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# HUMAN SECRETORY IMMUNOGLOBULINS

## II. SALIVARY SECRETIONS FROM INDIVIDUALS WITH SELECTIVELY EXCESSIVE OR DEFECTIVE SYNTHESIS OF SERUM IMMUNOGLOBULINS

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SUMMARY

Increased amounts of IgG were transmitted into the salivary secretions of patients with elevated serum levels of this immunoglobulin. Both its glandular and extra-glandular transfer apparently depended upon passive diffusion or epithelial 'leakage'.

High serum levels of IgM (in macroglobulinaemia), and especially local synthesis of this immunoglobulin (in IgA deficiency), enhanced its transfer into the saliva. The transmission through secretory epithelium (glandular transfer) seemed to be an active or selective process, probably dependent upon specific 'transfer sites' in the heavy polypetide chains of IgM.

The transmitted immunoglobulin (secretory IgM) was physicochemically and immunochemically similar to its 19S counterpart in serum. In IgA-deficient secretions no significant association between immunoglobulin components and secretory piece (SP) could be detected; the latter was physicochemically and immunochemically characterized as free SP.

## INTRODUCTION

Immunoglobulin A (IgA) is the major immunoglobulin in external secretions from normal individuals. It is mainly synthesized by glandular IgA-immunocytes, and is actively or selectively transported through secretory epithelia—probably because of specific 'transfer sites' in its heavy polypetide chains (Brandtzaeg, Fjellanger & Gjeruldsen, 1970). During the latter process it combines with a secretory protein through disulphide and non-covalent bonds (Brandtzaeg, 1970a,b). This component, termed secretory piece (SP) or 'S component'* apparently serves to stabilize the composite secretory IgA molecule, but its participation in glandular immunoglobulin transfer has not been fully excluded.

A 'pure' glandular secretion (e.g. parotid fluid) obtained from normal individuals contains only traces of IgG and IgM (Brandtzaeg *et al.*, 1970). Technical problems (e.g. har-

* An extension of the nomenclature for immunoglobulins, *Immunochemistry*, **7**, 497 (1970).

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vesting and isolation) have retarded the study of these secretory immunoglobulins, and very little is known about their mode of transmission through glandular epithelium. However, a preferential transfer of IgM is indicated by the normal secretion ratio of IgG:IgM (Brandtzaeg *et al.*, 1970).

In the present study IgG and IgM from parotid fluids of patients with hypergammaglobulinaemia or IgA deficiency were provisionally characterized. They were examined for possible complexing with secretory components such as SP, but apparently were similar to their counterparts in serum. By comparing the glandular and extraglandular transfer of IgG and IgM, it was verified that the secretory epithelium differentiates between these immunoglobulins by selectively transmitting IgM. The results render the participation of SP in glandular immunoglobulin transfer unlikely.

## MATERIALS AND METHODS

*Groups of individuals*

*Macroglobulinaemia (Waldenström).* Two patients with high serum levels of IgM and fairly normal levels of IgA were selected. L.H. was a 53-year-old female. Her gingivae were slightly inflamed with an average periodontal index (PI) score of 0·5 (Russell, 1956). T.H. was a 57-year-old edentulous male. He wore dentures, and his oral mucosa exhibited no overt signs of inflammation.

*Multiple myeloma.* H.A. was a 38-year-old female with G-myeloma. She wore an upper dental plate; the average PI score of the remaining ten teeth was 0·8. A.I. was a 66-year-old male with G-myeloma and a large extraosseous plasmacytoma adjacent to the left parotid gland. His gingivae were severely inflamed with an average PI score of 2·3.

*Immunoglobulin A deficiency.* J.H. was a 43-year-old female with selective lack of IgA and combined polyclonal and monoclonal increase of serum IgG. She suffered from autoimmune thrombocytopenia and severe haemorrhagic diathesis. She was edentulous and wore dentures. B.L. was a 53-year-old male with lack of IgA and polyclonal increase of serum IgG. He had several times been hospitalized for allergic rhinitis with nasal polyposis. He wore a complete upper dental plate, and his lower teeth had been extracted a week before the sample of whole saliva was obtained. G.B. (male) and L.J. (female) were two patients (age, 13–14 years) with ataxia telangiectasia. Case histories have been published elsewhere (Smeby, 1966). Their gingivae were slightly inflamed with average PI scores of 0·6 and 0·5 respectively.

*Normal control individuals and hypogammaglobulinaemic patients* were described in the preceding publication (Brandtzaeg *et al.*, 1970).

*Sera and secretions*

Serum, unstimulated whole saliva (ws), and stimulated parotid secretion (ps) were collected, treated and stored as detailed elsewhere (Brandtzaeg *et al.*, 1970). The parotid secretions were generally collected bilaterally, but for A.I. the fluids from the right (normal) and left (tumour) side were obtained and treated separately. Secretions from the normal and hypogammaglobulinaemic individuals served as control samples.

*Gel filtration*

Concentrated parotid secretions from normal individuals and from patients with hypogammaglobulinaemia, IgA deficiency or macroglobulinaemia, were chromatographed through columns of Sephadex G-100 or G-200 gels (Brandtzaeg *et al.*, 1970). Blue dextran (BD) and human albumin (Cohn fraction V; Hyland Laboratories, Los Angeles, California) were used as markers. The elution positions were determined by absorbancy at 622 nm for BD, by absorbancy at 280 nm and by single radial immunodiffusion (SRID) for albumin, and by SRID for the various immunoglobulin components. Typical experiments are described in Fig. 3.

*Ultracentrifugation*

Samples of serum (50 $\mu$l) and forty times concentrated parotid secretion (125 $\mu$l) from two IgA-deficient patients (J.H. and B.L.) were centrifuged in a 20–40% sucrose gradient for 18 hr at 4°C and 35,000 rev/min in a Spinco L-50 ultracentrifuge with a SW50 rotor. Fractions of eight drops (about 100 $\mu$l) were collected manually. $\alpha_2$-macroglobulin was used as a 19S marker (Schultze & Heremans, 1966); the parotid samples did not contain this protein and 5 $\mu$l of a serum rich in $\alpha_2$-macroglobulin, but containing only about 1% of the normal level of IgM, were added to these. The elution patterns of IgM, IgG and $\alpha_2$-macroglobulin were registered by SRID.

*Human immunoglobulins*

The preparations of IgG, F(ab')$_2$ and secretory IgA, were those previously used (Brandtzaeg *et al.*, 1970). Highly purified IgM was obtained by ultracentrifugation (cf. above) in a 10–40% sucrose gradient of 200-$\mu$l samples of an IgA-deficient serum (B.L.). Fractions containing no detectable IgG were pooled and IgM was precipitated twice by dialysis against deionized water at 4°C for 48 hr. The final sediment was washed in water and dissolved in isotonic phosphate-buffered saline (PBS), pH 7·5, containing 0·01% (w/v) merthiolate.

Individual preparations of partially purified IgM were obtained by Sephadex G-200 chromatography (pooled excluded fractions) of serum and parotid secretions from L.H., J.H., and B.L. The preparations of serum IgM were used unconcentrated; those of parotid IgM were concentrated about forty times (Curtain, 1964), lyophilized and dissolved in a small amount of deionized water. The protein concentrations were adjusted so that distinct precipitin lines occurred in double-diffusion tests against antisera to IgM.

*Antisera*

*General reagents.* Antisera to immunoglobulin light chains [anti-F(ab')$_2$], to IgG (anti-$\gamma$-chain), to IgM (anti-$\mu$-chain), to IgD (anti-$\delta$-chain), to IgE (anti-$\varepsilon$-chain), to IgA (anti-$\alpha$-chain), to secretory piece (anti-SP), to secretory IgA [anti-($\alpha$-chain + SP)], to free SP, and to $\alpha_2$-macroglobulin, were those used in a previous study (Brandtzaeg *et al.*, 1970).

*Individual reagents.* The following antisera were produced in rabbits against IgM from serum or parotid secretion of two IgA-deficient patients.

*Anti-serum IgM (B.L.).* R-117 was injected in multiple subcutaneous and intramuscular sites with 0·5 mg of a highly purified serum IgM preparation emulsified in Freund's complete adjuvant (Difco Laboratories Inc., Detroit, Michigan). 5 weeks later a similar amount of IgM was injected without adjuvant, and blood was drawn after a week. The antiserum,

adsorbed at equivalence with IgG, exhibited good activity to IgM (16 precipitating units; cf. Beutner, Holborow & Johnson, 1967), but also a faint activity to $\alpha_2$-macroglobulin.

R-136 was immunized intra- and subcutaneously with serum IgM precipitated from a partially purified preparation in micro-double-diffusion with R-117 (adsorbed with IgG). The precipitin bands from twenty six-well patterns (Die 6866A; LKB-Produkter AB, Stockholm, Sweden) were excised, washed, emulsified in adjuvant, and injected into multiple sites (Shivers & James, 1967). 6 weeks later precipitin bands from thirty six-well patterns homogenized in PBS were injected; and 3 weeks thereafter blood was drawn. The antiserum, adsorbed at equivalence with $F(ab')_2$, reacted strongly with IgM (16 precipitating units) and very faintly with $\alpha_2$-macroglobulin.

*Anti-parotid IgM* (*B.L.*). R-132 was immunized like R-136, except that partially purified parotid IgM was used in double-diffusion, and bands from only ten six-well patterns were available for reinjection. After adsorption with $F(ab')_2$, the antiserum was specific for IgM (4 precipitating units).

*Anti-serum IgM* (*J.H.*). R-137 and R-138 were immunized with precipitin bands produced between partially purified serum IgM and R-117. The schedule was as described for R-136, except that R-138 was not boosted. The antisera, adsorbed with $F(ab')_2$, contained 16 and 4 precipitating anti-IgM units, respectively, but only R-138 was monospecific (cf. Fig. 5).

*Anti-parotid IgM* (*J.H.*). R-133 and R-134 were injected with immunoglobulin precipitated immunologically from a partially purified parotid IgM preparation. The rabbits received an initial dose of agar-precipitin bands from twenty six-well patterns emulsified in adjuvant, and R-134 was reinjected with a similar dose in PBS. Both antisera were specific for IgM after adsorption with $F(ab')_2$ and contained 4–8 precipitating units.

### *Immunochemical techniques*

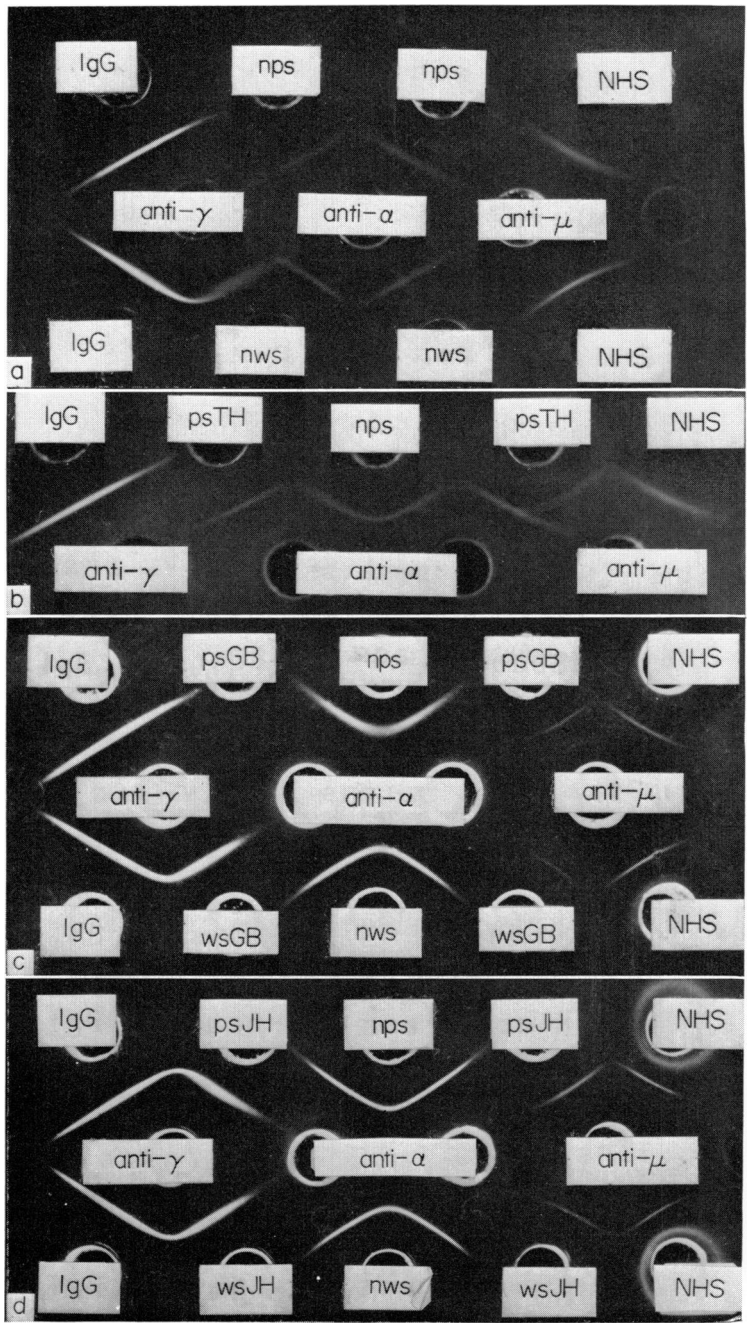
Micro-double-diffusion, immunoelectrophoresis, and single radial immunodiffusion (SRID) were carried out with the LKB equipment (Brandtzaeg *et al.*, 1970). In parotid secretions where the levels of IgM or IgG were too low to be quantitated directly by SRID, the values obtained with concentrated samples were divided by the concentration factor determined either for IgA or for IgM in the respective samples (cf. Table I and Brandtzaeg *et al.*, 1970). Secretion rates of immunoglobulins ($\mu$g/min/gland) were calculated for two patients (L.H. and T.H.) whose parotid flow rates had been determined.

## RESULTS

### *Immunochemical and physicochemical studies*

In normal parotid secretions concentrated 40 or 100 times only IgA was detected by

FIG. 1 (a, b, c and d). Identification of immunoglobulin components in secretions by gel diffusion. *Secretions*: nps, normal parotid secretion (concentrated 100 times); nws, normal whole saliva (concentrated twenty times); psTH, parotid secretion (concentrated forty times) from a patient with macroglobulinaemia; psGB, parotid secretion (concentrated forty times) from a patient with ataxia telangiectasia and IgA deficiency; wsGB, whole saliva (concentrated twenty times) from the same patient; psJH, parotid secretion (concentrated 100 times) from a patient with IgA deficiency and hyper-IgG-globulinaemia; and wsJH, whole saliva (concentrated twenty times) from the same patient. *Reference antigens*: IgG (0·8 mg/ml); and NHS (undiluted normal human serum). *Antisera*: anti-$\gamma$, specific for IgG; anti-$\alpha$, specific for IgA; and anti-$\mu$, specific for IgM.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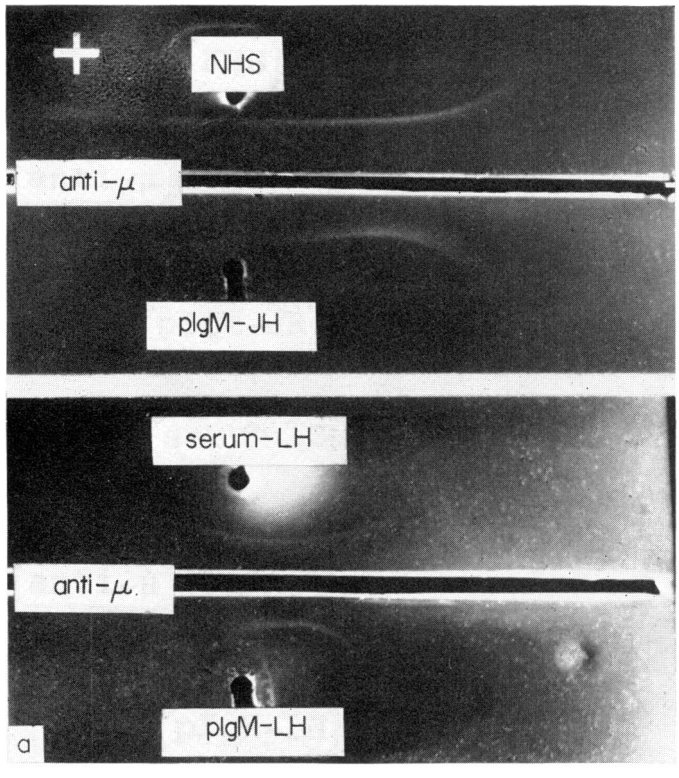
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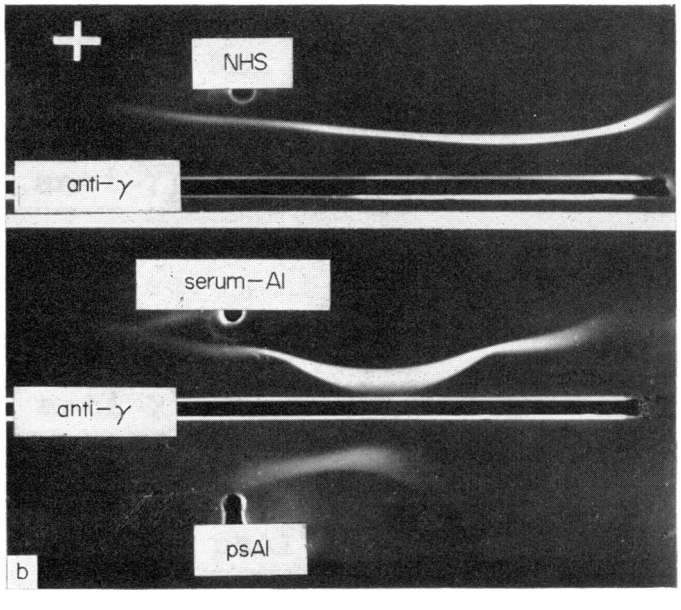
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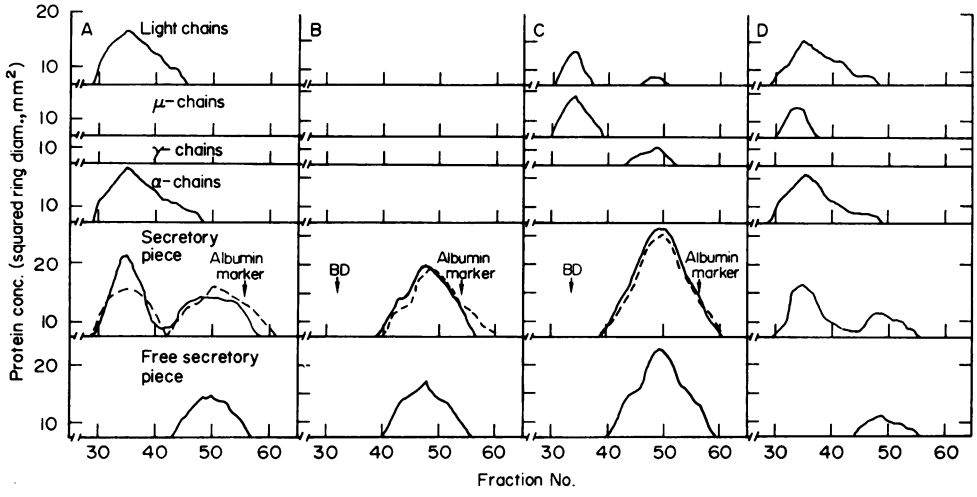


FIG. 3. Elution patterns of immunoglobulin components of parotid secretions after chromatography on Sephadex G-200. Column size, 2·5 × 37 cm; flow rate, 2·2 ml $cm^{-2}$ $hr^{-1}$; fractions, 2·4 ml. Samples: A, 1·6 ml of forty times concentrated normal secretion; B, 1·6 ml of forty times concentrated secretion from a hypogammaglobulinaemic patient; C, 1·6 ml of forty times concentrated secretion from an IgA-deficient patient (B.L.); and D, 0·8 ml of forty times concen trated secretion from a patient (T.H.) with macroglobulinaemia (in this experiment the fractions were concentrated three times before being tested). Elution positions of blue dextran (BD) and albumin marker added to the samples, are indicated by arrows. Distribution of immunoglobulin components was determined by SRID with antisera to light or heavy chain determinants, and to SP or free SP. The squared diameters of the precipitin rings were used as concentration estimates. In three experiments, the precipitates produced by a serum (R-77) from a rabbit immunized with bound SP (dashed line) were compared with those produced by a serum (R-123) from a rabbit immunized with free SP (solid line).

double-diffusion (Fig. 1a). Comparable samples from the patients with macroglobulinaemia, however, in addition produced a distinct IgM line (Fig. 1b). None of the IgA-deficient patients had detectable amounts of IgA in their secretions, but IgM was present in appreciable quantities (Fig. 1c, d). IgG was detected by double diffusion in the concentrated parotid secretions from the patients with G myeloma, as well as in those from the two IgA-deficient patients (B.L. and J.H.) who had high serum levels of IgG (Fig. 1d). Moreover, detectable amounts of IgG were present in all samples of concentrated whole saliva (Fig. 1). IgD and IgE could not be revealed by double-diffusion in concentrated parotid secretions, but a very faint precipitin line for IgD occurred with twenty times concentrated whole saliva from G.B. This patient had a seven to eight times elevated serum level of IgD. Also another IgA-deficient patient (B.L.) had a high serum level of IgD (two to

FIG. 2 (a and b). Immunoelectrophoretic characterization of IgM and IgG in parotid secretions. *Antigens*: NHS, normal human serum; pIgM-JH, partially purified parotid IgM from a patient with IgA deficiency; serum-LH, serum from a patient with macroglobulinaemia; pIgM-LH, partially purified parotid IgM from the same patient; serum-AI, serum from a patient with G myeloma; and psAI, parotid secretion (100 times concentrated) from the same patient. *Antisera*: anti-$\mu$, specific for IgM; and anti-$\gamma$, specific for IgG.

three times elevated), but the immunoglobulin could not be detected in his whole saliva by double-diffusion.

Parotid IgM obtained from a macroglobulinaemic patient exhibited immunoelectrophoretic properties similar to those of serum IgM from the same individual; both components appeared monoclonal, but only the latter produced extensive 'non-immunological' precipitation in the agar gel (Fig. 2a). Parotid IgM obtained from an IgA-deficient patient, on the other hand, appeared polyclonal by exhibiting an electrophoretic heterogeneity comparable to that of normal serum IgM (Fig. 2a). The immunoelectrophoretic behaviour of parotid IgG from a patient with G myeloma was similar to that of the monoclonal counterpart in serum (Fig. 2b).

IgM from serum as well as from parotid secretions of the patients with macroglobulinaemia, was excluded from Sephadex G-200 gels (Fig. 3D), indicating a size characteristic like that of normal 19S IgM. This was so also for parotid IgM from the patients with IgA deficiency (Fig. 3C). Parotid IgG from one of these patients (J.H.) was eluted similarly to serum IgG, whereas that from another (B.L.) was considerably (three fractions) more retarded (Fig. 3C).

Secretory piece (SP) was present in the secretions from all of the IgA-deficient patients. Its gel-filtration behaviour differed, however, from that of SP in IgA-containing secretions. SP is normally eluted from Sephadex G-200 columns in a bimodal pattern—the first peak representing IgA-associated or 'bound' piece, and the second one representing free piece (Fig. 3A). The IgA-deficient secretions generally seemed to contain only free piece (Fig. 3C) like those of patients with hypogammaglobulinaemia (Fig. 3B). Parotid fluid from J.H. in addition contained a minor population of larger SP-specific molecules, since the excluded fraction produced a hardly detectable reaction with antisera to SP. Whether this was due to a small proportion of IgM molecules combined with SP, or aggregates of free SP, was not established. The elution pattern of SP from IgA-deficient secretions was not compatible with its being associated with IgG or light-chain determinants (Fig. 3C). The distribution of immunoglobulin light chains conformed to those of IgM and IgG. Parotid IgM from patients with macroglobulinaemia was eluted in fractions containing bound SP. An association between the two components could therefore not be fully excluded (Fig. 3D); but the elution patterns indicated that SP was combined with IgA rather than with IgM.

In order to verify the apparent identity of the SP occurring in IgA-deficient parotid fluids with the free component present in hypogammaglobulinaemic and normal secretions, molecular size measurements were performed (Brandtzaeg *et al.*, 1970). The Einstein-Stokes radii determined by Sephadex G-100 chromatography of SP from the patients G.B. and J.H. were 42 and 43 Å, respectively, and similar to that previously estimated for free SP (42·5 Å). The radius determined by Sephadex G-200 chromatography of SP from patient B.L. was 41 Å and similar to a previous estimate (40·3 Å) of free piece with the same column (Brandtzaeg *et al.*, 1970). The Sephadex G-200 fractions of free SP from the three types of secretion were furthermore compared immunochemically in SRID and double-diffusion tests. With antisera active against the C, $A_1$, $A_2$ and I determinants of SP (cf. Fig. 23 in Brandtzaeg *et al.*, 1970), no antigenic difference was detected (Figs. 3A,B,C and 4). In addition the immunoelectrophoretic mobilities were comparable.

In order to verify the identity of IgM present in serum and parotid fluid, antisera to this immunoglobulin from both sources were produced (cf. Materials and Methods). With antigens from two patients, and the corresponding antisera, no immunochemical difference

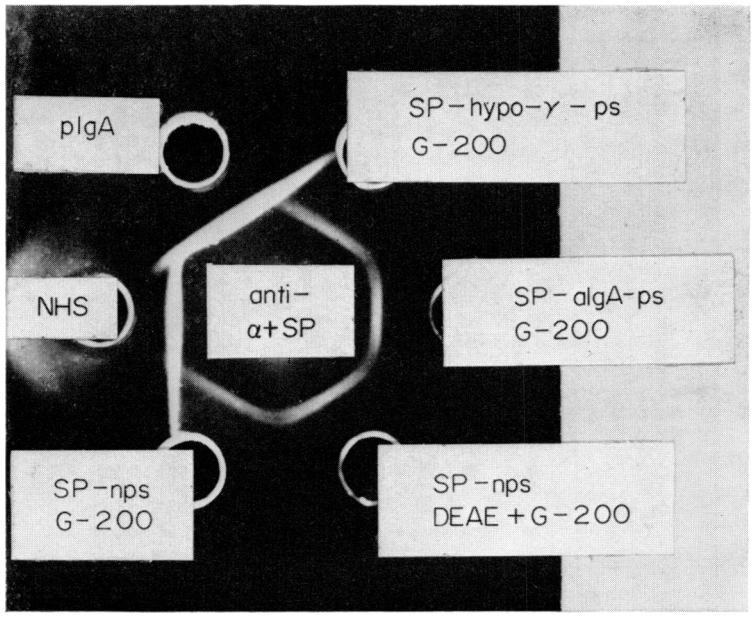


FIG. 4. Immunochemical comparison of free SP from different sources. The preparations were obtained by Sephadex G-200 filtration of a hypogammaglobulinaemic (SP-hypo-γ-ps G-200), an IgA-deficient (SP-αIgA-ps G-200) and a normal (SP-nps G-200) parotid secretion (cf. Fig. 3), as well as by filtration of a DEAE-cellulose chromatography fraction (SP-nps DEAE+G-200) of normal parotid secretion (cf. Fig. 10 in Brandtzaeg *et al.*, 1970). Fractions containing free SP, but no detectable bound SP, were pooled for each run and concentrated. *Reference antigens*: pIgA, purified parotid IgA containing bound SP; and NHS, normal human serum (1:4). *Antiserum*: anti-(α-chain+SP), R-51 adsorbed with $F(ab')_2$ and reacting with the C and $A_1$ determinants of SP and with the α-chains of IgA.

could be detected between serum IgM and parotid IgM (Fig. 5). One antiserum produced an apparent spur of IgM from serum over that from parotid fluid, but tests against a hypogammaglobulinaemic serum showed that the 'spur' was due to an antigen–antibody reaction unrelated to IgM.

Ultracentrifugation analyses of serum and parotid secretion (Fig. 6) likewise revealed no difference between the major IgM fractions. In serum from J.H. the immunoglobulin sedimented mainly as 19S polymers; only small amounts of heavier complexes and 7S monomers were present. In parotid fluid from the same patient, a 7S fraction was not observed, and the immunoglobulin consisted of 19S polymers and small amounts of heavier aggregates. Analyses of serum and parotid IgM from B.L. gave similar results, except that 7S IgM was not detected.

*Quantitative studies* (*Table* 1)

*Macroglobulinaemia.* Both patients had normal IgG and IgA levels and, consequently, normal IgG:IgA ratios in their parotid fluids. The parotid IgM levels, however, were six to eleven times elevated. L.H. had the highest serum concentration of IgM and also the highest parotid concentration. But her secretion rate of IgM (1·2 μg/min/gland) was lower

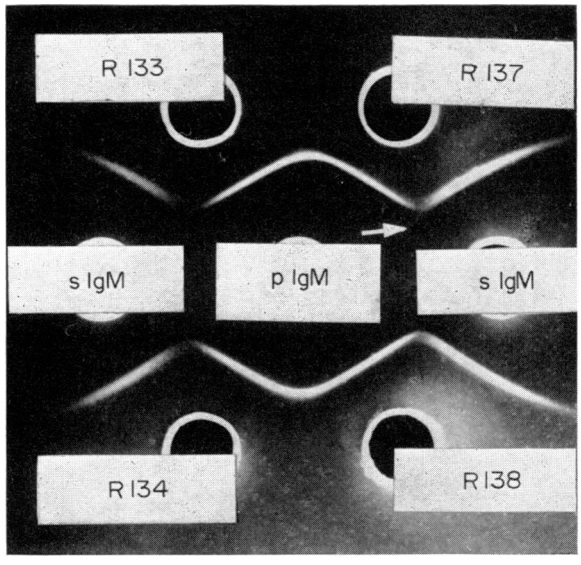


FIG. 5. Immunochemical comparison of secretory and serum IgM from one individual (J.H.). *Antigens*: pIgM, partially purified parotid IgM; and sIgM, partially purified serum IgM. *Antisera*: R-133 and R-134, antisera to parotid IgM from J.H., adsorbed with $F(ab')_2$; and R-137 and R-138, antisera to serum IgM from J.H., adsorbed with $F(ab')_2$. Arrow indicates false 'spur' (cf. text).

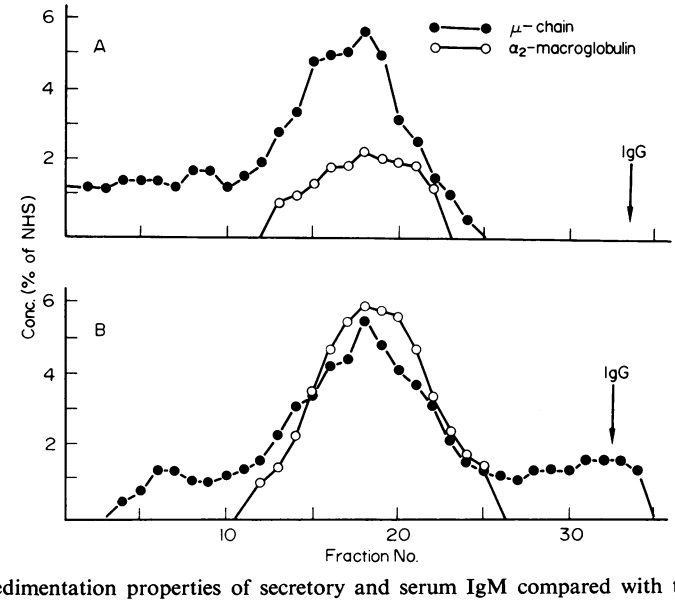


FIG. 6. Sedimentation properties of secretory and serum IgM compared with those of $\alpha_2$-macroglobulin. Samples: A, 125 $\mu$l of forty times concentrated parotid secretion from J.H. and 5 $\mu$l of an IgM-deficient serum; and B, 50 $\mu$l of serum from J.H. The distribution of IgM ($\mu$-chain) and $\alpha_2$-macroglobulin was determined by SRID analyses of ultracentrifugation fractions (100 $\mu$l) with monospecific antisera, the concentrations being plotted as a percentage of those in normal human serum (NHS). The elution position of IgG (arrow) was also determined by SRID.

TABLE 1. Immunoglobulin levels in serum, stimulated parotid secretion, and unstimulated whole saliva of patients with macroglobulinaemia, G-myeloma or IgA deficiency

| Disease classification | Subjects | Serum (mg/100 ml) | | | Serum Conc. ratios | | Parotid secretion (mg/100 ml) | | | Parotid Conc. ratios | | Whole saliva (mg/100 ml) | | | Whole saliva Conc. ratios | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | IgG | IgM | IgA | IgG: IgM | IgG: IgA | IgG* | IgM | IgA | IgG: IgM | IgG: IgA | IgG | IgM | IgA | IgG: IgM | IgG: IgA |
| Macroglobulinaemia | L.H. | 609 | 6329 | 87 | 0·1 | 7·0 | 0·047 | 0·462 | 3·53 | 0·102 | 0·013 | 3·15 | 13·73 | 19·60 | 0·23 | 0·16 |
| | T.H. | 1980 | 2784 | 338 | 0·7 | 5·9 | 0·025 | 0·251* | 3·23 | 0·100 | 0·008 | 0·89 | 1·39 | 36·53 | 0·64 | 0·02 |
| G myelomatosis | H.A. | 2349 | 69 | 131 | 34·0 | 17·9 | 0·157 | 0·069* | 3·20 | 2·275 | 0·049 | 5·90 | 0·26 | 18·42 | 22·69 | 0·32 |
| | A.I. | 9348 | 24 | 43 | 389·5 | 217·4 | 0·269$^{R}$ | 0·077* | 3·72 | 3·494 | 0·072 | 12·79 | 0·30 | 18·85 | 42·63 | 0·68 |
| | | | | | | | 0·418$^{L}$ | 0·063* | 2·98 | 6·635 | 0·140 | | | | | |
| IgA deficiency | J.H. | | | | | | | | | | | | | | | |
| | Mar. '66 | 3186 | 94 | 0 | 33·9 | N.D. | 0·357 | 0·847 | 0 | 0·421 | N.D. | 1·60 | 1·52 | 0 | 1·05 | N.D. |
| | Apr. '67 | 3284 | 96 | 0 | 34·2 | N.D. | 0·295 | 1·031 | 0 | 0·286 | N.D. | 4·06 | 3·47 | 0 | 1·17 | N.D. |
| | B.L. | | | | | | | | | | | | | | | |
| | Feb. '67 | 1870 | 153 | 0 | 12·2 | N.D. | 0·271 | 0·688 | 0 | 0·394 | N.D. | 7·77 | 2·18 | 0 | 3·56 | N.D. |
| | May '67 | 2423 | 211 | 0 | 11·5 | N.D. | 0·172 | 0·413 | 0 | 0·416 | N.D. | | | | | |
| | G.B. | | | | | | | | | | | | | | | |
| | Apr. '66 | 1439 | 125 | 0 | 11·5 | N.D. | 0·053 | 0·909 | 0 | 0·058 | N.D. | 2·78 | 2·81 | 0 | 0·99 | N.D. |
| | Feb. '67 | 1562 | 119 | 0 | 13·1 | N.D. | N.D. | 0·766 | 0 | N.D. | N.D. | 5·30 | 13·78 | 0 | 0·38 | N.D. |
| | L.J. | 1279 | 300 | 0 | 4·3 | N.D. | 0·048† | 0·255† | 0 | 0·188 | N.D. | 2·19 | 0·62 | 0 | 3·53 | N.D. |
| Normal | N | 1477 | 122 | 291 | 12·1 | 5·1 | 0·036 | 0·043* | 3·95 | 0·837 | 0·009 | 1·44 | 0·21 | 19·40 | 6·86 | 0·07 |
| | P | 1195 | 124 | 302 | 9·6 | 4·0 | | | | | | 6·97 | 0·76 | 37·14 | 9·17 | 0·19 |

* Value of concentrated sample divided by the concentration factor calculated for IgA (when present) or IgM in the same sample.

† Value of concentrated sample divided by an average concentration factor.

R, from the right (normal) side. L, from the left (tumour) side. N.D., not determined. N, nine subjects with healthy oral mucosa. P, thirteen patients with periodontitis.

than that in the other individual (2·1 μg/min/gland). L.H. also exhibited a relatively low secretion rate of parotid IgA (9·4 μg/min/gland) while that in T.H. (27·1 μg/min/gland) was normal (27·2±8·7 μg/min/gland). In his parotid fluid the IgG:IgM ratio was seven times decreased compared with the same ratio in his serum. In both patients the parotid IgG:IgM ratio was only $\frac{1}{8}$ of the normal figure.

Whole saliva from the two patients contained quantities of IgA within the normal range, but were unusual by containing more IgM than IgG—resulting in IgG:IgM ratios that were only 3–9% of the normal value. They were increased, however, compared with the parotid ratios in the same patients. The relatively low IgG and IgM levels in whole saliva of T.H. were probably explained by lack of immunoglobulin contribution from gingival pocket exudates, since this patient was edentulous.

*G-myeloma.* Parotid IgA and IgM levels were within the normal range in both patients; but increased quantities of parotid IgG resulted in ratios of IgG:IgM and IgG:IgA much higher than normal. It was noteworthy that although the left parotid fluid from A.I. contained decreased levels of IgM and IgA, the content of IgG was 50% higher than in the right secretion. This was probably due to local contribution of IgG from the plasmacytoma adjacent to his left parotid gland.

Whole saliva from the two patients contained normal amounts of IgM and IgA, but unusually high levels of IgG. The extremely high serum concentration of IgG combined with extensive oral inflammation (periodontitis) in A.I., probably accounted for the markedly raised transmission of IgG into his whole saliva. The ratios of IgG:IgM and IgG:IgA, were much higher than normal and almost 10 times higher than the parotid ratios in the same patients.

*Immunoglobulin A deficiency.* IgA was not detected in four times concentrated serum, 40–100 times concentrated parotid secretion, and twenty times concentrated whole saliva from these patients. When the respective serum elevations of IgG were considered, two subjects (J.H and B.L.) secreted relatively more of this immunoglobulin than the patients with G myeloma. The most striking finding, however, was a raised parotid IgM concentration in all of the IgA-deficient secretions, which on the average was fifteen times higher than normal. The parotid IgG:IgM ratio was thus much decreased, even in the two individuals with elevated parotid IgG levels. In the IgA-deficient patients there was no apparent relation between levels of IgM in serum and parotid secretion; and only one of them (L.J.) had a significantly elevated serum level. The patient (J.H.) with the highest parotid concentration of IgM had a rather low serum level.

The relatively high concentration of IgM in IgA-deficient whole saliva, could be ascribed mainly to enhanced glandular transfer of this immunoglobulin. Extraglandular transfer (determined by serum level of IgM and degree of mucosal inflammation (Brandtzaeg *et al.*, 1970)) accounted for only a minor fraction of salivary IgM in these patients. A possible exception was L.J. whose theoretical salivary IgM concentration, based solely on extraglandular transfer, was 0·4 mg/100 ml. One of the patients (J.H.) was edentulous, and this probably explained why her salivary IgG level was not as high as could have been expected in relation to her serum level. Exudation from extraction wounds probably contributed to the high whole saliva level of IgG in B.L., while those in G.B. and L.J. were in the expected range—periodontal status and serum IgG concentrations being taken into account.

## DISCUSSION

Although consisting of few patients, the three serologically defined groups exhibited distinct salivary immunoglobulin patterns (Fig. 1 and Table 1). The raised parotid levels of IgG in patients with high serum levels of this immunoglobulin indicated that glandular transfer of IgG is proportional to its availability from serum. However, two IgA-deficient individuals secreted relatively more IgG than those with G-myeloma if the respective serum levels are taken into account. The reason might be an additional contribution of IgG by local synthesis in the IgA-deficient patients. This was confirmed for one of them (B.L.) whose parotid tissue was examined by an immunofluorescence technique (Brandtzaeg, Fjellanger & Gjeruldsen, 1968). Glandular IgG-immunocytes were ten times more prevalent than in individuals with IgA synthesis. Moreover, at least some of his parotid IgG appeared to have a small size (Fig. 3C). This might be consistent with its derivation from glandular immunocytes—if it is true that local IgG synthesis favours certain subclasses (Rossen *et al.*, 1966).

The importance of local contribution of IgG to its glandular transfer was particularly evident in a patient (A.I.) with a plasmacytoma adjacent to the left parotid gland. Immunofluorescence (Brandtzaeg, unpublished) of histologically normal tissue from the same gland indicated an IgG concentration greatly exceeding that normally found in the stroma; and this was reflected by a high level of monoclonal IgG in the corresponding parotid secretion (Table 1 and Fig. 2b). Furthermore, immunofluorescence indicated that the local availability of IgG exceeded that of IgA; but the resulting secretion nevertheless contained about seven times more IgA than IgG (Table 1). Altogether the results suggested that while IgA is transferred through secretory epithelium by a selective or active mechanism, IgG is transmitted by a first order process such as passive diffusion.

Lack of selectivity in the glandular transmission of IgG was also apparent when the G-myeloma group was compared with the macroglobulinaemia group. Since synthesis of IgA in both groups seemed to be within the normal range, parotid production of IgG and IgM was most likely comparable and almost negligible as in normal individuals (Hurlimann & Zuber, 1968; Brandtzaeg, unpublished) rather than being enhanced as in IgA-deficient patients (cf. below). The monoclonal characteristic of the parotid IgM in macroglobulinaemia (Fig. 2a) agreed with its being derived from serum. However, only 25% of the IgM molecules leave the intravascular space, whereas approximately one-half of the IgG is distributed extravascularly (Waldmann & Strober, 1969). The average parotid tissue concentration of IgG in the G-myeloma group (disregarding the left gland of A.I.) was therefore probably two to three times higher than that of IgM in the macroglobulinaemia group. In contrast, the average IgM level in the parotid secretion of the latter was almost twice as high as the IgG level in the secretion of the former group (Table 1). This indicated that IgM, but not IgG, is transmitted through secretory epithelium like IgA by an active or selective process.

The relative concentrations of IgG and IgM in parotid secretion and colostrum from normal individuals similarly indicate a preferential glandular transmission of IgM (Brandtzaeg *et al.*, 1970). Moreover, the ratio of IgG:IgM in whole saliva is much higher than in parotid secretion. This is so in all groups of individuals (Table 1), and is hence a feature distinguishing extraglandular from glandular immunoglobulin transfer. The reason apparently is that the surface epithelium acts as a passive molecular sieve favouring trans-

mission of IgG, whereas the secretory epithelium is in addition provided with some selective transport mechanism specific for IgA and IgM.

Extraglandular transmission is highly dependent upon mucosal inflammation as well as upon the serum level of the respective immunoglobulin (Brandtzaeg *et al.*, 1970). The inflammatory exudate from gingival pockets thus seems to contribute a major fraction of the IgG and some of the IgM occurring in whole saliva (Brandtzaeg *et al.*, 1970). This is supported by the present study. In two edentulous patients extraglandular immunoglobulin transmission was apparently retarded. Kraus & Sirisinha (1962) reported that the absence of teeth did not lower the level of salivary immunoglobulins. Since they measured the total immunoglobulin fraction, however, quantitative variations of the major IgA component might have masked differences in the concentrations of IgG or IgM.

For the same reason it is not unexpected that total salivary immunoglobulin (Kraus & Sirisinha, 1962) and electrophoretic 'γ-globulin' (Green & Wilson, 1964) show no quantitative relation to hypergammaglobulinaemia. The present specific quantitation (Table 1), on the other hand, demonstrated that both glandular and extraglandular immunoglobulin transmission is enhanced by elevated serum levels of IgM or IgG. Gabl (1966) has previously used immunoelectrophoresis to demonstrate monoclonal immunoglobulins in whole saliva from patients with multiple myeloma; but the quantities of the components in serum and saliva exhibited no direct correlation. This result may be explained by the fact that extraglandular immunoglobulin transfer primarily depends on the mucosal status of the individual (Brandtzaeg *et al.*, 1970).

The absence of salivary IgA in patients lacking serum IgA, agrees with the results from several laboratories (Claman, Merrill & Hartley, 1967; Stobo & Tomasi, 1967; Collins-Williams, Lamenza & Kokubu, 1968; Hanson, 1968; Wilson *et al.*, 1968; Goldberg, Douglas & Fudenberg, 1969), although there has been one contradictory report (McFarlin *et al.*, 1965). Enhanced external transfer of IgM and IgG has also been described for such patients; but the quantities reported to be present in parotid secretions vary considerably (Table 2). Although it cannot be excluded that the quantitative differences are real, they

TABLE 2. Reported concentrations (mg/100 ml) of immunoglobulins in IgA-deficient stimulated parotid secretions

| No. of subjects | IgG | | IgM | | Ratio IgG:IgM | Authors |
|---|---|---|---|---|---|---|
| | Average | Range | Average | Range | | |
| 3 | 0·5 | (0–1·2) | 11·3 | (6·0–15·0) | 0·04 | Claman *et al.* 1967 |
| 4 | 0 | | 5·6 | (3·9–9·7) | | Stobo & Tomasi 1967 |
| 3 | 0·3 | | 0·3 | | 1·0 | Hanson 1968 |
| 4 | 0·162 | (0·048–0·326) | 0·646 | (0·255–0·939) | 0·25 | Present study |

may rather be due to difficulties in the determination of salivary immunoglobulin levels. Several relevant problems have been discussed elsewhere (Brandtzaeg *et al.*, 1970). The influence of flow rate on the immunoglobulin concentrations is one important variable which, unfortunately, was not recorded for the IgA-deficient patients.

The parotid levels of the different immunoglobulins are affected similarly by variations in flow rate (Brandtzaeg, 1970c). The estimation of concentration ratios therefore is a valuable

substitute for the determination of secretion rates (μg/min/gland) of immunoglobulins. Thus, the great differences in the IgG:IgM ratios between the four reported groups of IgA-deficient patients (Table 2) cannot be ascribed to variations in flow rate alone. Moreover, duplicate measurements after several months in three of our patients indicated that an individual's parotid immunoglobulin concentrations during 'maximal' secretory stimulation exhibit rather limited fluctuations (Table 1). Altogether it may be concluded that either the reported groups of patients (Table 2) were extremely dissimilar with regard to the parotid levels of IgG and IgM, or the quantitations performed in different laboratories are highly inconsistent and require standardization.

The fact remains, however, that IgA-deficient parotid secretions contain more IgG and particularly more IgM than normal parotid secretions. An increased level of IgG is not always present and may depend upon an elevated serum level alone or combined with glandular synthesis of this immunoglobulin (cf. above). The serum level of IgM, on the other hand, cannot explain the raised parotid IgM concentration which exceeds that found in macroglobulinaemia (Table 1). This leaves the possibility of local synthesis. From another biological situation it has been concluded that the only way a high level of IgM can be established in an extravascular compartment, is by local production (Hall *et al.*, 1969). One of our IgA-deficient patients (B.L.) was indeed found to have a prevalence of parotid IgM-immunocytes almost fifteen times higher than normal (Brandtzaeg *et al.*, 1968); and such patients generally have increased numbers of IgM-immunocytes in the intestinal and gastric mucosa (Crabbé & Heremans, 1966; Eidelman & Davis, 1968; Fjellanger, Brandtzaeg & Gjeruldsen, 1968).

In contrast, we did not find a definitely increased number of IgM-immunocytes in the nasal mucosa of two (B.L. and G.B.) IgA-deficient individuals (Brandtzaeg *et al.*, 1968). Nasal fluids from two other patients, however, apparently contained raised levels of IgG and IgM (Bellanti, Artenstein & Buescher, 1966). Since the serum levels of these immunoglobulins may be elevated in IgA-deficiency, their extraglandular transmission through an inflamed mucosa may be enhanced and jeopardize conclusions regarding the 'pure' secretion. Therefore it cannot at present be conclusively stated that the absent glandular IgA-immunocytes are generally replaced by IgM-immunocytes in IgA-deficient patients, although this seems to be the case along the digestive tract.

On the basis of the extravascular distribution of serum immunoglobulins (Waldmann & Strober, 1969), the concentration ratio of IgG:IgM in the parotid tissue of B.L. was estimated to be about 23:1. In addition, the ratio of local IgG-:IgM-immunocytes was 1:1·3 (Brandtzaeg *et al.*, 1968). In striking contrast was the concentration ratio of 1:2·5 for these immunoglobulins in the corresponding parotid secretion (Table 1), confirming that IgM is selectively transported through the secretory epithelium. The majority of the transmitted immunoglobulin was physicochemically and immunochemically similar to its 19S counterpart in serum (Figs 3, 5 and 6), and the possibility of its being associated with some sort of 'carrier protein' seemed unlikely. Only for an insignificant fraction of parotid IgM in one patient (J.H.; cf. Results) was complexing with SP indicated. The majority of SP in IgA-deficient secretions was physicochemically and immunochemically similar to the free component present in parotid secretions of hypogammaglobulinaemic and normal individuals (Figs 3 and 4).

It seems justified to propose that the selective or active glandular transport of IgM depends on 'transfer sites' in its heavy polypeptide chains, like the mechanism previously

suggested for the transport of IgA (Brandtzaeg *et al.*, 1970). External transfer of IgG, on the other hand, seems to depend on passive diffusion or epithelial 'leakage', and therefore mainly occurs through extraglandular sites. The same apparently applies to IgD which was only detected in the whole saliva of a patient with a highly elevated serum level of this immunoglobulin. Data concerning the transmission of IgE are scarce, but apparently suggest that a selective glandular process is involved (Johansson, Bennich & Foucard, 1970).

A common secretory mechanism for the external transfer of IgA and IgM may be of biological significance if IgM is able to act as a compensatory secretory immunoglobulin. Antibody functions normally held by secretory IgA may be exhibited by locally produced and transmitted IgM, as indicated by its isohaemagglutinin activity in IgA-deficient parotid secretions (Stobo & Tomasi, 1967); but little information is available regarding other possible activities of secretory IgM. As discussed above, there is moreover no definite evidence that glandular IgA-immunocytes of every secretory system are replaced by IgM-immunocytes in IgA deficiency.

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