



Title	A RADIOIMMUNOASSAY SYSTEM USING A SEROVAR-SPECIFIC LIPOPOLYSACCHARIDE ANTIGEN OF LEPTOSPIRA, AND STUDIES ON THE CHEMICAL NATURE OF THE ANTIGENIC SITES
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observed between the two lesions; however, there did appear to be some relation between their immunosuppressed condition and the incidence of MD lesions.

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LIPOPOLYSACCHARIDE ANTIGEN OF *LEPTOSPIRA*,  
AND STUDIES ON THE CHEMICAL NATURE OF  
THE ANTIGENIC SITES**

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A highly sensitive and serovar-specific radioimmunoassay (RIA) was established to implement studies on the chemical nature of leptospiral antigenic determinant. The serovar-specific lipopolysaccharide (TM) antigen extracted from *Leptospira interrogans* serovar *kremastos* strain Kyoto was labeled with tritium by a reduction using sodium boro [ $^3\text{H}$ ]-hydride after 1 h oxidation with periodate for the RIA. When 50 ng (625 cpm) of the labeled compound was used in the RIA system, 50% inhibition of the labeled antigen-antibody binding was obtained by the addition of 9 ng of the homologous TM antigen, whereas 5,000 times as much as the TM antigen from *hebdomadis*, which belongs to the same serogroup, was required. The TM antigens from different serogroups, such as *icterohaemorrhagiae*, *copenhageni* and *pomona*, showed no inhibitions in amounts up to  $3 \times 10^3$ ,  $2 \times 10^5$ , and  $2 \times 10^5$ , respectively.

The mild acid hydrolysis of *kremastos* Kyoto TM antigen using 0.5 N sulfonic acid or 2 N formic acid produced an antigen-active fraction which was dialysable. The chemical, physicochemical, and immunochemical properties of the fraction obtained by formic acid hydrolysis were then characterized. This fraction was eluted in a gel filtration at a molecular weight which was calculated at approximately 10,000 dalton. As compared with the original TM compound containing 10.5% protein and 1.5% fatty acid, this fraction lacked almost all of these components and was composed mainly of carbohydrates. However, the antigenic potency of this fraction diminished to 1/350 in the RIA. The carbohydrate nature of the antigenic determinant was also discussed.