

## Immunochemical Studies of Gibbon Anti-DNP Antibody

Principal Investigators :                   Stitaya Sirisinha, D.D.S., Ph.D.\*  
  Douglas R. Stutz, MAJ, MSC

Associate Investigator :                 Elisandro Rodriguez, SSG

**OBJECTIVE:** To study the affinity of antibody produced in gibbons against the hapten dinitrophenol (DNP).

**DESCRIPTION:** The gibbon has been used as an experimental animal for studies involving the immune response to various pathogenic organisms. Little is known about the kinetics of a specific antibody response. To learn more about antibody produced in the gibbon a series of experiments were designed using a hapten system for purposes of simplicity. Dinitrophenol (DNP) was selected as the hapten since this system has been defined in several other species. A test antigen of DNP-protein was prepared by linking DNP to bovine gamma globulin (BGG) by nucleophilic substitution using a sulphonic acid derivative, sodium dinitrobenzyl sulphate. The epsilon-NH<sub>2</sub> groups of lysine residues are essentially the only substitutions made in this system thus allowing better definition of the hapten-carrier combination.

Each animal was injected subcutaneously with 2.5 mg DNP-BGG (mixed 1:1 with Freund's complete adjuvant). A minimum of 4 injection sites was used. A second injection of DNP-BGG in Freund's incomplete adjuvant was administered 30 days after the initial dose. A third inoculation of alum precipitated DNP-BGG was administered 60 days after the initial injection. Each animal was bled once a week throughout the inoculation schedule.

**PROGRESS:** Five gibbons were selected for these experiments, and 3 have been immunized through the first 2 injections. Two gibbons were kept as controls and were bled according to the immunization schedule. Immunoabsorbents of DNP-Lysine conjugated to Sepharose have been prepared and a new technique for elution of purified antibody has been tested and found to be better than previous techniques (Hill, 1972)<sup>1</sup>. This particular technique has not been reported for a hapten-antibody system. Experiments have been designed for adsorbing anti-DNP antibody from each of the successive bleedings from each animal, and for determining the affinity of the antibody by the fluorescence quenching technique. The antibody affinity data obtained in these experiments will be correlated with the agglutinating, precipitating, and complement fixing activity of these same sera.

### REFERENCE:

1. Hill, R.J.: Elution of antibodies from immunoabsorbents: Effect of dioxane in promoting release of antibody. *J. Immunol. Methods.* 7: 231-245, 1972.

---

\* Dept of Microbiology, Faculty of Science, Mahidol University, Bangkok.