

## Agglutinins for complement-coated sheep erythrocytes

SALIVARY 'IgA Immunoconglutinins', which reportedly bind both altered and native C3, have generated considerable interest<sup>1,2</sup>. Unfortunately, the methods so far described for their purification are somewhat cumbersome, as they involve incubation of complement-coated sensitised sheep cells with saliva and subsequent elution of the 'IgA immunoconglutinins' with a chelating buffer. In our laboratory we have used a more classical approach, first fractionating parotid saliva on BioGel P100, then subfractionating each peak by DEAE-cellulose chromatography and testing the fractions for their reactions with complement and complement-coated cells (Fig. 1). (The details of the purification of salivary complement-reactive factors will be published elsewhere<sup>3</sup>.)

Our procedure has obvious advantages over that of Price *et al.*<sup>1</sup>. Since Ca<sup>2+</sup>-dependent IgA agglutinins for sheep cells are present in low titre in salivary secretions<sup>3,4</sup>, it is possible that those authors isolated secretory IgA attached not only to the human C3 on

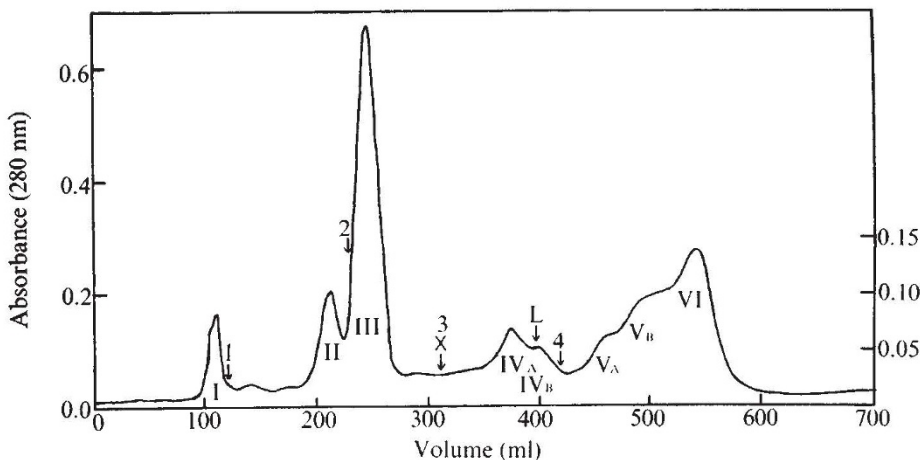
erythrocytes would tend to confuse qualitative and quantitative agglutination results. Furthermore, preliminary evidence suggests that salivary C3

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and C4 agglutinins can be separated by DEAE subfractionation of the BioGel P100 peaks, with some salivary fractions demonstrating agglutinating activity for complement-coated sensitised erythrocytes and yet containing no detectable  $\alpha$ -chain antigens.

Using purified saliva fractions we have found that saliva contains agglu-



**Fig. 1** Molecular sieve elution pattern of pooled parotid saliva chromatographed on BioGel P100. The elution buffer was 0.1 M Tris-0.2 M NaCl, pH 8.1, with a flow rate of approximately 6 ml h<sup>-1</sup>. Column bed dimensions were 2.5 × 96.5 cm with a volume of 475 ml. The saliva was dialysed against the column buffer using 3,000 m.w. cutoff tubing (Thomas) before concentrating (Amicon apparatus, UMO5). Five millilitres of sample was added to the column. Salivary fractions (SFP) are labelled with Roman numerals. Numbered arrows refer to marker protein elution volumes; the proteins used were bovine albumin, chymotrypsinogen A, insulin, and bovine trypsin inhibitor, for arrows 1-4 respectively. The arrow labelled L represents the elution volume of human lysozyme, which was verified independently, and X denotes the scale change position.

the sensitised erythrocytes but also to the erythrocytes themselves. Contamination with IgA antibodies and non-specific agglutinins for sensitised

tinins for IgG- and IgM-coated erythrocytes, located in SFPIV and SFPII respectively (Fig. 1).  $\alpha$ -chain antigens were found only in SFPI.

R. J. BOACKLE  
K. M. PRUITT  
H. HUGH FUDENBERG  
R. L. MILLER  
E. A. CARSGO  
W. F. HOWARD, JR

*Department of Basic and Clinical Immunology and Microbiology, Medical University of South Carolina, Charleston, South Carolina 29401*

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PRICE *et al.* REPLY.—The claim by Boackle *et al.*<sup>1</sup> that their purification procedure "has obvious advantages over that of Price *et al.*" is based on the erroneous assumption that calcium-dependent IgA agglutinins to sheep erythrocytes are present in high titre in salivary secretions. In support of this statement they refer to the work of Tönder and Larsen<sup>2</sup>. The IgA agglutinins described by these authors, however, were not calcium dependent and were directed against rabbit rather than sheep erythrocytes. Agglutinins to sheep erythrocytes were looked for and found in only four of 17 samples of saliva, the highest titre being 1:4. Other workers have shown that agglutinins to sensitised sheep erythrocytes are present in low titre in adult saliva, but these agglutinins are not calcium dependent<sup>3,4</sup>.

Contamination with IgA antibodies directed against the sensitised erythrocytes would therefore not occur when EDTA was used to elute the anti-C3 antibodies from the cell surface. A simple way of preventing possible contamination by calcium-dependent agglutinins would be to incubate the saliva with sensitised erythrocytes before incubation with complement-coated cells.

Other workers who may be interested in studying these antibodies will decide whether EDTA elution from washed complement-coated erythrocytes (a procedure which may be performed in 2-3 h) or fractionating on BioGel P100 and then subfractionating on DEAE-cellulose is the less cumbersome method of purification.

*Hospital for Sick Children and Guys Hospital, London, UK*

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