

## **Enterochromaffin Cells in the Rat Gastrointestinal Tract**

### *Aspects of factors influencing quantification*

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#### ABSTRACT

A quantitative study was made of the enterochromaffin (EC) cells, visualized with the Masson (slightly modified) and Falck-Hillarp techniques, in the rat gastrointestinal tract. Different factors influencing the quantification were analysed and the magnitude of methodological errors was estimated. The two histochemical techniques visualized the EC cells in different ways, influencing the quantification results. This means that a comparison of the quantitative results obtained by these techniques can only be made after correction by some factors.

The frequency of EC cells is given as the number of cells both per  $\text{mm}^3$  mucosa (cellular density) and per segment of mucosa with a base of  $1 \text{ mm}^2$ . The latter information takes into account both cellular density and mucosal thickness. The highest frequency of EC cells was found in the pyloric gland area of the stomach. On the border with the duodenum there was a marked decrease in this frequency and in the whole intestine the frequency was low, with only minor fluctuations of the mean values. The frequency was increased in the caecum, followed by a gradual decrease in the large intestine. The quantitative variation of the EC cells at the same gastrointestinal level was considerable, and was greater between animals than between serial sections within the same animal. The difference in EC cell frequency that was required to give significance was calculated. For quantitative studies the Masson method has some minor advantages over the Falck-Hillarp technique, as routine formalin-fixed deparaffinized sections can be used and no counterstaining is needed for the areal estimation.

#### INTRODUCTION

The chromaffin reaction in cells of the gastric mucosa was first described by Heidenhain in 1870 (23), and Ciaccio (1907) (5) suggested that these enterochromaffin (EC) cells might have an endocrine function. Masson (1914) (30) developed the argentaffin reaction and suggested that the silver-positive cells corresponded to the EC cells. The interest in these cells increased in 1952,

when Erspamer and Asero (9) found that they contained 5-hydroxytryptamine (serotonin). Some EC cells have been reported to contain substance P (24), motilin (33) or leu-enkephalin (1).

The two histochemical methods most frequently employed for visualization of the EC cells are the Masson (argentaffin) and Falck-Hillarp (formaldehyde-induced fluorescence) (FIF) techniques. The latter is considered to be more specific for biogenic amines than the former (cf 7). Since the completion of this study, serotonin antibodies have become available for immunocytochemical staining. The distribution of EC cells has been described in many species, including man (cf 34). It is difficult to evaluate these findings, however, as not only different staining techniques but also different methods of quantification have been used. Moreover, the degree of accordance between the staining results with the different methods is not clear. The aims of the present study were (1) to examine the influence of different staining characteristics and other factors in the Masson and in the Falck-Hillarp technique on the quantification of EC cells and (2) to examine the distribution and frequency of the EC cells in different parts of the gastrointestinal (GI) tract as visualized with the two methods.

#### MATERIAL AND METHODS

Six male rats of the Sprague-Dawley strain, weighing 250 to 300 g, were used. The animals were kept in a room with artificial lights switched on in the daytime (12 h). They were fed on a standard laboratory diet (Astra-Ewos laboratory animal feeds <sup>®</sup>).

After being starved overnight, but with water available ad libitum, the animals were killed between 9 a.m. and 1 p.m. by a blow on the neck.

Specimens were taken from different parts of the oesophagus and GI tract as illustrated in Fig. 1. Three consecutive specimens, each approximately 1 cm long, were removed from the proximal and distal parts of the small intestine. These specimens were marked with a silk suture in their proximal margin (see further below). From the remaining part of the small intestine five specimens, also about 1 cm long, were taken from the proximal half and three from the distal. The intervals between the former five specimens were equal, as were those between the latter three.

All the specimens were divided longitudinally into two pieces. Both pieces were then stretched flat, one on filter paper for fixation in 10% neutral-buffered formalin for 20-24 h, and the other on an aluminium foil with the mucosal surface upwards for freezing in a propane-propylene mixture cooled with liquid nitrogen. The formalin-fixed tissue was dehydrated, cleared in xylene and embedded in paraffin.

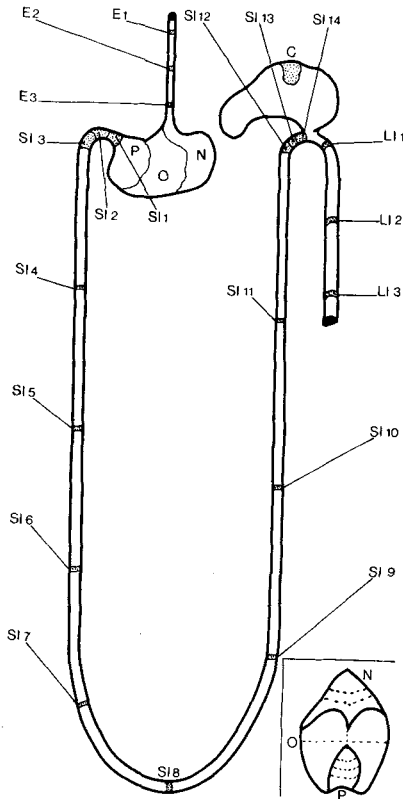


Fig. 1. Rat digestive tract- the shaded areas represent the different parts from which specimens were taken. E - oesophagus, N - non-glandular portion, O - oxyntic gland area, P - pyloric gland area, SI - small intestine, C - caecum, LI - large intestine. Inset - opened stomach. The dotted lines represent the delimitation of the collected specimens.

The frozen specimens were freeze-dried in a thermoelectric freeze drier (Edwards-Pearse type) for 48 h, exposed to formaldehyde gas (relative humidity 50-60%) for 1 h at 80°C (3, 10, 11) and embedded in Ralwax under vacuum.

During the paraffin embedding the specimens were oriented with the mucosal surface perpendicular to the cutting surface, and the suture-marked specimens were placed with their proximal end towards the frosted part of the glass slides.

From each formalin-fixed specimen four sections approximately 5 µm thick were cut, with an interval of 50 µm between each. These sections were stretched flat on a water surface before being attached to slides covered with glycerin-albumin. The formaldehyde-vapour-fixed specimens were cut in the same way, but on each level two consecutive sections were collected instead of one. One of these consecutive sections was attached dry to the glass slide and then transferred to a hot plate (+45°C) for a short time to allow it to spread out and stick to the glass surface and the other consecutive section was stretched flat on a water surface before it was attached to the slides. The formalin-fixed sections were cut with a sledge microtome and the vapour-fixed sections with

a rotary microtome. Slides with formaldehyde-vapour-fixed non-deparaffinized sections were stored in darkness at +4°C until examined within one week.

### Histochemical techniques

Formalin- and formaldehyde-vapour-fixed sections were stained with a slightly modified Masson technique (Fontana solution (22) preheated in the oven at 60°C for 1 h and the sections were placed in this solution for 1 h at the same temperature). A few silver-stained sections were counterstained with Mayer's haemalum.

Formaldehyde-vapour-fixed sections were deparaffinized in xylene, mounted in Entellan (Merck) and examined in a Leitz Orthoplan fluorescence microscope equipped with a Ploem illuminator, with filters selected to give a peak excitation at 390-490 nm.

From each of the parts of the GI tract some deparaffinized formaldehyde-vapour-fixed sections were examined in the fluorescence microscope before and after treatment with 0.1% sodium borohydride; the sections were then again treated in formaldehyde vapour and re-examined in the microscope (6).

### Quantitative studies

For quantification of the EC cells both the Masson and the Falck-Hillarp staining technique were used. The former was applied to formalin-fixed sections and the latter to sections fixed in formaldehyde vapour (attached dry to glass slides). In each part of the GI tract and with each stain, four sections at intervals of 50 µm were examined. In all sections the EC cells were counted at a total magnification of x 400 on two different occasions by the same observer, and the mean values of these counts were used. In the three proximal and in the three distal specimens of the small intestine each Masson-stained section was counted separately in three different parts in the proximal-distal direction. The EC cells were counted in both ideally and satisfactorily oriented parts of the sections (19). Ideally oriented sections meant that the mucosal surface lay perpendicular to the cutting surface, with intact stroma from the base of the mucosa to the top of the villi. By satisfactorily oriented parts of the sections is meant that the whole mucosa was included but with the villi and glandular lumen interrupted.

The size of each of the mucosal areas in which the EC cells were counted was estimated (duplicate measurements) with an image analyser system (Videovolumeter, Philips). With the Falck-Hillarp technique the sections were stained with haematoxylin-eosin before the areal estimations. Some of the sections, however, became detached during this staining procedure and areal estimations had to be performed on the consecutive sections.

The number of EC cells per unit volume ( $1 \text{ mm}^3$ ) of mucosa was calculated. The length of the ideally oriented mucosal parts of the sections was measured at the base of the glands on the monitor screen in the analyser system and the frequency of EC cells in a volume of a mucosal segment with a base of  $1 \text{ mm}^2$  was also calculated. Only EC cells with nuclei were counted. The Floderus formula (13) was used to calculate the frequency of EC cells whose nuclear centre lay inside the section. This formula takes into consideration the size of the nuclei, the smallest visible nuclear fragment, and the thickness of the section. The diameters of about 60 EC nuclear profiles (about 10 from each animal) from each level of the GI tract and with each staining method were determined by means of a micrometer scale in one of the oculars. In nuclei with an ovoid shape the geometrical mean ( $\sqrt{\frac{a \times b}{2}}$ , where a is the longest and b the shortest axis), was used as the diameter value (40). To calculate the error of nuclear size estimation, duplicate measurements were made. The observed profile distribution was completed for missing smaller observations by the method described by Weibel (38). To convert the distribution of the observed nuclear profile diameter to that of the true nuclear diameter the method described by Wicksell (39), extended to thick sections by Goldsmith (17), was used. The mean value of the diameters obtained after the Wicksell-Goldsmith transformation was used in the Floderus formula. The thickness of formalin-fixed Masson-stained sections and of formaldehyde-vapour-fixed haematoxylin-stained sections from each part of the GI tract was estimated with the aid of a Leitz Orthoplan light microscope at a magnification of  $\times 1000$  and the numerical aperture of the condenser optimally adjusted. By reading the micrometer screw scale when focusing the upper and lower section surfaces, the thickness could be roughly measured (1 scale unit =  $1 \mu\text{m}$  - information from Leitz). In each section the thickness was measured at three randomly selected points and the mean value of these determinations was used in the Floderus formula. To determine the error of the measurements of section thickness, 10 measurements were made at each of four well-defined points.

Tissue shrinkage. The frequency of EC cells was corrected for differences in tissue shrinkage between the formalin-fixed Masson-stained sections and the formaldehyde-vapour haematoxylin-stained sections. This difference in shrinkage was calculated by measuring the length of the muscularis mucosae occupied by 100 glands with a micrometer scale in one of the oculars. These measurements were performed at the base of the glands on the different levels of the GI tract of the six animals.

Statistical analysis. Coefficients of variation were used as a measure of errors of quantification techniques. In the comparison of the Masson and Falck-Hillarp techniques the most common used statistical test was the matched pairs t-test.

On the basis of the six animals and the four sections per animal investiga-

ted, the variance between animals ( $\sigma_A^2$ ) and the variance between sections ( $\sigma_S^2$ ), could be estimated by a standard hierarchical analysis of variance. Using this information it is possible to compute the accuracy of an estimated mean value based on  $k$  animals and  $n_0$  sections per animal for a given level of the GI tract. The variance for the estimate of the mean value  $\mu$ , which we denote  $\hat{\sigma}_\mu^2$ , is

$$\hat{\sigma}_\mu^2 = (n_0\sigma_A^2 + \sigma_S^2) / n_0k \quad (1)$$

If we assume that  $\sigma_A^2 = f_1\sigma_S^2$  ( $f_1$  is the ratio between  $\sigma_A^2$  and  $\sigma_S^2$ ) and further denote the coefficient of variation for sections, i.e.  $\sigma_S/\mu$ , by  $c_1$ , we can write this expression

$$\hat{\sigma}_\mu^2 = c_1^2 \mu^2 (f_1 n_0 + 1) / n_0k \quad (2)$$

If we compare two groups of animals and assume that the true mean value of the two groups are  $\mu$  and  $r\mu$ , the difference  $d$  between the estimated mean values  $\hat{\mu}_1$  and  $\hat{\mu}_2$  required for significance is

$$d > z_{1-\alpha/2} c_1 \mu \sqrt{(1+r^2) (f_1 n_0 + 1) / n_0k} \quad (3)$$

where  $z_{1-\alpha/2}$  is the standardized normal deviate and  $\alpha$  is the significance level (with  $\alpha = 0.05$  we obtain  $z_{1-\alpha/2} = 1.96$ ). It should be pointed out that formula (3) is based on the normal distribution, which requires that the variance is considered as known. If the variance is estimated from the data, a t-distribution must be used, which will slightly increase the difference required for significance.

## RESULTS

The sections from the different GI tract levels showed a normal histology. Argentaffin and yellow fluorescent cells were seen in all parts of the GI tract except in the oesophagus and the non-glandular part of the stomach. Stretching of the formaldehyde vapour-fixed sections on a water surface before attaching them to the glass slides somewhat decreased the fluorescence intensity compared with that in sections attached dry, but the frequency of EC cells in the sections stretched in these two ways did not differ significantly. Treatment of the sections with borohydride abolished the fluorescence of the EC cells in all parts of the GI tract except in the pyloric gland area, where the intensity only decreased and changed to yellowish green. After re-exposure

of the sections to formaldehyde vapour, the fluorescence in the EC cells was restored.

### Quantitative studies

#### Factors influencing the quantification

Nuclear size distribution. Counterstaining of the Masson-stained sections with Mayer's haemalum did not improve the visualization of the nuclear membrane in the EC cells.

In the respective staining technique no significant differences in nuclear profile size were observed at different levels of the GI tract. The distributions of measured (observed) nuclear profile sizes of EC cells in sections stained with the Masson and with the Falck-Hillarp method are illustrated in Fig. 2. Also presented in the figure are histograms of the converted (true) nuclear size.

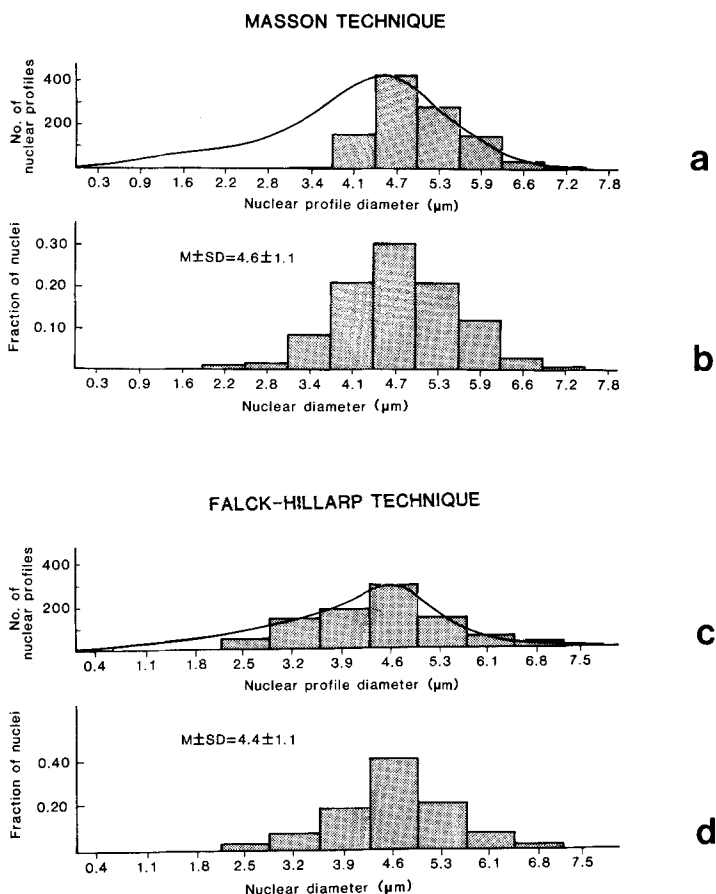


Fig. 2. a) The distribution of the observed diameters of the nuclear profiles of argentaffin cells in the Masson stain. The curve represents the smoothing for small undetectable nuclear profiles. b) The distribution of nuclear diameters after Wicksell-Goldsmith transformation. c) and d) Corresponding distribution of diameters of nuclear profiles and nuclei, respectively, in the Falck-Hillarp technique.

The means  $\pm$  standard deviations for the observed profile sizes were  $5.3 \pm 0.7 \mu\text{m}$  and  $4.6 \pm 0.9 \mu\text{m}$  for the Masson and Falck-Hillarp methods, respectively. The smoothing of the histograms for unobserved small profiles led to smaller means and larger standard deviations ( $4.2 \pm 1.3 \mu\text{m}$  and  $4.0 \pm 1.3 \mu\text{m}$ ). The Wicksell-Goldsmith transformation, finally, again increased the means to  $4.6 \pm 1.1 \mu\text{m}$  and  $4.4 \pm 1.1 \mu\text{m}$  (see Fig. 2 a-d). Although the difference between the staining methods was reduced, it was still significant ( $p < 0.01$ ).

Thickness of the sections. The thickness of the sections deviated from the  $5 \mu\text{m}$  for which the microtomes were adjusted, especially in the case of the rotary microtomes with which formaldehyde-vapour-fixed sections were cut. The mean thickness of the formalin-fixed Masson-stained sections was  $4.3 \pm 2 \mu\text{m}$  ( $M \pm SD$ ) and that of the vapour-fixed haematoxylin-stained sections  $3.2 \pm 1 \mu\text{m}$ .

Tissue shrinkage. For the formalin-fixed Masson-stained specimens the length occupied by 100 glands was  $4.26 \pm 0.4 \text{ mm}$  ( $M \pm SD$ ), while the corresponding figure for the vapour-fixed haematoxylin-stained ones was  $4.98 \pm 0.8 \text{ mm}$ . The difference is statistically significant (a paired t-test gave  $t(5) = 2.57$ ,  $p < 0.05$ ).

#### Errors of the quantification technique

The coefficient of variation of replicated readings of the nuclear profile size was approximately 5% for the Masson technique and approximately 2% for the Falck-Hillarp method. The error of estimation of the section thickness was about 10% for both types of microtome used. When three different points of the same section were measured, the coefficient of variation was about 15%. The error in the counting technique was about 5% for the Masson and about 2% for the Falck-Hillarp technique. The error of areal estimation was about 3% for both methods.

#### The effect of the correction of nuclear size and section thickness upon the frequency of EC cells

The frequency of argentaffin cells "increased" by 15% when the Wicksell-transformed values for nuclear size were used instead of the observed values of nuclear profiles. The corresponding finding for the FIF cells was 3%.

After correction of the thickness of the sections (observed contra expected thickness), the frequency of argentaffin cells "increased" by 9%, while that of the FIF cells "increased" by 21%.

#### Masson technique

The density of the argentaffin cells, i.e. the number of cells per  $\text{mm}^3$  mucosal volume, and the frequency of EC cells in the mucosa with a base of  $1 \text{ mm}^2$  (after correction with the Floderus formula), can be seen in Fig. 3. No EC cells were encountered in the oesophagus and in the non-glandular portion of the stomach. A few EC cells were observed in the oxyntic gland area, mainly in the bor-



der against the non-glandular area and in the vicinity of the oesophagus. The EC cells were most numerous in the pyloric gland area and showed an even distribution in its different parts, but decreased markedly at the border against the duodenum. The decrease in EC cells continued in the first 3 cm of the small intestine. A more detailed study of the first 3 cm of the small intestine in the proximal-distal direction showed a statistically significant downward trend in the frequency of the EC cells. No such trend was seen in similar studies of the last 3 cm of the small intestine. In the remaining parts of the small intestine the frequency of these cells was low, with minor fluctuations of the mean values. In the caecum and the proximal part of the large intestine there was a pronounced increase in EC cells, followed by a gradual decrease more distally. The four different parts of the pyloric gland area did not differ significantly with respect to the frequency of EC cells.

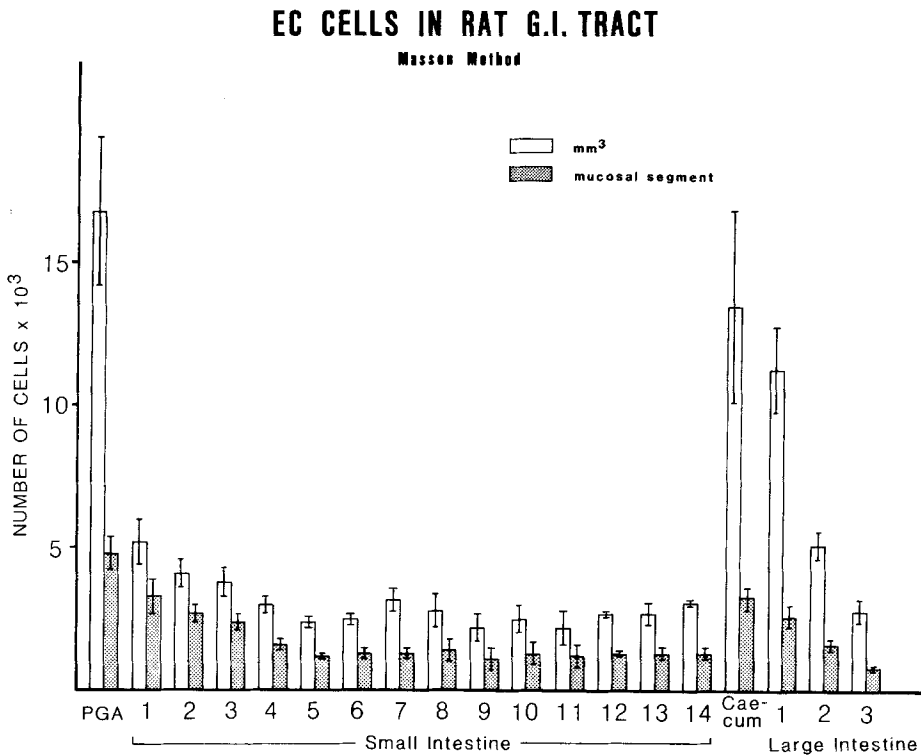


Fig.3. The frequency ( $M \pm SD$ ) of enterochromaffin (argentaffin) cells in different parts of the gastrointestinal tract from six rats. From each GI tract level in each animal four serial sections were examined. The unfilled bars represent the number of EC cells per  $\text{mm}^3$  mucosa, and the stippled bars the number of EC cells per mucosal segment with a base of  $1 \text{ mm}^2$ .

### Falck-Hillarp technique

Except in a few parts, the observed frequency of EC cells in the Falck-Hillarp technique was lower than that found in the Masson method. After correction of the frequency values with the Floderus formula the difference diminished and was statistically non-significant when shrinkage factor was taken into consideration.

### Statistical results

On the basis of the available data, the variance between animals was estimated as twice that between sections, while the coefficient of variation for sections was estimated as 0.25. Using our earlier notations this means that  $f_1 = 2$  and  $c_1 = 0.25$ . These values are representative of all the levels of the GI tract, as the estimated values did not differ greatly between these. From these values the difference required for significance at the 5% level, in the case  $r=1$ , for different combinations of  $n_0$  and  $k$ , was calculated (Table). Obviously, it is more efficient to increase the number of animals instead of the number of sections per animal. This conclusion holds irrespective of the value of  $r$ , while the required difference depend on  $r$ , as is clear from formula (3).

Table The difference (in per cent) in the frequency of EC cells required for significance when two groups of animals are compared at the 5% level as a function of the number of animals per group and number of sections per animal. Each figure represents a mean value for all GI tract levels.

No. of sections	No. of animals					
	2	4	6	8	10	20
1	85	60	49	42	38	27
2	77	55	45	39	35	25
4	73	52	42	37	33	23
6	72	51	42	36	32	23
10	71	51	41	36	32	23

### Distribution of EC cells at different levels of the mucosa

In the pyloric gland area the majority of EC cells (approximately 95%) were fairly evenly distributed between the middle and lower thirds of the mucosa. In the small intestine most of the EC cells were located in the crypts, while 20 to 30% were found in the villi.

Brunner's glands were found only in the first centimetre of the small intestine. In three of the six animals a small number of EC cells (3-8% of those

found in the overlying mucosa) were located adjacent to Brunner's glands. No EC cells were seen in this glandular area in the remaining rats.

In the caecum about 15% of the EC cells were observed in the upper third of the mucosa, whereas in the proximal, middle and distal parts of the large intestine the corresponding values were about 45, 25 and 20%, respectively. In the middle and lower thirds of the mucosa the EC cells were fairly evenly distributed.

## DISCUSSION

In a recent study Portela-Gomes and Grimelius (unpublished observations) found that the Masson-positive cells in the GI tract showed FIF and that FIF cells displayed an argentaffin reaction. Furthermore, the intensities of the respective histochemical reactions corresponded in the individual cells. In the intestinal tract, with few exceptions the FIF-argentaffin cells exhibited serotonin immunoreactivity. In the pyloric gland area, however, there were discrepancies; thus not all FIF-argentaffin cells showed serotonin immunoreactivity, and vice versa.

The observed frequency of cells is influenced not only by the choice of staining method but also by the cellular shape and size. Other factors having an impact on the quantification results are the thickness of the sections and tissue shrinkage. It is also important to know to what extent the errors of the quantification methods affect the results.

The influence of the cellular shape and size can be reduced if only those cells containing nuclei are counted, and can be abolished if only cells with their nuclear centre within the section are calculated (cf.25). This calculation can be done with the help of the formula proposed by Floderus (13), which also takes into consideration the size of the smallest visible nuclear fragment, as well as the thickness of the section. The size of a nuclear profile is influenced by the staining method used. The staining reaction in the perinuclear zone can more or less cover the nuclear membrane and the nuclear profile will then be the non-stained centre. This also means that the intensity of the staining reaction in the individual cells, especially in the perinuclear zone, will affect the size of the nuclear profile, and may also explain why nuclear staining apparently did not improve the appearance of the cellular membrane in most of the argentaffin cells. The nuclear profiles were larger with the Masson than with the Falck-Hillarp technique, also after the Wicksell-Goldsmith transformation. However, our estimation also showed that smaller nuclear fragments were detectable with the latter staining technique than with the former. Both these factors influenced the observed frequency of EC cells. This means that the quantitative results will differ even if the frequencies of EC cells in the two staining

methods are in reality the same. The same problem must arise when, for instance, the frequencies of endocrine cells demonstrated with the immunoperoxidase and immunofluorescence techniques are to be compared.

An important factor in quantification is the thickness of the section. In our study we used two different types of microtomes - the sledge microtome for paraffin-embedded specimens, and the rotary microtome for those embedded in Ralwax. The section thickness measured microscopically was smaller than that shown by the two microtome scales. There was also a difference in section thickness between the microtomes - Ralwax-embedded sections being thinner than the paraffin ones. The coefficient of variation in repeated measurements at the same point was about 10%, a variation which is in agreement with earlier reports (29). When the thickness was measured at three different points per section, the coefficient of variation was about 50% higher than that for repeated measurements at the same point. This implies that the thickness varies in different parts of the section, a finding reported by Glimstedt and Håkanson (16). As the section thickness influences the calculation of the cell frequency, an objective, exact and rapid method of thickness measurement is needed. The method used here is not satisfactory, the coefficient of variation being too high. Contributing to this variation is the accommodation power of the eye. As far as we know, however, no better method is available for routine estimations.

When using different fixatives, the shrinkage factor must also be taken into consideration. This shrinkage mainly influences the areal estimation and thereby the cellular density.

The errors of cell counting and areal estimation were small, but somewhat greater for the Masson than for the Falck-Hillarp technique. The frequency of EC cells was studied in relation to both mucosal volume ( $\text{mm}^3$ ) and mucosal segment (with a base of  $1 \text{ mm}^2$ ). The former value gives information about the density of the EC cells, while the latter also takes into account differences in mucosal thickness. The mucosal volume was calculated with the aid of an image analyser system (cf. 32). This is a rapid method, but it has the disadvantage that the cellular density can be influenced by factors that alter the mucosal volume, for instance oedema and inflammatory reactions. Other authors have related the number of endocrine cells to the number of villi and crypts (alt. 1) (8, 37), to the number of enterocytes (alt. 2) (15, 36), to a unit segment (alt. 3) (19, 27, 35), to a unit area (alt. 4) (4) or to a visible field of the microscope (alt. 5) (18, 21). All these alternatives except alt. 2 require optimally oriented sections. This exception is very time-consuming. Alternatives 4 and 5 are less useful for intestinal mucosa with its irregular villi. For all these alternatives except alt. 2 the thickness of the sections must be taken into account, as it influences the quantification.

In calculating the frequency of EC cells per mucosal segment, optimally

oriented sections are needed, which means that the mucosal surface must be perpendicular to the cutting surface. In this study problems arose in attempts to orientate the intestinal specimens optimally. Even when the specimens were fixed flat, they often curled somewhat during the fixation and dehydration and only part(s) of the mucosal segment could be used for this estimation. However, for estimations of cellular density, non-optimally oriented mucosal areas could be used, on condition that the sections included the whole mucosa.

In the present quantification the observed number of silver-positive cells exceeded that of FIF cells, but after taking the different factors analysed into consideration, the difference was non-significant.

The coefficient of variation of EC cells within the same animal and at the same GI level was about 25%. This variation can only be explained to some extent by errors of the quantification methods. The variance between animals was twice that within animals. This large interindividual variance means that it is difficult to detect small increases or decreases in the frequency of EC cells. As seen in Table, increasing the number of animals contributes more to the efficiency of the investigation than increasing the number of serial sections.

Both techniques give the same results when the different factors mentioned in this study are taken into consideration. The advantage of the Masson technique is that formalin-fixed sections can be used and no extra counterstain is necessary for the areal estimation. On the other hand, the Falck-Hillarp method showed smaller errors of quantification than the Masson technique.

Contrary to reports by some authors (12, 28), no EC cells were seen in the oesophagus. The highest frequency of these cells was observed in the pyloric gland area. In the small intestine, except in its most proximal part, the frequency was low, with minor fluctuations of the mean values. This distribution of EC cells differs somewhat from earlier findings in the rat. Some authors (20,26, 31) have reported a continuous decrease in EC cells in the distal direction, whereas Josephson and Altmann (27) and Thompson and Campbell (37) found a U-shaped distribution in the rat small intestine. This discrepancy may be explainable by the different staining methods used, as well as by different ways of quantification.

The majority of EC cells were located in the middle or deeper parts of the gastric mucosa and adjacent to the crypts in the small intestine. These locations are in good agreement with those found for the majority of the GI endocrine cells (cf. 14).

Different factors influence the quantification results in different degrees, but it is necessary to take them into consideration to avoid an over- or under-estimation. Regarding the EC cells there is an apparent variation of the frequency on the same GI tract level as well as a great variation between the animals. These variations in the EC cell frequency make it difficult to detect

small variations. Perhaps other factors such as sex, age, season and food composition may also influence the EC cell distribution and frequency.

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