PATHOGENICITY OF LEPTOSPIRA POMONA FOR HAMSTERS

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Joyce Elaine Trier

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PATHOGENICITY OF *LEPTOSPIRA POMONA* FOR HAMSTERS

By

Joyce Elaine Trier

AN ABSTRACT

Submitted to the College of Science and Arts Michigan State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

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The purpose of this study was to determine the effects of 4 different strains of Leptospira pomona upon young hamsters, especially the bacteriological, serological, and hematological aspects of the infection.

Four groups of 30 to 50 hamsters each, approximately 4 weeks of age, were infected with L. pomona, each group with a different strain of this serotype. One cc of a 1:5 dilution of guinea pig blood obtained at the febrile period of the infection was inoculated intraperitoneally into each animal. Control groups of animals were inoculated with a 1:5 dilution of normal guinea pig blood. At 2 or 3 days after infection and at suitable intervals thereafter groups of 5 animals were bled aseptically from the heart for duplicate cultures in modified Chang's media. Throughout the experiment, at intervals of several days, control hamsters were also sacrificed and hematological and serological studies performed on the bloods. The hamsters were then sacrificed and the jugular vein severed. One to 2 cc of this blood was introduced into approximately 10 mg of powdered heparin to be used for serological and hematological studies. The urine was observed for turbidity and in some cases the benzidine test was carried out. Normal hamster urine is turbid. It has been observed that urine of recently infected rodents becomes clear.

Hemoglobin, erythrocyte, and leucocyte values were determined from the blood and in some cases hematocrit levels were determined. Agglutination-lysis tests were performed on the plasma samples obtained from the remaining heparinized blood. After 2 and 4 weeks of incubation, the cultures were examined microscopically for the presence of leptospirae.

Strain Wickard produced a subclinical infection in hamsters with minor erythrocyte destruction and a mild leucocytosis. A high antibody titer was evidenced by day 13, which helped remove the organisms before extensive harm had occurred.

Strain Wickard (hamster lethal variant) caused widespread lysis of erythrocytes and a high mortality rate as early as day 6. Considerable leucocytosis occurred, but the antibody titer remained quite low in the early stages of the disease. The extreme lysis of erythrocytes with little antibody protection probably caused death.

Strain Brooks produced a subclinical infection in hamsters, and only minor hematological changes occurred. The high antibody titer by day 6 probably resulted in destruction of the leptospirae.

Strain Ohio was lethal, causing severe central nervous system disturbances and coma in all animals on day 7. There was severe lysis of erythrocytes and only minor leucocytosis. The lack of antibody development and the overwhelming effect of the organisms on the animals probably were the main factors causing death.

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INTRODUCTION

The leptospiroses, in the last 15 years, have received renewed and increased attention and interest throughout the world. In the United States, due to the prevalence of bovine and porcine Leptospira pomona infections, research efforts have been directed toward the elucidation of the host-parasite relationship, epizootiology, and public health aspects of this group of contagions.

Some reasons for increased interest in the leptospiroses are as follows: (1) rodents and domestic animals serve as resevoirs of infection, (2) there are many newly discovered species of Leptospira that infect both man and animals, (3) some diseases formerly thought to be viral in origin actually have been found to be due to leptospirae, (4) the leptospiroses may vary greatly in severity and assume bizarre clinical manifestations, and (5) the infections may be mistaken for brucellosis, typhoid fever, poliomyelitis, meningitis, and influenza.

LITERATURE REVIEW

Leptospirosis was first reported in farm animals in the United States less than 15 years ago (27). Today bovine and porcine leptospirosis is present in all states. However, long before this, other forms of leptospirosis had been found in various parts of the world. In 1905 Stimpson observed Weil's disease, or *Leptospira icterohemorrhagiae* infection of man (17). In 1923 canine leptospirosis was recognized in North America by Kirkwood and Horning (17). Schofield (30) described hemoglobinuria in dairy cows in Ontario, which he concluded was due to *Clostridium welchii*. Also in 1933 Rose and Edgar (29) described icterohemoglobinuria in calves in Australia and again concluded that *Cl. welchii* was the etiological agent. Further work failed to confirm these assumptions. These reports may have been dealing with bovine leptospirosis.

Michin and Azinov (21) in Russia reported spirochetal jaundice in cattle in North Caucasus during 1935. The organism which was isolated proved pathogenic for guinea pigs and mice. The authors indicated that infection was spread by the contamination of pastures, water, and salt licks by urine and feces, and that insects and rodents played a role in transmission. The agent was termed *L. icterohaemoglobinuriae*.

Brite, in 1942, reported in Kansas that a bovine hemoglobinuria of undetermined etiology affected cattle of all ages (4). The next year Smith (31) described an "idiopathic hemoglobinuria" of cattle in Oklahoma with a morbidity rate of 5 to 35 percent and a mortality rate which approached 100 percent. It, too, affected animals of all ages. The same year 3 "mysterious" deaths occurred in adult cattle in Connecticutt (17). The symptoms were anorexia, subnormal temperature, and convulsions on approximately the sixth day of illness. The livers had a "nutmeg" appearance. Multiple areas of sinusoidal congestion with hemorrhages and necrosis of the liver pyrenchyma were observed. Levaditi stained sections showed leptospiral-like bodies. This was the first report of bovine leptospirosis in the United States.

L. pomona, the organism under study in this paper, was first isolated in 1937 from a dairy farmer in Queensland, Australia by Clayton et al. (5). A mild type leptospirosis prevailed and in man was called the "seven day fever". The same organism was isolated from swine in Australia in 1942 by Johnson (16), and was incriminated as the etiological agent of Swineherd's disease by Gsell (12) in Switzerland.

Serological surveys have shown that L. pomona infections are widespread among cattle (7, 17, 22, 23, 28) and swine (3, 11, 23, 28). Isolations were made from a kidney

of an apparently healthy pig (3). Most porcine infections are subclinical; however, serious losses result in droves of pregnant sows which abort. Leptospirosis is communicable among swine and transmission readily occurs to other domestic animal species as has been shown experimentally. In addition to L. pomona, L. canicola and L. icterohemorrhagiae may be found as etiological agents in bovine (8, 15, 36) and porcine (9, 25) leptospirosis. It is, therefore, imperative that the infecting serotype be identified in all outbreaks of leptospirosis.

The course of leptospirosis is rather variable, but in general it follows a basic pattern in susceptible hosts. The incubation period is from 4 to 12 days, after which a demonstrable leptospiremia occurs. At this time there is a rise in body temperature to 104 to 106 F, depending on the species. Acute symptoms of diarrhea, anorexia, depression, and polypnea occur at this time. Hemoglobinuria and icterus occur at the end of the acute symptomatic phase. These clinical manifestations occur at about 7 to 12 days after infection. Antibodies also appear at this time and remain at high levels for about 3 weeks.

At day 12 to 14 a chronic leptospiruria begins and lasts about 3 months or longer, depending upon the host species, the infecting serotype, and the pH of the urine--pH 7.0 is optimal for the organisms. Kidney damage follows and grayish white foci up to 1 cm in diameter are found in

the cortices. Edema of the renal lymph nodes may also be observed, as reported by Langham et al. (18). Microscopically these foci contain lymphocytes, macrophages, and plasma cells. Some fibroblasts and collagenous fibers are also present. Degeneration and necrosis of the proximal and convoluted renal tubules is a common finding.

In pregnant animals abortion often occurs and does so at 20 to 30 days after exposure.

Morton (24), in 1942, first reported on the susceptibility of hamsters to leptospirae, in his work with L. canicola. These animals are most useful in isolating leptospirae from man and lower animals; however, Larson (19) indicated that L. canicola killed hamsters. In 1944 Randall and Cooper (26) reported that the hamster was the animal of choice for isolating leptospirae; guinea pigs and mice are relatively resistant, while laboratory white rats are totally refractory. Both L. canicola and L. icterohemorrhagiae killed hamsters in 5 to 10 days.

Hamdy and Ferguson (13) reported that hamsters survived infections with L. pomona, Hardacre strain, following 15 serial hamster passages. On the sixteenth passage the animals died on the fourth day after inoculation. A virulence decrease for cattle was observed on the twenty-third hamster passage.

According to Bauer (2), strain Wickard, which causes a mild infection in hamsters, becomes lethal after 4 pass-

ages in media. After 12 passages a steady decrease in virulence was noted. He also found that 4, 7, and 15 week old hamsters were very susceptible to L. pomona, and that the susceptibility of hamster erythrocytes to leptospira hemolysin was not associated with the hemolytic effect of the organisms upon the hamsters.

Very little work has been reported in the literature concerning hematological values of normal hamsters. Material for Table I was taken from a review of the literature by Gardner (10), as contributed by Stein (33, 34), Stewart (35), and Hu (14).

This thesis deals with the relationships between the following bacteriological, serological and hematological aspects of leptospirosis (L. pomona) in hamsters during the course of infection:

1. Hemoglobin, leucocyte and erythrocyte values of hamsters infected with the various strains.
2. Cultures of the heart blood which demonstrate presence or absence of leptospira in the blood.
3. Agglutination-lysis tests which indicate antibody formation.
4. Appearance of the urine, whether turbid or non-turbid, and examination for hemoglobin and for leptospirae.

TABLE I

NORMAL BLOOD VALUES OF HAMSTERS

Hamsters Age	No, Sex	Blood Values Erythrocytes[1] Mean	Range	Leucocytes[2]	Hemoglobin[3]	Reported By
20 days	2Male	5.06	5.03-5.08			Stein
33-34 da	5M	5.96	5.36-6.69			Stein
36-37 da	4M				17.04	Stein
1-2 mo	12M	7.0		7,470	16.4	Stewart
1-2 mo	11F	7.01		8,210	16.9	Stewart
40-43 da	3M	6.98	6.4-7.55			Stein
47-50 da	3M				18.3	Stein
57-62 da	6M	8.60	8.16-9.29			Stein
66-70 da	5M				18.98	Stein
85-100 da	5M	9.14	9.06-9.22			Stein
2-4 mo	25M	7.50		8,150	17.1	Stewart
2-4 mo	24F	7.10		7,870	16.4	Stewart
108-131 da	5M				15.65	Stein
140-226 da	M	8.8				Stein
9 mo plus	24M-F	7.5		8,560	17.6	Stewart
Adult	10	9.57		10,070		Hu
Adult	M				20.6	Stein

[1] Multiply by 10^6 for erythrocytes per cmm blood
[2] Leucocytes per cmm blood
[3] Grams hemoglobin per 100 cc blood

MATERIALS AND METHODS

In the first experiment 50 hamsters approximately 4 weeks of age were inoculated intraperitoneally with L. pomona, strain Wickard. The inocula consisted of 1 cc of a 1:5 dilution in sterile saline of infected guinea pig blood obtained at the height of the febrile response. Strain Wickard was isolated from the urine of an infected dairy cow in Wisconsin during 1953 by Morse et al. (22). The microorganism had been maintained in continuous guinea pig passage to assure retention of virulence. This strain was not lethal for hamsters. Thirty hamsters, which were similarly inoculated with 1 cc of a 1:5 dilution of normal guinea pig blood, served as controls for hematological and serological data.

In the second experiment 35 hamsters, approximately 4 weeks of age, were inoculated intraperitoneally with 1 cc of a 1:5 dilution of guinea pig blood infected with the Wickard strain (above) which had been found to be lethal for hamsters, as described by Bauer (2). Twenty additional hamsters were inoculated with 1 cc of a 1:5 dilution of normal guinea pig blood to serve as controls.

In the third experiment 30 hamsters, approximately 4 weeks of age, were inoculated intraperitoneally with 1 cc of a 1:5 dilution of guinea pig blood infected with L.

pomona, strain Brooks. This was the sixth guinea pig passage of this microorganism following isolation from bovine urine in Michigan. Similarly, 20 hamsters were inoculated with a 1:5 dilution of normal guinea pig blood to serve as controls.

In the fourth experiment 30 hamsters, approximately 4 weeks of age, were inoculated intraperitoneally with L. pomona, strain Ohio. The inocula consisted of 1 cc of a 1:5 dilution of infected guinea pig blood. Twenty hamsters, to serve as controls, were similarly inoculated with 1 cc of a 1:5 dilution of normal guinea pig blood.

Determinations as to the exact number of leptospirae present in guinea pig blood inocula were not made. However, previous work in this laboratory has shown that 10^4 to 10^5 leptospirae per cc are present in the blood of guinea pigs at the height of the febrile response (105-106 F).

In each of the four experiments, groups of five animals were sacrificed 3 days after inoculation and at appropriate intervals thereafter. The intervals depended upon the course of infection. All sick or moribund animals were killed as soon as detected. The hamsters were anesthetized with ether and then bled aseptically from the heart on the days when leptospiremia was expected. Blood was inoculated in modified Chang's media (23) which contained 10 percent sterile bovine serum and .01 percent hemoglobin (Difco). Two screw cap tubes, each containing 10 cc of media were inoc-

ulated with 0.02 to 0.1 cc of blood. The cultures were incubated at 30 C and examined for the presence of leptospirae at 2 and 4 weeks employing darkfield microscopy (590X).

Hamsters were exsanguinated and the blood collected in tubes containing approximately 10 mg of powdered heparin. Approximately 1-2 cc were obtained in this way for hematological and serological examinations.

The urine was collected where possible and examined for turbidity and the presence of leptospirae by darkfield microscopy (590X).

Hemoglobin values were determined by placing 0.02 cc of the heparinized blood in a tube containing 5 cc distilled water. A drop (0.025-0.04 cc) of ammonium hydroxide was added per tube and the tubes were shaken vigorously for 10 seconds. The tubes were then allowed to stand 10 minutes and were read on a Bausch and Lomb Spectronic 20 at 465 mu. This is an oxyhemoglobin method.

Erythrocyte counts were made using a Spencer Briteline counting chamber. Heparinized blood, 0.005 cc, was diluted to 1.01 cc with 0.85 percent saline or Hayem's solution in a red cell pipette. Five-hundredths cc of heparinized blood was diluted to 1.1 cc with a 1 percent acetic acid solution for counting leucocytes.

The agglutination-lysis test was conducted on all plasma samples. Living cultures of L. pomona, L. canicola,

and L. icterohemorrhagiae AB were used as antigens in the tests. The plasma was diluted from 10^{-1} through 10^{-8}, the antigen added, and the tubes were incubated in a 37 C waterbath for 2 hours. The mixture was then examined for agglutination-lysis using a microscope with an Abbe condensor fitted with a star diaphragm (100X). This provided a modified darkfield arrangement.

RESULTS

Part I. Strain Wickard

The Wickard strain produced only minor hematological changes following intraperitoneal inoculation in healthy young hamsters. During the first 13 days after inoculation, the hemoglobin and red cell values were quite constant, varying from 15.1 to 17.1 gm hemoglobin, and from 6,650,000 to 7,352,000 red blood cells. These were minimal and maximal averages of the groups of 5 sacrificed animals for the different days.

At the same time the controls ranged from 16.1 to 16.5 gm hemoglobin and 7,042,000 to 7,142,000 erythrocytes.

On the fifteenth day a decrease in the hemoglobin and erythrocyte values occurred for infected animals; 11.3 to 15.7 gm hemoglobin as the range and 13.5 as the average. The erythrocytes ranged from 4,230,000 to 6,430,000 with an average of 5,464,000 cells. On the twenty-first and twenty-fifth days the counts were slightly elevated (Table II).

The leucocyte counts showed considerable variation. The second day the values were very low for some unexplained reason, perhaps stress. The levels at this time averaged 3,825. Aside from this, they remained at about 6,000 to 7,000 until day 10 when an increase to 10,300 was obser-

ved. A gradual decrease occurred until day 25, when again an increase to 11,420 was noted. The controls remained quite constant at approximately 5,000 to 7,000 per cmm.

Antibody titers, as determined by agglutination-lysis (A-L) tests, rose slowly from day 7 until day 13, when a maximum of 10^{-6} was recorded. At this time there was some drop in red blood cells and hemoglobin. The titer decreased slightly in the following days (Table II). Plasma from the non-infected animals was used as a negative control and it remained serologically negative.

The cultures were examined at the indicated time intervals. Three of 5 animals were positive as early as day 2 following inoculation. On day 7 only one of five was positive, and thereafter no positive cultures were obtained. Therefore, 5 days before any drop in hemoglobin and erythrocytes, the leptospirae were not present in the blood and had presumeably become localized in the kidneys. However, no leptospirae were found in the urine by darkfield examination at any time. Hemoglobin was not found in the urine as determined by the benzidine test. The animals did not show any clinical symptoms or signs. The titers started to decline and the blood values approached normal. This would indicate that a subclinical disease occurred in hamsters infected with L. pomona, Wickard.

Part II. Strain Wickard (Hamster Lethal)

A variant of the Wickard strain, lethal for hamsters, was isolated by Bauer (2). This variant, when inoculated intraperitoneally into hamsters, gave rise to more pronounced hematological changes than the nonlethal parent strain. Hematological values until day 6 were fairly constant and compared closely with the controls. On day 7 the animals were moribund and one died. Hemoglobin values averaged 11.9 gm with an average of 5,587,000 erythrocytes which represented a decrease of 3.4 gm hemoglobin and 1,517,000 red cells from the averages for the animals sacrificed on the previous day. That evening 4 more animals were found sick and sacrificed. The hemoglobin and erythrocyte values decreased to 10.5 gm and 5,362,000 respectively, which was a significant depression in a few hours. One animal was ill on day 8; the red cell count was depressed to 3,980,000. For the next 2 days the hemoglobin and red blood cell counts rose to 21.1 and 9,250,000 respectively, due to hemoconcentration. These animals were moribund and very little blood could be obtained at sacrifice.

The leucocyte counts rose from an average of 8,090 on day 6 to 11,430 on day 7, and then to 28,300 on day 8. Then they decreased to averages of 7,120 and 6,300 on days 9 and 10 respectively, but increased slightly to over 9,000 on days 11 and 12. The 4 remaining animals appeared normal on the 2 last days of the experiment, days 11 and 12 (Table III).

On day 6 the urine of 4 of 5 animals appeared non-turbid and 2 samples were positive for hemoglobin. The turbid or normal urine of 1 hamster was negative for hemoglobin. It was not possible to obtain urine from 2 animals.

On days 7 and 8 urine samples were red in color; on days 9 and 10 urines were non-turbid again, but the benzidine tests were positive. On days 11 and 12 the urines were still partially cloudy or approaching the normal.

The antibody titers rose gradually to 10^{-7} on day 7 and 10^{-8} on day 9. They decreased to 10^{-5} and 10^{-4} on days 11 and 12 respectively. There were positive blood cultures on 4 of 5 animals on days 3, 5, and 6. It was not possible to obtain sufficient blood on succeeding days to make suitable cultures. The agglutination-lysis tests for the controls were negative throughout the experiment.

Part III. Strain Brooks

The Brooks strain of L. pomona produced slight hematological change in hamsters. The hemoglobin and red cell values and leucocyte counts compared closely with the controls through day 20. The average values of the infected as well as the control animals were somewhat lower than those of the hamsters used in Part I and Part II.

The antibody titers increased from 10^{-1} on day 3 to 10^{-6} on day 6. These receded to 10^{-5} on days 10, 15, and 20.

Media were inoculated with the blood from all animals but none proved to be bacteriologically positive. No clinical symptoms were evidenced and the hematocrit values compared quite well with those of the controls (Table IV).

Part IV. Strain Ohio

The Ohio strain, although lethal for hamsters, produced little observable change in the blood constituent values. However, on some days considerable variations among different animals did occur. Through day 6 the values were found to be quite normal; but on day 7 the range varied from 9.3 gm hemoglobin to 19.9 gm and from 4,910,000 to 9,320,000 red cells. These animals were moribund, and the hematocrit value for the animal having 19.9 gm hemoglobin was 63 percent. This was considerably higher than the expected normal of 46 percent, and indicated considerable hemoconcentration (Table V).

Other hamsters killed at 1:00 P.M. and 4:00 P.M. of day 7 had hemoglobin levels of 18.1 and 16.9 gm, while the red cell counts were 7,063,000 and 7,440,000 respectively. Controls on day 7 averaged 16.0 gm hemoglobin and 6,743,000 red blood cells. Again hemoconcentration was in evidence for the infected group.

The white cell count rose from 7,350 on day 6 to 16,040 on day 7 at 8:00 A.M., while the maximum was 26,000. The averages remained high for the remaining hamsters killed on that day.

The antibody titer rose to 10^{-5} on day 7 (4:00 P.M.). Cultures were positive in most cases, for 28 of 30 animals, from day 5 through day 7. The animals became moribund and died before the agent left the blood.

As early as the fifth day, the animals showed extreme central nervous system disturbances. The legs stiffened in extensor rigidity, the head was held back, and paralysis occurred. In some cases the animals trembled violently. The urine was non-turbid but did not contain visible hemoglobin.

DISCUSSION AND CONCLUSIONS

The hemoglobin values obtained using the Wickard strain as the infectious agent varied only slightly from the controls. The values remained constant until day 15, and then decreased to a minimum of 11.3 gm. Shortly thereafter a rise occurred, indicating that hemoglobin loss was slight, transient and probably minimal. The erythrocyte values dropped proportionately; 4,230,000 being the lowest noted on day 15 when again the normal level was gradually attained. This suggests that some degree of lysis of cells occurred around the fifteenth day, but this, too, was transient. This reaction was probably caused by the presence of leptospiral components in the blood which were released when the organisms were lysed by the antibody. The antibody titer rose significantly on day 13, which would tend to support this concept. It is assumed here that decreases in hemoglobin and erythrocytes is due to lysis of the cells; however it is possible that some of this may be caused by a depressed state of the blood forming organs due to the infection.

A leucocytosis was evidenced on day 10. This may have been stimulated by the migration and localization of leptospirae in the kidneys. The diffuse nephritis produced during renal residence would tend to lead to a leucocytosis. Approximately 3 days would allow for multiplication and development of renal inflammatory reactions.

Strain Wickard caused a subclinical infection in hamsters as shown by the following observations:

1. There was no apparent illness, and no apparent loss in body weight.
2. Leptospiremia was of low degree and insufficient organisms were present at day 15 to cause a significant drop in hemoglobin and red cell values. Hematuria or hemoglobinuria was absent.
3. A relatively mild leucocytosis occurred, which suggests that renal inflammatory reactions due to leptospiral colonization had occurred on day 15.
4. The high titer which developed by day 13 was responsible for removing leptospirae from the blood.

The infected hamsters reacted quite differently to Wickard strain which is lethal to hamsters. The hemoglobin levels remained quite constant until day 7, at which time the values fell rapidly to 11.9 gm. Within 9 hours the average levels were 10.5 gm; the lowest was 7.3 gm. The red cell counts decreased accordingly; the lowest was 4,100,000.

On day 6, 4/5 of the animals were positive and on day 7 in the morning, 2/3 were positive. Hereafter it was impossible to obtain enough blood for cultures; therefore, it was difficult to conclude whether the organisms were beginning to leave the blood on day 7. The enormous numbers of organisms in the animals probably led to the lysis of

erythrocytes, and the leucocytosis which reached 28,300 cells probably resulted from the severe nephritis.

Hemoconcentration was observed when the animals became moribund. This may have been a result of dehydration due to the toxic effects of the disease. Accurate hematological determinations were difficult.

The antibody titers increased, but not fast enough to counteract leptospiremia which eventually overwhelmed the animals.

It is suggested that death occurs with this strain due to:

1. Lack of development of antibody titer during the earlier phases of infection.
2. The considerable number of organisms which caused widespread lysis of erythrocytes.
3. Erythrocyte destruction was further evidenced by hemoglobinuria as indicated by positive benzidine tests and the red color of the urine.

The Brooks strain also produced a subclinical infection since the hamsters did not become visibly ill or moribund. Also significant decreases in hemoglobin or red cells were not noted, which suggests that there were insufficient organisms present to cause lysis of erythrocytes. On day 20 two samples were lower than normal. At this time leptospirae would not normally be present in the blood. The titer increased quickly to 10^{-6}. This may also have influ-

enced the course of the infection. Since cultures of blood were negative, it might be conjectured that the leptospirae were removed from the blood before day 3, and were localized in the kidneys.

Leucocyte counts remained low in this experiment which indicates that the kidney infection was probably minimal.

The Brooks strain was determined to be relatively non-pathogenic for hamsters as evidenced by:

1. Lack of lysis of erythrocytes.
2. Only minimal concentration of organisms in the kidney and probably a low grade renal damage as evidenced by a normal leucocyte count.
3. Rapid increase in antibody titer to 10^{-6} on day 6.

Strain Ohio was shown to be very pathogenic for hamsters, since death often occurred by day 7. The hemoglobin and erythrocyte values decreased to a low of 9.3 gm hemoglobin and 4,910,000 red cells on day 7, after normal values were recorded on day 6. Shortly thereafter increases to 20 gm hemoglobin and 9,300,000 erythrocytes were observed which indicated hemoconcentration. The hematocrit values substantiated this conclusion. The antibody titer increased to 10^{-5} on day 7. This lack of antibody may have hastened death by allowing the leptospirae to overwhelm the hamsters. Blood cultures were all positive through day 7, which indicated that the agent was still present in the blood at the time of death. In contradiction the average

white cell count rose, which may indicate kidney localization as well as severe generalized infection. Upon closer examination, however, it can be seen that the high white cell counts occurred only where hemoconcentration was evidenced.

Non-turbid urine, which is abnormal, was observed for hamsters which died. The urine would have undoubtedly become red due to the presence of large amounts of hemoglobin, had the animals survived. Death occurred before organisms left the blood and migrated with definite renal localization.

It is suggested that hamsters die of infection with Strain Ohio due to:

1. Lack of antibody development to prevent the overwhelming leptospiremia.
2. Lysis of erythrocytes.
3. Damage to the central nervous system, as evidenced by coma and paralysis. This may be due to toxic principles elaborated *in vivo*, but not demonstrable *in vitro*.

SUMMARY

The purpose of the investigation was to ascertain the hematological and serological effects caused by infection of hamsters with L. pomona, and to examine the differences in pathogenicity of various strains of this serotype.

Four experiments were conducted. In each 30 to 50 young hamsters were inoculated with L. pomona, strain Wickard, strain Wickard (hamster lethal), strain Brooks, or strain Ohio. At indicated intervals, hamsters were sacrificed and bacterological, serological, and hematological studies performed.

Strain Wickard caused only minor hematological changes, but marked antibody titers developed by day 13. Beginning on day 10 and continuing to the conclusion of the experiment, small decreases in erythrocyte and hemoglobin values occurred; and small increases in white cell counts were noted. This strain was shown to produce a subclinical infection in hamsters.

Strain Wickard (hamster lethal) produced much more severe hematological damage. The organisms produced death within 7 to 10 days. The blood constituents were as low as 7.3 gm hemoglobin and 3,980,000 red cells. The leucocyte counts increased moderately. Antibody titers of 10^{-7} were observed on day 7.

Strain Brooks produced very little erythrocyte lysis or observable illness in hamsters. Antibody levels increased to 10^{-6} by day 7, which may have had a limiting effect upon the infection. It is suggested that the very mildest of subclinical infections occurred.

Strain Ohio infections proved to be the most pathogenic and resulted in considerable hematological change. Minimal levels of 9.3 gm hemoglobin and 4,910,000 erythrocytes were observed. A leucocytosis occurred but this may have been the result of hemoconcentration. This strain was proved to be lethal for all animals by day 7. Severe central nervous system disturbances were noted. Failure of the host to produce agglutinin-lysins may have been responsible for, or at least contributory to, the lethal effect. The highest titers observed were 10^{-3}.

TABLE II

AVERAGE HEMATOLOGICAL AND AGGLUTINATION VALUES FOR HAMSTERS INFECTED WITH STRAIN WICKARD AND CONTROLS*

Day	Hemoglobin[1]	Erythrocytes[2]	Leucocytes[2]	Aggl'n Titer	Cultures[3]	Remarks
2	16.3	6,650,000	3,825	Neg.	3/5	
2C'	16.5	7,110,000	5,200	Neg.	---	
4	15.6	7,046,000	6,630	Neg.	0/5	
6	16.3	6,870,000	5,840	Neg.	3/5	
7	15.8	6,980,000	6,700	10^{-3}†	1/5	
8	17.1	7,352,000	6,430	10^{-3}	0/5	
8C	16.1	7,042,000	5,940	Neg.	---	
10	15.1	7,250,000	10,300	10^{-4}	0/5	
13	15.3	6,836,000	9,840	10^{-7}	---	
15	13.5	5,464,000	7,020	10^{-6}	---	
15C	16.2	7,142,000	6,950	Neg.	---	
21	15.0	6,910,000	7,970	10^{-6}	---	
25	14.3	6,107,000	11,420	10^{-6}	---	6 Hamsters
25C	16.2	7,384,000	6,590	Neg.	---	

* Values based on the average for 5 animals sacrificed on the various days except where noted.
1 Grams hemoglobin per 100 cc blood.
2 Number of cells per cmm blood.
3 Numerator--number of animals with positive cultures. Denominator--number of animals tested.
' C represents values for control animals.
† Majority of reactions in all tube dilutions 25% agglutination.

TABLE III

AVERAGE HEMATOLOGICAL AND AGGLUTINATION VALUES FOR HAMSTERS INFECTED WITH STRAIN WICKARD (HAMSTER LETHAL) AND CONTROLS*

Day	Hemoglobin[1]	Erythrocytes[2]	Leucocytes[2]	Aggl'n Titer	Cultures[3]	Remarks
3	14.9	7,126,000	6,410	10^{-1}	4/5	
3C'	15.8	6,940,000	6,640	Neg.	---	
5	16.0	6,898,000	6,940	10^{-2} †	4/5	
6	15.3	7,104,000	8,090	10^{-4} ‡‡	4/5	
6C	15.7	7,290,000	7,050	Neg.	---	
7 AM	11.9	5,587,000	11,430	10^{-5}	---	3 Hamsters, M
7 PM	10.5	5,362,000	10,900	10^{-7}	---	4 Ham., M
8	15.5	3,980,000	28,300	10^{-7}	---	1 Ham., M
8C	16.7	7,332,000	7,170	Neg.	---	
9	20.0	9,035,000	7,120	10^{-8}	---	2 Ham., M
10	21.1	9,250,000	6,300	10^{-8}	---	1 Ham., M
11	18.8	8,135,000	9,375	10^{-5}	---	2 Ham.
12	18.0	7,790,000	9,125	10^{-4}	---	2 Ham.
12C	15.2	6,560,000	7,110	Neg.	---	

* Values based on the average for 5 animals sacrificed on the various days except where noted.
1 Grams hemoglobin per 100 cc blood.
2 Number of cells per cmm blood.
3 Numerator--number of animals with positive cultures. Denominator--number of animals tested.
' C represents values for control animals.
† Reactions in all dilutions no greater than 25% aggl'n.
‡‡ Complete or 100% agglutination no higher than 10^{-2}.
M Moribund.

TABLE IV

AVERAGE HEMATOLOGICAL AND AGGLUTINATION VALUES FOR HAMSTERS INFECTED WITH STRAIN BROOKS AND CONTROLS*

Day	Hemoglobin[1]	Erythrocytes[2]	Leucocytes[2]	Aggl'n Titer	Cultures[3]
3	14.6	5,626,000	8,310	Neg.	0/5
3C'	14.4	6,103,000	7,370	Neg.	---
6	14.5	5,894,000	7,070	10^{-6}	0/5
6C	13.7	6,460,000	6,600	Neg.	---
10	13.6	5,532,000	6,920	10^{-6}	0/5
10C	15.3	6,513,000	7,600	Neg.	---
15	14.9	6,446,000	6,940	10^{-6}	0/5
15C	15.6	6,707,000	7,130	Neg.	---
20	14.9	6,541,000	7,183	10^{-6}	0/5
20C	16.0	7,533,000	6,470	Neg.	---

* Values based on the average for 5 infected animals and 3 control animals sacrificed on the various days.
1 Grams hemoglobin per 100 cc blood.
2 Number of cells per cmm blood.
3 Numerator--number of animals with positive cultures. Denominator--number of animals tested.
' C represents values for control animals.

TABLE V

AVERAGE HEMATOLOGICAL AND AGGLUTINATION VALUES FOR HAMSTERS INFECTED WITH STRAIN OHIO AND CONTROLS*

Day	Hemoglobin[1]	Erythrocytes[2]	Leucocytes[2]	Aggl'n Titer	Cultures[3]	Remarks
3	15.0	6,308,000	6,660	Neg.	1/5	
3C'	14.7	6,270,000	7,820	Neg.	---	
5	14.9	6,046,000	6,950	Neg.	5/5	
5C	14.9	6,573,000	6,830	Neg.	---	
6	15.8	6,636,000	7,350	10^{-1} †	4/5	
7 AM	15.5	7,287,000	16,040	10^{-2} †	5/5	Moribund
7 1PM	18.1	7,063,000	18,558	10^{-2} ††	7/7	7 Hamsters, M
7 4PM	16.9	7,440,000	16,610	10^{-2} ††	6/7	7 Ham., M
7C	16.0	6,743,000	7,280	Neg.	---	

* Values based on the average for 5 infected animals and 3 control animals sacrificed on the various days.
1 Grams hemoglobin per 100 cc blood.
2 Number of cells per cmm blood.
3 Numerator--number of animals with positive cultures; Denominator--number of animals tested.
' C represents values for control animals.
M Moribund.
† Reactions in all dilutions no greater than 25% aggl'n.
†† Only one serum had 50% at 10^{-1}; other reactions all 25% agglutination.

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APPENDIX

Detailed Data Pertaining to the Investigations

TABLE VI
HEMATOLOGICAL AND SEROLOGICAL VALUES--STRAIN WICKARD*

Day		Hemoglobin[1]	Erythrocytes[2]	Leucocytes[3]	Agglʼn Titer	Cultures[4]		Remarks
	H	17.1	7.03	4,900	Neg.	3/5	1(++)x	
2	M	16.2	6.65	4,400	Neg.		2(+-)	
	L	15.3	6.11	2,900	Neg.		2(--)	
	H	17.1	7.50	6,150	Neg.	---		
2C'	M	16.6	7.08	5,150	Neg.			
	L	15.7	6.75	4,350	Neg.			
	H	16.4	7.38	7,700	Neg.	0/5	5(--)	
4	M	15.7	7.02	6,600	Neg.			
	L	13.7	6.68	5,500	Neg.			
	H	17.1	7.48	7,200	10^{-1}	3/5	3(+-)	
6	M	16.2	7.01	5,500	Neg.		2(--)	
	L	15.3	6.06	4,450	Neg.			
	H	16.6	7.75	8,550	10^{-3}	1/5	1(+-)	
7	M	16.2	7.20	7,350	10^{-3}		4(--)	
	L	13.7	5.76	4,100	10^{-2}			
	H	18.1	7.60	8,750	10^{-3}	0/5	5(--)	
8	M	16.6	7.43	5,650	10^{-3}			
	L	15.3	6.78	4,400	10^{-3}			
	H	17.1	8.13	7,000	Neg.	---		
8C	M	16.2	7.02	6,250	Neg.			
	L	15.3	5.83	4,500	Neg.			
	H	16.2	7.89	11,750	10^{-4}	0/5	5(--)	
10	M	15.7	7.65	10,050	10^{-4}			
	L	13.0	6.41	9,600	10^{-4}			
	H	16.2	7.62	12,000	10^{-7}	---		
13	M	15.3	6.68	9,350	10^{-6}			
	L	14.5	6.25	7,650	10^{-5}			
	H	15.7	6.43	10,200	10^{-6}	---		
15	M	13.7	5.75	6,150	10^{-6}			
	L	11.3	4.23	4,000	10^{-5}			
	H	17.6	7.83	7,850	Neg.	---		
15C	M	15.7	7.55	7,000	Neg.			
	L	15.3	5.91	5,700	Neg.			
	H	15.7	7.90	9,050	10^{-6}	---		
21	M	15.0	6.70	7,950	10^{-6}			
	L	13.7	6.17	6,250	10^{-5}			
	H	16.2	6.98	14,500	10^{-6}	---		
25	M	14.5	6.03	13,550	10^{-6}			6 Hamsters
	L	12.0	4.83	6,750	10^{-5}			
	H	18.1	8.50	9,100	Neg.	---		
25C	M	16.6	7.68	6,700	Neg.			
	L	14.1	6.10	4,000	Neg.			

* Values represent the high, median and low values.
1 Grams hemoglobin per 100 cc blood.
2 Multiply X 10^6 for red cells per cmm blood.
3 Number of white cells per cmm blood.
4 Number positive animals/number animals tested.
x Duplicate tubes; number animals per tube combination.
' C represents values for control animals.

TABLE VII

HEMATOLOGICAL AND SEROLOGICAL VALUES--

STRAIN WICKARD (HAMSTER LETHAL)*

Day		Hemoglobin[1]	Erythrocytes[2]	Leucocytes[3]	Aggl'n Titer	Cultures[4]		Remarks
	H	15.3	7.55	8,550	10^{-1}	4/5	4(+-)[x]	
3	M	15.0	7.08	6,350	10^{-1}		1(--)	
	L	14.5	6.46	4,600	Neg.			
	H	16.6	7.32	8,750	Neg.	---		
3C'	M	15.7	7.08	6,200	Neg.			
	L	15.0	6.45	5,600	Neg.			
	H	16.2	7.18	9,050	10^{-2}	4/5	1(++)	
5	M	16.2	6.95	6,600	10^{-1}		3(+-)	
	L	15.3	6.40	5,800	10^{-1}		1(--)	
	H	15.7	7.35	8,800	10^{-5}	4/5	4(+-)	
6	M	15.3	7.20	8,300	10^{-4}		1(--)	
	L	15.0	6.43	6,900	10^{-2}			
	H	16.2	7.55	8,400	Neg.	---		
6C	M	15.7	7.40	6,600	Neg.			
	L	15.3	6.92	6,150	Neg.			
	H	13.7	6.48	12,650	10^{-6}	---		3 Hamsters
7 AM	M	12.0	5.78	11,500	10^{-6}			1 dead, 3 ill
	L	10.1	4.50	10,150	10^{-5}			Bloody urine
	H	14.1	7.65	12,500	10^{-8}	---		4 Hamsters
7 PM	M	10.1	4.55	10,850	10^{-8}			1 dead, 4 ill
	L	7.3	4.10	8,200	10^{-6}			Bloody urine
8		15.3	3.98	28,300	10^{-7}	---		1 ill Bloody urine
	H	17.1	7.75	8,900	Neg.	---		
8C	M	16.6	7.25	7,150	Neg.			
	L	16.2	7.02	5,800	Neg.			
9	H	20.4	9.57	7,900	10^{-8}	---		2 ill
	L	19.6	8.50	6,350	10^{-7}			Urine clear
10		21.1	9.25	6,300	10^{-7}	---		Hematocrit-62% 1 ill
11	H	19.9	8.45	9,750	10^{-5}	---		Not ill--2
	L	17.6	7.82	9,000	10^{-5}			Urine turbid
12	H	19.3	8.40	10,000	10^{-5}	---		Not ill--2
	L	16.3	7.18	8,250	10^{-4}			Urine turbid
	H	16.3	7.41	8,000	Neg.	---		
12C	M	15.3	6.62	7,800	Neg.			
	L	13.7	5.55	4,850	Neg.			

* Values represent the high, median and low values.
1 Grams hemoglobin per 100 cc blood.
2 Multiply X 10^6 for red cells per cmm blood.
3 Number of white cells per cmm blood.
4 Number positive animals/number animals tested.
x Tubes in duplicate; number animals per tube combination.
' C represents values for control animals.

TABLE VIII

HEMATOLOGICAL AND SEROLOGICAL VALUES--STRAIN BROOKS*

Day		Hemoglobin[1]	Erythrocytes[2]	Leucocytes[3]	Aggl'n Titer	Cultures[4]		Remarks
	H	15.7	6.27	9,250	Neg.	0/5	5(--)x	
3	M	15.0	6.01	8,650	Neg.			
	L	12.3	4.51	6,850	Neg.			
	H	15.0	6.71	8,800	Neg.	---		
3C'	M	15.0	6.42	7,000	Neg.			3 Hamsters
	L	13.3	5.18	6,300	Neg.			
	H	15.0	6.14	9,250	10^{-7}	0/5	5(--)	
6	M	14.5	6.05	6,750	10^{-6}			
	L	13.7	5.51	5,600	10^{-5}			
	H	14.1	6.75	7,700	Neg.	---		
6C	M	13.7	6.36	6,100	Neg.			3 Hamsters
	L	13.3	6.27	6,000	Neg.			
	H	--	5.78	8,750	10^{-6}	0/5	5(--)	
10	M	14.1	5.55	6,750	10^{-5}			Hematocrit--46%
	L	13.0	5.29	5,850	10^{-4}			
	H	16.6	6.86	9,900	Neg.	---		
10C	M	15.3	6.56	6,700	Neg.			3 Hamsters
	L	14.1	6.12	6,200	Neg.			
	H	15.7	6.90	8,300	10^{-7}	0/5	5(--)	
15	M	15.3	6.60	6,750	10^{-5}			
	L	13.3	5.81	5,550	Neg.			
	H	16.2	7.15	8,200	Neg.	---		Hemat.--52%
15C	M	15.7	6.63	8,150	Neg.			Hemat.--51%
	L	15.0	6.34	5,050	Neg.			3 Hamsters
	H	16.2	8.05	8,750	10^{-6}	0/6	6(--)	Hemat.--51%
20	M	14.5	6.55	7,600	10^{-5}			Hemat.--50%
	L	12.0	4.58	4,500	10^{-1}			6 Hamsters
	H	16.2	7.75	7,000	Neg.			Hemat.--45%
20C	M	16.2	7.65	6,200	Neg.			3 Hamsters
	L	15.7	7.20	6,200	Neg.			

* Values represent the high, median and low values.
1 Grams hemoglobin per 100 cc blood.
2 Multiply X 10^6 for red cells per cmm blood.
3 Number of white cells per cmm blood.
4 Number positive animals/number animals tested.
x Duplicate tubes; number animals per tube combination.
' C represents values for control animals.

TABLE IX

HEMATOLOGICAL AND SEROLOGICAL VALUES--STRAIN OHIO*

Day		Hemoglobin[1]	Erythrocytes[2]	Leucocytes[3]	Aggl'n Titer	Cultures[4]		Remarks
3	H	15.7	6.99	7,450	10^{-1}	1/5	1(+-)[x]	Hematocrit--45%
	M	15.3	6.75	6,850	Neg.		4(--)	
	L	13.7	5.23	5,800	Neg.			
3C'	H	15.7	6.78	10,050	Neg.	---		Hemat.--50%
	M	15.0	6.20	7,400	Neg.			3 Hamsters
	L	13.3	5.83	6,000	Neg.			
5	H	15.7	6.95	9,200	Neg.	5/5	2(++)	Urine clear
	M	15.0	5.83	9,000	Neg.		3(+-)	Nervous
	L	14.1	5.38	3,600	Neg.			symptoms
5C	H	15.7	7.02	7,250	Neg.	---		Hemat.--45%
	M	14.5	6.65	6,650	Neg.			3 Hamsters
	L	14.5	6.05	6,600	Neg.			
6	H	16.6	7.42	10,850	10^{-1}	5/5	5(++)	Urine clear
	M	16.2	6.49	7,950	Neg.			5 ill
	L	15.0	5.97	4,050	Neg.			
7 AM	H	19.9	9.32	26,050	10^{-2}	5/5	5(++)	Hemats.--
	M	16.2	7.08	16,800	10^{-1}			63, 55, 54%
	L	9.3	4.91	6,600	Neg.			Moribund
7 1 PM	H	20.4	7.60	22,500	10^{-2}	7/7	6(++)	7 Moribund
	M	18.1	6.93	18,700	10^{-1}		1(+-)	
	L	15.7	6.64	14,200	10^{-1}			
7 4 PM	H	18.1	8.55	23,250	10^{-4}	6/7	6(++)	7 Moribund
	M	17.1	7.62	18,250	10^{-1}		1(--)	
	L	15.3	6.18	6,900	10^{-1}			
7C	H	18.1	6.98	8,350	Neg.	---		Hemat.--49%
	M	16.2	6.80	7,200	Neg.			3 Hamsters
	L	13.7	6.45	6,300	Neg.			

* Values represent the high, median and low values.
1 Grams hemoglobin per 100 cc blood.
2 Multiply X 10^6 for red cells per cmm blood.
3 Number of white cells per cmm blood.
4 Number positive animals/number animals tested.
x, Duplicate tubes; number animals per tube combination.
C represents values for control animals.