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Relative growth of adenomatous polyps of the colon

Stereology and allometry of multiple polyposis

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Summary. The volume of the adenomatous mucosa (*V*), the area of the surface epithelium (*Ss*), the area of the glandular epithelium (*Sg*), and the *Sg*:*Ss* ratio were calculated in a series of 14 adenomatous polyps (*AP*s) of a case of multiple polyposis of the colon. The equation of simple allometry was used to study the relative growth of the four series of values. *Ss* grew isometrically with size; *Sg* overgrew *Ss* and accounted for most of the increase in *V*. The *Sg*:*Ss* ratio increased with *Sg* and *V*.

Key words: Adenomatous polyps – Colon – Relative Growth – Allometry – Morphometry

Introduction

Most works on the morphometry of adenomatous polyps (*AP*s) of the colon have relied on three-dimensional reconstruction and concerned the origin of the adenomatous mucosa. Lane and Lev (1963), by examining serial sections of a few *AP*s, have suggested that the adenomatous epithelium could originate from a central group of crypts of normal appearance and grow peripherally, displacing the normal epithelium. The minute adenoma, the precursor lesion of the *AP* consisting of a single or a few atypical glands, has been studied by serial sectioning of the colonic mucosa in non-polyposis coli cases (Oohara et al. 1981) and in cases of familial polyposis (Nakamura and Kino 1984). It appears to arise from the proliferative zone at the base and mid-portion of the normal crypt and to move with the surrounding epithelial cells to the upper portion of the mucosa. Elias et al. (1981) have compared the mucosa of *AP*s with the normal. They report that the mucosal volume, the epithelial surface, and the number of cells per epithelial area increase irregularly but steadily in *AP*s.

The purpose of this work was to study the relative growth of the mucosal components in a series of *AP*s of the colon through the assessment of morphometric variables and their allometric analysis.

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Material and methods

The material comprised 14 *AP*s from a case of multiple polyposis. The specimens were fixed in 10% buffered formaldehyde and embedded lengthwise in paraffin wax. Sections at 4 μm were chosen for morphometric studies only if they showed the fibro-vascular axes. Micrographs of the sections were taken at 2.5 magnification. In large specimens, multiple micrographs were combined to reconstruct the entire profile of the *AP*s.

The following variables of every *AP* were considered:
(a) Volume of the adenomatous mucosa (*V*), i.e. total volume of the *AP*s exclusive of their stalks and fibro-vascular axes;
(b) Area of the external epithelial lining (surface epithelium, *Ss*);
(c) Area of the epithelium of the glandular crypts of the *AP*s (glandular epithelium, *Sg*);
(d) *Sg*:*Ss* ratio.

(a) The *V* was measured as in Elias and Hyde (1983). This method, sketched in Fig. 1, assumes that the sections showing the fibro-vascular axes pass through the geometrical axes of the *AP*s and that transverse slices of the *AP*s are roughly circular. The areas (*A*) of the slices of adenomatous mucosa intercepted by a grid of equidistant lines were first calculated from the equation,

$$A = \pi(l/2)^2 \quad \text{mm}^2$$

where *l* was the length of the mucosal intercepts of each line. The *V* corresponded to

$$V = \mathrm{d}\sum A \quad \text{mm}^3$$

where *d* was the interlineal distance.

(b) The *Ss* corresponded to the equation,

$$Ss = V\,Ssv \quad \text{mm}^2$$

A grid of lines was used to measure the area of the surface epithelium per volume (*Ssv*). The grid, similar to that of Weibel

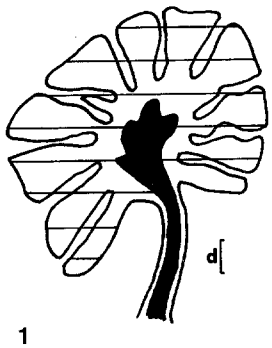

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Fig. 1. The method of Elias and Hyde (1983) for measuring the volume of the polyps is sketched. A grid of parallel lines at a known distance (*d*) was laid on whole-mount central sections of the polyps, and the combined length of the mucosal intercepts of the grid was calculated

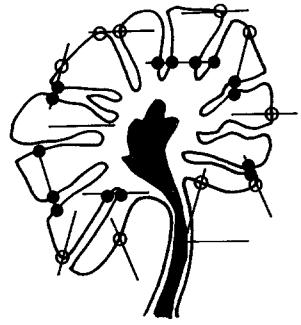

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Fig. 2. A grid with a pattern of lines consisting of segments at 60° angles was used to compensate for anisotropy in *PL* determinations. The *PLs* corresponded to the intersections of the grid lines with the surface epithelium (o) per unit length of the lines; the *PLg* corresponded to the intersections of the grid lines with the glandular epithelium (•) per unit length of the lines

(1963), included random segments forming 60° angles against each other in order to avoid artifacts caused by the anisotropic architecture of the specimens. *Ssv* was derived from the equation,

$$Ssv = 2PLs \quad \text{mm}^{-1}$$

where *PLs* was the number of intersections of the grid with the surface epithelium per unit length of the test lines. The unit length corresponded only to the segments of the test lines falling on the adenomatous mucosa of each *AP*, excluding its stalk and fibro-vascular axis. The number of grids (*n*) to be considered to ensure 95% confidence to the sample was assessed in every *AP* as follows:

$$n = (s/0.05\ PLs)$$

where *s* was the standard deviation of the *Ssv*.

(c) The *Sg* was calculated, like the *Ss*, from the area of the glandular epithelium per volume (*Sgv*),

$$Sgv = 2PLg \quad \text{mm}^{-1}$$

where *PLg* was the number of intersections of the grid with the glandular epithelium per unit length of the test lines.

The grid and the method used to calculate the *PLs* and *PLg* are sketched in Fig. 2.

(d) The *Sg:Ss* ratio, a dimensionless variable, was calculated directly, but corresponded mathematically to the index of folding (*F*) (Elias and Hyde 1983),

$$F = P/p$$

where *P* was the number of intersections of a grid of lines with the glandular surface of the *APs*, and *p* the number of intersections of the grid with the outer profile of the *APs*.

Allometry studies the differences in proportions correlated to changes in absolute magnitude of organisms or parts of them (Gould 1966). It is commonly expressed by the equation of simple allometry,

$$Y = aM^b$$

or, in logarithmic form,

$$\log Y = \log a + b \log M$$

where *b* is the slope of the log-log plot, and *a* the *y* value at $x=1$. *b*, the *y/x* ratio of specific growth rates, serves as a criterion of differential increase. When *b* is roughly equal to the *y/x* dimensional ratio (for instance, 1 for length/length ratios; 0.5 for length/surface ratios) geometrical similarity is maintained with size increase (isometry). If *b* is greater than the *y/x* dimensional ratio, there is a positive differential growth of *y* relative to *x* (positive allometry); if *b* is smaller, the *y/x* ratios decrease with increasing absolute magnitude (Gould 1966).

The relative growths of the above variables were studied with the allometric equation by log-log plotting of the values of each series.

Results

The *APs* showed histologically mild to moderate degrees of atypia and measured 1.43 mm to 2.04 mm in maximum diameter. The log-log plots and the allometric equations of the *Ss/V*, *Sg/V*, *Ss/Sg*, *Sg/Sg:Ss*, and *Sg:Ss/V* pairs with their *r* coefficients are shown in Fig. 3. Correlation was significant for the above pairs ($p<0.0001$ for *Ss/V* and *Sg/V*; $0.01>p>0.001$ for *Ss/Sg* and *Sg/Sg:Ss*; and $0.05>p>0.02$ for *Sg:Ss/V*). The *Ss/Sg:Ss* pair had the allometric equation,

$$y = 12.4X^{0.06} \qquad r = 0.06$$

the *r* being not significant.

The confidence limits (*CL*) of the *b* exponents were the following: *b*[*Ss/V*], 0.49–0.95; *b*[*Sg/V*], 0.91–1.39; *b*[*Ss/Sg*], 0.27–0.79; *b*[*Sg/Sg:Ss*], 0.52–1.60; and *b*[*Sg:Ss/V*], 0.11–0.83.

Discussion

Although the initial site of adenomatous transformation of the colonic mucosa has been recognized in the normal area of cell proliferation at the bases of the glandular crypts, the following stages of growth are controversial. In *APs* cell proliferation

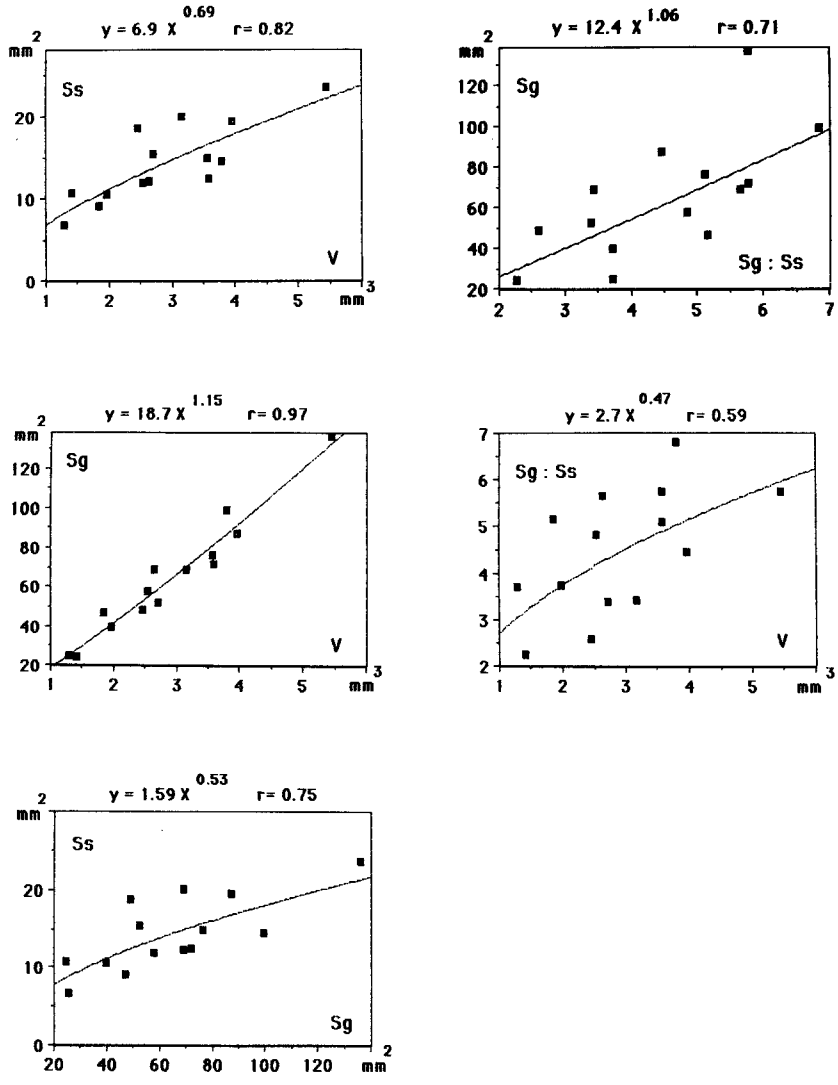

Fig. 3. Log-log plots, allometric equations and correlation coefficients of the *Ss/V*, *Sg/V*, *Ss/Sg*, *Sg/Sg:Ss*, and *Sg:Ss/V* pairs

shifts to the middle and upper portions of the crypts (Deschner 1983). This change has been demonstrated by autoradiography (Cole and McKalen 1983; Maskens and Deschner 1977) and mitotic counts (Wiebecke et al. 1974) in spontaneous *APs* of man and by autoradiography in 1,2-dimethylhydrazine-treated mice (Wiebecke et al. 1973). As a result, the highly proliferating adenomatous mucosa is three times as thick as the normal (Wiebecke et al. 1974).

Our data showed that the increase in *V* was related to a consensual growth of both the surface and glandular epithelium. *Ss* grew isometrically with size (b[*Ss/V*], 0.69; *CL*, 0.49–0.95; 0.66, expected isometry for L^2/L^3), suggesting that geometrical similarity of outer shape should be maintained with size increase. *Sg* was related to *V* by positive allometry (b[*Sg/V*], 1.15; *CL*, 0.91–1.39; 0.66, expected isometry) and accounted for most of its increase (r^2, 0.94). In addition, *Sg* overgrew *Ss* (b[*Ss/Sg*], 0.53; *CL* 0.27–0.79; 1, expected isometry), and showed negative allometry with *Sg:Ss* (b[Sg/*Sg:Ss*], 1.06; *CL*, 0.52–1.60; 2, expected isometry). Folding was related to *V* with relatively low dependence (r^2, 0.35), and appeared to grow at a slightly positive rate (b[*Sg:Ss/V*], 0.47; *CL*, 0.11–0.83; 0.3 expected isometry). In summary, *Ss*, *Sg*, and the *Sg:Ss* ratio increased consensually with size; *Sg* overgrew *Ss* and accounted for most of the increase in *V*.

These findings agree with those of Lane and Lev (1963), who have supported the concept that both the surface and glandular epithelium are actively proliferating in *APs*. According to Kaye

et al. (1971), the pericryptal fibroblast sheath of *APs* is as immature as the overlying epithelium; these authors have suggested that both components should grow abnormally. By contrast, Maskens (1977) and Deschner (1983) maintain that active stromal proliferation occurs only in villous polyps, where it gives rise to the typical mucosal outfoldings.

Imbalance of the superficial and glandular components with incomplete crypt formation has also been suggested by Cole and McKalen (1963), who have related it to the shift in the proliferating area in the adenomatous mucosa. According to them, the adenomatous mucosa grows exocentrically and produces infoldings. Maskens (1979) reports that the crypts opening at the mucosal surface of *APs* are more than those adjoining the muscularis mucosae and that the crypts never bifurcate at their bases. He infers that the colonic mucosa with focal adenomatous transformation should undergo enlargement and infolding of its upper portion, which could also justify the particular shape of small *APs*, a trapezoid with a convex upper side.

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