Virchows Archiv A Pathol Anat (1989) 416:97–103

Virchows Archiv A
Pathological Anatomy and Histopathology

Morphometric quantification of plasma cells in the intestinal mucosa of children

A comparative study between two sampling procedures

Marise Amaral Rebouças Moreira[1], Dorina Barbieri, and Antônio Sesso

[1] Department of Pathology, Faculty of Medicine of the Federal University of Goiás, Avenida Tocantins no. 1293, Setor Aeroporto, 74000 Goiânia, Goiás, Brasil
[2] Gastroenterology Unit, Children's Institute, Hospital das Clínicas, Faculty of Medicine of São Paulo University, Avenida Joaquim Eugênio de Lima no. 696, 01403 São Paulo, São Paulo, Brasil
[3] Molecular Pathology Laboratory, Department of Pathology, Faculty of Medicine of São Paulo University, Caixa Postal 8100, São Paulo, São Paulo, Brasil

Summary. In an attempt to obtain a sampling procedure of known accuracy for plasma cell quantification, applicable for routine analysis, we counted IgA, IgM and IgG producing cells in the lamina propria of the small intestine of six children by the method II of Aherne (1976), using two sampling procedures. In the first, we determined the number of cells in each one of 200 successive microscopic fields without specifying their localization in the mucosa. Proper tests upon this data showed that in order to estimate the number of IgA producing cells per mm^3 of lamina propria with a confidence of 95% that the mean of the sample would not differ by more than 5% from the mean of the population, it would be necessary to count 850–900 microscopic fields. With a confidence of 90% that the two means cited will not differ between them by more than 10%, the number of fields to be counted would lie in the range of 150 to 200. In the second procedure we arbitrarily divided the mucosa into upper, middle and lower segments, identifying and counting the number of cells in each segment. Consistent results for the number of IgA, IgM and IgG-containing cells were obtained by averaging the data of sufficient number of counts of 30 fields: 10 in the upper segment, 10 in the middle segment and 10 in the lower segment. Means obtained by stratified counts of IgA producing cells in 60 microscopic fields, 20 in each segment, differed, in 93% of the samples, by no more than 10% from the mean derived from the counts of 200 successive fields. Stratified sampling also enabled us to detect a segmental variation which had not thus far been quantified. In all cases, it was observed that the numerical concentration of IgA producing cells was greater in the lower region than in the middle zone, while for both IgM and IgG cells the larger numbers occurred in the middle and lower segments. The lowest concentration of IgA, IgM and IgG cells occurred at the top of the villi.

Send offprint requests to: M.A. Rebouças Moreira at the above address

Introduction

Immunocompetent cells have been quantified in the intestinal mucosa of children and adults in normal conditions and in diverse pathological states. Crabbé et al. (1965) were the first to quantify different plasma cell types in the mucosa in the digestive tract of adults. Their figures are utilized in the literature as normal values for adults. Using planimetric measurements over photographs of fluorescent areas, they managed to determine the number of IgA, IgM and IgG-containing cells per unit of the volume of lamina propria. It was possible to exclude, from the estimation of volume, structures such as epithelial cells, vessels and empty spaces. Due to technical difficulties, the authors chose the fields with the greatest numerical density and thus arrived at overestimated values.

Savilahti (1972, 1973); Savilahti and Pelkonen (1979), and Perkkio and Savilahti (1980), studied the jejunal mucosa of children by counting plasma cells directly over the sections and expressed their numerical density results in terms of mucosal area. In their work they do not explain how the microscopic fields were sampled, but mention the fact that the method does not allow for the exclusion

of empty spaces and areas occupied by ephithelium or vessels.

The introduction of the concept of the unit of mucosa by Baklien (1972); Brandtzaeg (1974); and Bradtzaeg and Baklien (1976), utilized by Maffei et al. (1979) among others, made possible the inclusion of all different mucosal components in the counts. Their results were expressed as the number of plasma cells per unit of mucosa.

In none of the previous studies has the accuracy of the counting procedures been assessed. In this paper we present the results of our efforts to standardize both an immunofluorescence technique for the detection of immunoglobulins and a sampling procedure for the relatively rapid evaluation of plasma cells in the jejunal mucosa of children by the morphometric method II of Aherne (1976).

Material and method

Six children from 15 months to 7 years of age were studied. Two of them had no history of diarrhoea and were free of intestinal parasites. They were of normal height and weight and had normal values for protein fractions, evaluated electrophoretically. The immunoglobulin A concentration in the serum was lower than one standard deviation from the mean value for the age (Table 1). The other 4 children had chronic diarrhoea but with a normal serum concentration of immunoglobulin A (Table 2).

Biopsies of the small intestine were made at the level of the angle of Treitz, utilizing the paediatric Crosby capsule. The material was fixed in 5% buffered formaldehyde, pH 7.0 for 4 to 6 h, then placed in 30% sucrose solution at 4° C for 12 to 18 h. Later the material was oriented on a piece of cork, frozen in hexane-liquid nitrogen and sectioned at 4 μ with a cryostat at −20° C. The slides were processed according to the standard technique for direct immunofluorescence (Nairn 1969) with the introduction of Evans blue as background stain. Details of the procedure are in the process of publication (Moreira, Barbieri and Castro).

We used antibodies anti-IgA, IgM and IgG, conjugated with fluorescein (Behringwerke A.G., Marburg W. Germany – Behring Institut) in the following dilutions: 1/50, 1/20 and 1/16, respectively.

The determination of the number of plasma cells IgA, IgM and IgG was made using the following morphometric relationship (method II of Aherne):

$$N=\frac{2\,n}{A\,(id+2\,\mathbf{t})}$$

where N is the number of cells per unit organ volume (mm^3); A is the area examined in mm^2; $i=c/n$; $c=$number of intersects between nuclear images of plasma cells in the lamina propria (whole nuclei and/or nuclear transections) and the parallel lines; $n=$number of nuclear images in the examined area (A); $t=$section thickness in mm and $d=$distance in mm between the parallel lines.

The area A of lamina propria was obtained from the number of points ("hits") falling over the lamina propria since each "hit" is associated with a given area. For these counts we used a Zeiss Kpl 8× eyepiece with a graticle containing a mesh of 100 points and a system of parallel lines (Fig. 1).

Table 1. Children without chronic diarrhoea and serum immunoglobulin A below the mean normal value for their age

Case	Age	Levels of serum immunoglobulin (mg%)		
		IgA	IgM	IgG
E.G.	2 yrs & 4 m	36	75	300
R.B.	7 yrs	55	262	950

Table 2. Children with chronic diarrhoea and normal serum immunoglobulin

Case	Age	Levels of serum immunoglobulin (mg%)		
		IgA	IgM	IgG
A.T.	4 yrs & 9 m	76	72	840
L.F.	15 m	38	225	950
F.T.	4 yrs & 9 m	72	76	1370
C.A.	5 yrs & 10 m	140	170	800

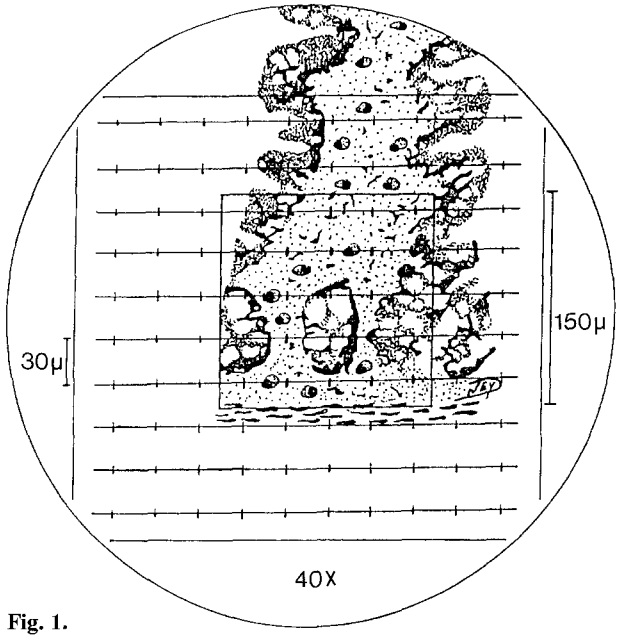

Fig. 1.

The measurements were carried out within the small square containing 25 points.

We effected the analysis of the material in a fluorescent microscope with epiillumination (Zeiss), using a 40× objective. At this magnification, each side of the small square measures 150 μ, therefore each "hit" corresponds to an area of 900 μ^2 (0.0009 mm^2).

The determination of the number of plasma cells IgA, IgM and IgG per mm^3 of lamina propria was made according to two sampling procedures in at least two slides, with 6 sections for each cell type. In the first procedure we made a count of plasma cells (A, M and G), in successive adjacent microscopical fields. We started from the upper left corner, descending verti-

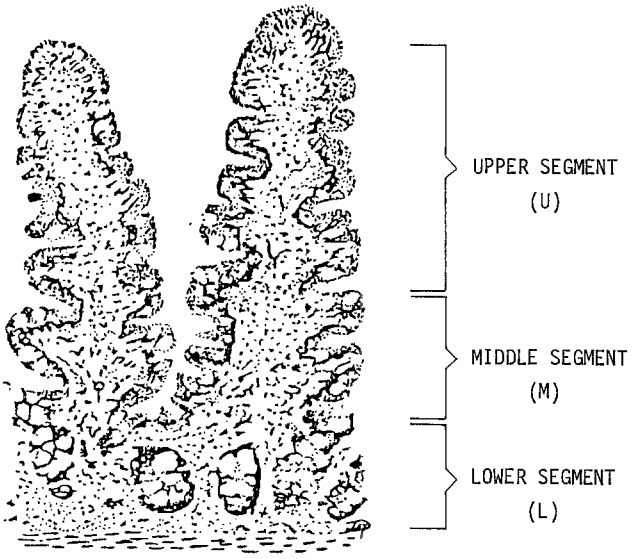

Fig. 2.

cally to the mucosal base. We moved to the adjacent next field and followed the vertical direction until all the extremities of the mucosa had been examined. Proceeding in this manner, we swept over all of the lamina propria microscopically, from above the "muscularis mucosae" to the top of the villi, without choosing fields, until a total of 200 fields was reached.

In the second procedure, programmed after analysis of the previous results, we divided the mucosa arbitrarily into upper, middle and lower segments. Cell counts were carried out in each segment until we obtained 200 fields. The position of the field, that is, whether it was in the upper, middle or lower region of the mucosa, was recorded. The upper segment went from the top of the villi to the lower extremity of the region representing the upper two thirds of the villi (U of Fig. 2); the middle segment extended from the higher limit referred to until the root of the glands of Lieberkühn (M of Fig. 2) and the lower segment from the previous lower limit to the "muscularis mucosae" (L of Fig. 2).

Results

In the six cases studied the number of plasma cells varied markedly from field to field. In some fields at the top extremities of the villi no cells were found, while fields at the bottom of villi and crypt regions exhibited up to 25 cells (Fig. 3).

Like the two other plasma cell types, the IgM producing cells appeared more concentrated in the lowest mucosal portion (Fig. 4). The IgG plasma cells were quite sparse, specifically at the top of the villi. There was a large quantity of fluorescent material in the interstices and basement membrane of the epithelium and blood vessels, which caused difficulty in the identification of cells containing IgG in the cytoplasm (Fig. 5).

After measurements made from 200 fields for each case, we found that the standard deviation for plasma cells IgA (first sampling procedure) varied most markedly (see Table 3). The variation range was close to the mean value and eventually

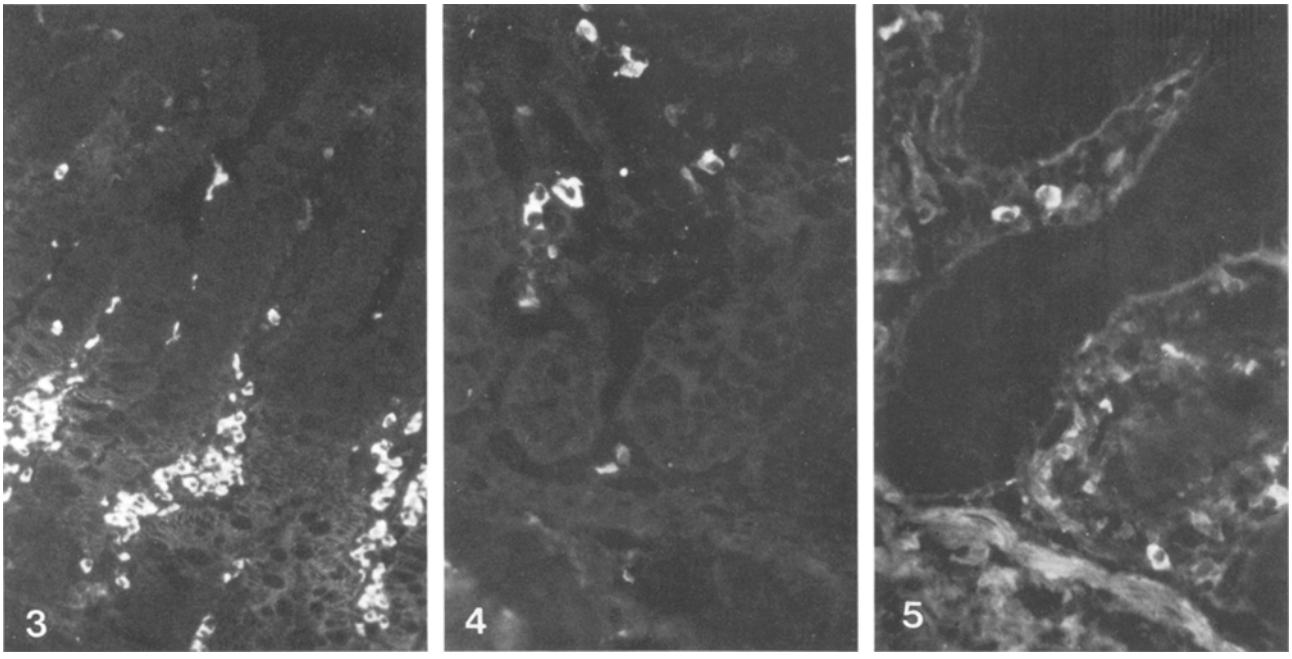

Fig. 3. Villi and crypt regions of the small intestine stained with anti IgA antibody. Original magnification × 100

Fig. 4. Crypt region of the small intestine stained with anti IgM antibody. Original magnification × 200

Fig. 5. Small intestine stained with anti IgG antibody. Original magnification × 200 (reduced to 90%)

Table 3. Average (μ) and standard deviation (σ) of IgA-containing cells (of numerical densities in the jejunal lamina propria) obtained after counting in 200 fields

Case	Cells IgA/mm^3	
	μ	σ
A.T.	52047	35986
E.G.	54625	52487
R.B.	49300	41144
L.F.	43244	34739
F.T.	57700	35986
C.A.	46206	56575

surpassed it. Through the relation $n_1 = s^2/(d/k)^2$ (e.g., Kish 1965) we obtain the number of sampling unit or microscopic fields (n_1) necessary to estimate with a confidence of say 95% ($k=1.65$) or 90% ($k=1.65$) that the mean of the sample will not differ from the mean of the population by a difference greater than 5% ($d=\bar{x}\cdot 0.10$); s^2 being the variance obtained in the sample. Applying the previous relation to the data in Table 3 it became apparent that it would be necessary to count between 850 to 900 fields to estimate the number of IgA producing cells with a confidence of 95% and $d=\bar{x}\cdot 0.05$. With a confidence of 90% and $d=\bar{x}\cdot 0.1$ the number of fields would lie between 150 to 200.

From the data of the 200 fields obtained with the second sampling procedure, we determined, for each type of plasma cell, groups of 10 fields by random allotments from the upper, middle and lower mucosal segments. The respective mean and standard deviations are shown in Tables 4, 5 and 6. Random allotment has been carried out in the same manner as in our state lottery.

Table 7 shows 20 collections of 3 values for IgA producing cells, each value representing cell counts in 10 microscopic fields of the upper, middle and lower mucosal segments made up artificially by random allotment from the 200 fields of the second sampling procedure. Thus in line 1 from Table 7 the numbers 97, 107 and 121 represent "hits" (H) over the lamina propria in 10 fields each, of upper, middle and lower segments, respectively. The numbers 14, 94 and 123 and 12, 74 and 94 represent nuclear images (n) and acrosses (c) of the parallel lines with nuclear images respectively, in the same 10 fields for each segment. These parameters were pooled and a single N value of 50300 IgA producing cells/mm^3 of lamina propria, representing the 3 mucosal levels, was obtained. In this manner we obtained 20 figures for each one of the cases studied. The corresponding means and standard deviations are 53165 ± 3959; 55075 ± 4937; 48410 ± 2831; 41930 ± 4043; 56115 ± 4482 and 46975 ± 4303. These values do not differ significantly ($P > 0.05$) from the corresponding cell count averages obtained with the counts of 200 fields utilized in the first procedure (Table 8). Similar results were obtained from plasma cells producing IgM and IgG.

Perusal of the data from Table 7 shows that the values 50300, 57800..., 51300, each one obtained by stratified sampling of 30 fields (10 in the upper, 10 in the middle and 10 in the lower muco-

Table 4. Mean values for plasma cells IgA for upper, middle and lower segments of the 6 cases obtained after counting 6 to 8 collections of 10 fields in each

Case	Upper	Middle	Lower
A.T.	13100	69800	75000
E.G.	12400	61900	86400
F.B.	11600	58200	71200
L.F.	11600	48000	65900
F.T.	14400	65300	87800
C.A.	11600	51200	73500
$\bar{X}$	12450	59067	76633
σ	1130	8333	8686

Table 5. Mean values for plasma cells IgM for upper, middle and lower segments of the 6 cases obtained after counting 6 to 8 collections of 10 fields in each

Case	Upper	Middle	Lower
A.T.	2100	15700	17800
E.G.	3000	16600	14200
R.B.	6400	18200	11900
L.F.	4000	14100	18100
F.T.	7900	21200	20900
C.A.	1200	10800	9100
$\bar{X}$	4100	16100	15333
σ	2583	3547	4396

Table 6. Mean values for plasma cells IgG for upper, middle and lower segments of the 6 cases obtained after counting 6 to 8 collections of 10 fields in each

Case	Upper	Middle	Lower
A.T.	4200	7900	5100
E.G.	600	9300	6400
R.B.	1300	7600	5500
L.F.	1500	5100	5000
F.T.	1300	4900	5400
C.A.	1200	7200	6600
$\bar{X}$	1683	7000	5667
σ	1270	1704	674

Table 7. Partial means and morphometric parameters (H = number of hits over lamina propria; n = number of nuclear profiles and c = number of crosses of the parallel lines with nuclear profiles) obtained in 20 collections of 30 fields

Number of draw	Upper			Middle			Lower			Total			Total (No. of IgA cells/mm³)
	n	H	c	n	H	c	n	H	c	n	H	c	
1	14	97	12	94	107	74	123	121	94	231	325	180	50300
2	28	104	20	97	106	65	128	123	94	253	333	179	57800
3	23	102	17	111	137	77	142	126	102	276	365	197	57300
4	4	91	3	120	119	98	113	110	92	237	320	193	50700
5	11	94	5	103	133	91	101	109	69	215	336	165	45800
6	17	87	9	117	134	93	179	149	126	313	370	250	58800
7	12	97	5	137	136	118	142	137	126	291	370	249	51900
8	14	97	12	137	136	118	142	126	102	293	369	232	57100
9	23	102	17	111	137	77	142	137	126	276	376	220	51100
10	14	97	12	97	106	65	179	149	148	290	352	225	58500
11	28	104	20	137	136	118	123	121	94	288	361	232	55100
12	11	94	94	94	107	74	113	110	92	218	311	171	49400
13	4	91	3	120	119	98	128	123	94	252	333	195	53900
14	11	94	5	120	119	98	123	121	94	254	334	197	54000
15	12	97	5	97	106	65	123	121	94	232	324	164	54500
16	28	104	20	103	133	91	142	126	102	273	363	213	53200
17	4	91	3	103	136	91	142	137	126	249	364	220	44100
18	11	94	5	120	119	98	123	121	94	254	334	197	54000
19	23	102	17	111	137	77	128	123	94	262	365	188	54500
20	23	102	17	117	134	93	101	109	69	241	345	179	51300
													$\bar{X} \pm$ 53165 3959

Table 8. Comparison between the averages obtained as stratified samples and the general average (μ) in each case, obtained with the first sampling procedure for plasma cells IgA

Case	General average μ (First procedure)	Stratified sample (Second procedure)		Test T
		$\bar{X}$	S	
A.T.	52047	53165	3959	1.26*
E.G.	54625	55075	4937	0.41*
R.B.	49300	48410	2831	1.41*
L.F.	43244	41930	4043	1.45*
F.T.	57700	56115	4482	1.58*
C.A.	46206	46975	4303	0.80*
Global mean	50520	50278		

* $P > 0.05$; Test "t"; $t = \frac{\bar{X} - \mu}{S/n}$

sal segments) relate in the following way to the average (Table 8 – 52047 cells/mm³) obtained by counting 200 fields successively. 65% (13 values) and 35% (7 values) of the data differ from the value 52047 in the ranges of 0–10% and 10–20% respectively. We have applied the procedure exemplified in Table 7 to the data of all the patients. Table 9 shows how frequently differences (D) between the means ($\bar{x}$) estimated through stratified sampling and the general average (μ) lie in the ranges of 0–10%; 10–20% or are greater than 20%, after random allotment of a given number of stratified microscopic fields.

The closeness (from 0.1 to 1.5%) of the two global means of cells number/mm³ of lamina propria obtained by the two sampling procedures (Table 9, column d) illustrates that stratified sampling is a reliable procedure for numerical density evaluation of the 3 cell types, once a sufficient number of microscopic fields are examined.

The pairs of means for IgA producing cells, obtained with both sampling methods in each one of the 6 patients (Table 8), differ one from another by 0.8–3%. This percentage difference for IgM and IgG producing cells varies from 1–5% and from 2–9%, respectively. The differences between the two sampling procedures become greater as the numerical densities decrease. This is more evident by examining, for the 3 cell types, the number of fields it is necessary to count by stratified sampling in order to obtain a low percentage of sample averages differing from that of the first sampling procedure by more than 10%. If one counts IgA cells in 20 fields in each one of the 3 mucosal segments, 93% of the means thus obtained will differ from the average of the first sampling procedure by figures between 0 and 10%. The numerical density of IgM and IgG cells will be estimated with even less accuracy by counts of 90 fields (30 in each segment (Table 9)).

Table 9. Distribution of the differences D, in percentage (1 = 100%), between the general average (μ) and the mean values ($\bar{x}$) obtained by stratified sampling

Cell type	Number of fields per segment (and total)	Range of D §	Occurrence of D §§	Cell number/ mm^3 – global means from 6 patients
	10	0 –0.1	0.77	
	(30)	0.1–0.2	0.21	
		0.2	0.02	
IgA				50520*
				50278**
	20	0 –0.1	0.93	
	(60)	0.1–0.2	0.07	
		0.2	0	
	30	0 –0.1	0.74	
IgM	(90)	0.1–0.2	0.26	12383*
		0.2	0	12578**
	30	0 –0.1	0.50	
				5217*
IgG	(90)	0.1–0.2	0.36	
				5221**
		0.2	0.14	

§ $D = (\bar{x} - \mu)^2/\mu$; e.g. in Table 3 the partial averages 57300 and 57800 (x) differ percentually from the average 52047 (μ) obtained in 200 successive microscopic fields, by 0.003 and 0.111, respectively

§§ Frequencies estimated in 6 patients, by alloting randomly from the stratified counts of the second sampling procedure, a total of 1200 microscopic fields for each one of the 3 mucosal segments

* and ** obtained respectively by the first sampling procedure and by stratified sampling, as indicated for IgA in Tables 7 and 8

Discussion

Although the counts of micrographs permit analysis by more than one person and can be easily revised after a long period, direct quantification of tissue has the advantage of being more economical and quicker.

We chose the method II of Aherne for cellular quantification because it is reliable (Mayhew 1972; Taga and Sesso 1978) and allows the acquisition of results of cellular profile density per area and of cell numbers per volume, which facilitates further comparisons. In order to carry out the Aherne II method one may utilize, as we did, a grid composed of a number of points regularly spaced over parallel lines. This grid is placed casually over the tissue to be examined. The number of points falling on any one of the components of the tissue gives a measure of the area examined. In this area it is possible to count the number of cell profiles, the results being expressed as cells per unit of volume (Skinner and Whitehead 1976). As shown in Fig. 1, using a Kpl 8× of Zeiss, with a graticle of 100 and 25 points, it is possible to work only with the points over the lamina propria excluding those over other structures. Previous quantifications have been expressed in different units, which renders comparison between them difficult. By providing data of the number of cells per unit volume of lamina propria it is possible to compare our data with those of Crabbé et al. (1965), also expressed on a per volume basis. However, the parameter n of Aherne's formula, expressing number of cell profiles per unit area of lamina propria, makes a comparison of our data with those of Savilahti (1972, 1973) and Perkkio and Savilahti (1980) possible.

The analysis of the counts of the first sampling procedure utilized in the present work showed that estimations, with a precision of 5%, of the numerical density of IgA producing cells would require the counting of the impractical number of 850–900 microscopic fields. To attain these numbers it would be necessary to sweep microscopically with a 40× objective about 50 jejunal mucosa biopsy sections. By working at the level of error of 10%, which may be acceptable in various instances, the number of fields to be counted is in the range of 150–200. The requirement of such a large number of fields derives from the great variation in the counts from field to field. The source of variation which markedly increases the standard deviation is the fact that many fields, mainly at the tops of the villi, possess few or no IgA cells. Another related source of variation is derived from the unequal thickness of the sections, which also affects the estimation of the number of cell profiles per unit area. To have an insight into the magnitude of this factor, which is inherent in all counting procedures (expressing the data either per area or per volume) we counted IgA cells in many successive cryostat sections of the same biopsy. The differences between the numerical density of cells in two successive sections varied from 0 to 20% with an average of 12%. These differences include both actual regional differences in cell numbers and differences in section thickness. Through stratified sampling of IgA cells it became apparent that with counts of 60 fields (20 in each segment) 93% of the estimates obtained differed by no more than 10% from the average cell number derived from the count of 200 successive fields.

The results obtained with the stratified sampling system utilized in the present work indicate that the mucosa of all children studied presented different numerical densities both at the top and

bottom of the villi and in the crypt regions. The concentration of IgA plasma cells per mm^3 of lamina propria was less in the highest region that in the middle; the greatest concentration was in the lower region (Table 4).

For IgM and IgG producing plasma cells, the major concentration occurred in the middle and lower regions. The number of these cells at the apex of the villi (Tables 5 and 6) was the lowest. Although it has been suggested in the literature that the concentration of plasma cells is greater in the lower region of the villi, there is no published data showing such segmental variation. The significance of this apparent stratification in plasma cell densities is still obscure. Furthermore, there are no means of evaluating the presence of abscence of transitional gradients between the regions measured.

In spite of having detected such segmental variation in children with partial IgA deficiency and in those with diarrhoe without this deficiency, it must be emphasized that this finding is not indicative that this pattern of stratification has a general occurrence. Two cases of intestinal pathology of identified aetiology and a number of cases of coeliac disease analysed separately by us, showed a total inversion of the values for IgA plasma cells with a diminution from the top to the bottom, and values of IgM plasma cells in the upper segment overlapped the cellular density of the middle and lower regions.

References

Aherne W (1976) Methods of counting discrete tissue components in microscopical sections. J Roy Micr Soc 87:493–508

Baklien K, Bradtzaeg P, Fausa O, Gjione E (1972) Mapping of the immunocyte distribution in human intestinal mucosa. Scand J Gastroent 16(Suppl.):13

Brandtzaeg P (1974) Mucosal and glandular distribution of immunoglobulin components: differential localization of free and bound SC in secretory epithelial cells. J Immunol 112:1553–1559

Brandtzaeg P, Baklien K (1976) Immunohistochemical studies of the formation and epithelial transport of immunoglobulins in normal and diseased human intestinal mucosa. Scand J of Gastroent 11 (Suppl. 36):1–45

Crabbé PA, Carbonara AO, Heremans JF (1965) The normal human intestinal mucosa as a major source of plasma cells containing γ-A-immunoglobulin. Lab Invest 14:235–248

Kish L (1965) Survey Sampling. New York, John Wiley

Maffei JL, Kingston D, Hill ID, Shiner M (1979) Histopathologic changes and the immune response within the jejunal mucosa in infants and children. Pediatr Res 13:733–736

Mayhew TM (1972) A comparison of several methods for stereological determinations of the numbers of organelles per unit volume of cytoplasm. J Microsc 96:37–44

Nairn RC (1969) Immunological Tracing: general considerations. Fluorescent protein tracing (Ed by RC Nairn) Edinburgh E, Livingstone, pp 129–148

Perkkio M, Savilahti E (1980) Time of appearance of immunoglobulin-containing cells in the mucosa of the neonatal intestine. Pediatr Res 14:953–955

Savilahti E (1972) Immunoglobulin-containing cells in the inestinal mucosa and immunoglobulins in the intestinal juice in children. Clin Exper Immunol 11:415–425

Savilahti E (1973) Immunoglobulin-containing cells in the intestinal mucosa, immunoglobulinas in secretions and serum IgA levels. Clin Exper Immunol 14:395–406

Savilahti E, Pelkonen P (1979) Clinical findings and intestinal immunoglobulins in children with partial IgA deficiency. Acta Paediatr Scand 68:513–519

Skinner JM, Whitehead R (1976) Morphological methods in the study of the gut immune system in man. J Clin Pathol 29:564–567

Taga R, Sesso A (1978) Avaliação do número de células de órgãos pela dosagem bioquímica de DNA em homogeneizados e por contagem direta através de métodos morfométricos. Ciênc e Cult 30(10):1232–1235

Weibel ER (1963) Principles and methods for the morphometric study of the lung and other organs. Lab Invest 12:131–155

Received December 2, 1988 / Accepted June 26, 1989