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Epithelial Proliferation and Morphogenesis of Hyperplastic Adenomatous and Villous Polyps of the Human Colon

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Summary. The morphogenetic mechanisms in the development of polyps were deducted from the structure of the mucous membrane and the distribution of the epithelial proliferation activity in various layers of the mucosa. Demonstration of the structure of the mucosa was effected by graphical-perspective partial reconstruction; the parameter used of proliferation activity was the mitotic index.

Hyperplastic polyps develop by lengthening and enlargement of the crypts due to increased supply of cells from an extended but normally located regeneration zone. The survival time of the cells may also be prolonged.

Adenomatous and villous polyps are based on a loss of epithelial differentiation, an extension of the proliferation zone up to the surface of the mucosa and a transposition of the main proliferation zone into the upper mucosal layers. In the case of adenomatous polyps this results in gland divisions and meandering with prevailing horizontal expansion.

In villous polyps, however, a predominantly vertical growth with villus formation takes place.

Because of the resemblance of the morphogenetic mechanisms in adenomatous and villous polyps the frequency of transitional forms is comprehensible.

There is no indication for the development of adenomatous or villous polyps from hyperplastic precursors.

In the course of the last few decades, the role of polyps in the carcinogenesis of the colon was the subject of numerous statistical and morphological examinations Although there was, of course, the question of the sequence of hyperplastic, adenomatous and villous polyps within a carcinogenetic development series, only few examinations were carried through on the formal pathogenesis of these polyps.

The opinion that adenomatous and villous polyps were tumours with different pathogenesis was supported by a theoretical concept of Dukes (1947), which was given much attention and according to which villous polyps develop by proliferation of the surface epithelium, whereas adenomatous ones are due to a proliferation of the deeper crypt sections. In contrast therewith, Sunderland and Binkley (1948) as well as Valdes-Dapena and Beckfield (1957) traced the origin of adenomatous polyps back to a proliferation of the upper crypt epithelium. The illustrations published show, however, marginal polyp sections. As already mentioned by Lane and Lev (1963) here the normal mucous membrane of the polyp pedicle generally appears to be superimposed by adenomatous mucosa

Lane and Lev (1963) showed in a very subtile serial section examination that adenomatous polyps may develop from one single or only few glands, in which—due to loss of the differentiating capacity—proliferating epithelium migrates to the upper crypt and to the surface. It was supposed that this undifferentiated epithelium invades the neighbouring crypts, displaces the normal epithelium and by increased proliferation leads to the lengthening and enlargement of the crypts and, as a consequence thereof, to polyp development. Authors supposed the zone of most intense cell proliferation to be in the lower half of the crypts as in normal mucous membrane.

In contrast therewith, autoradiographic findings of Cole and McKalen (1963) as well as of Deschner, Lewis and Lipkin (1963) showed that, in hereditary polyposis intestini, the most intense DNA synthesis takes place in the periphery of the adenomatous polyps. In addition, Cole and McKalen (1963) found in macroscopically unremarkable mucosa foci with a particularly intense DNA synthesis near the surface. They interpreted this transposition of the proliferation zone as the reason of polyp development.

It remained, however, unclear whether this phenomenon occurs only in hereditary polyposis or whether it is also the primary cause of solitary adenomatous polyps and applies to villous polyps, too. In addition, it remained unclarified whether and how the development of the typical adenomatous and villous polypous structures is linked with the changed epithelial proliferation.

It is the purpose of the present examinations, on parts of which reports have already been made (Wiebecke *et al.*, 1969, 1970), to describe the formal pathogenesis of the hyperplastic, adenomatous and villous polyps of the colon on the basis of objectively conceivable data.

The primary cause to be assumed for any development of polyps must be a change of epithelial proliferation. However, inductive effects between epithelium and mesenchyma may lead to considerable modifications of the structure and may thus also influence the macroscopical aspect. Therefore in all polyps, the size of the proliferation zone as well as the intensity of epithelial proliferation in several layers of the mucous membrane will be determined and put into relation with the structural characteristics covered by graphical-perspective reconstruction.

Material and Methods

The examinations are based on 6 hyperplastic, 6 adenomatous and 3 villous colonic polyps as well as 6 samples of normal colonic mucosa. The polyps were partly obtained bioptically, partly from resection specimens, where they were taken from the farther environments of carcinomas. Only such objects were used in which exactly orientated sections covering the muscularis mucosae were possible. The samples of normal colon mucosa were obtained from the section edges of resection specimens. None of the samples contained advanced epithelial atypias or focal carcinomas. The polyps examined were histologically "pure" forms. Mixed types, such as, for example, adenopapillary polyps, were not included in the examination. Hyperplastic and adenomatous polyps varied between 3 and 15 mm in diameter, villous polyps were larger. Large complete sectional series were made of all objects. Sections were cut vertically to the muscularis mucosae and in a longitudinal direction of the polyp pedicle. Sectional series covered small objects entirely, in larger polyps, however, no more than the central regions. Staining was done with haemalum eosin.

For a *quantitative comparison of epithelial proliferation* in the various layers of the mucous membrane, the only parameter available was the mitotic index. In sections from the central

region of the polyps, the entire mucosal height was subdivided into 10 zones of equal width and the mitotic index was determined separately for each zone. Measurements and counts were effected in 10 different places of each object.

After carrying through variance analysis the samples of all objects of each type were taken together according to their zones. In order to determine significant differences between the mitotic indices of the individual zones of the mucosa of each kind (maxima and minima), a combined T-test (standard deviation + comparison of two mean values) was applied. However, since there was no significant maximum for each type, neighbouring zonal indices of similar height were taken together in order to increase the number of n. The indices thus gained proved to be significantly higher as compared with other zones and may thus be regarded as maxima. This measure, which was partly also applied to show the minima, roughens the result but does not modify its principal importance.

Graphical-perspective reconstruction extends to individual glands and groups of glands and was effected along the lines of Barnett (1956) and Lebedkin (1926).

Results

Quantitative Findings of Epithelial Proliferation

Measurements of the mucosal height in a minor collective of hyperplastic adenomatous and villous polyps revealed a constant increase in this sequence. On an average, the hyperplastic mucous membrane was 1.5, the adenomatous one 3 times and the villous 6 times the height of the normal mucosa (Fig. 1). Whereas in normal mucous membranes, the measuring values are relatively close together, there is an increasing scattering width of the values from the hyperplastic to the villous polyps.

Determination of the mitotic index in the mucosa of the normal, hyperplastic, adenomatous and villous type subdivided into 10 zones each resulted in the following findings (Fig. 2a). In normal mucosa, mitoses of epithelial cells can only be demonstrated in the lower 6 zones, the mitotic index being highest in the zone 2 and decreasing constantly in the following 4 zones. In the hyperplastic mucosa, the regeneration zone is widened and reaches up to the 8th zone. The index maximum, however, being in the 2nd zone corresponds to normal conditions.

There is a decisive change in the adenomatous polyps. Mitoses are found in all layers of the mucous membrane, the proliferation zone is thus extended to the surface of the mucosa. Moreover, the highest mitosis indices are found in the upper zones of the mucous membrane. The frequency of cell divisions merely decreases in the zone immediately adjacent to the colonic lumen.

There is a very similar picture in villous polyps, however, the shifting of the increased mitotic activity to the surface is somewhat clearer still.

As the variation of the mitotic index was rather high especially in the adenomatous polyps, maxima and minima with statistically significant differences resulted only after an increase of the n-numbers by taking several neighbouring zones together (Fig. 2b). It is shown that the maximum of the mitotic index in normal and hyperplastic mucous membrane is close to the base, whereas it is unequivocally shifted to the zones close to the surface in adenomatous and villous polyps.

It is striking besides the displacements of the index maxima that the absolute heights of the zonal indices generally decrease from the normal mucosa to the villous polyps. This is also shown unequivocally by the mean mitotic index

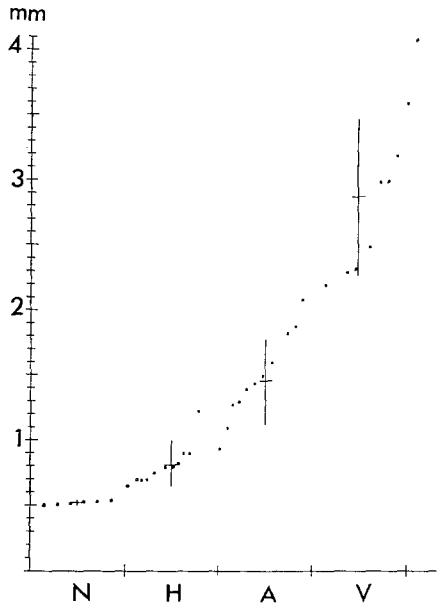


Fig. 1. Height of normal (*N*), hyperplastic (*H*), adenomatous (*A*), and villous (*V*) mucosa with standard deviation of the mean values. Each dot corresponds to one object

Table 1. Mucosal height, extension of the proliferation zone, and mitotic index related to the proliferation zone in normal mucosa and polyps (mean values). N = normal mucosa, H = hyperplastic polyp, A = adenomatous polyp, V = villous polyp

	Normal	Hyper-plastic	Relation H:N	Adeno-matous	Relation A:N	Villous	Relation V:N
Mucosal height	500 μ	800 μ	1.6	1500 μ	3.0	2900 μ	5.8
Extension of proliferation zone	300 μ	640 μ	2.1	1500 μ	5.0	2900 μ	9.7
Mitotic index in proliferation zone	2.2%	1.1%	0.5	1.1%	0.5	0.8%	0.4

related to each proliferation zone (Table 1). It is only 0.8% in villous polyps as compared with 2.2% in normal mucosa. However, as the proliferation zones are considerably enlarged in the polyps, in villous polyps by factor 9.7, the total result is a strong cell increase.

Structural Findings

In *hyperplastic polyps*, the epithelium reveals—with increasing differentiation in the middle and upper crypt as well as at the surface—a characteristic clustering. Here, the elongated nuclei are crowded and pushed one over the other in several layers (Fig. 3a). Graphical reconstruction of a hyperplastic gland shows that

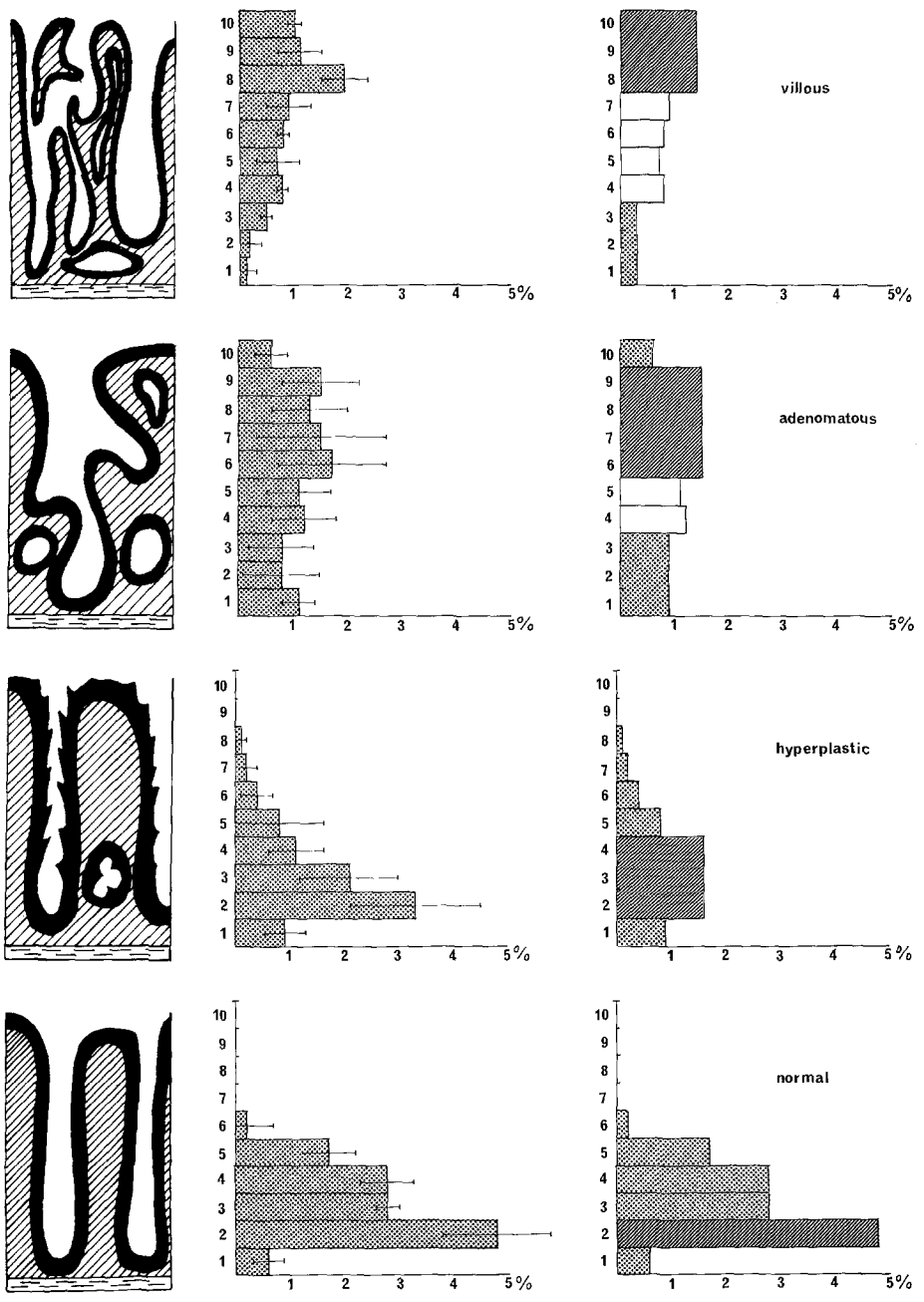


Fig. 2a and b. Mitotic index related to zones of always 1/10 of the mucosal height in normal, hyperplastic, adenomatous, and villous mucosa (a). Maxima (dark) and minima (moderate shading) of the mitotic indices with statistically significant differences obtained by fusion of several adjacent zones. $p < 0.05$ (b)

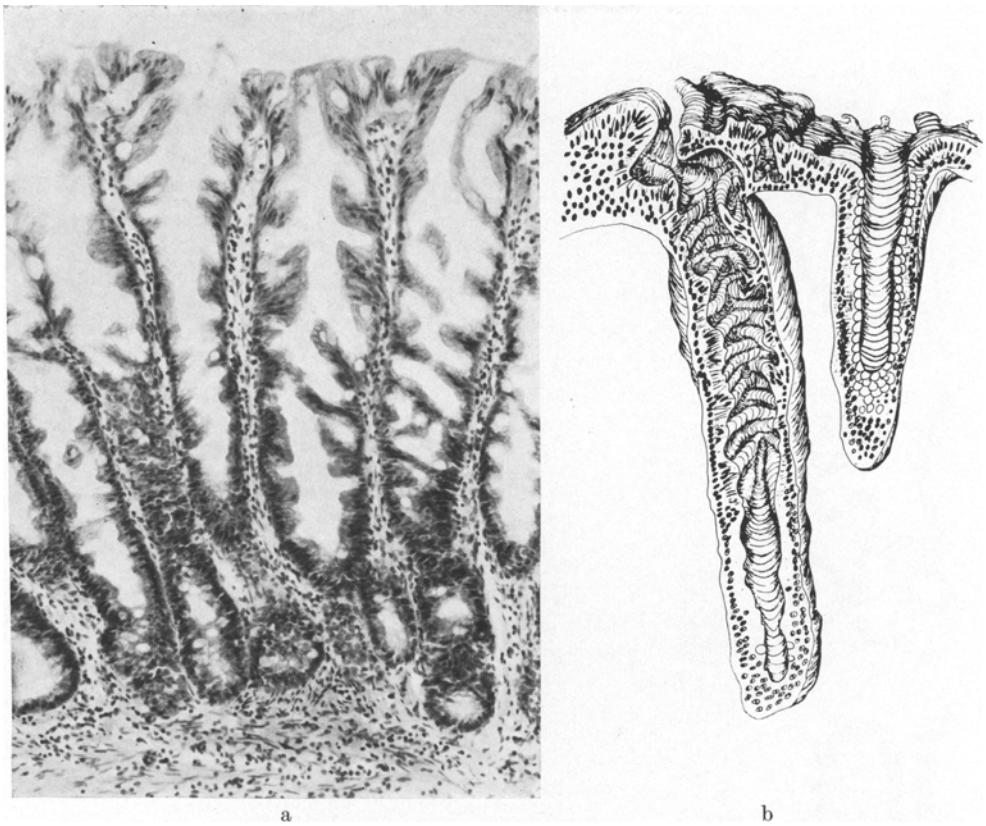


Fig. 3a and b. Hyperplastic polyp. Slight meandering of the lower crypts and characteristical epithelial folding in the middle and upper crypt regions (a). Graphic reconstruction shows the folds to be semicircular and oblique (left). At the right side part of a normal crypt (b)

this phenomenon is caused by semicircular or oblique folds (Fig. 3b). This plication is exclusively restricted to the epithelium, the basal membrane is not involved in its formation.

It must remain open whether these foldings-up can be regarded as an expression of a congestive pressure because of increased cell regeneration in the cryp fundus. The slight meandering of the lower cryp sections is, however, explained easily as a consequence thereof, all the more so since crypt divisions are occasionally observed. The latter event, however, seems to play a minor role in the morphogenesis of the hyperplastic polyp whereas lengthening of the crypts and dilatation of the upper crypt regions are of greater importance in this respect. Thus thickening and lateral expansion of the mucous membrane effect a polypous bulging.

By the predominant localisation of hyperplastic polyps on mucosal folds the polypous bulging becomes more prominent (Fig. 4). Hyperplasia of the mucous membrane entails increased sprouting of vascular connective tissue, which may possibly lead to short pediculation with peristaltic powers involved.

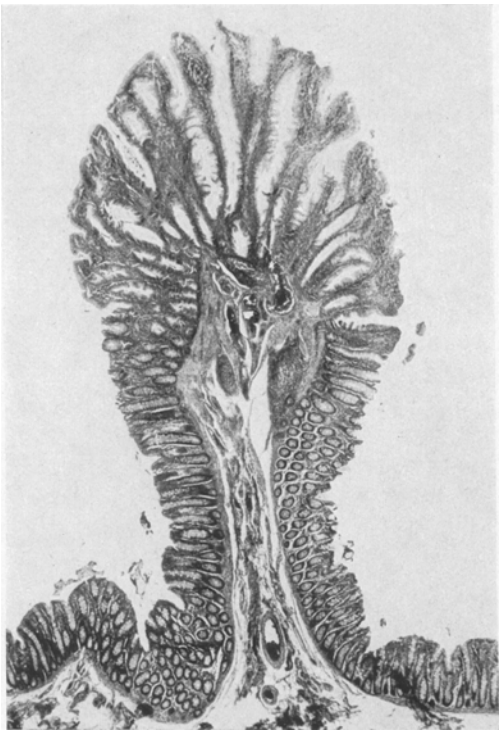


Fig. 4. Hyperplastic polyp located on a mucosal fold which is extended to a short stalk. Marked elongation and dilatation of the crypts

In *adenomatous polyps*, the course of the glands is not doubtlessly recognisable, not even in orientated histological sections. Partial reconstruction should, therefore, clarify whether adenomatous gland proliferation is merely a lengthening of the crypts and glomerulation or whether there are essentially glandular divisions and at which layers of the mucous membrane.

Partial reconstruction of an individual gland (Fig. 5) from a central section series shows an irregular course of the glandular tube with numerous bulgings and a division relatively densely underneath the surface.

From the reconstruction of a gland group (Fig. 6), it becomes clear that glandular divisions occur in all layers of the mucous membrane, most frequently, however, in the upper layers, which are identical to the zones of the most intense epithelial proliferation.

The growth and the further development of the adenomatous polyps is easily explained when we assume that the glands are divided by stromal septa growing inductively from below to the surface. As represented schematically in Fig. 7, the division procedures take place in the course of a generally upwardly orientated growth of glands and stroma, so that there are—besides an increasing lengthening of the glands—continuously new ramifications close to the surface. This increase of glands in the upper mucosa, causes a horizontally directed growth pressure,

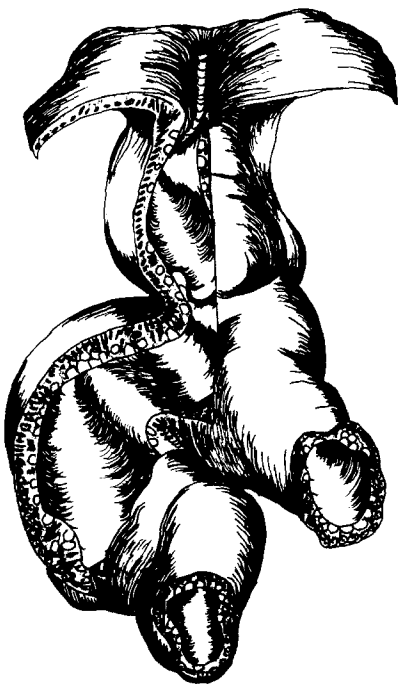


Fig. 5. Graphic reconstruction of a solitary gland of an adenomatous polyp with marked meandering and bulging as well as a division of the gland. Parts of the wall and the lower crypt ends are cut off to get insight

and thus a fanshaped spreading of the adenomatous focus and development of a typical fungiform polyp with laterally overhanging edges (Figs. 7, 8).

The fan-shaped expansive growth effects a protrusion of the mucous membrane, which necessarily involves the muscularis mucosae and submucosa. Short pediculation of the polyp is, therefore, already existent in statu nascendi.

In the non hereditary polyps, examined systematically in the present work, there was, in each case, the fan-shaped expansive growth mode presented herein, so that the borderline between the adenomatous and normal mucous membrane is approximately linear and corresponds to the radius (Fig. 7d).

Two polyps obtained from cases of hereditary polyposis and examined for the purpose of comparison displayed a variant of this picture. Whereas the adenomatous glands in the central sector of the polyp originated from the basal mucosa, they superimposed the normal mucosa at the periphery, as it were as an expression of a particularly rapid proliferation and spreading of the adenomatous epithelium at the surface (Fig. 9). Wherever the adenomatous glands located at the surface were connected to the local normal crypts, there was an abrupt transition between undifferentiated and differentiated epithelium. The location of these connecting places corresponded approximately to the position of the normal crypt orifices (Fig. 9). In rather central polyp regions, there was occasionally also intrusion of adenomatous epithelium into the preexisting crypts.

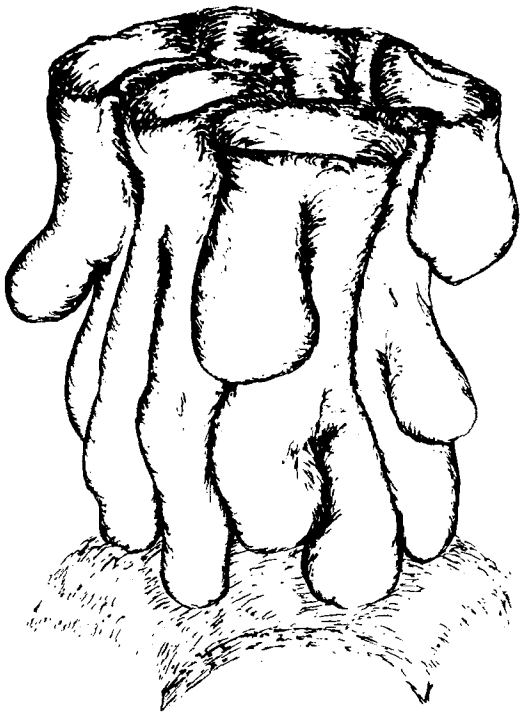


Fig. 6. Graphic reconstruction of a gland complex with several divisions, most of which are in the upper half

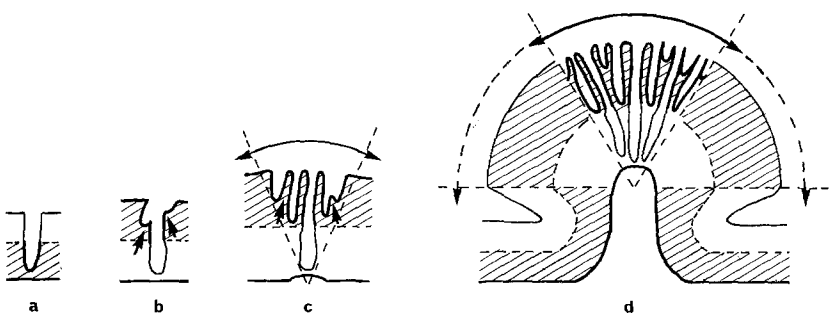


Fig. 7a—c. Morphogenesis of adenomatous polyps. Increased cell proliferation causes progressive lengthening of the crypts. Gland divisions within the zone of main proliferative activity (shaded) which is transposed to the upper mucosa results in a fan-like expansion of the adenomatous area. Small arrows indicate inductive stroma proliferation in gland divisions (b, c). With the course of the polyp development the muscularis mucosae is lifted simultaneously, thus indicating beginning stalk formation. a Normal crypt

The structure of *villous* polyps is not clearly understandable from the normal histological section. The villi, which frequently have ramifications, or connections, appear as slender formations with little stroma (Fig. 10a). Partial reconstruction shows that the villi are really foliaceous formations (Fig. 10b), which develop

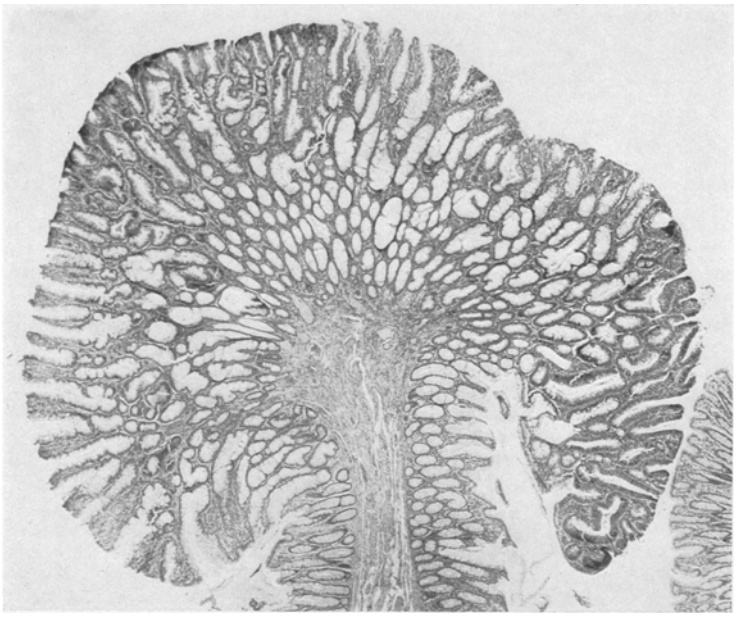


Fig. 8. Central section through an adenomatous polyp. Despite of many cross sections the glands display a roughly radial arrangement. Note increased number of glands at the periphery compared with the base

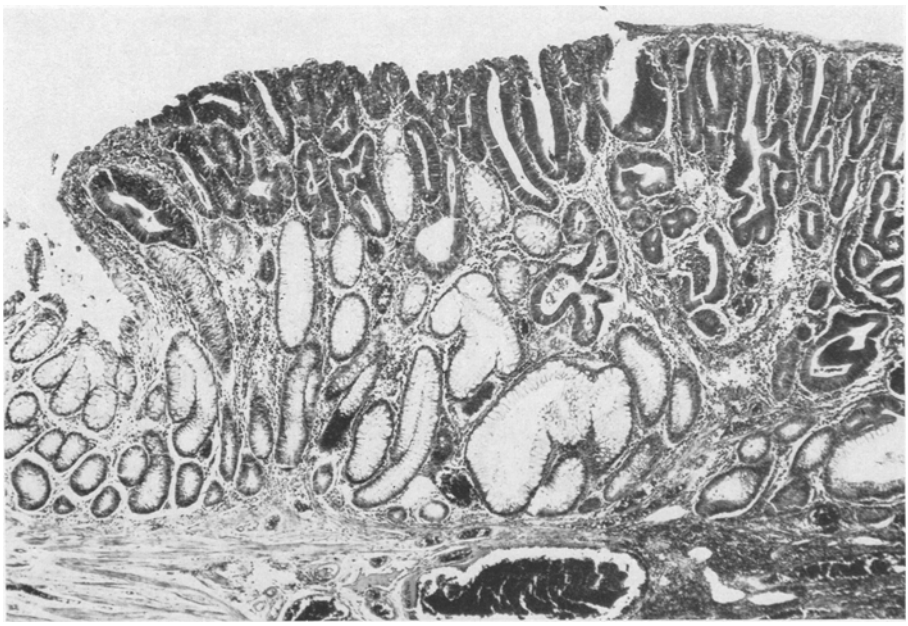
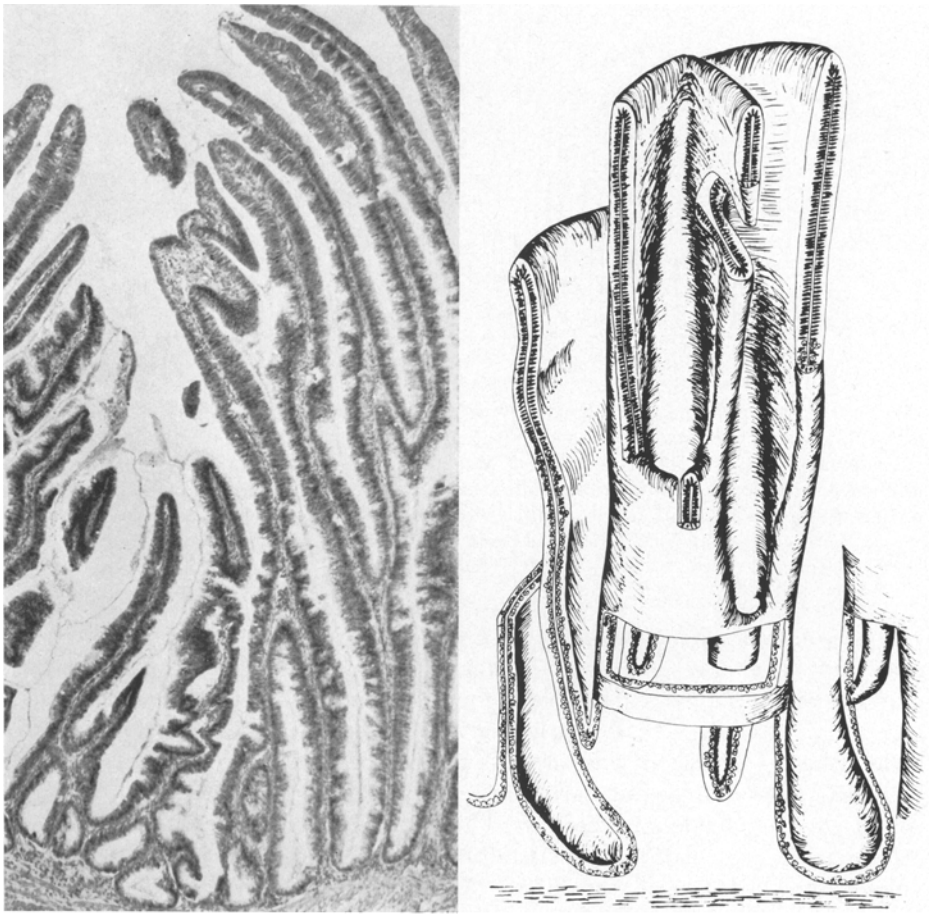


Fig. 9. Detail of an adenomatous polyp from familial polyposis. Near the top of the polyp the mucosa is completely composed of adenomatous glands (right). Spreading towards the periphery the adenomatous tissue superimposes the normal mucosa forming a second layer. Obstructive dilatation of some underlying normal crypts



a b

Fig. 10. a Microscopic appearance of a villous polyp. b Partial reconstruction shows leaf-shaped villi sometimes representing groove-like elongations of the dilated crypts. The reconstruction indicates that the histologic appearance is largely dependent on the direction of sections

by vertical growth of the mucosal crests limiting the crypt orifices. These foliaceous formations are of different sizes. Contrary to those of the small intestine, they often envelope individual or groups of crypts—entirely or partly—, so that they may appear as tubular or groove-like lengthenings of the crypts. It becomes comprehensible from the reconstruction that oblique or horizontal sections variate the histological picture.

The schematical representation on the morphogenesis of villous polyps (Fig. 11) makes clear that there are, in this case, the same basic phenomena as in the development of adenomatous polyps. Shifting of the main epithelial proliferation zone into the upper half of the mucosa effects—presupposedly again by simultaneous inductive stroma proliferation—the formation of villi. As in

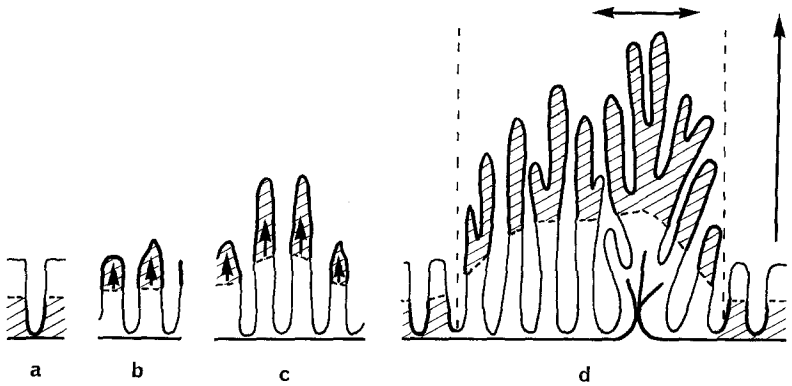


Fig. 11a—d. Morphogenesis of villous polyps. Transposition of the main proliferative activity (shaded) to the upper mucosa causes a prevailingly vertical growth, villous formation, and thus development of a sessile tumor (b, c, d left). Multiple branchings of the villi result in a polypous formation with slight additional horizontal expansion (d right). Secondary stalk formation by increasing vascularisation with splitting of the muscularis mucosae and drawing up of muscular strands. Arrows indicate growth direction

comparison with adenomatous polyps crypt or villus divisions are generally rarer, the result is a prevailingly vertical growth of villous polyps. The muscularis mucosae ist not involved so that planar tumours are a typical development here (Fig. 11d left). In contrast therewith, numerous villus divisions effect a horizontal expansion and necessarily entail—due to an increase in the epithelial mass—an augmentation of supplying vascular connective tissue, so that a papillary bulging results (Fig. 11d right). Villous polyps of this type may also undergo pediculation by peristaltic forces (Fig. 12). Most of the examined villous tumours revealed the mixed planar and polypous type of growth represented in Fig. 11d.

Discussion

In the present investigation on the formal pathogenesis of hyperplastic, adenomatous and villous polyps, the expectation that the decisive morphogenetic mechanisms are closely linked up with the epithelial proliferation behaviour was substantiated. The extension of the proliferation zone, found in hyperplastic polyps, just represents a quantitative modification of normal conditions, since the differentiating capacity of the epithelial cells is fully maintained. According to Kaye *et al.* (1973), cell differentiation already starts earlier and in deeper cryp sections. This is not necessarily in contrast with our findings of an enlarged proliferation zone. As the loss of cell divisibility only occurs upon complete differentiation, there may be a prolongation of cell maturation. The decrease of the mean mitotic index as compared with the standard is even unclear when assuming a constant mitosis period. It may, on the one hand, be based on the fact that the growth fraction becomes smaller by premature differentiation of cells; hyperplasia of the mucous membrane would then have to be referred to a lengthened lifetime of the cells. On the other hand, index reduction may be

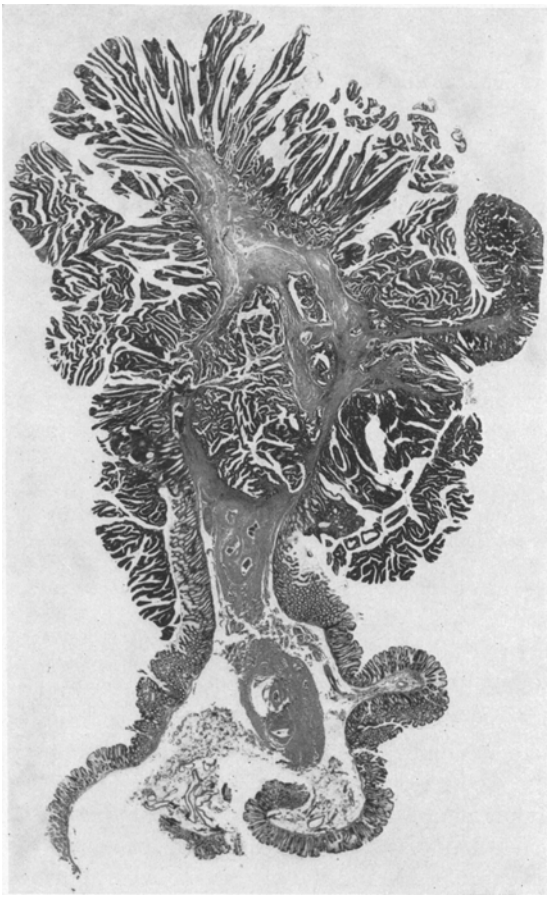


Fig. 12. Survey of a pedunculated villous polyp. Increased vascularisation of the pedicle which is covered with normal mucosa

based on a lengthening of the generation period. The latter is more probable since the mitotic index is even reduced in the main proliferation zone, in which there are only undifferentiated cells.

In neoplastic polyps, a prolongation of partial phases of the generation cycle has already been proved (Lipkin *et al.*, 1969; Lipkin, 1971), so that the reduction of the mitotic index can easily be explained thereby. However, whereas cell proliferation and differentiation correspond, in principle, to normal conditions in hyperplastic polyps, there is a fundamental difference in adenomatous and villous polyps. The loss of the differentiating capacity brings about an extension of the proliferation zone up to the surface of the mucosa. As, at the same time, the zone of most intense cell proliferation is shifted to the upper mucosal layers the proliferation behaviour is changed, not only quantitatively but also qualitatively.

This changed proliferation behaviour—first of all observed only as an autoradiographic phenomenon in hereditary polyposis (Cole and McKalen, 1963)—is,

as the present results show, a fundamental event in the development of adenomatous but also of villous polyps. There is also full conformity with our quantitative autoradiographical findings at various developmental stages of experimentally induced colonic polyps of the rat (Wiebecke and coworkers, 1969, 1970, 1973). As a primary change, there was a loss of epithelial differentiation and a shifting of the main proliferation activity to the upper layers of the mucosa, which also continued in fully developed adenomatous and villous polyps. On the whole, these proliferation-kinetic changes can thus be regarded as pathognomonic of adenomatous and villous polyps.

Shifting of the main proliferation zone in an upward direction is somewhat more marked in villous polyps than in adenomatous ones. It must be left open whether this statistically not significant difference is causal for the respective structural development of the polyps. The findings prove, however, that adenomatous and villous polyps are variants of a common basic pathogenetic process: The increase of epithelial proliferation close to the surface induces a secondary stroma proliferation, in adenomatous polyps, with the result of glandular division, in villous ones, with that of the formation of villi. Details of the epithelio-mesenchymal interactions are as yet unknown, the resemblance of the morphogenetic mechanisms makes, however, the frequency of adenovillous mixed and transitional forms comprehensible. This does not allow the conclusion, that villous polyps regularly develop from adenomatous precursors. Probably most polyps are villous, adenomatous, or of mixed type from their inception.

No indication can be derived from our findings to support the assumption that adenomatous or villous polyps may arise from hyperplastic precursors (Goldman *et al.*, 1970). In accordance with Lane *et al.* (1971) it must be stressed, that there are fundamental differences between hyperplastic and neoplastic polyps. In typical adenomatous and villous polyps, the different prevailing growth direction is expressed in a difference of the mean mucosal thickness which is highest in villous polyps. The distributions indicated in Fig. 1 correspond to a random collective of specimens. Since small, clinically symptomless adenomatous and villous polyps are not included the scattering widths are not fully representative. The compilation, however, gives a useful survey on the development of sizes.

It is observed quite frequently in neoplastic polyps that undifferentiated epithelium invades normal crypts from the surface. This procedure has, however, hardly any significance—in contrast with the opinion of Lane and Lev (1963)—for the spreading of the adenomatous foci. The present combined examination of structure and proliferation shows clearly that the development of polyps is first of all based on a glandular division and tissue expansion. As was observed in hereditary polyposis, neoplastic foci may occasionally spread more rapidly on the surface of the mucosa so that the normal mucosa is superimposed by an additional adenomatous layer. In contrast therewith, the undifferentiated epithelium invades the underlying normal crypts at a delayed rate only.

The expansive growth of the adenomatous polyps brings about welldefined borders. The rather planar, villous polyps, on the contrary, are often ill defined, thus explaining the higher rate of recurrences after removal.

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