# Secretion of Immunoglobulins and Plasma Proteins from the Jejunal Mucosa

**Transport Rate and Origin of Polymeric Immunoglobulin A**

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**A**bstract. Parameters of secretion of IgA and several other plasma proteins from the jejunal mucosa were investigated in 11 individuals who had a normal distribution of Ig-containing cells in the lamina propria and in one patient who was totally deficient in jejunal IgA and IgM plasmacytes. Jejunal samples were collected during segmental gut perfusion. The following results were obtained: (a) The secretion of polymeric IgA (p-IgA, mean equals 217 $\mu$g/40 cm per min) exceeded those of albumin (132 $\mu$g), IgG (35 $\mu$g), and monomeric IgA (m-IgA, 15 $\mu$g, or 6.4% of total IgA). About 35% of IgA was IgA2 in the jejunal secretion, compared with ~23% in serum. This closely corresponds to the 35 and 24% of IgA2 plasmocytes in jejunal mucosa and peripheral lymph nodes, respectively. (b) For each protein, a relative coefficient of excretion (RCE) was calculated (jejunum to serum concentration ratio expressed relative to that of albumin). RCEs of 1.41 for orosomucoid, 1.0 for albumin, 0.83 for IgG, and 0.74 for IgE and, in the deficient patient, of 0.64 for m-IgA and 0.016 for IgM were obtained. This was inversely related to the molecular weight of these proteins and indicated their predominantly passive transport into the jejunum. However, in normal individuals,

the RCE of transferrin (~1.11 > 1, $P > 0.05$), $\alpha_2$-macro globulin (~0.77), m-IgA (~1.98), and p-IgA (~218) exceeded the value expected from simple seepage from plasma, thus pointing to an additional role of either local gut synthesis and/or active transepithelial transport. (c) Approximately 98% of p-IgA, ~99% of IgM, and ~68% of m-IgA in jejunal secretions were derived from local production in the gut wall, as determined by $^{125}$I-p-IgA specific activities and/or by comparison between the RCE values of the deficient patient to the values of controls. Therefore, the jejunal production of p-IgA (~312 mg/d per 40 cm vs. ~54 mg/d from bile) contributes the majority of upper intestinal IgA in humans. The active transport of plasma p-IgA across the intestinal mucosa (~0.08 mg/40 cm per kg per d) contributes <2% of the total amount of p-IgA (4.5 mg/kg per d) that is cleared daily from plasma.

## Introduction

The parameters of secretion of Ig and plasma proteins from intestinal mucosa are still poorly characterized. Most plasma proteins are believed to reach the gut lumen as a result of passive leakage from lamina propria capillaries (1). In addition, other mechanisms such as local protein synthesis in the gut lamina propria and receptor-mediated active transepithelial transport also contribute to the secretion of some proteins. This is well documented for polymeric IgA (p-IgA)[1] and IgM (2–6). However,

1. *Abbreviations used in this paper:* Alb, albumin; d-IgA, dimeric IgA; FITC, fluorescein isothiocyanate; IRMA, immunoradiometric assay; $\alpha_2$M, $\alpha_2$-macroglobulin; m-IgA, monomeric IgA; ORO, orosomucoid; PEG, polyethyleneglycol 4,000; p-IgA, polymeric IgA; RCEs, relative (to albumin) coefficient of excretion(s); SC, secretory component; SDGU, sucrose density gradient ultracentrifugation; TRITC, tetramethylrhodamine isothiocyanate.

the respective contributions of plasma IgA vs. gut-synthesized IgA are still unknown.

The intestinal lamina propria is populated by a wealth of plasmocytes, of which 80% produce IgA and 15% produce IgM (7, 8). Over half of this IgA is in polymeric form (9–11), and ~35% of it is IgA2 (12). In contrast, plasma IgA is predominately monomeric (m-IgA), and of the IgA1 subclass (5, 13), and is mostly produced in the bone marrow (11, 14). Only p-IgA (and IgM), which is locally produced or comes from plasma (12%) (13), has a high affinity for the secretory component (SC) (15, 16), which is a glycoprotein that acts on the basolateral membrane of epithelial cells as a transmembrane receptor for polymeric Ig (17–19). The SC initiates the active translocation of p-IgA and IgM across the epithelial cells from the lamina propria to the gut lumen (3, 4, 19). So far, the transport of p-IgA from plasma into intestinal secretions has not been studied.

Therefore, this study analyzes the jejunal secretion rate of IgA and of other plasma proteins and pays particular attention to the different molecular sizes (m- and p-), subclasses (IgA1 and IgA2), and origins of IgA. Pure jejunal secretions were collected by segmental perfusion of the gut, which thus avoided protein and proteolytic enzyme contamination. The transport rate of p-IgA from plasma into the jejunum and the contribution of plasma p-IgA to the total jejunal p-IgA were assessed radioisotopically. We also studied a patient who was totally deficient in IgA and IgM plasmacytes in the jejunal lamina propria. In this individual, it was assumed that all IgA and IgM present in the gut lumen was of plasma origin.

## Methods

### Subjects

12 subjects (eight caucasians and four blacks; ten males and two females) with a mean age of 40 yr (28–52 yr) were included in the study. They comprised 11 controls with a normal gastrointestinal status and one patient who lacked IgA- and IgM-containing plasma cells in the jejunal lamina propria.

*Controls.* The control group included two healthy volunteers and nine patients who were referred to the Hospital Saint-Lazare because of cryptogenic abdominal complaints. After complete investigation, which included jejunal biopsy, none of these patients showed evidence of organic gastrointestinal or immunological abnormality. All these individuals were submitted to jejunal perfusion. Jejunal biopsy was obtained after perfusion under endoscopic control. Two of these patients were also injected intravenously with 5–10 μCi each of ^{131}I-albumin and ^{125}I-p-IgA. Informed consent was obtained from all individuals, and the protocol of the study was approved by the Ethics Committee of the Hospital Saint-Lazare.

*Patient lacking lamina propria IgA and IgM plasmacytes.* This Chadian patient (33 yr old) had suffered from mild diarrhea for several years. Complete gastrointestinal investigations and immunofluorescence studies that were performed on three separate occasions revealed a complete absence of IgA- and IgM-containing cells in his jejunal lamina propria, which was associated with low serum levels of IgA and IgM. This patient was also submitted to jejunal perfusion after informed consent had been obtained. His jejunal biopsy sample revealed no evidence of villous atrophy or inflammation.

### Jejunal perfusion

Segmental perfusion of the jejunum (20, 21) was performed according to Rambaud et al. (22), using a four-lumen tube. The tube was comprised of a proximal segment that had a lumen opening in the duodenum, an inflatable balloon that was able to occlude the duodeno-jejunal junction, a distal segment with an upper infusion point, and a distal collection point that was 40 cm below the infusion point. The tube was set in place 16 h before the perfusion. The infusion point was located near the duodeno-jejunal junction by using fluoroscopic control just before perfusion. The gut was perfused with a 115 mM NaCl, 10 mM KCl, and 35 mM mannitol solution that was supplemented with polyethyleneglycol 4,000 (PEG) (lg/l), at a rate of 10 ml/min. In order to rinse the secretions that were present in the jejunal lumen, a 60-min equilibration period was allowed after the inflation of the balloon with 50–80 ml of air. After the equilibration period, four 20-min samples were collected by continuous free siphoning and kept at 4°C. 1 mM diisopropylfluorophosphate, a potent serine protease inhibitor, was added to each sample. A serum sample was obtained from each patient after the second 20-min period. Serum and perfusate samples were stored at −20°C.

During the perfusion, duodenal contents were continuously collected and discarded. Contamination of the jejunal samples by duodenal fluids bypassing the balloon was controlled by using two semiquantitative methods: detection of bromosulfophtalein, which was infused above the balloon into the duodenum, and detection of chymotrypsin or lipase activity in perfusates. Five 20-min samples from three patients were excluded after detection. Absence of blood contamination was confirmed in all samples (Hemotest, Ames, France).

*Protein concentrations in serum and jejunal perfusate.* In general, the protein concentrations in serum and jejunal samples were measured using the same methods, reagents, and standards as described by Delacroix et al. (23). Specific concentrations of albumin (Alb), transferrin, IgG, IgA, and IgM in serum were measured by immunonephelometry (24). IgA in the serum of the deficient patient, $\alpha_2$-macroglobulin ($\alpha_2$M), and orosomucoid (ORO) in all sera, and all proteins in jejunal samples, including SC, were measured by immunoradiometric assay (IRMA) (23, 25). Goat serum ($\alpha_2$M and IgM IRMA) or horse serum (all other IRMA) were added to jejunal samples in order to keep them in a protein-rich buffer.

These aliquots were assayed at four serial dilutions (minimal dilution was 1/12.5 for $\alpha_2$M). Results for the dilutions falling within the standard ranges (4–100 ng/ml) always yielded curves closely parallel to the standard curves. For the deficient patient, jejunal samples were concentrated 100-fold by vacuum ultrafiltration before protein measurements. For both immunonephelometry and IRMA, the same pool of normal serum (1,000 blood donors) was used as standard.

IgE was assayed in concentrated (20–80 times) intestinal fluids and in serum by particle counting immunoassay (26). Before the assay, intestinal samples were diluted with 1 vol of normal rabbit serum to minimize the effect of protein concentration in the agglutination process, and were digested with pepsin. In 30 min, pepsin releases the Fc″-fragment (D1-determinant) from IgE or its partially fragmented components, whereas already existing Fc′-fragments are not further degraded to any major extent (27). The Fc′-fragment was then assayed by its agglutinating activity towards latex particles that were coated with anti-IgE-Fc″ antibodies. Serum IgE was assayed in the same way after pepsin digestion of diluted serum. The degree of concentration of jejunal samples was always measured by comparing Alb and IgG levels in the concentrated and unconcentrated samples.

*Ultracentrifugation and measurement of m- and p-IgA.* The proportions of m- and p-IgA in all sera and perfusate samples were measured

after sucrose density gradient ultracentrifugation (SDGU). Samples were centrifuged for 16 h at 38,000 rpm ($\omega^2 t = 9.11 \times 10^{11}$) on isokinetic (5–21%) sucrose gradients using the same 7S and 10.5S markers as previously described (23, 28). The amounts of serum (4–6 $\mu$l) and secretion (50–300 $\mu$l) applied to the gradients were based upon their respective IgA concentrations. For the deficient patient, 60 $\mu$l of serum and 300 $\mu$l of the 100-fold concentrated (vacuum dialysis) perfusate were applied. IgA concentrations were measured (IRMA) in the 30 fractions of 0.4 ml that were eluted from SDGU after dilution 1:9 in 20% horse serum. Percentages of m- and p-IgA were measured by planimetry. Absolute concentrations of m- and p-IgA were calculated from the total IgA levels multiplied by the percentages determined above. The influence of the size of IgA in IRMA (25), but not in nephelometry (29), was taken into account by multiplying all results obtained by IRMA by a factor of 2 for p-IgA in secretions, or of 1.5 or 2 for dimeric and heavier IgA, respectively, in serum (23).

The proportions of free and bound SC in each perfusate sample, and the molecular size of other proteins in a 100-fold concentrated pool of perfusate samples from controls, were determined according to the same methods as for IgA. Appropriate IRMA and dilutions were used.

*IgA subclasses in serum and perfusate.* IgA1 and IgA2 (30–32) concentrations in serum and perfusate were measured by IRMA and performed as previously described (33), with modifications using monoclonal antibodies. Three mouse monoclonal antibodies, anti-$\alpha$1, anti-$\alpha$2 and anti-$\alpha$-chain, were obtained in our laboratory.[2] Their specificity was verified by means of various highly sensitive immunoassays, using 10 human monoclonal IgA proteins of each subclass. In these preliminary experiments,[2] we used both m(1) and m(2) allotypes of IgA2 (34), both isotypes of light chains, and both Fab and Fc fragments of IgA1. Anti-subclass antibodies were used to coat the solid phase, and the purified anti-$\alpha$-chain antibody was labeled and used as second antibody. Standards (5–150 ng/ml) were made with a serum pool of 1,000 blood donors. The pool contained 21% IgA2 and 79% IgA1, as previously measured (13, 33). The influence of the size of IgA1 and IgA2 in these assays[2] was also carefully assessed, using highly purified monomeric and dimeric monoclonal IgA1 and IgA2 as controls in all assays. Results were expressed as (IgA2)/(IgA1 + IgA2) × 100. The sum of (IgA1 + IgA2) concentrations averaged 115±18% of total IgA concentration in serum and 94±20% in perfusate.

## Immunochemistry

Jejunal biopsies were obtained in nine control patients and in the patient lacking jejunal IgA and IgM plasmocytes. To compare the IgA-subclass distribution in the jejunal lamina with that in the peripheral lymph nodes, three histologically normal lymph nodes were obtained from patients operated on for laryngeal and breast cancer.

*Jejunal biopsies.* In each case, five tissue samples were obtained using a forceps that was passed through an endoscope. They were fixed in Bouin's fluid, dehydrated, and embedded in paraffin within 12 h. For each patient, a standard staining procedure allowed selection of the two best-oriented specimens. Serial 3-$\mu$m thick sections were then processed for immunohistochemistry.

*Immunohistochemical procedures.* Before applying antisera, all sections were incubated for 60 min with a 100 times dilution (in phosphate-buffered saline [PBS]) of a normal serum of the species corresponding to the conjugated antiserum used.

IgA-, IgM- (goat antisera used in IRMA), and IgG (fluorescein isothiocyanate [FITC] conjugated rabbit antiserum, Dako, Denmark)-containing cells were stained on serial sections using an indirect staining

procedure for IgA and IgM (FITC-conjugated rabbit antigoat IgG antiserum, Nordic, Netherlands) and a direct procedure for IgG. Each antiserum was applied for 60 min at room temperature. IgA1- and IgA2-containing cells were simultaneously stained on the same section using a double-tracing technique. Sections were simultaneously incubated with the mouse monoclonal anti-IgA2 antibody[2] and with a rabbit anti-IgA1 antiserum (33) for 60 min, washed in PBS for 30 min, further incubated for 60 min with FITC anti-mouse and tetramethylrhodamine isothiocyanate (TRITC) anti-rabbit antisera (Nordic), then washed again for 30 min.

Fluorescence was observed with a Wild-Leitz Ortholux II equipped with a Ploem-type vertical illuminator, and 4–20 objective fields (× 25) (mean equals six fields for IgA, IgA1, IgA2, and IgM and 15 for IgG) were micrographed on Kodak Ektachrome 200 daylight film (Eastman Kodak Co., Rochester, NY) (final 100 times magnification), using appropriate filters. In the double-tracing procedure, the same fields were successively micrographed for IgA1 (red) and IgA2 (green). Both red and green fluorescence were also simultaneously micrographed on the same section.

*Controls.* Controls included: specific inhibition experiments by antiserum absorption with appropriate antigens, absence of staining when the second labeled antisera were directly applied on sections, and absence of red staining when the TRITC-goat anti-rabbit antiserum was applied on sections that were incubated with mouse anti-IgA2 antibodies.

*Morphometry.* The micrographs were projected on a semiautomatic image analyzer (Nacher, NS 1000, Levallois-Peret, France) and the following parameters were recorded: surface of the analyzed mucosa, corresponding lamina propria surface, and the number of green- or red-stained plasmocytes. The absolute and relative distributions of IgA, IgM, and IgG, as well as those of IgA1 and IgA2 plasmocytes, were calculated.

## Radioisotopic studies

[125]I-dimeric IgA and ^{131}I-albumin were simultaneously injected intravenously into two control patients 16 h before jejunal perfusion. Specific radioactivities were simultaneously measured in serum and perfusate in order to determine the proportion of p-IgA or of Alb in perfusate that was derived from plasma. Immunochemical and biological characteristics as well as the labeling procedure for the injected dimeric polyclonal IgA have been previously described in detail (13, 23, 28). This Ig was essentially pure (28), was not complexed to SC (28), contained an appropriate amount of J-chain (13), was able to bind SC, and was transported at a high rate from plasma into bile of rats and rabbits (23, 35). ^{131}I-albumin was purchased from Sorin Biomedica s.p.a. (Saluggia, Italia) and was homogeneous in size when submitted to SDGU. The following procedures were used to measure specific activities in serum and perfusate. Two samples of serum, obtained before and after the jejunal perfusion, were pooled. All 20-min perfusion samples were pooled after an aliquot of each had been frozen. The pools of perfusate (250 and 450 ml, respectively, in the two patients) were concentrated by vacuum ultrafiltration and adjusted to 3 ml. Radioactivities in both serum (3 ml) and concentrated perfusates (3 ml) were counted for 20 min before and after precipitation with TCA at 100 g/l final concentration. Concentrations of p-IgA and Alb in serum and concentrated perfusate were measured according to standard procedures. In one of the two patients, the concentrated perfusate (300 $\mu$l) was submitted to SDGU in order to compare the size profile of ^{125}I-radioactivity to the profile of the cold IgA (IRMA) in the sample. Specific activity ratios were calculated from: (TCA precipitable counts/ mg of p-IgA or Alb/20 min) in perfusate/(TCA precipitable counts/mg of p-IgA or Alb/20 min) in serum.

*Mathematical and statistical analysis.* Net movements of water, electrolytes, and proteins in the jejunal test segments are listed in Table

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2. Delacroix, D. L., et al., manuscript in preparation.

## Table I. Transintestinal Water and Electrolyte Movements during Jejunal Perfusion

|  | Mean* | Range |
|---|---|---|
| Perfusion flow rate $(ml \cdot min^{-1})$ | 10.08 | 9.20–11.43 |
| [PEG]p/[PEG]s‡ | 0.952 | 0.872–1.124 |
| Net water flux $(ml \cdot min^{-1} \cdot 40\ cm^{-1})$ | −0.48 | −1.21–+1.28 |
| Net Na flux $(mmol \cdot min^{-1} \cdot 40\ cm^{-1})$ | −0.02 | −0.10–+0.06 |
| Net K flux $(mmol \cdot min^{-1} \cdot 40\ cm^{-1})$ | −0.03 | −0.05–+0.03 |
| Net Cl flux $(mmol \cdot min^{-1} \cdot 40\ cm^{-1})$ | −0.08 | −0.17–+0.03 |
| Net bicarbonate flux $(mmol \cdot min^{-1} \cdot 40\ cm^{-1})$ | +0.01 | +0.01–0.02 |

* Arithmetic mean value ($n = 12$).
‡ Concentration of PEG at the perfusion point (p) and the sampling point (s), respectively.

I. They were calculated as follows: (a) FRs = FRp × (PEG)p/(PEG)s, where FRs and (PEG)s are the fluid flow rates and PEG concentration at the sampling point, whereas FRp and (PEG)p are fluid flow rate and PEG concentration at the perfusion point. (b) Net water flux $(ml \cdot min^{-1} \cdot 40\ cm^{-1})$ = FRp − FRs. (c) Net electrolyte flux $(mmol \cdot min^{-1} \cdot 40\ cm^{-1})$ = (FRp × electrolyte $p$) − (FRs × electrolyte $s$), where electrolyte $s$ and electrolyte $p$ are electrolyte concentrations at the sampling and perfusion points, respectively. (d) Protein secretion rate $(\mu g \cdot min^{-1} \cdot 40\ cm^{-1})$ = FRs × protein $s$, where protein $s$ is the protein concentration at the sampling point.

The results of the protein measurements will be presented according to three mathematical approaches: (a) The measured protein concentrations in serum and jejunal perfusates. (b) The absolute protein secretion rate across or from the jejunal mucosa was calculated as detailed above.

## Table II. Numbers and Distributions of Ig-containing Cells in the Jejunal Lamina Propria of Control Patients

|  | Number of counted cells | Observed surface | Number of cells/mm² | Percentage of all stained cells |
|---|---|---|---|---|
|  |  | *mm²* |  |  |
| **Classes** |  |  |  |  |
| IgA | 388* | 0.18 | 2,282 | 79.6‡ |
|  | 188–685§ | 0.11–0.22 | 1,268–3,478 | 59.2–92.2 |
| IgM | 84 | 0.20 | 444 | 16.0‡ |
|  | 30–126 | 0.08–0.35 | 153–770 | 4.9–30.8 |
| IgG | 54 | 0.50 | 126 | 4.4‡ |
|  | 5–85 | 0.22–0.79 | 22–124 | 1.1–10.0 |
| **Subclasses** |  |  |  |  |
| IgA1 | 236 | 0.16 | 1,439 | 64.9‖ |
|  | 122–413 | 0.07–0.26 | 928–2,067 | 61.3–69.6 |
| IgA2 | 132 |  | 771 | 35.1‖ |
|  | 58–240 |  | 547–1,100 | 30.4–38.7 |
| IgA1 + IgA2 | 368 |  | 2,210 |  |
|  | 180–653 |  | 1,498–3,617 |  |

* Arithmetic mean per subject ($n = 9$), and for the two tissue specimens.
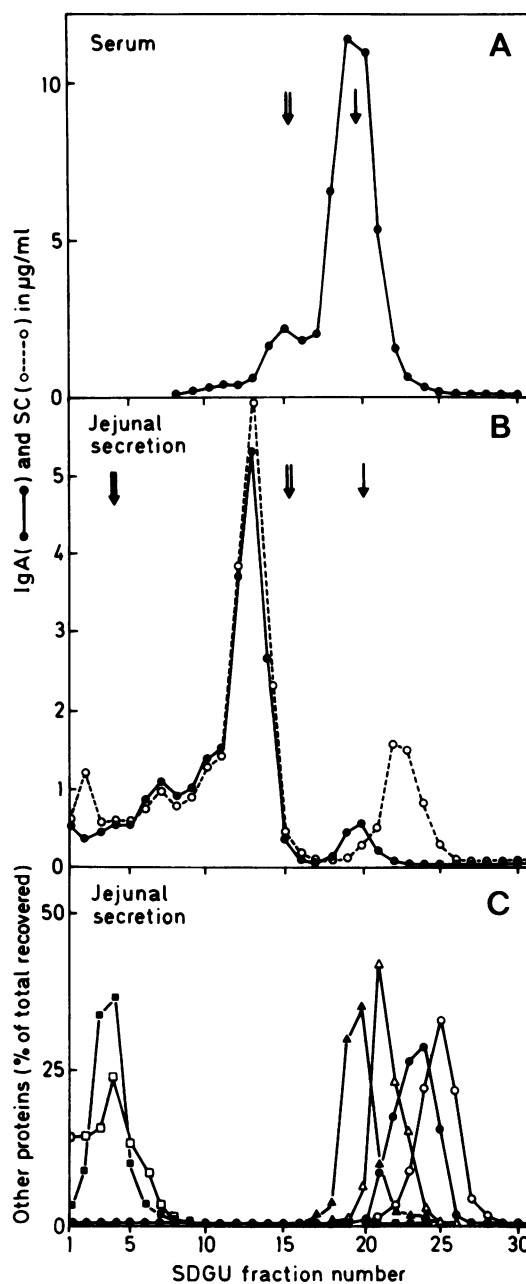‡ Percentage of IgA + IgM + IgG. § Range. ‖ Percentage of IgA1 + IgA2.



*Figure 1.* Simultaneous ultracentrifugation of serum (one control patient) and pooled jejunal perfusates of controls ($n = 11$). In serum (A) and jejunal secretions (B), the profile of IgA (● —— ●) measured by IRMA is corrected for the influence of the size of IgA; SC, (o----o). The lower profiles (C) are those for the other proteins (ORO, o —— o; Alb, ● —— ●; transferrin, △ —— △; IgG, ▲ —— ▲; $\alpha_2$M, □ —— □; and IgM, ■ —— ■). The arrows indicate the sedimentation levels of 7S m-IgA (→), 10.5S d-IgA (→), and 19S IgM (→).

The value is the mean value of the four 20-min periods of perfusion after the 1 h equilibration period. (c) The relative coefficient of excretion (RCE) of proteins is obtained from: RCE = [(protein in perfusate)/

Table III. Concentrations of Proteins in Serum and in Perfusate in Normal Individuals

| | Alb | ORO | Transf | IgG | IgE | m-IgA | p-IgA | SC* | α₂M | IgM |
|---|---|---|---|---|---|---|---|---|---|---|
| Serum (mg/ml) | 41.3‡ 36.3–46.0§ | 0.8 0.5–1.7 | 3.2 2.6–4.2 | 13.1 8.9–19.3 | $0.6 \times 10^{-3}$ $0.06 \times 10^{-3}$–$17.3 \times 10^{-3}$ | 2.2 1.4–4.7 | 0.3 0.1–1.4 | — | 2.0 1.3–3.7 | 1.0 0.6–1.8 |
| Perfusate (µg/ml) | 14.9 4.9–56.8 | 0.4 0.1–1.2 | 1.3 0.5–3.6 | 3.8 1.9–9.7 | $0.2 \times 10^{-3}$ $0.02 \times 10^{-3}$–$4.05 \times 10^{-3}$ | 2.0 0.5–4.8 | 29.6 4.0–54.7 | 28.5 4.8–89.9 | 0.5 0.3–1.5 | 2.2 0.6–4.5 |

* Total free and Ig-bound SC.  ‡ Antilog of mean of log values ($n = 11$).  § Range.

(protein in serum)]/[(albumin in perfusate)/(albumin in serum)]. RCE was calculated for each 20-min period. Mean values were then calculated for the entire period. This coefficient, already used by Delacroix et al. (23) to analyze the parameters of secretion of proteins in human bile and saliva, and in dog, rat, and rabbit bile (36), is by definition 1 for Alb. It expresses the secretion rate independently from the concentration of proteins in the serum and from the net water output in the secretion. If all serum proteins were secreted according to the same transport mechanism as for Alb, without any influence of their individual physical or chemical properties, RCE should always average one.

All data are presented as geometric mean and range, except for water and electrolyte movements and immunohistochemistry, where arithmetic means±SD were used. Results were compared by means of the non-parametric Wilcoxon's test for paired values (37).

## Results

*Immunohistochemical studies.* The distribution of IgA-, IgA subclasses-, IgM-, and IgG-containing cells in the jejunal lamina propria are listed in Table II. Densities were measured per mm² of lamina propria. The lamina propria accounted for ~43% of the total mucosa. The sum of IgA1- plus IgA2-containing cells averaged 89–139% (mean equals 99%) of the IgA-containing cells that were counted on serial sections of the same tissue specimen. Less than 10 out of 3,250 cells were found double stained using the double tracing procedure. In three peripheral lymph nodes, IgA2 represented 24.2, 25.2, and 25.7%, respectively, of the total number of IgA-containing cells.

*Molecular size of proteins and IgA subclasses in jejunal secretion of controls.* IgA in control sera resolved in a minor peak (7.2–22.4%, mean equals 11.5%) of 10.2S dimeric-IgA (d-IgA) and a predominant peak of 7S m-IgA (Fig. 1 A). The perfusate showed a major peak (80.4–97.5%, mean equals 92.5%) of 11S and heavier p-IgA (Fig. 1 B). SC in perfusate samples (Fig. 1 B) sedimented with p-IgA (83.3–99.3%, mean equals 91.5%) and in the 4.5S position corresponding to free SC (mean equals 8.5%). SC-free p-IgA could not be detected in the perfusate.

All other proteins studied in the perfusate (Fig. 1 C) sedimented as homogeneous peaks, with sedimentation coefficients similar to those usually observed in the serum. Thus, there was no evidence of proteolysis. IgA2 contributed from 15 to 31% (mean equals 23%) of total IgA in the serum vs. 27–48% (mean equals 35%) in the perfusate (P < 0.01). For each individual, the percentage of IgA2 was greater in the perfusate than in the serum.

*Protein concentrations and absolute jejunal secretion rates in controls.* Protein concentrations in serum and perfusate samples are listed in Table III. In the perfusate, SC and p-IgA were present at greater concentration than all other proteins (P < 0.01). Polymeric-IgA contributed 79% of total Igs in the perfusate, (IgG/p-IgA = 0.13) but contributed only 2% in the serum (IgG/p-IgA = 44). Absolute secretion rates of proteins from the jejunal mucosa are shown in Fig. 2. About 217 µg of p-IgA, 35 µg of IgG, and 15 µg of m-IgA were secreted per minute into the 40-cm long perfused segment. Within the successive 20-min period samples from the 11 controls, the mean coefficient of variation of secretion rates ranges from 13% (6–32%) for IgM to 20% (6–39%) for IgG.

*RCE of proteins in controls.* Two groups of proteins could be recognized when protein RCEs were calculated (Fig. 3). In the first group of proteins (Fig. 3, open columns and dotted
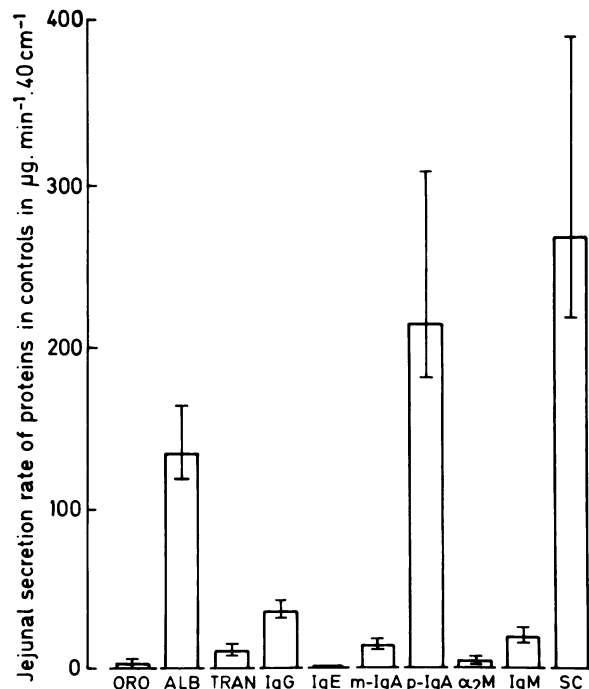


Figure 2. Jejunal secretion rate of various proteins in controls ($n = 11$) measured by segmental perfusion of the jejunum. Columns represent geometric mean±SE of the geometric mean.

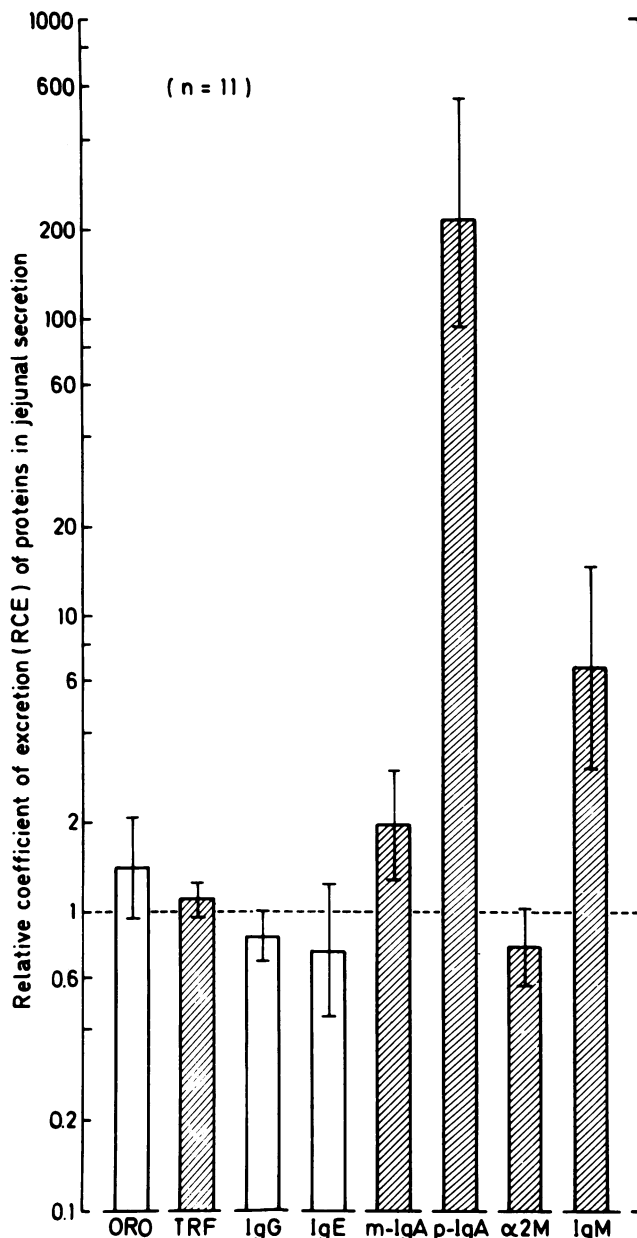*Figure 3.* RCE of various proteins in jejunal secretion. Geometric means±SD are shown by columns. The horizontal dotted line represents the RCE of albumin, taken as a unit. Empty columns represents the proteins for which RCE values appear inversely related to their molecular weight. TRF, transferrin.

line), RCE values decreased as the protein molecular weight increased. The RCE values obtained were: 1.41 for ORO, which was >1.0 for Alb, and >0.83 for IgG, which was $\cong$0.74 for IgE (all $P < 0.05$). However, for all other proteins studied, no inverse relationship between RCE values and MW was observed. For example, transferrin (85,000 mol wt) had an RCE value of 1.11 greater than the RCE of Alb (65,000 mol wt, $P < 0.05$). Similarly,

other RCE values observed were: 1.98 for m-IgA, which was >IgG ($P < 0.001$), 0.77 for $\alpha_2$M, which was $\cong$IgG and IgE, and 6.7 for IgM and 218 for p-IgA, which was >RCEs of all other proteins of lower molecular weight ($P < 0.001$).

*Radioisotopic studies.* As shown for patient 2 in Fig. 4, the major part (~70%) of the ^{125}I-radioactivity in the perfusate which had been ultrafiltrated corresponded to the peak of unlabeled p-IgA seen in SDGU. The results for the two individuals are listed in Table IV. For Alb, specific activities in serum and perfusate were similar (specific activity ratios equal 1.11 and 0.90), whereas for p-IgA, specific activities in perfusate were much lower than in serum (specific activity ratio equals 0.048 and 0.013). In other words, ~100% of Alb in jejunal secretion was derived from plasma, whereas plasma p-IgA accounted only for 4.8 and 1.3% of jejunal p-IgA.

When the RCE for p-IgA was calculated for these two individuals, considering only their plasma-derived p-IgA, values of 9.19 and 2.92 were obtained. This indicates that their plasma-to-jejunum transport of p-IgA was approximately nine and three times more rapid than the transport of Alb.

*Jejunal secretion of Igs in the patient lacking lamina propria IgA and IgM plasmacytes.* The sedimentation profiles of IgA and SC from the deficient patient are shown in Fig. 5. In serum, p-IgA contributed 6% of total IgA, a figure slightly lower than the lowest value obtained in controls. In the perfusate, IgA resolved in a major peak of m-IgA (82%) and a minor peak of 11S p-IgA (18%). SC occurred predominantly (98%) in the free form. These profiles are in sharp contrast to those observed in controls (Fig. 1).
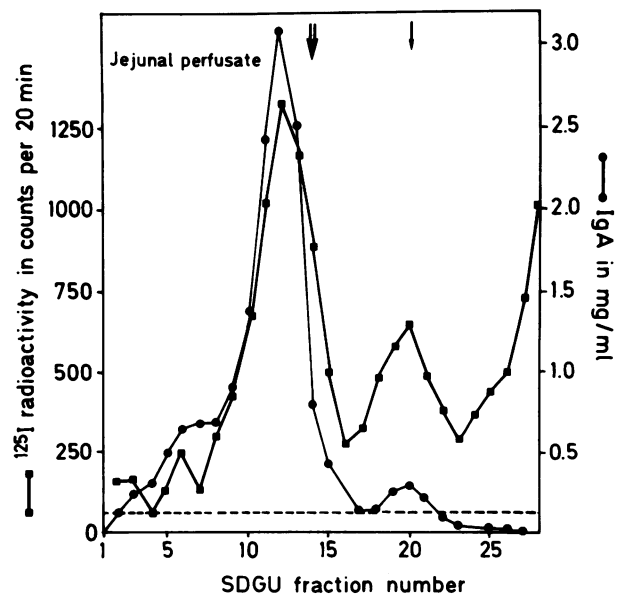


*Figure 4.* Sedimentation profile of unlabeled IgA (●) and ^{125}I-radioactivity (■) recovered in the jejunal perfusate of patient 2. Arrows indicate the sedimentation levels of unlabeled m-IgA (7S, →), and d-IgA (10.5S, →) tracers). Dashed line: 2 SD above the ^{125}I background.

*Table IV. Serum Vs. Local Origin of p-IgA and Albumin in Jejunal Secretion: Isotopic Experiments*

| | Patient 1 | | Patient 2 | |
|---|---|---|---|---|
| | Albumin ($^{131}$I) | p-IgA ($^{125}$I) | Albumin ($^{131}$I) | p-IgA ($^{125}$I) |
| **Serum:** | | | | |
| TCA precipitable radioactivity | | | | |
| (Counts/20 min/3 ml)* | 177,090 | 14,228 | 98,519 | 86,116 |
| Concentration (*mg/ml*) | 41.46 | 0.37 | 36.33 | 0.31 |
| Specific activity (Counts/20 min/mg) | 4,271 | 38,454 | 2,712 | 276,013 |
| **Perfusate‡:** | | | | |
| TCA precipitable radioactivity | | | | |
| (Counts/20 min/3 ml)* | 8,129 | 5,431 | 3,615 | 9,950 |
| Concentration (*mg/ml*) | 1.72 | 2.96 | 1.48 | 2.84 |
| Specific activity (Counts/20 min/mg) | 4,726 | 1,835 | 2,444 | 3,507 |
| **Perfusate/serum specific activity** | 1.11 | 0.048 | 0.90 | 0.013 |
| **Relative coefficient of excretion** | | | | |
| Total RCE§ (local + serum derived) | 1 | 193 | 1 | 225 |
| Serum-derived RCE‡ | 1.11 | 9.19 | 0.90 | 2.92 |

* In serum, TCA precipitable counts were >88% of total radioactivity. In perfusates, precipitable counts accounted for >80% of total counts for Alb and 59 (patient 1) and 64% (patient 2) for p-IgA. ‡ 100-fold concentrated sample. § Total RCE equals ([protein] perfusate/[protein] serum)/([Alb]perfusate/[Alb]serum). ‡ Serum derived RCE equals (total RCE) × (perfusate/serum specific activity).

Serum concentrations and jejunal secretions rates for Alb, IgG, $\alpha_2$M, SC, IgA, and IgM in the deficient patient are listed in Table V (all in absolute values and in percentage of mean control values). In the serum, IgA and IgM concentrations were mildly decreased. By contrast, jejunal secretion rates of p-IgA and IgM were strongly depressed at 0.2% of the control values. The secretion rate of m-IgA was less depressed at 13% of the controls. Secretion rates of other proteins, including SC, were in the upper range of control values, which are shown in Fig. 2. IgA2 percentages (Table V) were below the control range in both serum and perfusate and were similar in both fluids, which contrasted with results obtained in controls.

When the RCEs for p-IgA as well as m-IgA and IgM were calculated in this deficient individual (Table V), values of 1.24, 0.64, and 0.016 were obtained, respectively. Thus, only p-IgA was found to be selectively transported from plasma, as compared with Alb, whereas the transport of plasma IgM appeared to be greatly affected by the molecular weight of this protein. RCE values for p-IgA and IgM were ≅0.5% of control values. This again indicates that in normal individuals, local production of p-IgA as well as that of IgM contributes the majority of these Ig in the jejunal lumen. Fig. 6 summarizes the RCE data that was obtained in the deficient patient, (shaded columns for m-IgA, p-IgA, and IgM), in radioisotopic studies (shaded columns for Alb and p-IgA), and in controls (points). Plasma-to-jejunum transport of Alb, m-IgA, and IgM were inversely related to their molecular weights, as observed in controls for ORO, Alb, IgG, and IgE. By contrast, plasma-to-jejunum transport of p-IgA was ≅3.2 times (mean RCE in the deficient individual and in isotopic

studies) more rapid than the transport of Alb, exceeding by ∼15 times the transport rate (RCE equals 0.2) expected from its molecular weight (see regression line). It is interesting to notice that $\alpha_2$M's RCE (0.77) in controls also greatly exceeded the value expected (0.02) from its molecular weight.

## Discussion

This study allowed the assessment of three main parameters of protein secretion from the jejunal mucosa: the absolute protein secretion rate, the relative coefficient of protein excretion, and the relative contributions of plasma-derived and locally produced IgA (and IgM). These parameters were characterized with greatly reduced sources of errors. The distribution of Ig-containing cells in the lamina propria of our control group was similar to that previously observed in normal individuals (8, 38). The use of an occluding balloon in the segmental perfusion of the gut prevented contamination or reflux of the perfusate samples. In our procedure, only minor movements of water and electrolytes were induced. The artefacts, due to the occlusion of the gut lumen with alteration of gut vascularization, were found unsignificant in a preliminary study (22). No proteolysis was observed in the perfusate, and protein measurements were reproducible over successive periods of time. Both m- and p-IgA were considered separately, with appropriate correction for the influence of their size in IgA immunoassays.

Analysis of protein concentrations in jejunal perfusates of controls demonstrated a large predominance of p-IgA and SC. Polymeric-IgA accounted for 92% of jejunal IgA and was entirely
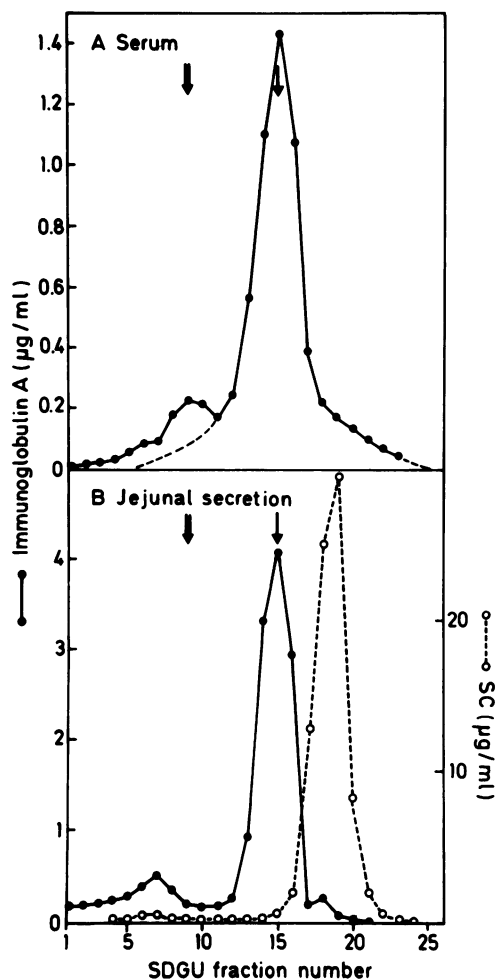
Figure 5. Simultaneous ultracentrifugation of serum and jejunal perfusate of the patient lacking IgA and IgM plasmacytes in his jejunal lamina propria. Arrows indicate the sedimentation levels of 7S m-IgA (—) and 10.5S d-IgA (—). The jejunal IgA and SC profiles show that m-IgA and free SC are in large excess compared with the control group (see Fig. 1).

bound to SC. SC was also present in the free form. The serum concentration of p-IgA was ~50 times lower than that of IgG, whereas the p-IgA concentration in jejunal perfusates was eight-fold greater than that of IgG, and usually exceeded that of Alb. This is in contrast to results of previous studies (1, 39). In these studies, IgG and IgA concentrations in upper bowel secretions were roughly similar, and lower than that of Alb. This may have been due to a contamination with bile, which contains similar amounts of IgG and IgA and a larger amount of albumin (23). The separate measurement of m- and p-IgA, with correction for size effects in IgA IRMA, could also explain the difference between the present and previous studies (40).

In the deficient patient, although jejunal concentrations of p-IgA and IgM were greatly reduced, SC concentration was

similar to the mean control value. However, SC in the perfusate of this patient occurred almost entirely in the free form. This observation again indicates that the jejunal secretion of SC occurs independently of the amount of polymeric Ig present at the basolateral surface of the gut epithelial cells (41).

The absolute secretion rates of proteins into the jejunum could be calculated from the perfusate protein concentration and the perfusion flow rate at the sampling point. In the control individuals, the total amount of p-IgA secreted from 40 cm of perfused jejunum under fasting conditions averages 217 $\mu$g/min, or about 312 mg/d. This value is 1.5 and 6.5 times greater, respectively, than the total secretion rates of p-IgA measured in saliva (~193 mg/d) and bile (~54 mg/d) using the same assays and standards (23). It can therefore be concluded that jejunal secretion provides the largest amount of p-IgA present in the upper small bowel, with only a minor contribution of bile. This conclusion illustrates another aspect of the species differences that was observed between the IgA systems of humans and rats (23, 13, 36). Rat hepatocytes actively transport p-IgA from plasma into bile (42), and deliver to the intestine 50 times more p-IgA (35 mg/kg per d) than does human bile (36). Due to this liver pathway, plasma-derived biliary p-IgA in rat contributes the majority (~90%) of total IgA in upper intestinal washings (43).

We further analyzed our data using the RCE calculation. This parameter expresses the protein secretion rate independently of the critical influence of the serum protein concentration. It allowed the distinction of two main groups of proteins in the jejunal perfusate with respect to the mechanism of their secretion. For ORO, Alb, IgG, and IgE in controls, and for m-IgA and especially IgM in the deficient patient, RCE values (Fig. 6) pointed to a predominant secretion by molecular weight-affected seepage from plasma. This observation for IgE contrasts with previous suggestions of a more selective secretion of this Ig (44, 45). For another group of proteins, transferrin, m-IgA, $\alpha_2$M, p-IgA, and IgM, RCE in controls exceeded the value expected from a molecular weight-affected transport from plasma. RCE of transferrin (1.11) slightly but significantly exceeded that of the smaller Alb. The RCE of $\alpha_2$M (0.7), similar to that of the smaller IgG, was 50-fold greater than that of IgM in the deficient patient. Local production of transferrin (46, 47) and of $\alpha_2$M (48) and receptor-mediated transepithelial transport of transferrin (49) could play a role in the more selective secretion of these proteins from the jejunal mucosa. Further analyses are needed to assess the reality and physiological relevance of these factors. According to their RCE, m-IgA, IgM, and p-IgA in controls were, respectively, secreted from the jejunal mucosa twofold, sixfold, and 216-fold more selectively than Alb. These results demonstrate that local production in the lamina propria and SC-mediated transepithelial transport of p-IgA and IgM play a major role in enhancing the secretion of these Ig from the jejunal wall.

Analysis of the distribution of IgA subclasses demonstrated a higher proportion of IgA2 (35%) in all control perfusate samples when compared with the corresponding serum samples. This

*Table V. Parameters of Jejunal Secretion of Proteins in the Patient Lacking Jejunal IgA and IgM Plasmacytes*

| | Alb | IgG | m-IgA | p-IgA | $\alpha_2 M$ | IgM | SC | IgA2* |
|---|---|---|---|---|---|---|---|---|
| Protein concentration in serum | | | | | | | | |
| (*mg/ml*) | 38.2‡ | 10.2 | 0.44 | 0.05 | 1.3 | 0.5 | — | 0.13 |
| | (92) | (78) | (20) | (17) | (64) | (52) | | (55) |
| Protein jejunal secretion rate | | | | | | | | |
| $\mu$g/min/40 cm | 248.8 | 66.0 | 2.0 | 0.4 | 8.0 | 0.05 | 327.6 | 0.12 |
| | (187) | (186) | (13) | (0.2) | (158) | (0.2) | (120) | (36) |
| Protein RCE | 1 | 0.99 | 0.64 | 1.24 | 1.00 | 0.016 | — | — |
| | (100) | (119) | (32) | (0.6) | (130) | (0.5) | | |

* Ratio of IgA2/(IgA1 + IgA2). ‡ Mean value of the four 20-min samples in the deficient patient. Values in parentheses are percentages of the mean value obtained in control individuals.

observation, although demonstrating less disparity between IgA1/IgA2 ratios in serum and secretions than frequently stated, extends results already obtained for milk (32) and several other external secretions (33). Similarly, the proportion of IgA2-con-

taining cells in the jejunal lamina propria was also greater than that in peripheral lymph nodes, as already observed when gut lamina propria (49) was compared with bone marrow (50, 51) using single-staining procedures. The similarity between the figures observed in jejunal secretion vs. serum, and in cells of lamina propria vs. peripheral lymph nodes or bone marrow, indirectly points to a large contribution of locally produced IgA to the IgA present in the gut lumen.

The contributions of plasma vs. local intestinal production of IgA (and IgM) in jejunal secretions were directly investigated with radioisotopes or were derived from comparison between RCE results in the deficient patient and those obtained in controls. Calculation of the specific activities of ^{125}I-p-IgA in serum and perfusate simultaneously demonstrated that only a minority (2 and 5%) of jejunal p-IgA is derived from plasma, vs. $\cong$100% for Alb. In the deficient patient, RCE of p-IgA was only 0.6% of the mean control value, which again indicated that in normal individuals almost all jejunal p-IgA ($\sim$98%) comes from local production in the gut wall. In the present study we calculated an RCE for plasma-to-jejunum transport of p-IgA of $\sim$3.2 (mean from isotopic experiments and from the deficient patient). This value is close to those previously recorded for the transport of plasma p-IgA into bile (RCE equals 4.5) and into saliva (RCE equals 4.4) (23). It demonstrates a small, selective plasma-to-jejunum transport of p-IgA ($\sim$5-fold more rapid than for
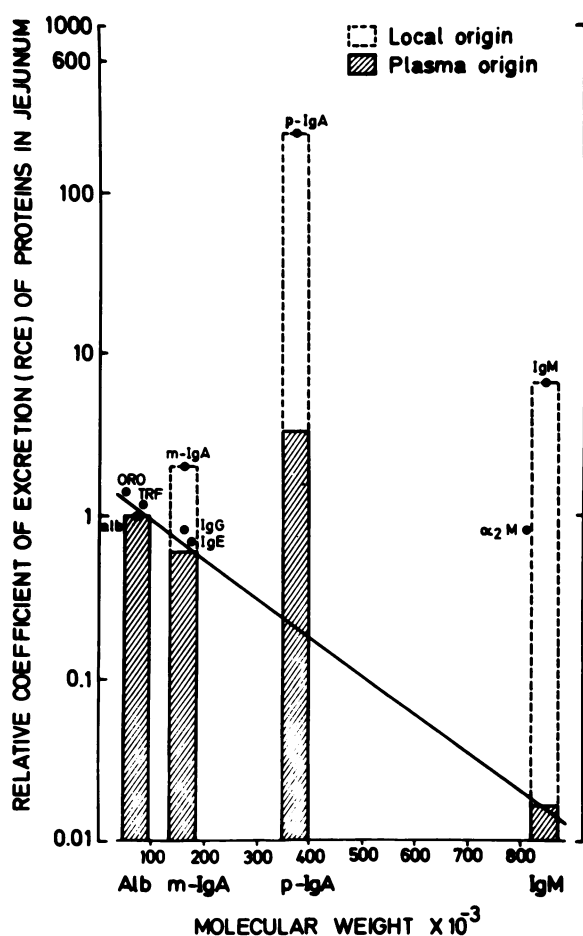


*Figure 6.* Transport of plasma proteins into the jejunum as assessed by their RCE. RCE values for ORO, Alb, transferrin (TRF), IgG, IgE, m-IgA, p-IgA, $\alpha_2 M$, and IgM in controls are shown by points (●). RCE calculated for plasma-derived Alb, m-IgA, p-IgA, and IgM are shown by striped columns. Their values were obtained in radioisotopic studies (Alb and p-IgA), and from the patient deficient in jejunal IgA and IgM plasmacytes (p-IgA, m-IgA, and IgM). Open columns represent the contribution of locally produced p-IgA, m-IgA, and IgM. A regression line ($r$ = 0.83) joins the RCE values that were obtained for ORO, Alb, IgG, IgE, and plasma-derived m-IgA and IgM to outline the influence of the molecular weight of these proteins on their transport from plasma across the jejunal mucosa.

m-IgA). Its absolute amount can be estimated at ~6 mg/d or ~0.08 mg/kg per d for the first 40 cm of jejunum. This is <2% of the fraction of the intravascular pool of p-IgA that is cleared daily from plasma (4.5 mg/kg per d) (13). In the deficient patient, the RCE for m-IgA (0.6) was similar to that of IgG and accounted for 32% of the mean control value. Thus m-IgA does not appear to be selectively transported across the jejunal wall, and its local production in the lamina propria probably contributes for greater than half of its total secretion. For IgM, the RCE in the deficient patient was extremely low (0.016), 400-fold smaller than in controls. This result indicates that the plasma-to-jejunum transport of IgM, despite the presence of epithelial SC, is dramatically affected by its molecular weight, and that local synthesis in the gut wall contributes >99% of the jejunal secretion of IgM in normal individuals.

## Acknowledgments

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