

# This Week's Citation Classic

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Vaitukaitis J L, Robbins J B, Nieschlag E & Ross G T. A method for producing specific antisera with small doses of immunogen. *J. Clin. Endocrinol. Metab.* 33:988-91, 1971. [Reprod. Res. Br., Nat. Inst. Child Health Hum. Develop., NIH, Bethesda, MD]

**A method for generating antisera with small doses of immunogen is described. With this technique, 100 ug or less of immunogen induced specific antibody production in rabbits injected intradermally. Moreover, animals injected with a single dose of the immunogen continued to produce antisera for several months in response to the single immunizing dose. [The SC<sup>P</sup> indicates that this paper has been cited over 535 times since 1971.]**

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"In the early 1970s, Griff Ross and I were studying structure-function relationships of human chorionic gonadotropin in terms of both its biologic and immunologic activities. This work was carried out in collaboration with Robert Canfield and Frank Morgan at the College of Physicians & Surgeons in New York City. The New York laboratory was purifying and biochemically characterizing hCG and had worked out a technique to separate hCG into its two subunits. A limited amount of highly purified subunit preparations was available to us for study. Moreover, the characterization of the subunits' amino acid composition and sequence was limited at that time. We thought the most rapid way of attaining some understanding of the structure-function relationship of the subunits was with an immunologic approach.

"Since relatively small quantities of the hCG subunits could be used for immunization, we decided to try our hand at generating antibody with small amounts of immunogen. Our laboratory and others had generally used mg quantities of antigens for immunization up to this time. Consequently, John Robbins, a microbiologist at NICHD, was consulted, since he too

used a variety of immunization techniques in his studies. Since no techniques incorporated microgram quantities of immunogen, we collectively decided to modify our immunization technique because of our restricted, precious supply of highly purified hCG subunits. In short, we rationalized that if an animal were injected intradermally over a wider area with increased concentrations of heat-killed tubercle bacillus in an emulsion containing microgram quantities of the subunit, antibody with high affinity and specificity might result. Since our initial studies were fruitful, we wished to know whether haptens conjugated to carrier proteins would also induce similar responses in animals immunized with 100 ug or less of conjugated haptens. Consequently, E. Nieschlag was invited to participate in our study with a steroid conjugated to bovine serum albumin or keyhole limpet hemocyanin.

"In short, the technique worked with generation of antibody of high titer and affinity being harvested between eight to 16 weeks after the primary immunization. Because the technique was so successful in our hands, we wished to share it with our scientific colleagues and, consequently, published it as a Rapid Communication. Just as highly purified hormonal preparations became available in the early 1970s, small quantities of highly purified preparations of non-hormonal substances were generated with the newer separation techniques. Consequently, investigators in other fields wished to generate antibody to their highly purified preparations and, consequently, used our approach.

"I continue to receive phone calls from investigators around the country who wish to use that technique to generate antibody to their precious, limited quantities of substances ranging from proteins extracted from viruses to subcellular enzymes. At the time we developed the technique, we had no idea how widely that approach would be utilized."