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Heated Sheep or Horse Serum Substitute for Rabbit Serum in Culture Media for *Leptospira*

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Culture media for growing leptospires, especially for pathogenic varieties, usually contain approximately 10% rabbit or guinea pig serum. Other mammalian sera, such as horse or sheep sera, are used occasionally, but do not usually support growth of most strains. Antileptospiral activity of serum (R. C. Johnson and L. H. Muschel, J. Bacteriol. **91**:1403, 1966) was shown to be mediated by a heat-labile IgM natural antibody (S. Faine and J. N. Carter, J. Bacteriol. **95**:280, 1968).

Assuming that the unsuitability of some mammalian sera might be due to their natural antibody content, samples of modified Korthof medium containing 10% sheep or 10% horse serum were heated at 65, 68, and 70 C for 1 hr. These cultures and similar, but unheated, controls were inoculated with *Leptospira icterohaemorrhagiae* or *L. biflexa* (Patoc) strains, susceptible to natural antibody, and with *L. pomona* strains (EP-F and B), not susceptible to natural antibody (Faine and Carter, J. Bacteriol. **95**:280, 1968), and were incubated at 30 C. In the unheated controls, the susceptible leptospires grew in agglutinated clumps, but later, after approximately 10 days, developed unagglutinated growth in addition to the clumps. Growth was profuse in 4 days and free from clumping in media heated for 0.5 to 1 hr at 68 to 70 C. The insusceptible strains showed minimal clumping in unheated controls. Thus, sera antagonistic to leptospiral growth can be made suitable by heating.

Practical application allows cultivation of large quantities of leptospires in serum media without the need for rabbit serum. It is advisable to heat the serum before dilution to conserve water-bath space. Also, protein precipitation occurs, especially in hemolyzed sera, above 65 C, causing excessive turbidity. The very slight precipitate at 65 C settles during incubation and does not interfere with macroscopic or microscopic observation of cultures. Because the precipitate may be enough to clog sterilizing filters quite quickly, it is best to sterilize serum before heating.

The technique used in this laboratory now is to obtain sheep serum from the slaughter house, sterilize it by filtration, dispense it into convenient volumes, and heat it at 65 C for 2 hr. The heated serum is added aseptically to Korthof medium base. The complete medium is checked for sterility and inoculated as usual. Growth with minimal or no auto-agglutination is comparable with growth in medium with rabbit serum.

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