Antigenic Similarity of Bovine Strains of *Leptospirae* (United States) and *Leptospira pomona.* (17853)

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(Introduced by Joseph E. Smadel.)

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Sera from horses affected with recurrent iridocyclitis contain antibodies against *Leptospira pomona* and the New Jersey C164 strain of bovine leptospira(1). *L. pomona* was originally isolated in 1936 from a dairy farmer in Australia(2) and has since been found in Italy, Savoy, and Switzerland(3), but has not as yet been reported to occur in the United States.

Methods. The strains of leptospira used in these studies were: *L. icterohaemorrhagiae,** *L. canicola,*† *L. pomona,** *L. bovis (Baruch Strain),*‡ New Jersey bovine leptospira strain C164,* and New York bovine leptospira strain A.§ The positive sera employed included one from a patient who acquired a natural infection with *L. pomona* in Malaya, bovine serum from a naturally infected steer in Kansas, and hyperimmune sera produced experimentally in rabbits. Of the latter, one serum, "Lapin 97", hyperimmunized against *L. pomona,* was obtained from the Pasteur Institute in Paris through the courtesy of Mme. Kolochine-Erber. Three to 4-day-old actively growing cultures of leptospirae cultivated in Schüffner's medium incubated at 30°C were used as antigens in microscopic agglutination-lysis tests which

TABLE I.
Cross Agglutination-Lysis Tests.

Serum	Antigen	Titer 10	50	100	500	1000	5000	10000	50000
Kansas Steer No. 190	*L. icterohaemorrhagiae*	+	0	0	0	0	0	0	0
	L. canicola	0	0	0	0	0	0	0	0
	L. bovis (Baruch)	0	0	0	0	0	0	0	0
	N.J. C164	+	+	+	+	+	+	±	0
	N.Y. A	+	+	+	+	+	+	0	0
	L. pomona	+	+	+	+	+	+	0	0
Hyperimmune Rabbit L97 Pasteur Inst.	*L. icterohaemorrhagiae*	+	±	0	0	0	0	0	0
	L. canicola	+	0	0	0	0	0	0	0
	L. bovis (Baruch)	0	0	0	0	0	0	0	0
	N.J. C164	+	+	+	+	+	+	+	0
	N.Y. A	+	+	+	+	+	+	+	±
	L. pomona	+	+	+	+	+	+	±	0
Human Patient R, Malaya	*L. icterohaemorrhagiae*	0	0	0	0	0	0	0	0
	L. canicola	0	0	0	0	0	0	0	0
	L. bovis (Baruch)	0	0	0	0	0	0	0	0
	N.J. C164	+	+	+	±	0	0	0	0
	N.Y. A	+	+	+	±	0	0	0	0
	L. pomona	+	+	+	±	0	0	0	0

+, All organisms agglutinated or lysed.
±, Many organisms agglutinated or lysed.
0, few to no organisms agglutinated or lysed.

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* Obtained October 1949 from the National Institutes of Health, Bethesda, Md.

† Isolated October 1948 at the Army Medical Dept. Research and Graduate School.

‡ Obtained February 1950 from the Hebrew University, Jerusalem.

§ Obtained February 1950 from Dr. Karl Reinhard, Cornell University, Ithaca, N. Y.

TABLE II.
Cross Absorption Tests.

Serum of rabbit hyperimmunized against:	Agglutination-lysis test antigen	Titer					
			Absorbed with:				
		Not absorbed	*L. icterohem.*	*L. canicola*	*L. pomona*	New Jersey C164	New York A
L. icterohemorrhagiae	*L. icterohemorrhagiae*	100000	5000	100000	100000	100000	100000
	L. canicola	10000	1000	0	1000	1000	1000
	L. pomona	1000	0	0	0	0	0
	N.J. C164	1000	0	0	0	0	0
	N.Y. A	0	0	0	0	0	0
L. canicola	*L. icterohemorrhagiae*	5000	0	0	1000	0	0
	L. canicola	500000	100000	0	100000	100000	100000
	L. pomona	0	0	0	0	0	0
	N.J. C164	0	0	0	0	0	0
	N.Y. A	0	0	0	0	0	0
L. pomona	*L. icterohemorrhagiae*	0	0	0	0	0	0
	L. canicola	0	0	0	0	0	0
	L. pomona	100000	100000	50000	0	1000	1000
	N.J. C164	100000	50000	50000	0	0	0
	N.Y. A	50000	50000	50000	0	1000	0
N.J. C164	*L. icterohemorrhagiae*	10000	0	10000	0	1000	1000
	L. canicola	0	0	0	0	0	0
	L. pomona	10000	10000	10000	0	1000	0
	N.J. C164	100000	10000	50000	0	0	0
	N.Y. A	100000	10000	10000	0	0	0

were performed by the technic of Schüffner and Mochtar(4). Results were recorded as + when all organisms were agglutinated or lysed; ± when many organisms were agglutinated or lysed; and 0 when few to no organisms were agglutinated or lysed. Immune rabbit sera were absorbed with 5 different strains of leptospirae and retested for agglutinins. The absorption technic of Schüffner and Bohlander(5) was employed with the following modifications: living leptospirae grown in Stuart's medium were collected by centrifugation for 10 minutes at 16,000 rpm, and resuspended in buffered saline solution equal to one tenth the original volume of culture. Each of the immune sera was diluted before absorption so that its agglutination-lysis titer was approximately 1:50,000. In general 3 ml of a concentrated suspension of leptospirae were added to 2 ml of immune sera and the mixtures were incubated for 18 hours at 30°C, after which the leptospirae were removed by high speed centrifugation. Such an absorption procedure was repeated twice more before the sera were tested for residual antibodies.

It has been found in this laboratory that the New York A strain of bovine leptospira is pathogenic for weanling hamsters when injected intraperitoneally and can be maintained readily in this host by passage of heart's blood taken when the animals are moribund on the 4th or 5th day after inoculation. In order to determine the protective value of antisera against artificial infection with this strain of leptospira, a hamster protection test, modeled after the mouse potency test used in evaluating anti-swine-erysipelas serum(6) was employed. Groups of weanling hamsters were injected subcutaneously with 1 ml of immune rabbit serum. Twenty-four hours later, they were inoculated intraperitoneally with 0.5 ml of a virulent culture of New York A strain of bovine leptospira.

Results. Cross agglutination-lysis tests were performed on 3 sera from widely different sources, a naturally infected human being in Malaya, a naturally infected bovine in Kansas, and an experimentally immunized rabbit in France. These sera gave the same titration end points with *L. pomona* as with the American strains of bovine leptospira, but did not react significantly with *L. icterohaemorrhagiae, L. canicola,* or *L. bovis (Baruch Strain).* Results of these cross agglutination-lysis tests are shown in Table I.

TABLE III.
Hamster Protection Test.

Group	Immune serum	Hamsters surviving
1.	*L. icterohaemorrhagiae*	0/4
2.	*L. canicola*	0/4
3.	*L. pomona*	4/4
4.	N.J. C164	4/4
5.	None—control	0/4

Absorption tests clearly indicate that antibodies against *L. icterohaemorrhagiae, L. canicola, L. pomona,* and the New Jersey C164 strain of bovine leptospira were removed by absorption with homologous organisms, and that the first 2 leptospirae listed were unrelated to the last two. However, *L. pomona* and the New Jersey C164 and New York A strains of bovine leptospira were indistinguishable on the basis of cross absorption studies. The results of these tests are shown in Table II.

Hamsters passively immunized by the subcutaneous injection of *L. pomona* immune serum and hamsters similarly immunized with New Jersey C164 bovine leptospira immune serum were protected against infection with the virulent New York A strain of bovine leptospira. No protection was afforded hamsters receiving either *L. icterohaemorrhagiae* immune serum or *L. canicola* immune serum. It is worth mentioning that the initial infecting agent was recovered from each of the animals which succumbed but not from those which survived. The results of this test are shown in Table III.

Summary. The results of agglutination-lysis, absorption, and animal protection tests indicate that the strains of bovine leptospira of American origin designated as New Jersey

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C164 and New York A are indistinguishable from *L. pomona*. These findings stress the need for reclassification of the American strains. They also indicate that *L. pomona* or an antigenically indistinguishable organism is present in North America.

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Protective Effect of Para-Aminopropiophenone Against Lethal Doses of X-Radiation. (17854)

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Yeasts(1), plants(2,3) and both normal tissues and tumors of mammals(4-9) have been protected against radiation injury by reducing the oxygen tension during the period of exposure to radiation. The reduction of oxygen tension has been accomplished in a variety of ways. Tumors have been rendered hypoxic by *in vitro* enclosure in a nitrogen atmosphere during radiation(6), and portions of intact animals have been made hypoxic by occlusion of blood vessels by either pressure or ligation(4,5,8). Hypoxia during the period of irradiation has been produced in the entire animal by strapping the chest to interfere with respiration(8), by bleeding the animal immediately prior to irradiation (7), and by enclosure in a low oxygen, high nitrogen atmosphere(9). These methods are somewhat cumbersome and with one exception the evaluation of the degree of protection afforded has depended on estimations of radiation damage based on gross or histologic observations. In the instance where animals were exposed in a low oxygen atmosphere weight changes and 30-day mortality were used as criteria for the evaluation of protection. Cyanide has been shown to exert some protective action against X-radiation in mice, but this effect was not necessarily attributed to the histotoxic anoxia produced(10). The present study was undertaken to determine whether or not hypoxia produced by other chemical means during the period of exposure to X-radiation would protect intact animals against radiation damage. Para-aminopropiophenone (PAPP),† a compound shown to produce methemoglobinemia of relatively long duration(11), was

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