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Determination of Immunoglobulin A Concentrations in Human Nasal Secretions with a Serum Immunoglobulin A Standard

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By quantitative immunodiffusion tests, nasal secretion immunoglobulin A was underestimated by approximately 5.6-fold when the serum 7S immunoglobulin A standard was employed.

The concentration of immunoglobulin A (IgA) in human nasal secretion specimens is usually determined by single radial immunodiffusion tests. These assays are usually performed with commercially available kits which employ 7S IgA as a reference standard (1, 12). Concentrations of IgA in secretions determined by this procedure are grossly underestimated. The IgA in these specimens is predominantly of larger molecular size than the 7S standard. This increased molecular weight is due not only to the presence of additional polypeptide chains, secretory piece and J chain, but also to its polymeric nature (2, 13).

In this study, secretory IgA was purified from pooled human nasal secretions and was compared with commercial 7S IgA standards in immunodiffusion plates. Nasal washes were obtained from 50 adult volunteers and concentrated as previously described (9-11). The secretions were then pooled and chromatographed on Sephadex G-200 to remove low-molecular-weight contaminants. Those fractions which contained IgA by immunoelectrophoresis and eluted near the void volume were pooled and concentrated as described above. The pool was then chromatographed on Sepharose 4-B, and the IgA peak was again pooled and concentrated. The purified secretory IgA (50 to 75 μg) gave a single precipitant arc with goat anti-human whole serum which was identical to that observed with goat anti-human IgA. Although low levels of contaminating proteins may be present, none was detected by this procedure. The protein concentration of this pool was 5.3 mg/ml as determined by the method of Lowry et al. (4), and ultracentrifugation in sucrose density gradients revealed the sedimentation pattern expected for secretory IgA (i.e., a major peak at 11S and minor amounts at 13 to 19S). The secretory IgA pool was aliquoted and stored at −70°C.

Plates for immunodiffusion tests were prepared in our laboratory by using a 1:32 dilution of goat anti-human IgA (Meloy Labs, Springfield, Va.) mixed with 2% Noble agar. The antiserum-agar solution was mixed at 50°C and poured quickly into square plastic phage plates (Falcon Plastics, Oxnard, Calif.), taking care to uniformly cover the bottom to a depth of 1.5 mm. A 1.5-mm steel agar punch (immunoelectrophoresis kits; LKB Instruments Inc., Rockville, Md.) was used to punch holes in the agar plates at 8-mm intervals. Plates were kept at 4°C in humidified chambers before use.

The relationship of the nasal secretion IgA to commercially obtained monospecific 7S IgA serum standards (Meloy Labs) was tested both in our laboratory-prepared plates and in commercial plates (low-level IgA; lot AP18, Meloy Labs). The sedimentation characteristics for two of the commercial standards were confirmed by ultracentrifugation in sucrose density gradients. The immunodiffusion plates were charged and incubated at room temperature. Although completion of diffusion may require several days, all precipitin diameters were measured at 18 h because of the distinct precipitin lines and the linear relationship observed (6).

Twofold dilutions were made of each IgA preparation and tested in duplicate on each immunodiffusion plate. Five separate experiments were performed, and the mean value for each dilution is given in Fig. 1. The dose-response curves for the secretory and 7S IgA preparations were parallel in both immunodiffusion plates, which suggests that the two responses were related by a constant factor over the range of concentrations tested. A linear-regression equation was determined for the relationship be-

tween the nasal secretion IgA and the serum IgA standard in both types of plates (Fig. 2). The regression equation ($y = a + bx$) with laboratory plates was as follows: milligrams of nasal secretion IgA = −5.5 + 5.5 (concentration of 7S IgA). With a commercial plate, the equation was as follows: milligrams of nasal secretion IgA = 2.0 + 5.8 (concentration of 7S IgA). Values for a in the regression equation will vary depending upon the individual plates employed and are not a component of the relation of 7S to 11S IgA concentration (3).

When the commercial low-level IgA immunodiffusion plates were used, the measured diameters at 18 h were comparable (±5%) for the commercial serum standard (38 IU of IgA per ml) and the WHO immunoglobulin reference preparation 67/97 which contained 95.3 IU of IgA per ml (kindly provided by P. Ogra, Buffalo, N.Y.).

A simultaneous comparison of the nasal secretion IgA with commercial serum IgA standard in commercially prepared and laboratory-prepared immunodiffusion plates provided a factor for conversion of 7S IgA values to the concentration of IgA in a nasal secretion preparation. The nasal secretion preparation provided an IgA concentration 5.5 to 5.8 times that provided by a serum 7S IgA standard.

Several studies have attempted to employ secretory IgA as well as 7S serum IgA in measurements of mucosal surface antibodies (7, 8, 14, 15). In one recent report, underestimation of IgA concentration in tears was found when a commercially prepared 11S IgA preparation was compared with a commercial 7S serum IgA standard (5). However, there are no previous reports comparing a purified human nasal secretory IgA preparation with a commercial 7S serum IgA standard.

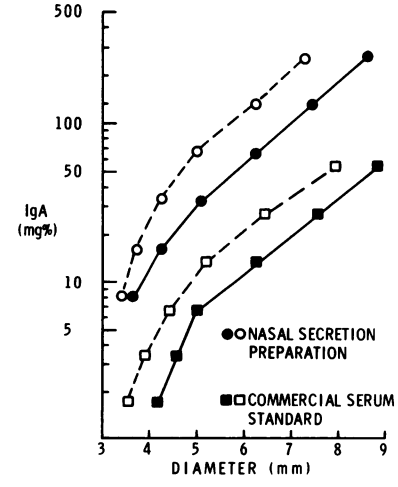

FIG. 1. *Standard curves of the nasal secretion preparation on laboratory (○) and commercial (●) immunodiffusion plates versus the commercial serum IgA standard on laboratory (□) and commercial (■) immunodiffusion plates.*

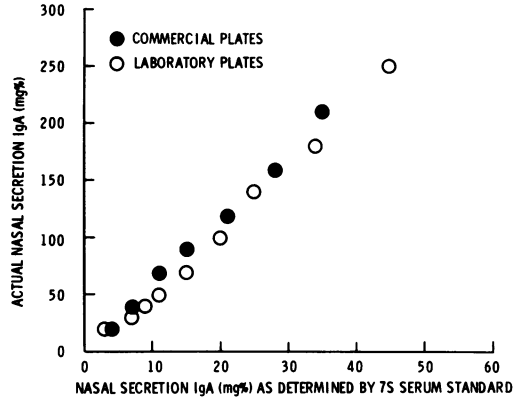

FIG. 2. *Relationship of nasal secretion IgA to a 7S serum IgA standard on immunodiffusion plates.*

Rossen et al. have shown that the sedimentation characteristics and concentration of nasal secretion IgA vary from person to person, but that the bulk of nasal secretion IgA sediments in the 9 to 14S region (10). Indeed, when 15 individual nasal secretions were analyzed for secretory IgA by sucrose density centrifugation, the major peak was always found in the 10 to 12S region. This is in agreement with the nasal secretion IgA preparation used in our comparative experiments. Although differences can be found depending on the commercial plate and standard employed, the conversion factor reported here should allow for close approximation of the secretory IgA concentration in an individual nasal secretion, provided the 7S IgA preparation used is standardized against the international standard (3).

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LITERATURE CITED

1. **Brunner, H., H. B. Greenberg, W. D. James, R. L. Horswood, R. B. Couch, and R. M. Chanock.** 1973. Antibody to *Mycoplasma pneumoniae* in nasal secretions and sputa of experimentally infected human volunteers. Infect. Immun. **8**:612-620.
2. **Butler, W. T., R. D. Rossen, and T. Waldman.** 1967. The mechanism of appearance of immunoglobulin A in nasal secretions in man. J. Clin. Invest. **46**:1883-1893.
3. **Hosty, T. A., M. Hollenbeck, and S. Share.** 1973. Intercomparison of results obtained with five commer-

cial diffusion plates supplied for quantitation of immunoglobulin. Clin. Chem. **19**:524–526.

4. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
5. **McMurray, D. N., H. Rey, L. J. Casazza, and R. R. Watson.** 1977. Effect of moderate malnutrition on concentrations of immunoglobulins and enzymes in tears and saliva of young Colombian children. Am. J. Clin. Nutr. **30**:1944–1948.
6. **Mancini, G., A. O. Carbonara, and J. F. Heremans.** 1964. Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry **2**: 235–254.
7. **Mandel, M. A., K. J. Dvorak, L. W. Worman, and J. J. DeCosse.** 1976. Immunoglobulin content in the bronchial washings of patients with benign and malignant pulmonary disease. N. Engl. J. Med. **295**:694–698.
8. **Reynolds, H. Y., and H. H. Newball.** 1974. Analysis of proteins and respiratory cells obtained from human lungs by bronchial lavage. J. Lab. Clin. Med. **84**:559–573.
9. **Rossen, R. D., W. T. Butler, T. R. Cate, C. F. Szwed, and R. B. Couch.** 1965. Protein composition of nasal secretion during respiratory virus infection. Proc. Soc. Exp. Biol. Med. **119**:1169–1176.
10. **Rossen, R. D., W. T. Butler, W. E. Vannier, R. G. Douglas, Jr., and A. G. Steinberg.** 1966. The sedimentation and antigenic properties of proteins in nasal and other external secretions. J. Immunol. **97**:925–938.
11. **Rossen, R. D., A. L. Schade, W. T. Butler, and J. A. Kasel.** 1966. The proteins in nasal secretion: a longitudinal study of the gamma-A-globulin, gamma-G-globulin, albumin, siderophilin, and total protein concentrations in nasal washings from adult male volunteers. J. Clin. Invest. **45**:768–780.
12. **Smith, C. B., R. H. Purcell, J. A. Bellanti, and R. M. Chanock.** 1966. Protective effect of antibody to parainfluenza type 1 virus. N. Engl. J. Med. **275**:1145–1152.
13. **Tomasi, T. B., and H. M. Grey.** 1972. Structure and function of immunoglobulin A. Prog. Allergy **16**:81–213.
14. **Waldman, R. H., J. M. Cruz, and D. S. Rowe.** 1971. Immunoglobulin levels and antibody to Candida albicans in human cervicovaginal secretions. Clin. Exp. Immunol. **9**:427–434.
15. **Waldman, R. H., J. P. Mach, M. M. Stella, and D. S. Rowe.** 1970. Secretory IgA in human serum. J. Immunol. **105**:43–50.