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Use of 8-Azaguanine to Differentiate Leptospires Isolated from Iowa Surface Waters

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Definitive classification of leptospires according to serotype rests with use of the microscopic agglutination and agglutinin-absorption tests. R. C. Johnson and P. Rogers (J. Bacteriol. **88**:1618, 1964) observed inhibition of pathogenic serotypes by the purine analogue 8-azaguanine, whereas saprophytic serotypes were able to grow. This report is concerned with the effect of 8-azaguanine on growth of leptospires isolated from Iowa surface waters. A preliminary study of the applicability of 8-azaguanine to a field situation was undertaken because the complex and time-consuming serotype definition of saprophytes unnecessarily complicates epidemiological studies of human cases with a potential water exposure.

Leptospires for testing were isolated from water obtained at 12 sampling points on five Iowa streams during August to December 1965. A pathogen control, *Leptospira icterohaemorrhagiae*, was obtained from Mildred M. Galton, Communicable Disease Center, Atlanta, Ga., and a saprophyte control, *L. patoc* I, was obtained from the National Animal Disease Laboratory, Ames, Iowa.

Stuart's liquid medium without phenol red (Difco) with 10% rabbit serum (Pel-Freeze Biologicals, Rogers, Ark.) was used as the growth medium. This medium was sterilized by Seitz filtration and dispensed in 9-ml amounts in 20 × 125 mm screw-cap tubes. The 8-azaguanine (2-amino-6-oxy-8-aza-purine; Calbiochem, Los Angeles, Calif.) was used in a final concentration of 100 μg/ml of medium. All batches of media were used within 3 weeks of preparation.

All cultures were incubated at 30 C, with maintenance transfers at 4-day intervals. A 1% inoculum of 4-day-old culture was used to initiate growth for test sets. Each set consisted of duplicate tubes of plain medium and medium containing 8-azaguanine; three trials were conducted for each organism.

Growth was measured by use of a Coleman no. 9 Nepho-colorimeter as a nephelometer. H. C. Ellinghausen, Jr. (Am. J. Vet. Res. **20**:1072, 1959) suggested that growth, as mirrored by relative leptospiral cell turbidities, could be adequately determined by nephelometry. Nephelos readings were made for all cultures at the end of 2 and 4 days of incubation; the cultures were examined microscopically on inoculation and at the end of the 4-day incubation period.

Nephelos values which were obtained are summarized in Table 1. Both types of media gave base-line nephelos values of three immediately after the addition of the 1% inoculum. The pathogen control grew noticeably slower than the saprophyte control. The saprophyte control was growing well at the end of 2 days in the 8-azaguanine medium, whereas the pathogen control was almost completely inhibited; the differences in growth in the 8-azaguanine medium are more marked at the end of 4 days of incubation. Although the saprophyte control grew well,

TABLE 1. *Nephelos values[a] observed from cultures of leptospires grown in plain media[b] and in media[b] containing 100 μg of 8-azaguanine per ml*

Organism	Incubation period			
	2 days		4 days	
	8-Aza-guanine	Plain	8-Aza-guanine	Plain
Saprophyte control (*Leptospira patoc* I)	24	36	42	56
Pathogen control (*L. icterohaemorrhagiae*)	6	7	8	20
Eighteen water leptospires[c]				
Mean	24	31	43	55
Median	22.5	34.5	38	53
Range	7–42	9–49	22–61	35–73

[a] Average of three trials.
[b] Stuart's Liquid Medium Without Phenol Red (Difco).
[c] Obtained from five Iowa streams.

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diminution of nephelos values between growth in plain medium as compared with the response to 8-azaguanine medium is evident.

One of the 18 water isolates grew to nephelos values which were rather low in comparison with the other 17; the values for this isolate are represented by the low figure for each range listed in Table 1. The other 17 water leptospires demonstrated nephelos values which were much more closely aligned to the saprophyte control values than to the pathogen control values.

Leptospiral serotypes isolated in Iowa include: *L. ballum* (W. F. McCulloch et al., Pacific Science Congr., 11th, Univ. of Tokyo, Japan, 1966); *L. grippotyphosa* (S. L. Diesch, et al., Bull. Wildlife Disease Assoc. **2:15**, 1966); *L. pomona* (S. L. Diesch and W. F. McCulloch, Public Health Rept. U.S. **81:**299, 1966); and *L. hardjo* (*L. sejroe*; *unpublished data*, Institute of Agricultural Medicine, Iowa City, Iowa, 1967). The 18 water isolates were tested with 12 antisera (Difco), representing serogroups for the four serotypes which have been demonstrated to occur in Iowa in addition to other serogroups of leptospires for which there is cultural or serological evidence of occurrence in the United States (M. M. Galton, Ann. N.Y. Acad. Sci. **98:**675, 1962). The isolates did not react with these 12 antisera in the microscopic agglutination test.

In vivo titrations were not attempted with the water isolates; however, the available cultural and serological evidence suggests that the 18 isolates be given a tentative saprophytic classification. If this classification is correct, the findings of this study lend support to the previously cited observations of R. C. Johnson and P. Rogers (J. Bacteriol. **88:**1618, 1964). A selective tool for use with isolates obtained during epidemiological studies of human cases associated with water exposure is clearly desirable; thus, additional studies on recent leptospiral isolates with 8-azaguanine as a selective tool are indicated.

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