Immunology, 1975, **29**, 933.

Secretory Component of the Guinea-pig

J.-P. VAERMAN, MICHÈLE C. NACCACHE-CORBIC AND J. F. HEREMANS

Department of Experimental Medicine, Institute of Cellular Pathology, Avenue Hippocrate, Bruxelles, Belgium

(*Received* 15*th January* 1975; *accepted for publication* 28*th April* 1975)

Summary. Free secretory component (FSC) was purified from guinea-pig milk by gel-filtration and immunoabsorption on anti-SC antibodies. Guinea-pig FSC cross-reacted with antisera to human FSC, and the reverse. Guinea-pig and human FSC resembled each other in molecular size, electrophoretic mobility and heterogeneity, as well as by the existence of antigenic determinants restricted to the unassociated form of the molecule. Guinea-pig FSC associates *in vitro* with guinea-pig IgM. Disruption of disulphide links is required to set free guinea-pig FSC from secretory IgA.

INTRODUCTION

'Secretory component' (SC) is a glycoprotein believed to mediate the transepithelial transfer of IgA polymers containing J chain (and to a lesser extent of IgM) from the intercellular fluid of glands and mucosae into external secretions, by acting as a specific receptor protein on the membrane surface of the epithelial cells (Brandtzaeg, 1974a, b; Poger and Lamm, 1974). The mechanism of this transfer is, however, far from being unravelled.

The SC of the secretory IgA (SIgA) system has been identified in various animal species, including the dog (Ricks, Roberts and Patterson, 1970; Reynolds and Johnson, 1971; Vaerman, 1973), bovine (Mach and Pahud, 1971; Butler, 1971), goat and sheep (Pahud and Mach, 1970), pig (Bourne, 1969, 1974; Porter and Allen, 1970), horse (Pahud and Mach, 1972), rabbit (O'Daly and Cebra, 1971a, b, c) and rat (Vaerman, Heremans, Bazin and Beckers, 1975). The availability of anti-SC reagents to study the transfer of IgA in secretions of other small laboratory rodents is clearly desirable. The present report deals with the purification and partial characterization of the SC of the guinea-pig.

MATERIALS AND METHODS

Materials

Guinea-pig serum, milk, saliva, tears and urine were obtained as described previously (Vaerman and Heremans, 1972).

Guinea-pig SIgA was isolated from milk as described earlier (Vaerman and Heremans, 1972). A final gel-filtration through a 2 m-long column of Sepharose 6B was added in order to remove small amounts of contaminating IgM.

Guinea-pig IgM was purified by gel-filtration on Sepharose 6B of the euglobulin precipi-

Correspondence: Dr J.-P. Vaerman, Department of Experimental Medicine, I.C.P., Avenue Hippocrate 75, B-1200 Brussels, Belgium.

tate (0·02 M Sørensen phosphate buffer, pH 6·0) obtained from serum deprived of its β-lipoproteins (Burstein and Samaille, 1959).

Human free secretory component (FSC) was purified according to Kobayashi (1971).

Anti-guinea pig SIgA. A rabbit (number 555) and a goat (number 595) were injected with purified guinea-pig milk IgA. The antisera were absorbed with small amounts of serum and with an α-globulin of high molecular weight obtained by preparative electrophoresis of the second Sephadex G-200 peak from milk whey (see Results section). These antisera reacted both with α-chains and with SC determinants.

Anti-guinea pig IgA, IgM, IgG1, IgG2, albumin, transferrin, and anti-whole milk whey or serum, were the same as used previously (Vaerman and Heremans, 1972).

Anti-guinea pig FSC. A rabbit (number 709) and a goat (number 629) were injected with guinea-pig FSC purified from milk (see Results section). The antisera were absorbed with small amounts of serum and with a milk protein eluted from G-200 slightly after FSC to render them monospecific.

Anti-human-FSC was obtained as reported earlier (Kobayashi, Vaerman and Heremans, 1973).

Methods

Preparative electrophoresis was performed on 1 cm-thick Pevikon blocks, as detailed elsewhere (Vaerman, 1970).

Anti-guinea pig FSC immunoabsorbent. The IgG fraction, obtained from goat serum (number 595) by elution from DEAE–cellulose at pH 8·0 in 0·1 M Tris–HCl buffer, was coupled (20 mg/ml) to 6 aminohexyl-Sepharose 4B activated by glutaraldehyde, according to Cambiaso, Goffinet, Vaerman and Heremans (1975). The non-retained proteins were removed by washing with alternating aliquots of buffered saline and of 2 M NaCl. The antigen was eluted with 3 M ammonium thiocyanate at pH 6·0.

Analytical ultracentrifugation was performed in a Beckman–Spinco model E ultracentrifuge at 20° in saline at a speed of 56,000 rev/min, using Schlieren optics.

Density gradient ultracentrifugation was carried out in the SW 65 Ti-rotor of the Beckman-Spinco model L ultracentrifuge according to Vaerman (1970).

Gel electrophoresis in SDS–urea was performed as outlined previously (Kobayashi, Vaerman and Heremans, 1973) at pH 7·0 in 6 per cent gels containing 8 M urea.

Single radial immunodiffusion was carried out according to Mancini, Carbonara and Heremans, 1965.

Semi-quantitative Ouchterlony analyses were performed on microscopic slides in agarose gels using a standard '12 wells-1 trough' pattern. Equal volumes of successive eluates from gel-filtration experiments were allowed to diffuse against various specific antisera in the troughs. The antigen content of all tubes was estimated by the intensity, position and aspect (antigen excess, equivalence, antibody excess) of the precipitin line. Elution curves were constructed, but without ordinates, as the relative amounts of a given protein amongst successive eluates should not be compared to those of another protein in absolute concentration. Therefore, the ordinates of these elution curves have no scale. They merely serve to localize the eluate fractions containing a given protein (see Fig. 1).

RESULTS

Guinea-pig milk whey was fractionated on Sephadex G-200 (Fig. 1) and the eluates were tested for the presence of α chains, SC, IgM, IgG, albumin and transferrin. The

agarose gel electrophoreses and immunoelectrophoreses of the concentrated Sephadex fractions are illustrated in Fig. 2a and 3b, respectively. When an immunoelectrophoresis of whole milk whey was developed with anti-SIgA antiserum, two precipitin lines of nearly similar mobility, could be visualized (Fig. 3a). The line closest to the antibody reservoir was found to represent FSC, diffusing faster than SIgA. This conclusion was based on the finding that the low-molecular-weight Sephadex fraction 5, although devoid of IgA (as observed earlier: Vaerman and Heremans, 1972), contained a β-component reacting

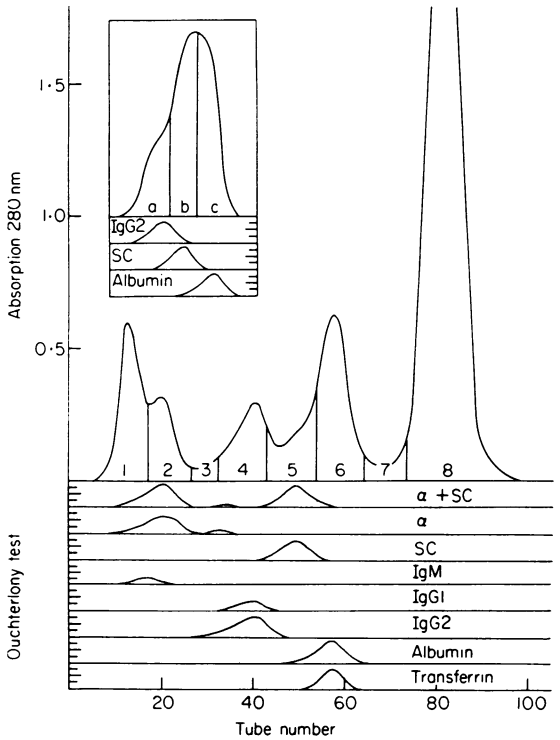

FIG. 1. Gel-filtration of guinea-pig milk whey on Sephadex G-200. Elution pattern and semi-quantitative immunochemical distribution by Ouchterlony analysis of IgA (α), SC, IgG1, IgG2, IgM, albumin and transferrin. The ordinates for individual protein concentrations were all arbitrarily divided into four units corresponding to increasing strengths of reaction in the Ouchterlony tests (+ to + + + +). Insert shows the refiltration of fraction 5.

with anti-SIgA antiserum, but not with the same antiserum absorbed with purified SIgA. SIgA was mainly eluted in Sephadex fractions 1 and 2, but small amounts of α chain determinants were also found in fractions 3 and 4. The SIgA from fraction 2 was separated from an unidentified α globulin by Pevikon electrophoresis. Three fractions were recovered: a slow fraction containing the bulk of SIgA, a fast fraction containing only the milk α globulin, and an intermediate fraction containing both SIgA and α globulin (Fig. 2b). This SIgA still contained trace amounts of IgM, which could be removed by gel-filtration through Sepharose 6B (not shown).

The antigenic relationships of guinea-pig SIgA, serum IgA and FSC (fraction 5, Fig. 1) are illustrated in Fig. 4 using anti-SIgA number 595. SIgA spurred over both FSC and

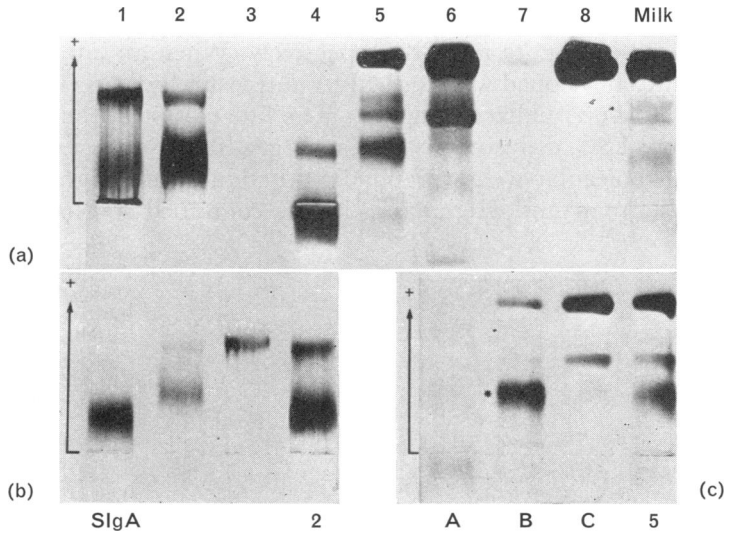

FIG. 2. Agarose gel electrophoreses of the following. (a) Whole guinea-pig milk whey and the concentrated Sephadex fractions 1–8 of Fig. 1. (b) The three fractions obtained after preparative electrophoresis performed on Sephadex-fraction 2. From left to right: SIgA, mixture of SIgA and α globulin, milk α globulin and starting material. (c) Fraction 5 from Fig. 1 and subfractions 5a (A), 5b (B) and 5c (C) from Fig. 1, insert. Asterisk marks major non-FSC milk protein.

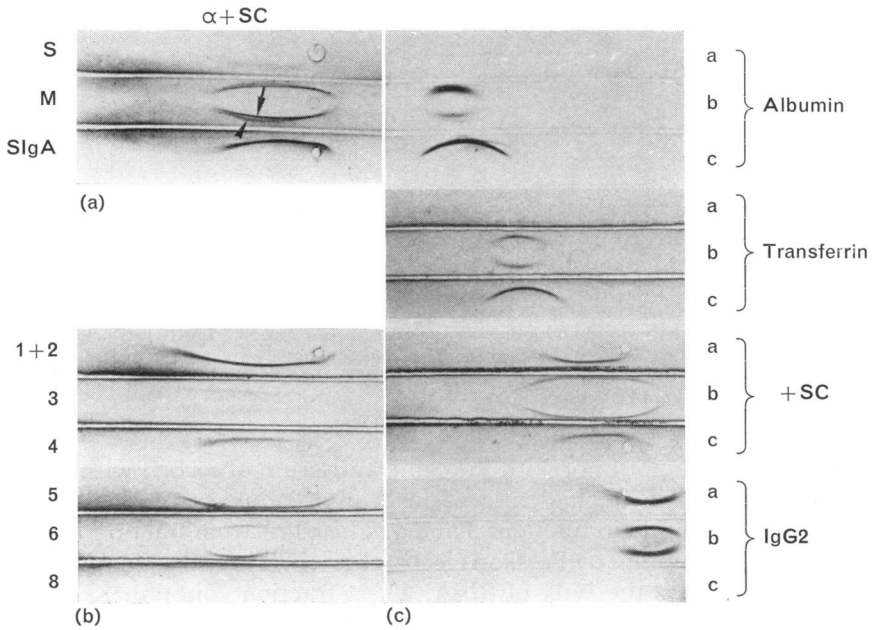

FIG. 3. (a) Immunoelectrophoreses of guinea-pig whole serum (S), milk whey (M) and SIgA developed with anti-SIgA (α+SC) antibodies. The arrow head points to presumed FSC, and the arrow to SIgA. (b) Immunoelectrophoreses of concentrated fractions 1 to 8 from Fig. 1, developed with anti-SIgA antiserum. (c) Immunoelectrophoreses of subfractions 5a (a), 5b (b) and 5c (c) from Fig. 1, insert, developed with anti-albumin, anti-transferrin, anti-SIgA (α+SC) and anti-IgG2 antisera. The anode is to the left.

serum IgA. The FSC line crossed the serum IgA line, giving a pattern of non-identity. When a concentrated high-molecular weight fraction of serum ('heavy' serum IgA; fractions 5 and 6 of Fig. 6 from Vaerman and Heremans, 1972) was similarly analysed, it produced two precipitin lines: the major one, closest to the antibody reservoir, reacted similarly to 'light' serum IgA, whereas the minor line merged with those of SIgA and FSC, indicating the presence of SIgA in the high molecular weight fraction of serum IgA.

In order to further purify FSC, Sephadex fraction 5 was refiltered on G-200 (Fig. 1, insert). The agarose gel electrophoreses and immunoelectrophoreses of subfractions 5a, 5b and 5c are shown in Figs 2b and 3c, respectively. Subfraction 5b was richest in FSC, but it still contained appreciable amounts of albumin, transferrin, IgG and a major contaminant of fast β mobility (Fig. 2c, asterisk). Subfraction 5b was then applied to an anti-SIgA immunosorbent column. The retained FSC was eluted with 3 M ammonium thiocyanate, dialysed against borate-buffered saline, pH 8·4, concentrated and cen-

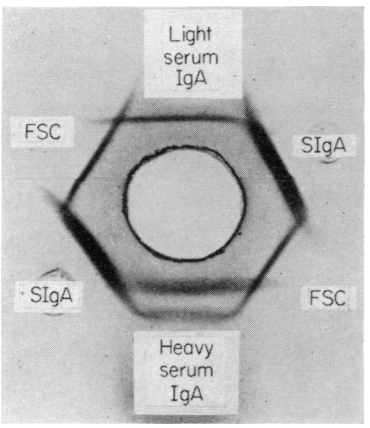

FIG. 4. Antigenic relationships of guinea-pig serum IgA, SIgA and FSC, revealed by anti-SIgA antibodies (central well). Heavy serum IgA refers to a concentrated high molecular weight fraction of serum, containing both IgA dimer and SIgA (see text). Light serum IgA comprises monomeric serum IgA.

trifuged to discard a precipitate. Fig. 5 illustrates the electrophoretic and immunoelectrophoretic purity of this FSC, as compared to whole serum and milk whey. The preparation appeared as a double component of fast and slow β mobility. It was contaminated only by small amounts of guinea-pig IgG, and by traces of goat immunoglobulins which hampered the accurate measurement of protein concentration of this sample. A final purification step consisted in another gel-filtration on Sephadex G-200. The sample was first mixed with dextran blue® and with 5 μg of human FSC labelled with ^{125}I, in order to directly compare the elution of guinea-pig and human FSC (Fig. 6). The effluent was monitored for optical density, radioactivity, and reactivity with anti-guinea-pig SIgA. The second OD peak coincided exactly with the radioactivity of the human FSC and with the reactivity to anti-guinea-pig SIgA. This second peak was concentrated to about 1 ml and ultracentrifuged (Fig. 7a). A single boundary was observed, yielding an uncorrected sedimentation coefficient of 3·72S for guinea-pig FSC, as compared to 4S for its human counterpart. Upon SDS gel-electrophoresis (Fig. 7b), the purified FSC displayed a single component, migrating at a position very close to that of human FSC. By comparison

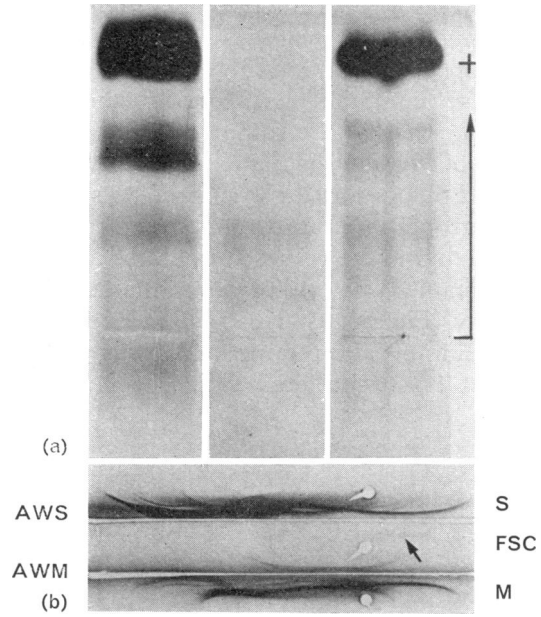

FIG. 5. Agarose electrophoresis (a) and immunoelectrophoresis (b) (anode to the left) of guinea-pig FSC purified by elution from anti-SIgA immunoabsorbent, as compared to whole serum (S) and milk whey (M). AWS = anti-whole serum; AWM = anti-whole milk-whey. The arrow in (b) points to trace of guinea-pig IgG2.

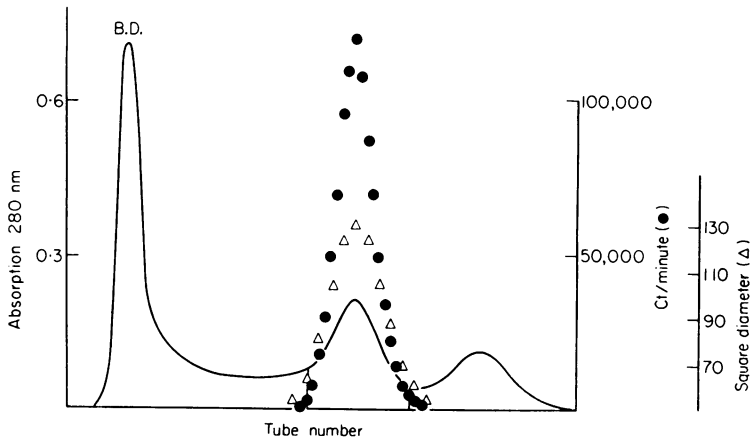

FIG. 6. Gel-filtration, on Sephadex G-200, of guinea-pig FSC obtained by immunoadsorption. The sample was mixed with dextran blue (B.D.) and a trace of ^{125}I-labelled human FSC (●). Guinea-pig FSC (△) was located by radial immunodiffusion. Square diameter refers to squared diameters (in mm) of precipitin rings in the Mancini plate containing anti-guinea-pig SC antiserum. The last absorption peak represented fragments of the immunoglobulin coupled to the immunoabsorbent column.

with human IgG, bovine serum albumin monomer and dimer, and ovalbumin, the calculated apparent molecular weights of guinea-pig and human FSC were 88,000 and 93,000, respectively.

The nature of the bonds linking SC to guinea-pig IgA was investigated in milk SIgA. After reduction with 0·02 M dithiothreitol DTT and alkylation with 0·06 M recrystallized

iodoacetamide at pH 8·0, immunoelectrophoreses of SIgA (Fig. 8) revealed two components, the slower of which migrated slightly behind untreated SIgA, and reacted with anti-SC but not with anti-α antibodies. The faster precipitin line of the reduced material only reacted with anti-α antibodies, suggesting that the splitting between SC and IgA upon these conditions of reduction–alkylation was almost complete.

The existence of the so-called 'I' antigenic determinants (I = determinants of SC inaccessible in the SIgA molecule) was investigated by means of antisera raised against the purified guinea-pig FSC. Upon reaction with one of these (rabbit number 709), FSC clearly spurred over SIgA (Fig. 9a). After absorption of this antiserum with native SIgA, it still reacted with FSC, but no longer with SIgA (Fig. 9b), demonstrating the

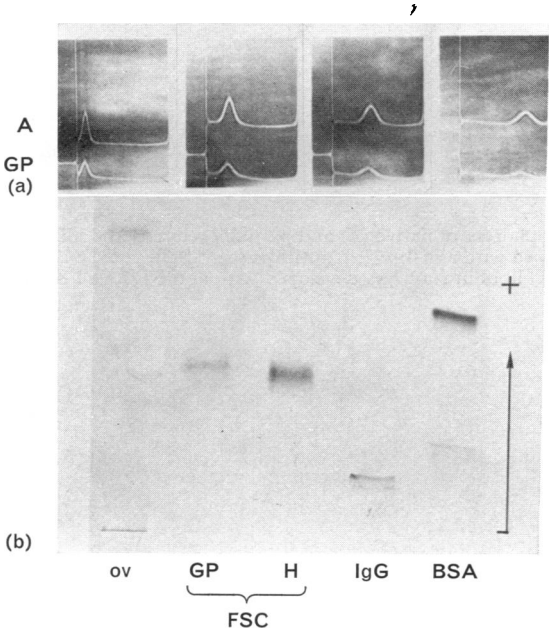

FIG. 7. (a) Analytical ultracentrifugation of human (H) and guinea-pig (GP) FSC (fraction between vertical bars in Fig. 6). Sedimentation from left to right. Photographs taken at 16, 48, 80 and 136 minutes after reaching 56,000 rev/minute. Phase plate angle at 65° and 55°, respectively, for first and last pictures. Protein concentrations: 7 and 4 mg/ml, respectively, for H and GP. (b) SDS–acrylamide gel electrophoresis of ovalbumin (OV), human IgG, bovine serum albumin (BSA) monomer and dimer and guinea-pig (GP) and human (H) FSC. The anode is at the top.

existence of the 'I' determinants on guinea-pig FSC. In addition, when SIgA was first dialysed against 6 M urea at pH 3·5 for 16 hours at 4°, and then against buffered saline, the 'I' determinants became exposed in the SIgA molecule, as shown by their reaction with anti-'I' antibodies (Fig. 9c, d). FSC was also detected, using specific anti-'I' antibodies, in urine and saliva after concentration, and in tears (not shown).

Human and guinea-pig FSC were found to cross-react with their respective antisera (Fig. 10). Cross-reactions were also observed with canine FSC (not shown).

In vitro combination of guinea-pig FSC with guinea-pig serum IgM was attempted by mixing 90 μl of a solution, in pH 8·2 buffered saline, of purified serum IgM (1·1 mg), with 10 μl (about 100 μg) of ^{125}I-labelled semi-purified guinea-pig FSC (obtained by two-gel filtrations on Sephadex G-200 and preparative electrophoresis, but not further purified).

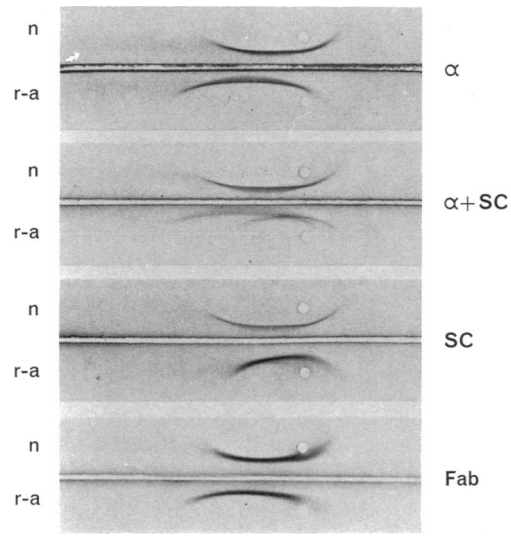

FIG. 8. Immunoelectrophoresis of native (n) and partially reduced and alkylated (r-a) guinea-pig milk SIgA (see text) developed with anti-IgA (α), anti-SIgA (α+SC), anti-FSC and anti-Fab antisera. Note crossing of IgA and SC lines in r-a SIgA developed with anti-SIgA (α+SC).

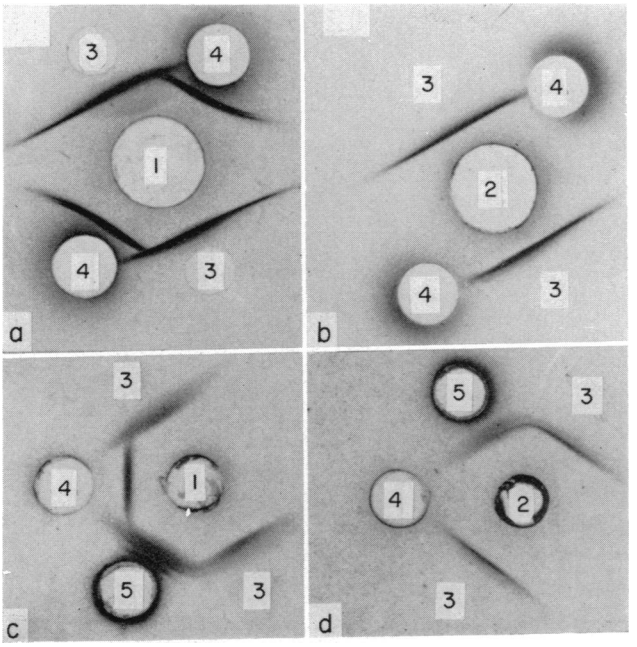

FIG. 9. (a) Spurring of FSC (3) over SIgA (4), due to 'I' determinants as revealed with anti-FSC number 709 (1). (b) Absorption of anti-FSC with native SIgA gives rise to antiserum (2) specific for 'I' determinants of FSC, absent from SIgA. (c) Spurring of denatured (8 M urea at pH 3·0 for 16 hours) SIgA (5) over native SIgA (4) and identity with FSC (3), using anti-SIgA (1). The nature of the small additional precipitin line given by (5) remains conjectural. (d) Specific anti-I (2) reacts only with FSC (3) and denatured SIgA (5).

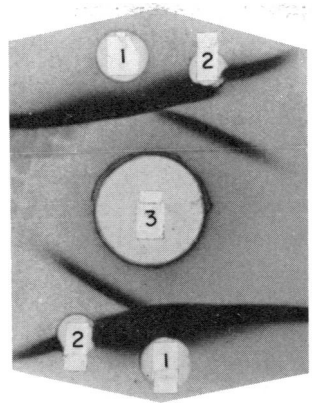

FIG. 10. Cross-reaction between human (1) and guinea-pig (2) FSC, demonstrated by anti-human FSC antiserum (3).

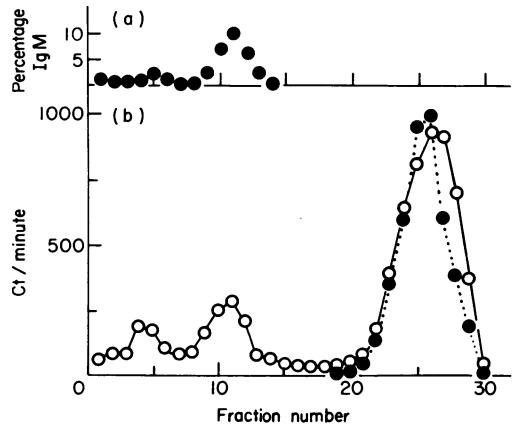

FIG. 11. *In vitro* binding of ^{125}I-labelled guinea-pig FSC to guinea-pig serum IgM, as shown by density gradient ultracentrifugation. (a) IgM distribution, as a percentage of the standard, by radial immunodiffusion. The IgM distribution was not affected by the admixture of ^{125}I-labelled guinea-pig FSC.) (b) Distribution, in ct/minute, of FSC alone (· · · ·) or mixed with IgM (—). The bottom of the gradients is to the left.

After incubation for 1 hour at 37° and overnight at 4°, the mixture was layered over a sucrose gradient and centrifuged for 17 hours at 35,000 rev/min. Two additional gradients were run in parallel, one containing only the IgM, and the other only the labelled FSC. The mixture of IgM and FSC (Fig. 11) displayed two main radioactive peaks, the lighter one corresponding to FSC and the heavier one (fractions 8–13) to IgM, as detected by radial immunodiffusion. Fractions 8–13 from the gradient containing labelled FSC+ IgM were pooled and divided into four equal aliquots. One aliquot was counted as such (standard). Two others were mixed with an excess of anti-FSC or anti-IgM antibodies, and with small amounts of cold carrier FSC or IgM, respectively. The resulting precipitates were washed twice with cold saline and counted. Anti-FSC and anti-IgM precipitated 96 and 82 per cent of the standard counts, respectively. The last aliquot was mixed with 0·5 mg of horse spleen ferritin and an excess of its antiserum, in order to give a control immune precipitate similar in size to those of the two preceding aliquots. Only 4 per cent of

the standard counts were found in this control, indicating that radioactivity was not non-specifically adsorbed.

DISCUSSION

In a preceding report (Vaerman and Heremans, 1972), the existence of guinea-pig SC was inferred from the presence of additional antigenic determinants on the IgA found in secretions as compared to serum IgA, and from the existence of a milk protein which, although devoid of immunoglobulin antigenic determinants, reacted with anti-SIgA antibodies and eluted on Sephadex G-200 at a position comparable to various mammalian FSC.

Here, guinea-pig FSC has been clearly identified by its cross-reaction with human FSC (Fig. 10). In addition, guinea-pig FSC was found to co-elute exactly with human FSC from Sephadex G-200 (Fig. 6). The uncorrected S values for guinea-pig and human FSC reported in this study roughly agree with $S^{\circ}_{20,w}$ values of 4·3 (van Munster, Stoelinga, Clamp, Gerding, Reijnen and Voss, 1972); of 4·6 (Haupt and Baudner, 1974) and 5·0 (Kobayashi, 1971) published for human FSC. By SDS–gel electrophoresis, our molecular weight estimates of guinea-pig and human FSC were 88,000 and 93,000, respectively; comparing well with values of 80,000 (Lamm and Greenberg, 1972), 85,000 (Mach, 1970), 90,000 (Mestecky, Kulhavy and Kraus, 1972) and 115,000 (Haupt and Baudner, 1974), obtained for human FSC by others using a similar technique.

Judging by its immunoelectrophoretic patterns, guinea-pig FSC has a heterogeneous β electrophoretic mobility. Similar electric charge heterogeneity has also been reported for human FSC, both on the basis of electrophoretic mobilities (Brandtzaeg, 1974a; Haupt and Baudner, 1974; Vaerman, unpublished data) and by elution from ion-exchangers over a range of ionic strengths (Kobayashi, 1971; Haupt and Baudner, 1974; Brandtzaeg, 1974c). The purified guinea-pig FSC seemed to have a bimodal electrophoretic mobility (Fig. 5). The origin of this phenomenon may be related to two observations made on human purified FSC. First, human FSC is very susceptible to proteolytic degradation (Kobayashi, Vaerman and Heremans, 1973). Fragments of SC have also been observed during purification of human FSC from milk (Haupt and Baudner, 1974; Kobayashi and Vaerman, unpublished observations). The fragments still react with antibodies to FSC, yet have a different electrophoretic mobility, giving rise to apparent immunoelectrophoretic heterogeneity. Secondly, human FSC is sensitive to neuraminidase treatment which significantly lowers its anodal electrophoretic mobility (Haupt and Baudner, 1974).

Small amounts of SIgA could be detected in a fraction of serum IgA of high molecular weight, as also observed in humans (Waldman, Mach, Stella and Rowe, 1970), ruminants (Mach and Pahud, 1971; Pahud and Mach, 1970), pigs (Bourne, 1974), and dogs (Vaerman, 1973).

The antigenic relationships between SIgA, serum IgA and FSC were comparable to those reported for other mammalian secretory components. Guinea-pig FSC possessed the 'inaccessible' (= I) antigenic determinants (inaccessible in native SIgA) of FSC which have been described for human (Brandtzaeg, 1968), canine (Vaerman, 1973), bovine (Butler, 1971) and rat FSC (Vaerman *et al.*, 1975). In addition the I determinants could be exposed on SIgA by exposure to acid urea, as shown for human (Brandtzaeg, 1970) and rat SIgA (Vaerman *et al.*, 1975).

The bonds between guinea-pig SC and IgA were apparently split by reduction with 0·02

M DTT (Fig. 9) suggesting their disulphide nature. A similar situation seems to prevail in humans (Tomasi and Bienenstock, 1968), dogs (Vaerman, unpublished data) and rats (Vaerman *et al.*, 1975) where reduction of disulphide bonds is required for the complete dissociation of SC from SIgA. Rabbits, in contrast, appear unique in the sense that their SC is linked essentially by non-disulphide non-covalent interactions (O'Daly and Cebra, 1971a, c; Halpern and Koshland, 1970; Underdown, 1972). However, non-covalent bonds presumably play a role in the *in vitro* binding between guinea-pig serum IgM and labelled guinea-pig FSC (Fig. 11). Such *in vitro* binding has been demonstrated between human J chain containing IgM, and human FSC (Eskeland and Brandtzaeg, 1974). The guinea-pig IgM used did contain J chains (Kobayashi, Vaerman, Bazin, Lebacq-Verheyden and Heremans, 1973). By analogy with with the human situation, non-covalent association of SC with J chain-containing compounds presumably occurs first, whereafter stabilization of the complex is achieved by disulphide interchange.

The great similarity between the human and guinea-pig SC and SIgA systems is obvious. Recently, very similar data were reported for the rat (Vaerman *et al.*, 1975). These two common rodent species may now be considered as convenient experimental models for the study of the mechanism and function of the SIgA system.

ACKNOWLEDGEMENTS

This work was supported by grants numbers 1192 and numbers 10.013, from the 'Fonds de la Recherche Scientifique Médicale' and the 'Fonds de la Recherche Fondamentale Collective', Brussels, respectively, as well as by Convention number 74.7.0487 from the 'Délégation Générale à la Recherche Scientifique et Technique', Paris.

The technical assistance of Mrs N. Ghion-David, A. Langendries-Deryck and T. Van den Bergh-Briet was highly appreciated. The authors wish to thank Mr M. Delory for his competent editorial work.

REFERENCES

Bourne, F. J. (1969). 'IgA immunoglobulin from porcine serum.' *Biochem. biophys. Res. Comm.*, **36,** 138.

Bourne, F. J. (1974). 'Structural features of pig IgA.' *Immunol. Comm.*, **3,** 157.

Brandtzaeg, P. (1968). 'Hidden antigenic determinant in secretory immunoglobulin A.' *Nature (Lond.)*, **220,** 292.

Brandtzaeg, P. (1970). 'Unfolding of human secretory immunoglobulin A.' *Immunochemistry*, **7,** 127.

Brandtzaeg, P. (1974a). 'Mucosal and glandular distribution of immunoglobulin components: differential localization of free and bound SC in secretory epithelial cells.' *J. Immunol.*, **112,** 1553.

Brandtzaeg, P. (1974b). 'Mucosal and glandular distribution of immunoglobulin components. Immunohistochemistry with a cold ethanol-fixation method.' *Immunology*, **26,** 1101.

Brandtzaeg, P. (1974c). 'Human secretory component. I. Purification of free secretory component from colostrum.' *Scand. J. Immunol.*, **3,** 579.

Burstein, M. and Samaille, J. (1959). 'Nouvelle méthode de séparation et de dosage des lipoprotéines de faible densité.' *Scand. J. Immunol.*, **3,** 579.

Butler, J. E. (1971). 'Physicochemical and immunochemical studies on bovine IgA and glycoprotein-a.' *Biochim. biophys. Acta (Amst.)*, **251,** 435.

Cambiaso, C., Goffinet, A., Vaerman, J.-P. and Heremans, J. F. (1975). 'Glutaraldehyde-activated, 6-aminohexyl–Sepharose as a new versatile immunoabsorbent.' *Immunochemistry*, **12,** 273.

Eskeland, T. and Brandtzaeg, P. (1974). 'Does J chain mediate the combination of 19S IgM and dimeric IgA with the secretory component rather than being necessary for their polymerization?' *Immunochemistry*, **11,** 161.

Halpern, M. S. and Koshland, M. E. (1970). 'Novel subunit in secretory IgA.' *Nature (Lond.)*, **228,** 1276.

Haupt, H. and Baudner, S. (1974). 'Isolierung, Kristallisation und Eigenschaften der freien secretorischen Komponente aus human-Kolostrum.' *Behring Inst. Mitt.*, **54,** 9.

Kobayashi, K. (1971). 'Studies on human secretory IgA: comparative studies of the IgA-bound secretory piece and the free secretory piece protein.' *Immunochemistry*, **8,** 785.

Kobayashi, K., Vaerman, J.-P., Bazin, H., Lebacq-Verheyden, A. M. and Heremans, J. F. (1973). 'Identification of J-chain in polymeric immunoglobulins from a variety of species by cross reaction with rabbit antisera to human J-chains.' *J. Immunol.*, **111,** 1590.

Kobayashi, K., Vaerman, J.-P. and Heremans, J. F. (1973). 'Studies on human secretory IgA. II. Comparative studies on a fragment of secretory

component derived from secretory IgA and fragments obtained by enzymatic digestion of free secretory component.' *Immunochemistry*, **10,** 73.

LAMM, M. E. and GREENBERG, J. (1972). 'Human secretory component. Comparison of the form occurring in exocrine immunoglobulin A to the free form.' *Biochemistry*, **11,** 2744.

MACH, J.-P. (1970). '*In vitro* combination of human and bovine free secretory component with IgA of various species.' *Nature* (*Lond.*), **228,** 1278.

MACH, J.-P. and PAHUD, J. J. (1971). 'Secretory IgA, a major immunoglobulin in most bovine external secretions.' *J. Immunol.*, **106,** 522.

MANCINI, G., CARBONARA, A. O. and HEREMANS, J. F. (1965). 'Immunochemical quantitation of antigens by single radial immunodiffusion.' *Immunochemistry*, **2,** 235.

MESTECKY, J., KULHAVY, R. and KRAUS, F. (1972). 'Subunit structure of secretory IgA.' *J. Immunol.*, **108,** 738.

MUNSTER, P. J. J. VAN, STOELINGA, G. B. A., CLAMP, J. R., GERDING, J. J. T., REIJNEN, J. C. M. and VOSS, M. (1972). 'The molecular weight and the amino acid and carbohydrate compositions of free secretory component (SC).' *Immunology*, **24,** 249.

O'DALY, J. A. and CEBRA, J. J. (1971a). 'Rabbit secretory IgA. I. Isolation of secretory component after selective dissociation of the immunoglobulin.' *J. Immunol.*, **107,** 436.

O'DALY, J. A. and CEBRA, J. J. (1971b). 'Rabbit secretory IgA. II. Free secretory component from colostrum and its specific association with IgA.' *J. Immunol.*, **107,** 449.

O'DALY, J. A. and CEBRA, J. J. (1971c). 'Chemical and physico-chemical studies of the component polypeptide chains of rabbit secretory immunoglobulin A.' *Biochemistry*, **10,** 3843.

PAHUD, J. J. and MACH, J.-P. (1970). 'Identification of secretory IgA, free secretory piece and serum IgA in the ovine and caprine species.' *Immunochemistry*, **7,** 679.

PAHUD, J. J. and MACH, J.-P. (1972). 'Equine secretory IgA and secretory component'. *Int. Arch. Allergy*, **42,** 175.

POGER, M. E. and LAMM, M. E. (1974). 'Localization of free and bound secretory component in human intestinal epithelial cells. A model for the assembly of secretory IgA.' *J. exp. Med.*, **139,** 629.

PORTER, P. and ALLEN, W. D. (1970). 'Intestinal IgA in the pig.' *Experientia* (*Basel*), **26,** 90.

REYNOLDS, H. Y. and JOHNSON, J. S. (1971). 'Structural units of canine serum and secretory immunoglobulin A.' *Biochemistry*, **10,** 2821.

RICKS, J., ROBERTS, M. and PATTERSON, R. (1970). 'Canine secretory immunoglobulins: identification of secretory component.' *J. Immunol.*, **105,** 1327.

TOMASI, T. B. and BIENENSTOCK, J. (1968). 'Secretory immunoglobulins.' *Advanc. Immunol.*, **9,** 1.

UNDERDOWN, B. J. (1972). 'Studies on the subunit structure of rabbit secretory IgA.' *J. Immunol.*, **109,** 227.

VAERMAN, J.-P. (1970). *Studies on IgA immunoglobulin in man and animals*. Thesis, Sintal, Louvain.

VAERMAN, J.-P. (1973). 'Comparative immunochemistry of IgA.' *Research in Immunochemistry and Immunobiology* (ed. by J. B. G. Kwapinski), volume 3, p. 93. University Park Press, Baltimore, London, Tokyo.

VAERMAN, J.-P. and HEREMANS, J. F. (1972). 'The IgA system of the guinea pig.' *J. Immunol.*, **108,** 637.

VAERMAN, J.-P., HEREMANS, J. F., BAZIN, H. and BECKERS, A. (1975). 'Identification and some properties of rat secretory component.' *J. Immunol.*, **114,** 265.

WALDMANN, R. H., MACH, J.-P., STELLA, M. M. and ROWE, D. S. (1970). 'Secretory IgA in human serum.' *J. Immunol.*, **105,** 43.