LEPTOSPIRAL COLONIAL MORPHOLOGY

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ABSTRACT

STALHEIM, O. H. V. (University of Wisconsin, Madison) AND J. B. WILSON. Leptospiiral colonial morphology. J. Bacteriol. 86:482-489. 1963.—A sequence of apparent colonial types was observed with colonies of *Leptospira pomona*, *L. canicola*, *L. icterohaemorrhagiae*, and *L. grippotyphosa* in agar medium. Although some colonies of these serotypes had a different appearance initially, they eventually developed the mature or final appearance characteristic of the serotype. Colonies of freshly isolated, virulent cultures of *L. pomona*, *L. canicola*, and *L. icterohaemorrhagiae* were similar in appearance to colonies of avirulent strains of the same serotype. Additional studies of three stable and distinct colonial types of a laboratory strain of *L. autumnalis* revealed no differences in antigenicity, catalase activity, or mouse infectivity; however, differences in susceptibility to lysis by oleic acid were found. Although the colonial variants were stable during several in vitro variations, including growth in the presence of homologous antiserum and mutation to growth in a chemically characterized medium, rapid dissociation in vivo was found.

The first report of the growth of leptospires as isolated colonies in laboratory media was made by Cox and Larson (1957), who used a medium containing 10% rabbit serum, rabbit erythrocyte lysate, 0.2% Tryptose Phosphate Broth (Difco), and 1% agar. They reported that two types of colonies were generally observed. The first (type 1) were smaller and more opaque; the second (type 2) were larger, more translucent, and veil-like in appearance. Upon prolonged incubation, type 1 colonies were observed to eventually resemble type 2 colonies with opaque centers. Qualitative and quantitative variations of the medium were reported to inhibit colonial growth but their effect on colonial morphology was not described. Their preliminary conclusion that colonies of leptospires arose from single cells was subsequently supported by the report of Larson et al. (1959).

Armstrong and Goldberg (1960) used an agar medium containing sheep serum, sheep erythrocyte lysate, Tryptose Phosphate Broth, and cycloheximide for a study of leptosomal colonial morphology. They reported that 15 cultures characteristically displayed two or more of six proposed colonial types as well as numerous variations, and advanced the opinion that leptosomal colonies have variable morphology depending upon such environmental factors as agar concentration, pH, autolysins, oxygen tension, surface tension, and the inherent motility of the organisms. They found no catalase activity when leptosporial colonies were flooded with 1% *H₂O₂*.

The purpose of this work was to investigate leptosomal colonial morphology and, if possible, to correlate colonial types with virulence, antigenicity, or some physiological attribute as, for example, has been done with the brucellae.

MATERIALS AND METHODS

Organisms. The sources of the cultures used in this investigation were as follows: *Leptospira pomona* strains Wickard and Iowa from the Department of Veterinary Science, University of Wisconsin, Madison, strains S-91, C-164, CB, V-42, and T-262 from the WHO/FAO Leptospirosis Reference Laboratory, Division of Veterinary Medicine, Walter Reed Army Institute of Research, Washington, D.C., strain LT540 from the Communicable Disease Center, U.S. Public Health Service, Atlanta, Ga., and strain LC34 from the Department of Microbiology, University of Missouri, Columbia; *L. canicola* strains Hond Utrecht IV and Dog L Reinhard from the State Laboratory of Hygiene, Madison, Wis., strain Moulton from WHO/FAO Leptospirosis Reference Laboratory, and strain LT562 from the Communicable Disease Center; *L. icterohaemorrhagiae* strains CF-1, LT351, and M20 from WHO/FAO Leptospirosis Reference

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Laboratory, and strain Wijnberg from the University of Missouri; *L. grippotyphosa* strain 13 from the State Laboratory of Hygiene, strain Moscow V from the University of Missouri, and strains 2168 and 2188 from the Department of Veterinary Science, Louisiana State University, Baton Rouge; *L. autumnalis* strains Fort Bragg, LT136, and 1033 from the WFO/FAO Leptospirosis Reference Laboratory; *L. andaman*, *L. alexi*, and *L. semaranga* from the University of Missouri; and *L. biflexa* from the WFO/FAO Leptospirosis Reference Laboratory. The cultures were not purified by single-colony isolations before use.

The cultures of strains Moulton and CF-1 were lethal for hamsters when received from the WHO/FAO Leptospirosis Reference Laboratory. Additional virulent cultures were derived from the Dog I. Reinhard strain of *L. canicola* and the Iowa strain of *L. pomona* by several blind passages through young hamsters at intervals of 4 to 5 days.

*Media.* Leptospiral cultures were maintained routinely at 30°C in the 10% rabbit serum-phosphate buffer medium of Johnson and Wilson (1960) and transferred at intervals of 10 to 14 days by use of a 10% inoculum. Other liquid media used included Cox's (1955) medium, the boiled serum medium of Yanagawa and Wilson (1962), and the chemically characterized medium of Vogel and Hutner (1961).

The solidified medium of Cox and Larson (1957) was used routinely; however, the lysate of rabbit erythrocytes was omitted when commercial rabbit serum (Pel-Freeze Biologicals, Inc., Rogers, Ark.) was used and heat inactivation was not practiced. Cycloheximide (100 µg/ml) was added to some lots of medium and found to have no effect on colonial morphology at this concentration. Other agar media included Stuart's (1946) medium and Fletcher's (1927) medium, solidified by the addition of agar to a concentration of 1%, and the following experimental medium: sodium acetate, 50 mg; ammonium sulfate, 800 mg; ferrie ammonium citrate, 55 mg; thiamine hydrochloride, 0.4 mg; Na2HPO4, 2 g; KH2PO4, 0.6 g; Difco agar, 10 g; and distilled water, 800 ml. The pH was adjusted to 7.3 ± 0.1. The basal medium was autoclaved, cooled to about 45°C, and 200 ml of oleic albumin complex (Difco) were added (Ellinghausen and McCullough, 1962).

Each liter of complete medium was poured into 30 150-mm petri dishes and incubated overnight at 37°C. Plates of sterile medium not more than 4 days old were either streaked for isolated colonies or 0.1-ml volumes of a suitable dilution of a liquid culture were applied and spread over the surface of the plates with a sterile, bent glass rod. After incubation for 24 hr at 30°C in a humidified environment, the plates were inverted and incubation was continued, usually for 30 days. Well-isolated colonies were subcultured by transferring with a sterile wire loop or by first propagating the organisms for a few days in a tube of liquid medium and then streaking onto an agar medium.

The colonial morphology of virulent leptospires was observed and compared to the colonial morphology of avirulent cultures of the same serotype. Colonies of virulent and avirulent organisms were homogenized in phosphate buffer (pH 7.3 ± 0.1), centrifuged to sediment the agar, and, after determining the cellular density by microscopic counting, appropriate numbers of leptospires were injected into hamsters and white mice. The average numbers of leptospires in typical colonies of the colonial morphological variants of *L. autumnalis* were determined in a similar manner.

A Petroff-Hausser bacterial counting chamber with dark-field illumination was used for direct microscopic counting of leptospires. The sample to be counted was placed in the chamber and, after counting the leptospires in 80 small squares, the number of organisms per ml of sample was calculated.

*Stability tests.* The colonial variants of *L. autumnalis* were grown in Cox's (1955) medium with increasing concentrations of homologous type LOD rabbit antiserum which had a titer of 1:12,800. During 22 successive transfers, the initial 1:200 antiserum concentration was increased to a final concentration of 1:20. The cultures were periodically inoculated on plates of agar medium for observations of colonial morphology.

The effect of mutation on the colonial morphology of *L. pomona* and the *L. autumnalis* colonial variants was studied by selecting mutant strains capable of growth in boiled serum medium and chemically characterized medium. More than 10º leptospires from each of the four cultures were inoculated into each medium and
subcultured at least three times. Mutant organisms were inoculated on agar medium and their colonial morphology observed.

**Catalase activity determinations.** The quantitative procedure of Herbert (1955) was used at pH 7.0 as recommended by Faine (1960). Young cultures of the *L. autumnalis* variants were counted and 1-ml volumes were added to 5 ml of 0.01 M H3O2. After 1, 5, and 15 min at room temperature, the reactions were stopped by the addition of sulfuric acid and the remaining H2O2 was titrated iodometrically. The results were calculated in μlTERS of O2 produced and adjusted to 10⁶ leptospires/sample.

**Lysis by oleic acid.** The susceptibility of the colonial variants of *L. autumnalis* to lysis by oleic acid was compared by direct microscopic counts of organisms suspended in chemically characterized medium containing 2 mg/100 ml of grade A oleic acid (Calbiochem) substituted for monolein. The leptospires in 10 ml of mature variant cultures were sedimented by centrifugation (10,000 × g for 30 min) and resuspended in 10 ml of the oleic acid medium. Counts were made immediately after resuspending the organisms and after incubation for 2, 24, and 48 hr at 30 C.

**Animal passage.** Cultures of the three colonial variants of *L. autumnalis* were passed through adult hamsters by the intraperitoneal injection of more than 10⁶ organisms of each colonial type per animal. After 7 days, the animals were anesthetized and bled by cardiac puncture. The kidneys were removed aseptically and one kidney from each animal was homogenized in a Ten Broeck tissue grinder with 10 ml of buffer. Quadruplicate plates of agar medium were streaked with a loopful of the citrated blood and homogenized kidney from each animal, and then were incubated. Further decimal dilutions through 10⁻⁴ were made of the blood and homogenized kidneys in phosphate buffer; after the addition of sterile rabbit serum (10%), the tubes were incubated at 30 C. Seven days later, a leptospiral culture obtained from the blood and kidney of each hamster was diluted appropriately, and approximately 100 leptospires were spread over the surface of quadruplicate plates of agar medium containing cycloheximide. The resulting colonies were classified as to morphological types after 40 days of incubation.

**Virulence determinations.** The virulence of leptospiral cultures was estimated by their hamster LD₅₀ values and by the mouse immune-response method of Yanagawa et al. (1959). Mature leptospiral cultures were diluted appropriately and 0.2-ml volumes were injected intraperitoneally into Charles River white mice (20 to 22 g) supplied by the Department of Veterinary Science, University of Wisconsin, Madison. The mice were bled from the femoral artery 18 days later and the sera examined for leptospiral agglutinins by the microscopic agglutination (MA) test. Prior to use the mice were bled orbitally (Riley, 1960) and the sera did not agglutinate *L. autumnalis* and *L. ballum* antigens.

**Serology.** Rabbits were immunized with either live or formalinized (0.5%) cultures of *L. autumnalis* colonial variants by several intravenous injections at intervals of 4 to 5 days in doses increasing from 1 to 5 ml. The serum obtained 10 days after the last injection was sterilized by Seitz filtration and stored at -20 C.

The MA test was performed on twofold dilutions of serum. To 0.2 ml of each serum dilution was added 0.2 ml of live, mature leptospiral culture. After 3 hr of incubation at room temperature, drops of the antigen-serum mixture were examined on glass slides without cover slips by means of a 20 × objective with dry, dark-field illumination. The titer of the serum was taken as the reciprocal of the highest final dilution in which at least 50% clearing with agglutination occurred as compared to the control.

Agglutinin cross-absorption was performed. The leptospires were sedimented from 30 ml of culture by centrifugation (10,000 × g for 30 min) and resuspended in 2 ml of serum which had been diluted 1:25 in phosphate buffer. The mixture was incubated at room temperature for 2 hr and at 5 C overnight. After centrifugation (10,000 × g for 30 min), the supernatant fluid was tested for agglutinins by the MA test.

**Results**

When cultures of leptospira were applied to the moist surface of an agar medium, the resulting colorless colonies could be classified by their relative size and opacity, and the definitiveness of their borders. Because we wished to avoid the use of terms which connote virulence or antigenicity, we decided to adopt the descriptive terminology originally proposed by C. D. Cox (personal communication), in which L = large, 2 mm or larger in diameter; S = small, 1 mm or less in diameter; T = translucent; O = opaque,
generally smaller; D = defined or distinct border; H = hazy or indefinite border; and I = irregular border, often with daughter-colony formation.

Colonies of L. biflexa and L. semaranga became visible in plates of agar medium after 4 to 6 days of incubation. Colonies of L. semaranga continued to enlarge and became confluent. An additional 7 to 10 days of incubation were required for the appearance of colonies of pathogenic leptospires. The variability of the initial appearance of colonies of L. canicola after 16 days of incubation is shown in Fig. 1. Most colonies were of the mature morphological type (LTD); some colonies were opaque, with hazy or defined borders (OH and OD types). When individual opaque colonies were observed at intervals of 2 to 3 days, a sequence of further development to the mature, translucent colonial type was found, i.e., OH → OD → LTD. Usually about 7 days of incubation beyond the 11 to 16 days were required for colonial maturation. Sequential colonial maturation was characteristic of L. biflexa, L. andaman, L. alexi, L. icterohaemorrhagiae, L. pomona, L. canicola, and L. grippotyphosa. However, in a high percentage of the plates, colonies continued to appear for several days. The tardy appearance and maturation of some colonies often resulted in the presence in those plates of apparent colonial types unless repeated observations were made (Fig. 2). Immature (OH) and mature (LTD) leptospiral colonies were subcultured in plates of agar medium. When these were observed at intervals of 16, 20, and 26 days, no differences in the proportion of mature and immature colonial types were found.

Virulent leptospires from mature colonies in agar medium had hamster LD₅₀ values of less than ten organisms and mouse ID₅₀ values of less than ten organisms. Colonies of virulent leptospires could not be distinguished from colonies of avirulent leptospires by colonial morphology or by the time of incubation required for their appearance.

When all three of our cultures of L. autumnalis were found to have three distinct and stable colonial types, investigations of leptospiral colonial morphology were confined to this serotype (Fig. 3). The colonial variants were distinguished by differences in size and opacity as follows: smaller, 1 mm or less in diameter, opaque with distinct borders (SOD); larger, 1 to 2 mm in diameter, opaque with distinct borders (LOD); and large-translucent, 3 to 5 mm in diameter, translucent with distinct borders (LTD; Fig. 4, 5, and 6). When well-isolated colonies were transferred to liquid medium, incubated for a few days, and again grown in agar medium, more than 95% of the resulting colonies were of the parental type during incubation periods of up to 60 days.

Cultures of the colonial variants in the early and late stages of logarithmic growth and in the stationary phase of the growth cycle were appropriately diluted and inoculated onto agar medium. The resulting colonies became visible at about the same time, and more than 95% of the colonies conformed to the parental morphology.

Dissociation of cultures of the colonial variants during storage was studied. The organisms were maintained at room temperature in tubes of Cox's (1955) medium containing 0.4% agar, without transferring for 9 months. When subcultured in plates of agar medium, the resulting colonies conformed to the parental morphological type as follows: 80% for type LOD, 50% for type SOD, and 40% for type LTD. By a single subculturing procedure, greater than 95% type specificity was restored.

Microscopic observation of cells from cultures of the colonial variants revealed no morphological differences. Their generation times were 8.5 ± 0.5 hr in Cox's medium. The average number of cells per colony × 10⁶ was LOD, 2.8; SOD, 1.0; LTD, 2.8.

Stability tests. Several unsuccessful attempts were made to modify the colonial growth of the three variants of L. autumnalis by variations of the medium. Although growth decreased and ceased as the pH was varied beyond a range of 6.8 to 8.0 and as the agar concentration was increased above 1.2%, colonial specificity was not lost. When the osmotic tension of the agar medium was increased by the addition of sucrose (1 to 2%), LTD colonies were more uniform and distinct.

The specificity of the three colonial variants was not lost when the organisms were inoculated onto medium made with agar which had been extracted with absolute methanol for 96 hr in a Soxhlet apparatus; nor was it lost upon the addition to agar medium of 40 mg/100 ml of oleic acid, triolein, or polyoxyethylene sorbitan mono-oleate (Tween 80). Type-specific colonies developed in Stuart's and Fletcher's media solidified
FIG. 1. *Leptospira canicola* colonies 16 days after inoculation, showing the morphological variations of young colonies.

FIG. 2. *Leptospira grippotyphosa* colonies 24 days after inoculation, showing mature colonies and, near the center of the plate, a single immature colony which appeared 21 days after inoculation.

FIG. 3. *Leptospira autumnalis* colonies 30 days after inoculation; note the morphological variations among colonies. Three stable and different colonial types were isolated from a similar plate of colonies.

FIG. 4. *Leptospira autumnalis* colonies type LOD; larger, opaque colonies, with distinct borders.

FIG. 5. *Leptospira autumnalis* colonies type SOD; smaller, opaque colonies, with distinct borders.

FIG. 6. *Leptospira autumnalis* colonies type LTD; larger, translucent colonies, with distinct borders.
with 1% agar; however, leptospires could not be routinely grown as isolated colonies in the experimental medium containing oleic albumin complex.

As Yanagawa, Hiramune, and Fujita (1963) have reported recently, colonial leptospiral growth was not obtained in the absence of CO₂ or in the presence of 10% or more CO₂ in the air. When the colonial variants were grown in a semisolid medium containing 0.4% agar, no differences were found in the number or distribution of bands of growth (Dinger’s bands).

The colonial morphology of the three colonial variants was not modified by the addition of NaCl (0.15 M) to the agar medium. No differences were found in their ability to grow in the presence of crystal violet, thionine, and basic fuchsin. Sterile paper discs were placed on the surface of agar plates 10 days after inoculation and saturated with dye (1:1000). Similar zones of growth inhibition were observed.

The colonial morphology of variant cultures of *L. autumnalis* was not modified by two subcultures at 37°C, by growth in the presence of homologous antiserum, or by selection of mutants which grew in boiled serum medium and chemically characterized medium.  

*Animal passage.* Mixtures of colonial morphological types were observed to develop in plates of agar medium inoculated with leptospires isolated from the blood and kidneys of hamsters. Approximately equal numbers of type LOD, SOD, and LTD colonies were observed in plates inoculated with leptospires isolated from the blood of three hamsters which had each been inoculated with a different colonial type and from the kidney of the hamster injected with type LOD organisms. Cultures isolated from the kidneys of hamsters injected with type SOD and type LTD leptospires produced 25% LOD, 75% SOD, and 10% LTD and 25% LOD, 25% SOD, and 50% LTD type colonies, respectively, when inoculated on agar medium.

* Catalase activity determinations. Because quantitative evidence of catalase activity was found when leptospiral colonies were flooded with 10% H₂O₂, a quantitative procedure was applied to cultures of the *L. autumnalis* colonial variants. The results showed the reaction rates to be linear for at least 10 min. The μlators of O₂ produced in 5 min by 10⁶ colonial variant leptospires were: LOD, 57.0; SOD, 54.1; and LTD, 51.0.

* Lysis by oleic acid. Rapid lysis by oleic acid of organisms from cultures of the LOD and SOD colonial variants was found by direct counts of organisms suspended in an oleic acid medium, whereas cells of the LTD variant were quite resistant to lysis. The percentage of cells remaining after incubation periods of 2, 24, and 48 hr, respectively, was: LOD 50 (102), 2 (90), 0 (70); SOD 50 (100), 0 (90), 0 (75); and LTD 80 (105), 75 (100), and 80 (90). The numbers in parentheses are the percentages of cells remaining in the control medium without oleic acid. The absence of cells of types LOD and SOD and the viability of LTD cells after 48 hr of incubation was confirmed by subculture in liquid medium. A type SOD mutant subcultured in chemically characterized medium and in boiled serum medium had increased resistance to lysis by oleic acid; the corresponding percentages were 60, 40, and 40.

*Virulence trial.* There was no visible evidence of disease in mice injected with the colonial variants of *L. autumnalis*. Serum antibodies were detected by the MA test after the injection of

<table>
<thead>
<tr>
<th>Colonial type</th>
<th>No. organisms injected X 10⁴</th>
<th>No. of sera positive/no. of mice injected</th>
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</thead>
<tbody>
<tr>
<td>LOD</td>
<td>1</td>
<td>0/4</td>
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<tr>
<td></td>
<td>2</td>
<td>0/4</td>
</tr>
<tr>
<td></td>
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<tr>
<td></td>
<td>8</td>
<td>0/4</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>4/4</td>
</tr>
<tr>
<td>Control†</td>
<td>16</td>
<td>0/3</td>
</tr>
<tr>
<td>SOD</td>
<td>1</td>
<td>0/4</td>
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<td></td>
<td>2</td>
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<td>16</td>
<td>3/4</td>
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<tr>
<td>Control†</td>
<td>16</td>
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<tr>
<td>LTD</td>
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<tr>
<td>Control†</td>
<td>16</td>
<td>0/4</td>
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</table>

* The mice were bled 18 days after injection and the sera examined by the microscopic agglutination test.
† Control organisms were killed by heating to 56°C for 30 min.
TABLE 2. Microscopic agglutination titers of colonial type rabbit antisera before and after absorption with colonial type antigens*

<table>
<thead>
<tr>
<th>Colonial type antisera</th>
<th>Colonial type antigens</th>
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<tr>
<td></td>
<td>LOD</td>
</tr>
<tr>
<td>LOD</td>
<td>12,800</td>
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<tr>
<td>LOD absorbed with</td>
<td></td>
</tr>
<tr>
<td>LOD cells</td>
<td>100</td>
</tr>
<tr>
<td>SOD cells</td>
<td>100</td>
</tr>
<tr>
<td>LTD cells</td>
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</tr>
<tr>
<td>SOD</td>
<td>12,800</td>
</tr>
<tr>
<td>SOD absorbed with</td>
<td></td>
</tr>
<tr>
<td>LOD cells</td>
<td>200</td>
</tr>
<tr>
<td>SOD cells</td>
<td>200</td>
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<tr>
<td>LTD cells</td>
<td>200</td>
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<tr>
<td>LTD</td>
<td>51,200</td>
</tr>
<tr>
<td>LTD absorbed with</td>
<td></td>
</tr>
<tr>
<td>LOD cells</td>
<td>400</td>
</tr>
<tr>
<td>SOD cells</td>
<td>400</td>
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<tr>
<td>LTD cells</td>
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* Titers were taken as the reciprocal of the highest final serum dilution showing at least 50% clearing with agglutination after 3 hr of incubation at 30 C.

approximately 8000 or more organisms of each colonial type (Table 1). Because Yanagawa et al. (1959) showed that leptospiral infection in mice is reflected by an immune response, we concluded that there were no differences in virulence for mice among the colonial variants of L. autumnalis.

Serology. Rabbit antisera produced by injections of living cultures of the three colonial variants had identical titers when tested with the three colonial type antigens. (Table 2). By reciprocal agglutinin absorption, uniform decreases in titers were obtained, indicating no major antigenic differences among the colonial variants. Similar results were obtained with antisera to formalinized cultures, except that the titers were lower.

DISCUSSION

When plates of agar medium containing young colonies of leptospires were observed after 11 to 16 days of incubation, variations in colonial morphology were noted. Although most of the colonies in an individual plate were of the final or mature colonial type (LTD), a varying percentage were hazy or immature (OH). During continued incubation, immature colonies were observed to develop definite borders and to assume the mature colonial morphology characteristic of the species. Colonial maturatiion was characteristic of our cultures of L. pomona, L. canicola, L. icterohaemorrhagiae, and L. grippophosa.

Our observations of sequential colonial maturation elaborate the original report of Cox and Larson (1957) but are in disagreement with the report of Armstrong and Goldberg (1960). Because the latter authors did not state when their classifications were performed, it may be concluded that three of the colonial types which they propose (button-like, rough-opaque, and rough-translucent) are transitional or immature types (OH or OD), and that two others (ringworm-like and asteroid) are minor variations of mature, translucent colonies (LTD). In addition to differences in time of observation, the variations in leptosporal colonial morphology which they reported could be due to consistently poorer growth in their medium (made with sheep serum) than in medium made with rabbit serum. Boyd (1959) found that cultures of L. pomona could not be maintained beyond three subcultures in Chang's medium containing 10% sheep serum. We find that some lots of commercial rabbit serum support poor leptosporal colonial growth; the colonies have hazy or irregular borders and maturation is delayed or incomplete. Johnson and Gary (1963) reported that the addition of NHCl improved the growth-supporting ability of certain deficient lots of rabbit serum.

The disagreement between our results and those of Armstrong and Goldberg (1960) as to the catalase activity of leptosporal colonies can be explained in part by differences in the concentrations of H2O2 employed. We found slight but definite evidence of catalase activity when plates were flooded with 1% H2O2; a more copious liberation of O2 followed the use of 10% H2O2.

When it became clear that antisera to living cultures of the colonial variants may not be colonial type-specific because of in vivo dissociation, studies on the antigenicity of the colonial variants of L. autumnalis were repeated with antisera to formalinized cultures (A. D. Alexander, personal communication). No difference in virulence or antigenicity among three distinct and stable colonial variants of L. autumnalis was demonstrated. The only difference found was the relative resistance of cells of the "spreading" colonial type (LTD) to concentrations of oleic acid which rapidly lysed cells of colonial types LOD and SOD. Because an oleic acid-resistant mutant of colonial type SOD showed no colonial
morphological change, it is not likely that the LTD variant is selected by in vitro interaction of leptospiral lipase (Kemenes and Lovrekovich, 1959) and the “buffering” capacity of serum albumin (Davis and Dubos, 1947).

Because our cultures of colonial variants commonly contained a few organisms (1 to 2%) of one or both of the other colonial types, the results of the hamster passage experiment are difficult to evaluate. Recovery of approximately equal numbers of all three colonial types from infected hamster blood may reflect selection of mutants or an equilibration process. The establishment of colonial variants of brucellae in vivo has been reported by Berman, Redfearn, and Simon (1955), who suggested that local accumulations of metabolites may favor the establishment of variants. The role of immunity in the selection of colonial variant brucellae was studied by Redfearn, Simon, and Berman (1956). Our limited results contain an indication that the small, opaque, colonial type organisms may predominate in infected kidneys. We were unable to distinguish virulent and avirulent leptospires by their colonial morphology; an in vitro method for estimating the virulence of leptospiral cultures remains to be found. Morphologically different types of leptospiral colonies probably have no significance in the production of immunizing agents or diagnostic antigens, but their association with stages of leptospiral infection warrants further study.

Acknowledgments

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Literature Cited


