

Thesis Title A Study on the Pepsin Digesting of the
 Horse Anti-snake Venom

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ABSTRACT

A study was carried out aiming at optimizing the pepsin digestion reaction of horse antiserum against snake venoms. Rabbit antibodies, specific to horse antivenom IgG/IgG(T), horse F(ab')₂ and horse Fc, were prepared and purified by using different affinity adsorbents. These rabbit antibodies were used to develop ELISA's which, when used together, quantitated horse IgG/IgG(T) and horse F(ab')₂ over a linear concentration range between 5-50 x 10⁻⁵ mM.

Horse antivenom was digested with pepsin under the following 6 conditions: Condition A, pepsin 0.15% (w/v), pH 3.5; Condition B, pepsin 0.15% (w/v), pH 3.0; Condition C, pepsin 0.15% (w/v), pH 3.25; Condition D, pepsin 0.15% (w/v), pH 3.75; Condition E, pepsin 0.1125% (w/v), pH 3.5

and Condition F, pepsin 0.1875% (w/v), pH 3.5. The reaction temperature was 25^o C and the digestion was carried out over a period of 24 hours. Aliquots, from each reaction Condition, were taken at various time intervals to assess the extent of digestion by measuring the concentrations of the immunologically active antivenom IgG/IgG(T) and the F(ab')₂ product. It was found that the digestion rate was faster at lower pH or at higher enzyme concentration. Under most of the conditions, the starting IgG(T) were all digested within 2 hours with the concomitant appearance of F(ab')₂. Using 0.15% pepsin, digestion at pH 3.5 (Condition A), the highest level of immunologically active F(ab')₂ was observed with a recovery of antibody activity of about 60%.

This study should lead to the optimization of the pepsin digestion reaction of horse antivenom for the preparation of antivenom refined globulin.