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**ANTIGENIC VARIANTS OF LEPTOSPIRAS SELECTED BY IN VITRO  
AND IN VIVO LEPTOSPIRICIDAL ACTIVITY TEST MEDIATED  
BY ANTISERUM PLUS COMPLEMENT**

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The presence of antigenic variants in a culture of leptospiras was shown by applying in vitro and in vivo leptospiricidal activity test mediated by the homologous antiserum plus complement.

Isolation of the antigenic variants by applying in vitro leptospiricidal activity test done as follows. A *copenhageni* Shibaura culture was mixed with guinea pig complement (1:20) and the homologous antiserum (1:2000) showing 99% leptospiricidal activity, incubated for 3 hours at 37°C, and inoculated onto the solid serum medium. 0.09% colonies developed from the treated culture, whereas no antigenic variants were isolated from the untreated culture of the same strain. The precipitin absorption test revealed that 86% of the colonies which developed from the treated culture were antigenic variants; the rate of antigenic variants in the culture of this strain was estimated to be 0.08% ( $0.09 \times 0.86$ ). The antigenicity of the variants was found to be unstable as they reversed after 3-5 passages in the liquid serum medium to the antigenicity of the parent. Antigenic variants were also isolated from the *icterohaemorrhagiae* RGA culture by applying in vitro leptospiricidal activity test.

Isolation of the antigenic variants by applying in vivo leptospiricidal activity test was done as follows. In vivo leptospiricidal activity test was performed in the peritoneal cavity of guinea pigs immunized with *copenhageni* Shibaura. The guinea pigs showing agglutinin titers 1:320-640 after immunization were inoculated intraperitoneally with  $10^9$  of the leptospiras, which were killed after 1, 3 and 5 hours, and the ascites was inoculated onto the solid serum medium. The number of colonies which developed from the ascites of the immunized guinea pigs was 0.02% of that from the ascites of the unimmunized guinea pigs irrespective of the incubation period in the peritoneal cavity. No antigenic variants were found in the colonies developed from the ascites of the unimmunized guinea pigs. Of the colonies which developed from the ascites of the immunized guinea pigs, 60% were found to be antigenic variants by the precipitin absorption test.

The presence of the unstable antigenic variants in the culture of leptospiras was thus confirmed in vitro and in vivo using a new selection method, treatment with the homologous antiserum plus complement.