipitation Test.

This test is based on the use of specific soluble substances on the antigens. Schuffner (1934) gives a detailed discussion of this method of diagnosis in leptospiral infections.

In serological tests for the early diagnosis of the specific leptospiral agent, the leptospiroae isolated from infected test animals, after inoculation with the patient's blood, may be tested against specific antisera. The antiserum that demonstrates the
greatest degree of agglutination, especially after adsorption, may be reported as the aetiological agent.

(e) Spinal Fluid Examination.

Cargill and Besson (1947), working on the assumption that there are occasional infections of humans with Weil's disease whose principle clinical manifestations is meningitis, studied the spinal fluid of fourteen persons suffering from *Leptospira icterohaemorrhagiae*. Thirteen of the fourteen patients were icteric. Diagnosis of the disease was made positive agglutination of the patient's sera with viable leptospiroae. They concluded that the only significant spinal fluid finding was xanthochromia which was present in ninety per cent of the cases.

Treatment

The treatment of leptospirosis is chiefly concerned with those leptospiroae that affect man and dogs.

(a) General Treatment.

Symptomatic treatment, as in other illnesses, is the rule also in leptospirosis. Intravenous infusions of five per cent glucose in 0.85 per cent saline are indicated to treat the hyperpyrexia, acidosis from vomiting, and the nephritis. Supportive treatment for liver damage may be undertaken by giving methionine (1.0 mg./kilogram of body weight). Wylie (1946) was able to prevent liver damage in ninety-five percent of the guinea-pigs that he had administered methionine prior to infection with
Leptospira icterohaemorrhagiae.

(b) Chemotherapy.

Walch - Sorgdrager (1939) found prontosil to have no effect on the course of Weil's disease. Ashe, et al. (1941) found sulfanilamide of no avail in the treatment of Leptospira icterohaemorrhagiae infection in humans.

Walch - Sorgdrager (1939) stated that sodium bismuth tartrate impeded the development of Weil's disease in guinea-pigs and prompted recovery. The recovered pigs demonstrated antibodies for Leptospira icterohaemorrhagiae, and were immune to later inoculations of the aetiological agent. Immunity in these animals would suggest that the bismuth salt has an inhibiting effect on the leptospira rather than a leptocidal effect, especially since antibodies were elicited in a high enough titer to render the guinea-pigs immune.

(c) Serum Therapy

Walch - Sorgdrager (1939) stated that the effect of serum therapy is difficult to judge since there is such a great variability in the pathogenicity of the organisms. The effectiveness of antiserum seems to vary greatly with the strain that elicit them.

Inade, et al. (1918) were able to immunize passively a group of forty-one patients with Leptospira icterohaemorrhagiae antiserum prepared in rabbits. It was found that immune serum had definite leptospiricidal and leptospirilytic effects upon the organisms. This was determined by observing the leptospirae in
the blood at intervals after the antiserum was administered. In
general, it seemed that immune serum had to be administered before
the seventh day of illness for beneficial results. The degree of sym-
ptoms was lessened. The intravenous route of administration was
more beneficial than the subcutaneous route. Mortality decreased
from 57.1 per cent to 40 per cent in a group of patients treated with
immune serum by subcutaneous administration, and to 38.5 per cent in­
travenously treated patients. In another group, there was a reduction
in mortality from 30.6 to 18.3 per cent in twenty-three treated patients.
There was a reduction of haemorrhage and the course of the disease was
shortened. Doses of high titer antiserum was given over a period of
five hours in 60 ml. total dosage.

Kaneko and Okuda (1918) treated four cases of human
Weil’s disease with immune serum. The results were compared with
four untreated cases. Leptospirae were as numerous in the kidneys of
both groups, but there was a reduction of leptospirae in the in­
ternal organs of the treated cases.

Noguchi (1920) prevented infections in guinea-pigs when
antiserum (prepared in horses) was administered. The control group
of guinea-pigs succumbed to *Leptospira icterohaemorrhagiae*.
Human subjects showed beneficial effects from immune serum on the
progress of Weil’s disease.

Larson (1943) was able to protect guinea-pigs against
infection with *Leptospira icterohaemorrhagiae* when antiserum was
administered on or before the fourth day of infection. Mortality
was reduced to sixteen per cent, but when antiserum was administered
after the fourth day of infection, the mortality was fifty-four per cent. Young mice (*Mus musculus*) were completely protected against infection with *Leptospira icterohaemorrhagiae*. Immune rabbit serum (titer 1:10⁶), and human convalescent serum (titer 1:10,000) was administered to the mice on the fourth day. The untreated controls showed a total mortality.

Larson (1944) was able to completely protect two groups of hamsters, inoculated with *Leptospira canicola* and *Leptospira icterohaemorrhagiae*, with immune serum that had a titer of 1:100,000 and 1:1,000,000 respectively.

(d) Antibiotics.

(1) Penicillin.

Chang (1946) tested the effects of penicillin, in concentrations up to 1:5000, for its effect in *Leptospira icterohaemorrhagiae* in distilled water at ten, twenty-three, and thirty-seven degrees Centigrade. The penicillin seemed to have no leptospiricidal effect, but reproduction was halted. Control tubes of leptospiroaiae in distilled water, at these same temperatures, showed proliferation of the organisms. In fluid medium, there was a leptospiristatic effect noted when the penicillin concentration was 0.4 units per milliliter. Higher concentration of penicillin exhibited no significant changes in the leptospiristatic effect. Leptospiristatic effects were also observed in infected guinea-pigs by the disappearance of leptospiroaiae from the blood three to five days after inoculation, when eight hundred Oxford units /day of penicillin were administered,
with a serum level of 0.2 U./ml. However, the liver was not cleared of leptospirae. The penicillin seemed to have its best effect when given before the onset of icterus.

Wylie and Vincent (1947) tested the sensitivity of different species of leptospirae in vitro. They found a variance of sensitivity from 0.1 - 70.5 Units of penicillin per milliliter among four different strains of Leptospira icterohaemorrhagiae. Leptospira canicola was sensitive to 0.5 U./ml., Leptospira grippotyphosa to 0.25 U./ml., and Leptospira bataviae to 0.1 U./ml. On a weight basis penicillin was more effective than streptomycin. None of the organisms tested produced penicillinase.

Larson and Griffith (1945) found penicillin therapeutically effective against hamsters and white mice, provided that an adequate concentration of the antibiotic could be established early in the infection and thereafter maintained.

Wylie and Vincent (1947) showed that penicillin (1500 units/day) must be given during the first twenty-four hours of the disease (Leptospira icterohaemorrhagiae) in order to be effective in guinea-pigs.

Heilman and Herrel (1944) found penicillin effective in human subjects with Weil's disease if administered early in the disease, i.e., before the appearance of icterus.
It would seem that the main criterion for effective therapy in leptospirosis is early treatment in adequately maintained doses. The therapeutic agents, whether they be immune sera, chemotherapeutics, or antibiotics, must exert their untoward effects upon the leptospiroae before they settle in the liver and kidneys. The therapeutic effects that penicillin exerts in guinea-pigs, with Leptospira icterohaemorrhagiae, should be encouraging for treatment in man since the former is far more susceptible to the effects of this organism.
Epidemiology

It is the purpose here to present some of the studies that have been done with reference to the host-parasite relationship in leptospirosis, and on the various factors that affect the incidence of this disease in humans. Proper understanding and evaluation of these studies is necessary before attempting to prevent the disease.

1. Season.

Leptospirosis occurs in Europe chiefly from July to October. Schuffner (1934) noted that leptospirosis was endemic in Holland throughout the year, but was noted in epidemic proportions during the summer season. Inada, et al., (1916) reported that leptospirosis reached epidemic proportions in Japan during the months of September to November. Fletcher (1927) noted that leptospirosis was particularly increased during the period of the southwest Monsoon. Its incidence was noted in males engaged in out-of-door occupations. Ashe, et al. (1941) stated that the epidemic season in the United States paralleled that of Europe.

Stokes, et al. (1917) noted that the incidence of Weil's disease seemed to vary with the weather. There were few cases in the dry weather of the summer. When rainy weather set in (August and September) the incidence of leptospirosis increased and many cases were admitted to the army hospital.

Alicata (1947) stated that high rainfall and the abundant rodent population in Micronesia seem to favor the spread of Weil's disease.
2. Reservoir Hosts.

(a) Rats.

Attention has been focussed on the wild rat since the work of Ido, et al. (1917) and Noguchi (1917) who succeeded in isolating the organisms \( \text{Leptospira icterohaemorrhagiae} \) from the kidneys of rats. The former demonstrated that 40.2 per cent of 149 rats (\textit{Rattus norvegicus}), and 0.8 per cent of twenty-four Rats (\textit{Rattus alexadrinus}), carried the aetiological agent in their kidneys. These workers showed that the rat passes the organisms in its urine. Ridlon (1931) found a thirty-four per cent infection with \textit{Leptospira icterohaemorrhagiae} among the \textit{Rattus norvegicus} population in the slaughterhouse district of San Francisco, California. Fletcher (1927) cited an incidence of twenty-six per cent amongst the \textit{Rattus alexadrinus} of Singapore. These rats carried \textit{Leptospira hebdomadis}, the seven-day leptospirosis of Japan. Lewis (1942) found a ten per cent incidence of \textit{Leptospira icterohaemorrhagiae} among the \textit{Rattus norvegicus} in Philadelphia. Schuffner (1934) cited incidences of leptospirosis (\textit{Leptospira icterohaemorrhagiae}), in various parts of Western Europe, among the brown rats (\textit{Rattus norvegicus}). Amsterdam showed an average infection of seventeen per cent among the rat population. Rotterdam had an incidence of seven per cent among the waterworks rats, and forty per cent among the slaughterhouse rats.

Alicata (1947) showed a twenty per cent infection of \textit{Leptospira icterohaemorrhagiae} and the rats of the Truk Atoll and the Carolina Islands.

Walch - Sorgdrager (1939) listed the rats that were proven
to be carriers of leptospirae in all parts of the world. These reports covered the period of 1917 to 1939.

1. America Incidence ranged from 7 to 44 per cent.
   - Rattus norvegicus.
   - Rattus rattus.
   - Rattus alexandrinus.

2. Africa.
   Same species as are seen in America.

3. Asia Incidence ranged from 0 to 28.5 per cent.
   - Rattus norvegicus.
   - Rattus alexandrinus.
   - Rattus rattus.
   - Microbis montebellai (Leptospira autumnalis).
   - Rattus diardi.
   - Rattus grisewester (Leptospira hebdomadis and Leptospira icterohaemorrhagiae
   - Nesobei spei.
   - Gunomys varnir (India).
   - Nesobei biugaleusis (India).

4. Europe. Incidence 3 to 42 per cent.
   - Rattus norvegicus.
   - Rattus rattus.
   - Field mice.
   - Rattus alexandrinus.
   - Spondemus sylvaticus.
   - Mus musculus (16.7 per cent)
Arvicola arvicolae (13.3 per cent).

5. Australia Incidence 2.3 to 23 per cent.

Rattus norvegicus.

Rattus alexandrinus.

Rattus rattus rattus.

Hence, it has been shown that rats are the carriers of leptospirae in all parts of the world. In each case, the leptospirae were isolated only from the kidney and the urine.
(b) Dogs.

Fletcher (1927) has shown that *Leptospira hebdomadis* was epidemic in dogs in Malaya.

Raven (1941) reported the following serologically proven incidence of *Leptospira canicola* in dogs with past or present infection. Serological surveys in the Netherlands showed a 39.6 per cent infection; 44 per cent in Belgium; 8.6 per cent in Germany; and 3.6 per cent in Italy. In the United States, New York dogs showed a 11.7 per cent infection; 14.3 per cent in Santa Rosa; and a 34 per cent infection in San Francisco, California; rural Pennsylvania showed a 38.1 per cent infection; and urban Pennsylvania showed a 28 per cent infection. The sampling in Europe ranged from 100 to 290 dogs, while the sampling in the United States ranged from 28 to 111 dogs.

Schuffner (1934) found, in a survey of fifty dogs in Holland, that seventeen were infected with *Leptospira icterohaemorrhagiae* and the remaining twenty-eight infected with *Leptospira canicola*.

Walch-Sorgdrager, in the 1939 paper, noted that pathogenic leptospirae have been isolated from hogs (*Leptospira icterohaemorrhagiae*), foxes, horses, cats, and mice.

Occupations:

It can be readily ascertained that occupations that are associated with rat infested areas show the greatest incidence of
infections (past or present) with pathogenic leptospires. Meyer, et al. (1939) noted that veterinarians are prone to infections with *Leptospira canicola*.

**Miners.**

Ito (1918) noted the high incidence of Weil's disease among the coal miners in Japan. It was shown that the rats that inhabited the wet mines were carriers of *Leptospira icterohaemorrhagiae*. Buchanan (1927) reported that miners were suffering with Weil's disease. It was demonstrated that these mines in Scotland were moist and rat infested. The rats were shown to be carriers of *Leptospira icterohaemorrhagiae*. *Leptospira icterohaemorrhagiae* were isolated from the slime that was deposited on the walls and floors of the coal mines.

**Sewer workers.**

Alston and Broom (1935) showed that nine out of twenty-four workers had agglutination titers of 1:100 when their sera was tested with *Leptospira icterohaemorrhagiae* antigen. The sewer rat (*Rattus norvegicus*), which inhabits the sewers of England, has been shown to harbor the disease.

**Fish workers.**

Davidson and Smith (1936) studied forty cases of Weil's disease among fish workers. These investigators found twenty-two persons with *Leptospira icterohaemorrhagiae* in their blood. Twenty-six of the forty cases showed positive agglutinations with *Leptospira icterohaemorrhagiae* in their sera on or before the tenth day of illness. The establishments where these fish workers were em-
employed were infested with rats.

**Tripe Workers.**

Stuart (1938) studied leptospiral infections occurring among women who were employed to clean, scrape, and prepare tripe. The working conditions were wet. Rats were numerous. The workers were very liable to minor cuts and abrasions of the hands, from the instruments used in their work. Washings from the benches and floors of these establishments were injected into guinea-pigs. The test animals showed no signs of leptospiral infection. Nine out of twelve rats (*Rattus norvegicus*) that were trapped in the working places showed agglutinating titers of 1:30 to 1:30,000 when their sera were treated with *Leptospira icterohaemorrhagiae*. Leptospirae were found in the kidneys in eight of the nine serologically positive rats. There were masses of leptospiroa seen in the lumina of the convoluted kidney tubules. Such rats, it seems, must have been excreting a large number of organisms.

**Rice Field Workers**

Havens, *et al.* (1941) discussed an epidemic of Weil's disease that broke out among the workers in rice fields in Italy. The water in the fields contained rat urine. No mention was made of studies of the rat population.

**Miscellaneous Occupations.**

Tiffany and Martorana (1942) did a study of a group of individuals in New York City. The results of their work is indicated below.
Sera examined for presence of agglutinins for Leptospirae.

<table>
<thead>
<tr>
<th>Source</th>
<th>1:320</th>
<th>1:160</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>New York Health Department</td>
<td>1</td>
<td>23</td>
<td>1</td>
<td>614</td>
</tr>
<tr>
<td>Sewer Workers</td>
<td>6</td>
<td>11</td>
<td>6</td>
<td>498</td>
</tr>
<tr>
<td>Fish Workers</td>
<td>3</td>
<td>8</td>
<td>4</td>
<td>91</td>
</tr>
<tr>
<td>Canine Contact</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>58</td>
</tr>
</tbody>
</table>

It should be noted from the above table that the stronger positive serological reactions were found among those occupations that brought the workers in proximity to rat infested areas.

**Soldiers.**

Stokes, et al., (1917) pointed out that trench warfare with its wet sodden conditions of the skin, and the presence of rats provided favorable conditions for the incidence of Weil's disease. The cases of Weil's disease were always among the "trench" troops.

**Barge Men or Fishermen.**

Schuffner (1934) reported on the cases of Weil's disease that were associated with a group of persons who worked on or about the rat infested canals of Holland. Here one can readily see the ease in which an individual may come in contact with rat urine containing leptospiroae. The high incidence of leptospiroae among the canal rats of Holland has been mentioned elsewhere in this paper.
Transmission:

There has been a good deal of interest in the problem of how the leptospiroae enter the host and set up their infective processes. There have been many studies carried out since Inada (1916), their prime purpose being the demonstration of conditions conducive to the entrance of the leptospiroae into a susceptible host. Questions of how the leptospiroae enter a susceptible host, the speed with which they spread through the tissues, and where they settle and multiply have still not been answered to the satisfaction of all.

Skin and Mucous Membranes.

The leptospiroae apparently have the ability to pass through moist skin. When the keratinized skin has been water-soaked, the epidermis loses its ability to hold back infective agents. It is more often the case however, that the skin has been abraded before entrance to the aetiological agent. Schuffner managed to infest one of twelve guinea-pigs with *Leptospira icterohaemorrhagiae* when he immersed them in Dutch canals containing rat urine. Davidson and Smith (1936) and Stuart (1938) have shown that fish-workers and Tripe-workers have contracted Weil's disease, through their abraded hands, when they came in contact with work benches that were contaminated with leptospiroae-containing rat urine. Wassworth, *et al.* (1922) reported a laboratory infection with *Leptospira icterohaemorrhagiae*, in a laboratory worker, after the skin had been abraded with a needle contaminated with the infective agent. Walch - Sorgdrager (1939) reported that seventy-eight per cent of the patients in Holland were infected by bathing in rat infested canals. Havens, *et al.* (1941) stated that the most common
mode of infection with Weil's disease in Great Britain was by bathing in water polluted with rat excreta.

A small outbreak of seven cases of Weil's disease in young men who had bathed in a pool, that was adjacent to a cattle barn, was reported in Philadelphia. One of the seven victims died. Leptospira icterohaemorrhagiae was cultured from this fatal case. The cattle barn was shown to be rat infested.

Schuffner (1934) philosophically noted that attempted suicides by drowning in Dutch canals failed, but the inhalation of polluted water (rat urine containing leptospires) caused death later in the form of leptospirosis.

Evans and Jones (1946) reported the case of a farmer who was infected with Leptospira icterohaemorrhagiae. A few days before the onset of symptoms, the farmer recalled that a rat had run up his trouser leg and had scratched the inner aspect of his right thigh. Undoubtedly the rat had urinated and infected the farmer through the abraded skin.

Stavitsky (1945) succeeded in infecting one of three guinea-pigs by oral inoculation of Leptospira icterohaemorrhagiae.

Rosenbaum (1946) reported the case of a man who became ill with canicola fever. This human case had been in close contact with a dog with leptospirosis.

Walch - Sorgdrager (1939) have reported cases of infections through the ingestion of rat urine contaminated food and water. The pH of the stomach would lead one to believe that invasion by the organisms took place through the buccal or pharyngeal mucosa.
Contact.

There has been no evidence, to date, to support any claim that transmission of pathogenic leptospirae from man to man can take place. This seems strange in view of the fact that leptospirae are passed in the urine of humans with leptospirosis.

Insects.

There is no evidence of transmission of pathogenic leptospirae by insect bites. Stokes, et al. (1917) allowed 350 lice to feed on eight guinea-pigs in various stages of Leptospira icterohaemorrhagiae infection. The lice were then crushed, suspended in saline, and injected intraperitoneally into guinea-pigs. The guinea-pigs remained healthy.

Rats.

The numerous cases of human and dog infections from the excreta of rats have been mentioned. Mason and Liverp (1937) reported that one of twelve guinea-pigs, in a laboratory, became jaundiced and died. Leptospira icterohaemorrhagiae was isolated and proven serologically. The dealer who supplied these guinea-pigs later admitted that rats had gained access to his cages and had killed two or three guinea-pigs. It was assumed that the guinea-pig infection was transmitted from the rats.

Molner and Kaspar (1938) reported cases of Leptospira icterohaemorrhagiae among young dogs whose kennels were rat infested.

Schuffner (1934) pointed out that the infective leptospirae pass from one rat to another by way of food contamination with urine from infected rats. Rats probably infect one another
through sexual intercourse.

Food.

It is easy to see that food can become contaminated from the urine of infected rats.

Soil.

Ido, et al. (1917) noted that the soil and water in mines, where the pH was just above the acid range, was infective.

Havens, et al. (1941) reported hundreds of cases of Leptospira grippotyphosa in the rice fields of Italy and the fields of Bavaria. The soil and water of these fields contained rat urine.

Incidence:

Since the isolation of the aetiological agent by Inada, et al. (1914), the infection has been recognized to occur in all parts of the world in varied forms and hosts.

Humans.

It is not difficult to see that even though many cases of leptospirosis have been diagnosed, the proportion of infections with pathogenic leptospiroae do not approximate the incidence of some of the other diseases like tuberculosis, typhoid and diphtheria. Havens, et al. (1941) state that the low reported incidence is due to a lack of interest in subclinical cases, especially in the United States, rather than the actual incidence, as has been previously noted in this paper. Many past infections have been discovered as the result of serological tests on survey groups.
Bruno, et al., (1943) have noted the following incidence of human leptospirosis.

Netherlands......1924 - 1937....808 cases diagnosed.
France.............1924 - 1932....262 cases diagnosed.
Great Britain.......1922 - 1939....248 cases diagnosed.
North America..... up to 1940.... 73 authentic cases.

**Age and Sex Distribution.**

For the most part, men are more often infected than women; the age group being from ten to forty years. However, as has been shown in the section on occupations, the sex incidence depends upon the type of work, the sex usually employed, and the proximity of infected rats.

Davidson, et al., (1934) showed that in 210 fish-workers, the ratio of males to females (with Weil's disease) was 19.5 to 3 where the ratio of all employees was 10 males to 3 females.

Walch - Sorgdrager (1939) showed that in 363 cases of Weil's disease, 320 were males and 40 were females. In nonoccupational cases of Weil's disease, the proportions of women increase. The disease is apparently rare in children.

**Dogs.**

Raven (1941) summarized the incidence of canine leptospirosis over the period of 1930 to 1940, as follows:

Netherlands------212 dogs showed a 39.6 per cent infection.
Belgium.........100 dogs showed a 44 per cent infection.
Germany.........290 dogs showed a 8.6 per cent infection.
Rome, Italy.....112 dogs showed a 3.6 per cent infection.
New York............. 111 dogs showed an 11.7 per cent infection. 
Santa Rosa, Cal......... 28 dogs showed an 14.3 per cent infection. 
Pennsylvania (Rural)... 105 dogs showed a 38.1 per cent infection. 
Pennsylvania (Urban)... 50 dogs showed a 28.0 per cent infection. 
San Francisco.......... 47 dogs showed a 34.0 per cent infection. 

The animals in these serological surveys were older dogs for the most part. Males were in the majority, which is perhaps due to their habit of sniffing the excreta of other dogs.

Rats.

Since it has been repeatedly shown that the wild rat (Rattus species) is an asymptomatic carrier of Leptospira icterohaemorrhagiae, who excretes infective leptospirae wherever and whenever it may, it is of epidemiological importance to note the incidence of the disease among rat populations.

As Schuffner noted in his 1934 paper, rat surveys teach very little unless the sampling is kept localized. The density of the local rat population is very important since they must be in great enough numbers to distribute numerous leptospirae about. Large numbers of organisms increase the chances for transmission to humans.

Tiffany and Martorana (1942), summarizing world surveys, have shown that wild rats are infected with Leptospira icterohaemorrhagiae, in percentages of ten per cent to thirty per cent. The work of Ridlon (1931), Schuffner (1934), Meyer et al. (1939), Walch-Sorgdrager (1939) and Lewis (1943) all show figures of rats infected with Leptospira icterohaemorrhagiae that approximate this rate.
Prophylaxis:

With the knowledge of the nature of the aetiological agent, the intermediate hosts, the mode of transmission, and susceptibility of human beings, one should be able to institute prophylactic measures that will decrease the incidence of leptospirosis to a marked degree.

**Human Case.**

The excreta of the patient, especially the urine, should be sterilized.

**Rats.**

Rat extermination combines with rat-proofing of buildings such as bakeries, slaughter houses, fish-cleaning establishments, and swimming pools must be religiously enforced. Rat proofing may be done by closing all accesses to sewers and water. The ground around a building may be surrounded with a substance like cement which will discourage the burrowing of rats.

Food and other substances that may attract rats should be eliminated whenever possible.

Benches, floors and utensiles in slaughter houses and fish cleaning establishments should be kept scrupulously clean. The floors, benches, and other places that may become contaminated should be washed down twice a day with a disinfectant. Davidson and Smith (1936) found that sodium hypochlorite (12.5 per cent available chlorine) solution killed virulent leptospirae (Leptospira icterohemorrhagiae) in five minutes. A solution of 1:64 is very effective for cleaning places contaminated with rat urine.
Dogs.

Dogs are very difficult to control since many people are prejudiced when it comes to exterminating dogs in great numbers. Yet one has only to review the incidence of latent infections in dogs to note their danger as infective carriers of the disease. The only compensating factor in the problem of controlling leptospirosis in dogs is the fact that *Leptospira canicola* is comparatively rare in man.

Occupations.

Fish workers and slaughter-house employees should protect their hands by wearing rubber or plastic gloves.

Laboratory workers should be especially careful when handling infectious material. Rubber gloves should be worn at all times when infected animals are being worked upon.

Water-tight boots should be worn by field-workers, especially where the incidence of *Leptospira hebdomadis* or *Leptospira grippotyphosa* is endemic.

Drainage should be done in agricultural districts to discourage the growth and viability of excreted leptospiroae.

Bathers should avoid water where Weil's disease is endemic. The head should not be submerged if a water accident should occur since the mucosa of the mouth, nose, and eyes act as portals of entry for pathogenic leptospiroae.

Active Immunization.

Active immunization of persons whose occupations are such that leptospirosis is endemic and, at times, epidemic, has been done
from time to time since the early Japanese work on the disease. The usual method is to inject phenolized cultures (liquid) of pathogenic leptospiroae in two doses of one and two ml. at intervals of one week. Active immunization in Japan decreased the morbidity rate from 1.12 per cent in unvaccinated cases to 0.3 per cent in vaccinated individuals.

**Serum Prophylaxis.**

Since it has been shown that inoculation with high titered leptospiral anti-sera will prevent leptospirosis in test animals if given before the icteric stage, this sort of prophylaxis may be instituted in man. When cases are first reported in an occupational group, the entire remaining group who have not as yet shown symptoms may be given immune serum (Inada et al., 1918).

Mass vaccination of entire groups is not at all feasible as a general thing since leptospirosis is not as prevalent to indicate such procedures.

**Health Education.**

Awareness as to how one contracts leptospirosis and how it may be avoided should be fervently stressed where sporadic outbreaks of the disease have been evidenced in the past. Occupational groups should be taught the danger of contamination and ways of insuring that they will not fall victim to leptospiral infection.

It should be compulsory, by law, that all cases be reported to the local health officer. New outbreaks may be noted early and checked before too great a toll has taken place. Reporting of cases also provides information for the epidemiologist in the compilation of data and the study of leptospirosis.
Suggestions for Further Study

Although the scientific literature contains countless studies on the various aspects of leptospirosis and its etiologic agents, there are many questions left unanswered. The two outstanding questions center around the mode of toxicity of the pathogenic leptospirae and the reason for the related antigenicity found among the many species.

It seems that some of the reasons for the variations in the antigenicity of the leptospirae might have light shed upon them if attention were turned to the phenomenon of bacterial dissociation.

Variability or dissociation has been recognized in practically all groups of microorganisms. When microorganisms dissociate, many or all their demonstrable characteristics may be affected. A change in morphology means a change in the chemical structure of the cell, and hence its immunologic specificity. Addition and/or deprivation of growth factors has been known to change the ability of microorganisms to oxidize, hydrolyze and utilize new substrates. A change in the ability to synthesize growth factors and metabolic products may result in the ability to invade and produce disease. It has been repeatedly shown that the virulence of some microorganisms can be gradually increased by repeated passages through a guinea-pig or other suitable test animal.

In attempting to understand the diverse results that one observes in studies of the antigenicity of the various pathogenic leptospirae, certain assumptions must be made. It seems logical to assume that the numerous leptospirae are in reality a very small group of organisms that have had their characteristics changed in some way. The evidence in favor of diverse variability among the pathogenic leptospirae can be
seen in their identical morphology, related antigenicity, and similar pattern of pathogenicity. It is of interest to note that the greatest variability in antigenicity and pathogenicity is present only in the various leptospiroae found in India, Australia and the East Indies. Perhaps the geographical isolation of these areas has favored the development of variable strains.

Apparently there have been no attempts made to establish a clear cut relationship among the leptospiroae, or the basis of antigenicity and pathogenicity. This work could be undertaken by painstaking and diligent studies. One could collect type cultures from the different types of leptospirosis that have been noted—especially the pathogenic leptospiroae reported from the Far East. The first step in such a study would be to inoculate each of these diverse strains of leptospiroae into groups of guinea-pigs. Repeated passages would be necessary until each type of organism manifested a constant degree of pathogenicity. The organisms should then be isolated from the test animals and grown in liquid culture medium. Antisera for each of these strains should then be prepared in leptospirosis-free rabbits, and the organisms tested with homologous as well as heterologous antisera. Adsorption testing should follow. This method of studying the leptospiroae should throw much more light on their relationships to one another, since a good deal of reversibility, especially among the so-called miscellaneous leptospiroae, to the more orthodox strains might be noted.

Another problem that remains unanswered in connection with leptospiral diseases, is the pathogenesis of the organisms. Basile (1921), Stavitsky (1945) and many others have attempted to demonstrate the
endotoxin that causes the adverse effects in the host. To date, there has been no toxic agent isolated. The answer to the isolation and demonstration of the toxic agent lies in the field of chemistry. Until methods are devised to isolate the agent in pure form, the question of pathogenesis will remain in its present status. Perhaps more light will be shed on the treatment of leptospirosis once the nature of the toxicity is known.
Summary and Conclusions

Through the ages, a disease that has usually been associated with epidemics, and frequently described by such names as "typhus icterode", "yellow fever", and "acute atrophy of the liver" was shown by Inada, et al. (1916) to be leptospirosis.

Noguchi (1917) demonstrated that the classical organism was carried by wild rats (Rattus norvegicus). He showed that this organism was morphologically and immunologically identical with the organism isolated by Inada. The organism was shown to belong to the order Spirochaetales; hence, named Leptospira icterohaemorrhagiae. It has been demonstrated that the leptospiroae are a group of organisms that are morphologically identical. Their differences can be noted by their pathogenicity and antigenicity. One member of the group (Leptospira biflexa) was shown to be immunological distinct and unique in its nonpathogenic qualities.

Metabolic studies of the pathogenic leptospiroae have shown them to be facultative aerobes, and very fastidious in their growth requirements. In vitro cultures require one or more of the thermolabile factors from the serum of rabbits, horses, guinea-pigs or humans. Optimal growth occurs at a pH of 7.2 and a temperature range from thirty to thirty-seven degrees Centigrade. The enzyme systems of the pathogenic leptospiroae allow them to utilize proteins exclusively. Optimum growth requires a period of seven days to reach its peak in contrast to twenty-four hours for most of the other microorganisms.

The organisms are readily demonstrated by darkfield (from
liquid cultures) as fine filaments with tapering ends, which may closely coil upon themselves to form S-shapes and figures-of-eight. They do not stain with the usual aniline dyes, but can be demonstrated with Romanovsky stains and silver impregnation methods.

Immunological studies show that the pathogenic leptospires may arbitrarily be placed in two main groups. The first group may be called the Cosmopolitan Group, which includes *Leptospira icterohaemorrhagia*, *Leptospira canicola* and *Leptospira grippotyphosa*. The second group may be called the Far East Group, which includes *Leptospira hebdomadis*, *Leptospira autumnalis*, and *Leptospira febrilis* (*Leptospira pyrogenes*). The specificity of these organisms, from immunological aspects, is a matter of relativity since they show varying degrees of antigen-antibody reactions in their heterologous antisera.

The pathogenic leptospires also demonstrate varying degrees of pathogenicity depending both upon the species (and even strains) in question and the host infected. In general, the organisms are more virulent when they attack the liver, kidneys, and capillaries, causing icterus, uremia and haemorrhage in the susceptible host. This phenomenon can be correlated with the severity of the disease of the same organism strain in different hosts, and diverse leptospiral agents on the same host group. *Leptospira hebdomadis*, *Leptospira grippotyphosa*, and the miscellaneous leptospires seen in Asia and the Far East seldom cause jaundice or the severe symptoms that the other leptospiral diseases produce in man and test animals.

In leptospiral infections, the aetiologic agent may be
demonstrated in the blood during the early or toxemic stage. The organisms settle in the liver and kidneys in the second or toxic stage. This stage is manifested by icterus, haemorrhage into the subcutaneous tissues, and uremia while the host is alive. In fatal cases, the leptospirae may be demonstrated in the liver and kidneys. Haemorrhages are seen throughout the internal organs. Antibodies are elicited in detectable quantities during the convalescent stage (seventh to twentieth day) of the disease, and can be detected in increasing titers for weeks thereafter.

*Leptospira canicola* is a primary infection of dogs, but has been found in man. The other leptospiral agents infect man in varying degrees of pathogenicity.

Lasting immunity is established in the host after an attack of leptospirosis. However, reinfection can occur by introduction with other groups of leptospirae. As a rule, reinfection occurs when the leptospira is only slightly related antigenically to the initial organism. Past infections may be detected serologically as long as twenty years after the initial infection.

Diagnosis of a leptospiral infection may be confirmed by darkfield examination and culture of the blood or emulsions of the kidney or liver; inoculation of these tissues reproduce the disease in guinea-pigs, hamsters, young mice (*Mus musculus*), and puppies. Antibodies may be demonstrated during convalescent and latent periods.

The treatment of leptospirosis, in practice, is usually none too satisfactory. Effective treatment must be started before the onset of icterus if the best response is to be obtained. Serum
therapy and penicillin are the most effective agents, but they must be given before the onset of icterus (first seven days) in order to be effective. The main problem here is that the disease usually isn't diagnosed until the toxic stage is manifested. General treatment consists of intravenous fluids and administration of agents that will ameliorate the symptoms.

Leptospirosis is endemic in Japan, Holland, South Central Europe, England, and in parts of the United States. The presence of the disease is seen in occupational groups that are in contact with the infective agent. Leptospira canicola infects dogs and those who are closely associated with dogs, i.e., veterinarians and dog owners. Weil's disease is most prevalent in those whose occupations bring them in proximity to infected rats. Leptospira grippotyphosa and Leptospira hebdomadis are infective for field-workers who contract the disease from viable leptospiroae in the water and soil where they labor. Chance infections are contracted by bathers and water-accident victims where leptospiroae have been deposited in the water via rat excreta.

Prophylactic measures should include rat-proofing and extermination; cleanliness and disinfection in working establishments that are rat-infested; protective covering for the hands and feet of workers; active immunization in highly endemic localities; and health education of all persons who are likely to come in contact with pathogenic leptospiroae.
Abstract

The leptospiral infections from the aspects of the characteristics of the aetiologic agents, pathogenicity, immunity, diagnosis, treatment and epidemiology have been discussed. The type organism (Leptospira icterohaemorrhagiae) which belongs to the order Spirochaetales and family Treponemataceae is a strikingly flexible, motile microorganism measuring seven to fourteen microns in length with exceptionally long forms measuring from thirty to forty microns due to delayed transverse fission. The organisms are finely coiled with regular spirals measuring 0.45 to 0.5 micron in amplitude, and 0.3 micron in depth. There are one of more undulations throughout the entire length and the body culminates in finely pointed ends. The organism may be demonstrated as a fine tapering filament which may assume the forms of semicircles, figures-of-eight, and S-shapes from a liquid culture medium, and serpentine, undulating or bent forms from a semi-solid medium. The leptospira may be stained by Giemsa's or Fontana's silver impregnation methods. The leptospirae are morphologically undistinguishable. They are facultative aerobes and require one or more of the thermolabile factors in the serum of rabbits, horses, guinea-pigs or humans for growth. Optimal growth occurs at a pH of 7.2 and a temperature range from thirty to thirty-seven degrees Centigrade. The pathogenic leptospirae live exclusively on proteins. Optimum growth requires a period of seven days to reach its peak in contrast to twenty-four hours for most of the other microorganisms. The media in use today are either liquid or semi-solid that contain a 10 per cent concentration of suitable animal serum and are buffered to a pH of 7.2. Examples of these are Schuffner's...
liquid and Chang's (1947) semi-solid media. Studies of the antigenicity of the leptospirae show that they may arbitrarily be placed into two groups. The first group is called the Cosmopolitan group, which includes Leptospira icterohaemorrhagiae, Leptospira canicola, and Leptospira grippotyphosa. The second group is called the Far East Group, which includes Leptospira autumnalis, Leptospira hebdomadis, and Leptospira febrilis (Leptospira pyrogenes). The organisms are all antigenically related since they show varying degrees of antigen-antibody reactions in their heterologous antisera. One species of leptospirae (Leptospira biflexa) is unique in its non-pathogenicity, simple growth requirements, and distinct antigenicity. The pathogenicity of the two groups of leptospirae depends upon the species (and even strains) in question and the host infected. The wild rat (Rattus species) is an asymptomatic host of the organisms. The organisms attack the liver, kidneys, and capillaries causing icterus, uremia, and haemorrhage in the susceptible host. Guinea-pigs, hamsters, and young albino mice (Mus musculus) are susceptible hosts to Leptospira icterohaemorrhagiae, and Leptospira canicola. They may be used as test animals for diagnosis of these agents. Leptospira canicola and Leptospira grippotyphosa, and the miscellaneous leptospirae of Asia seldom show icterus or the severe symptoms that the other leptospiral agents demonstrate in man and test animals. In leptospiral infections, the aetiological agent may be demonstrated in the blood during the primary toxicemic stage. The organisms are found in the liver and kidneys in the secondary stage. This stage is manifested by icterus, haemorrhage into the subcutaneous tissues, and uremia. Postmortem examination shows
haemorrhages of the internal organs. Agglutinins may be demonstrated in the tertiary stage (seventh to twentieth day of infection), and can be detected in increasing titers for weeks thereafter. Lasting immunity is established after an attack, and agglutinins have been detected as long as twenty years after the initial infection. The diagnostic agglutinin titer is 1:300 and may run as high as 1:30,000. Diagnosis of a leptospiral infection is confirmed by darkfield examination or culture of the blood during the primary stage. Inoculation of emulsions of kidney or liver from infected hosts into test animals will reproduce the disease. Antibodies may be detected during convalescent and latent periods. Effective treatment must be started before the onset of icterus for the best therapeutic response. Serum therapy has been used experimentally to protect guinea-pigs, hamsters and young white mice (Mus musculus) from infection. Penicillin in concentrations of 0.4 u/ml. has been shown to have a leptospiristatic effect on the organisms in vitro. Leptospira icterohaemorrhagiae has been shown to have a sensitivity variance to 0.1 - 70.5 u./ml. of penicillin when tested in vitro. Leptospira grippotyphosa was found to be sensitive to 0.25 u./ml., Leptospira canicola to 0.5 u./ml., and Leptospira bataviae to 0.1 u./ml. when tested in vitro. Guinea-pigs have been effectively treated against Leptospira icterohaemorrhagiae when 1500 units of penicillin a day was given early and in maintained doses. General treatment consists of intravenous fluids and the administration of agents that will ameliorate the symptoms. Leptospirosis is endemic in Japan, Holland, South Central Europe, England, and in parts of the United States. The disease prevails in groups that are in proximity to the infective agents. Lepto-
Leptospira canicola infects dogs and those who are closely associated with dogs, i.e., veterinarians, and dog owners. Weil's disease is seen in persons who are in proximity to infected rats. Leptospira grippotyphosa and Leptospira hebdomadis are noted among field workers who contact the disease from infective soil. Wild rats (Rattus species) have been shown to be infected with Leptospira icterohaemorrhagia in incidences of ten to thirty per cent from surveys conducted in Japan, Holland, England, and some of the major cities in the United States. Prophylactic measures should include rat proofing and extermination. Occupational groups should wear gloves to protect themselves from leptospiroa-containing urine. Field workers should protect their feet with boots. Rat excreta may be sterilized with sodium hypochlorite 1:64. Health education should be stressed in endemic areas.
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