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Morphological and Autoradiographical Investigations on Experimental Carcinogenesis and Polyp Development in the Intestinal Tract of Rats and Mice

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Summary. Most types of neoplastic lesions seen in human intestinal mucosa were reproduced experimentally in rats and mice after application of 1,2-dimethylhydrazine. Tumor distribution displayed dose-related variations. The relative tumor frequency in the small intestine rose after higher dosage of the carcinogen, with preponderance of the upper segments. Furthermore there were certain species specific differences between rats and mice concerning the tumor distribution within the large intestine.

Primary nonpolypous carcinomas with early infiltration developed as a consequence of rapid epithelial dedifferentiation and malignant transformation, predominantly in the small intestine. Benign and malignant polypous tumors were much more frequent in the large than in the small intestine.

Benign adenomatous and villous polyps of the colon develop after preceding mucosal hyperplasia due to partial loss of epithelial differentiation. As demonstrated by quantitative autoradiographical findings, the proliferation zone in these lesions is extended to the mucosal surface. Additionally, the zone of maximal epithelial proliferation is transposed to a mucosal layer near the surface. This qualitative change in the mode of epithelial proliferation compared with normal and hyperplastic mucosa is the essential factor in the development of benign neoplastic polyps.

In 1967 Druckrey and coworkers described the carcinogenic effect of 1,2-dimethylhydrazine in rat intestines. This was the first real chance for systematical investigations of intestinal tumor development in the experiment. Indeed, there had been several reports before about the experimental production of intestinal carcinomas (Laqueur, 1964, 1965; Spjut and Spratt, 1965; Schoental and Bensted, 1968). But in contrast to the carcinogens used by these authors 1,2-dimethylhydrazine is characterized by marked organotropism and certainty of tumor production. As we have published earlier (Wiebecke and coworkers, 1969), it is not only effective in rats but also in mice.

In the paper presented now we studied the changes of the intestinal mucosa of rats and mice during cancerisation with dimethylhydrazine. Besides morphological aspects of the carcinomas and precancerous lesions our interest concerned the basic disturbances of the epithelial proliferation. Special attention was paid to the morphogenesis of the colonic polyps and their significance with regard to the carcinogenetic process. Therefore quantitative autoradiographical investigations were carried out in the colonic lesions.

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Materials and Methods

Animals (Zentralinstitut für Versuchstierzucht, Hannover): 50 female Wistar rats, initial weight 150–200 g and 40 female NMRI mice, initial weight 25–30 g. The animals had standardized compressed food and free access to water.

The rats received 1.2-dimethylhydrazine (DMH) in a dosage of 14 and 21 mg/kg body weight and week (two experimental groups). Because the LD 50 is considerably lower in mice (about 42 mg DMH/kg body weight; Löhrs and coworkers, 1969) than in rats (215 mg/kg; Druckrey and coworkers, 1967), the mice were treated with a weekly dose of 14 mg DMH/kg body weight only. The injection solution was applicated subcutaneously and always freshly prepared: 0.35% solution of DMH (related to the base) adjusted to pH 6.5 with NaOH and stabilized with EDTA.

In order to get as many precancerous lesions as possible animals were killed successively beginning with the 3rd month of the experiment. Injections were stopped at least 14 days before sacrifice to avoid cytotoxic effects we had seen in previous experiments (Löhrs and coworkers, 1969).

40 minutes before sacrifice the animals received an intraperitoneal injection of tritiated thymidine (3 H-Tdr; NEN Chemicals, Boston, specific activity 2.0 C/mMol) in a dosage of 2 μC/g body weight.

Bowel specimens were routinely obtained from duodenum, jejunum, ileum, ascending and descending colon and additionally from every lesion, which was macroscopically visible.

After formol fixation, embedding and cutting of the bowel specimens the slides were covered with photoemulsion (Ilford K2) and exposed to the darkroom for 21 days. After photographical development the slides were stained with hemalum and eosin.

Quantitative autoradiographical analysis was carried out in 8 stalked adenomatous polyps without signs of malignant degeneration, furthermore in hyperplastic mucosa of 3 and in normal mucosa of 4 rats. For this purpose the mucosa in each specimen was divided into 5 horizontal zones of equal breadth and, for each zone the labelling index was determined separately. Only vertical sections were evaluated, which, in polyps were taken from the central portion axially cut through the stalk.

Results

Tumor Induction and Distribution

Already after 3 months the first tumors occurred in the large bowel of those animals, which had received the higher dosage of 21 mg DMH/kg weekly. After application of a total dose of 300 mg DMH/kg and an induction time of 200 days intestinal tumors could be found in every case. On the average, tumor induction time was longer in those animals, which had received the lower single dose of 14 mg DMH/kg.

Table 1. Frequency of tumor affection of the bowel segments in 3 experimental groups after application of 14 respectively 21 mg DMH/kg and week. N = total number of animals evaluated

	Duod.	Jejun.	Ileum	Col. asc.	Col. desc. + rect.	N
Mice						
14 mg/kg	0	1	0	8	18	19
Rats						
14 mg/kg	4	2	0	9	9	10
21 mg/kg	16	10	3	15	19	22

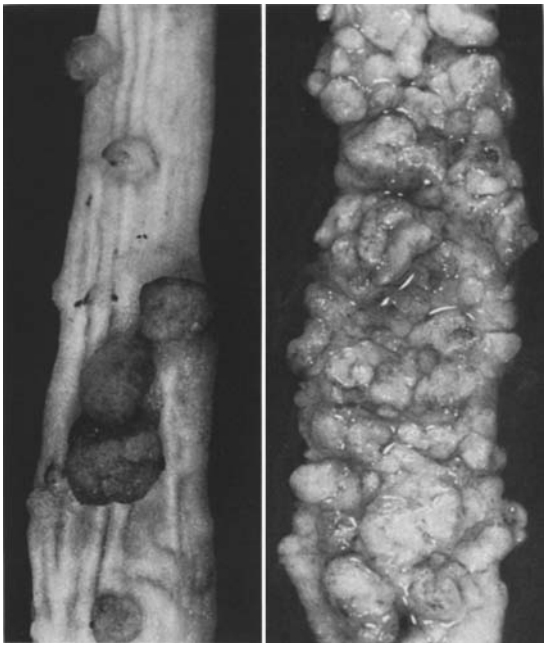


Fig. 1. a Rectosigmoid of a rat with isolated polyps of various size some of which are stalked. b Rectosigmoid of a mouse with a widely extended flat poypous tumor

Tumor distribution revealed species specific and dosage dependant differences. In rats the tumors of the large bowel were mostly isolated and often short-stalked, whereas mice tended to develop broad-based and widespread tumors (Fig. 1).

A comparison between rats and mice equally treated with 14 mg DMH/kg shows, that in mice the descending colon inclusive the rectum is much more often affected than the ascending colon whereas in rats the frequency of tumor development is nearly the same in these two bowel segments.

In animals treated with 14 mg DMH/kg tumors of the small bowel are rare compared with the colon, but their number rises considerably after treatment with 21 mg DMH/kg. However, tumor distribution in the small intestine revealed a strong preponderance of the duodenum, whereas only few tumors occurred in the ileum.

In advanced tumor stages we noted several causes of death: intussusception ileus due to polypous tumors, bleeding to death out of exulcerated carcinomas, peritoneal carcinosis, lymphogeneous and hematogeneous metastases especially of undifferentiated and mucus producing carcinomas.

In four cases we found squamous cell carcinomas of the anus combined with rectal carcinomas. Extraintestinal tumors occurred in eight cases as keratinized squamous cell carcinomas of the external auditory canal.

Histological and Autoradiographical Findings

Small Intestine. The earliest changes occurring during carcinogenesis are more or less localized mucosal hyperplasias with remarkable enlargement of villi and

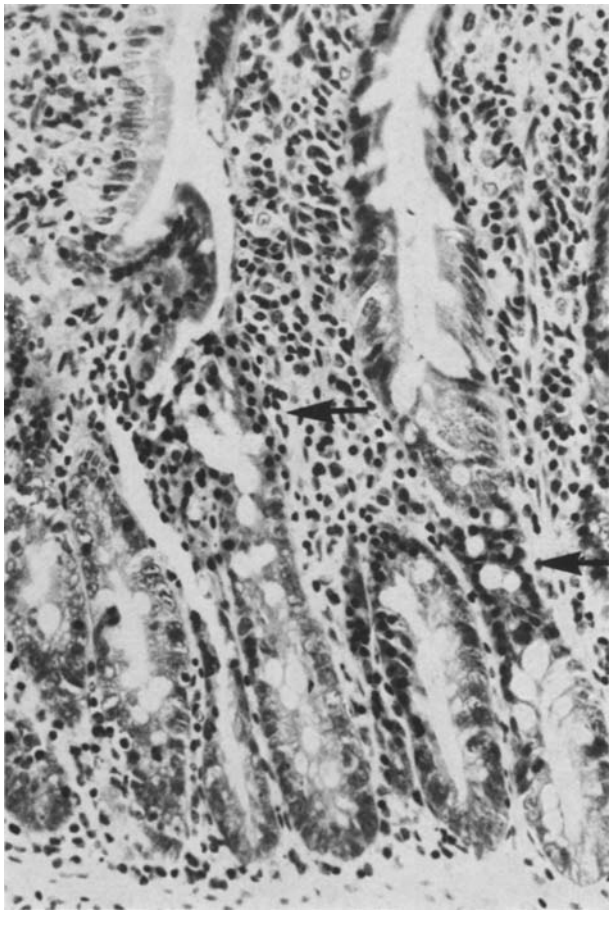


Fig. 2. Jejunum, rat: Undifferentiated cells especially in the neck of the crypts. Note atypical cells with small compact nuclei (arrows). Epithelial defect at the base of a villus (middle)

some irregularity of villus shape. An increased cell extrusion can be seen on the villus tips, in some cases with shedding of large epithelial sheets (Fig. 3). The increased cell turnover is maintained by an augmented cell supply out of elongated crypts, which contain numerous DNA-synthetizing cells.

In the course of the experiment we sometimes observed an abrupt loss of epithelial cell differentiation in circumscript areas of the small bowel. Especially in the neck of the crypts, the region of physiological cell differentiation, groups of cells were found, lacking alkaline phosphatase in the brush border. They also differed from normal epithelial cells by loss of the cylindrical shape, a strongly basophilic cytoplasm and small compact nuclei (Fig. 2). Sometimes they seemed to form syncytiums. Most of these cells do not move on the villi and parts of them obviously become necrotic.

Because of diminished cell supply the villi develop epithelial defects (Fig. 2). Neighbouring villi having epithelial defects on their opposite sides fuse with each

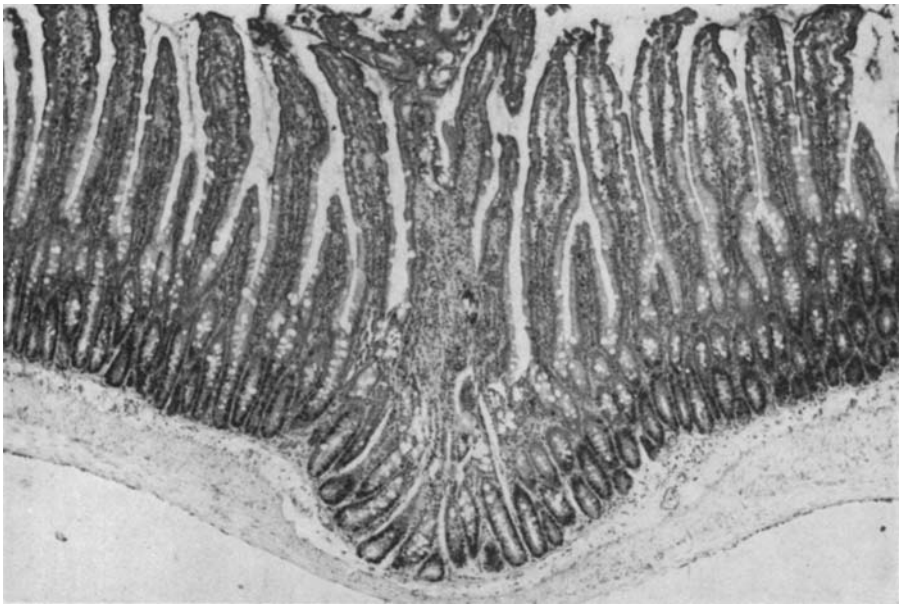


Fig. 3. Duodenum, rat: Fusion of several villi (middle) with inclusion of crypts. Autoradiogram, labelled cells black

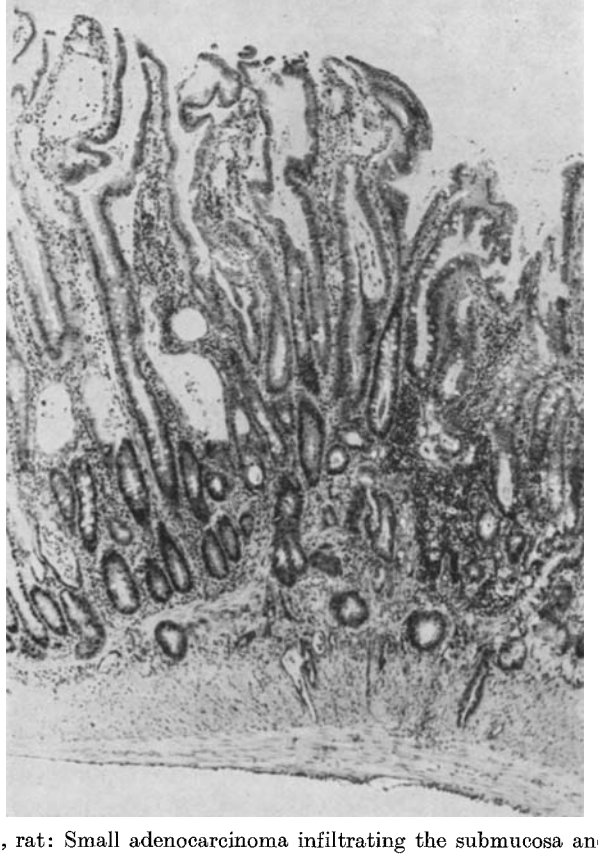


Fig. 4. Jejunum, rat: Small adenocarcinoma infiltrating the submucosa and the muscularis propria

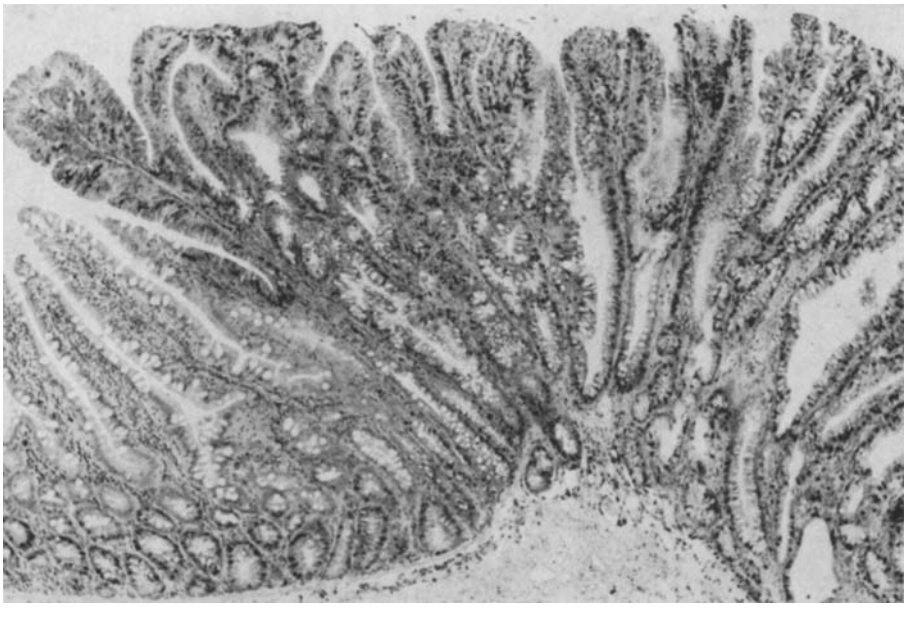


Fig. 5. Ileum, rat: Small villous polyp with dense ^{3}H-labelling near the surface. Differentiation of goblet cells in lower mucosal layers preserved. Autoradiogram

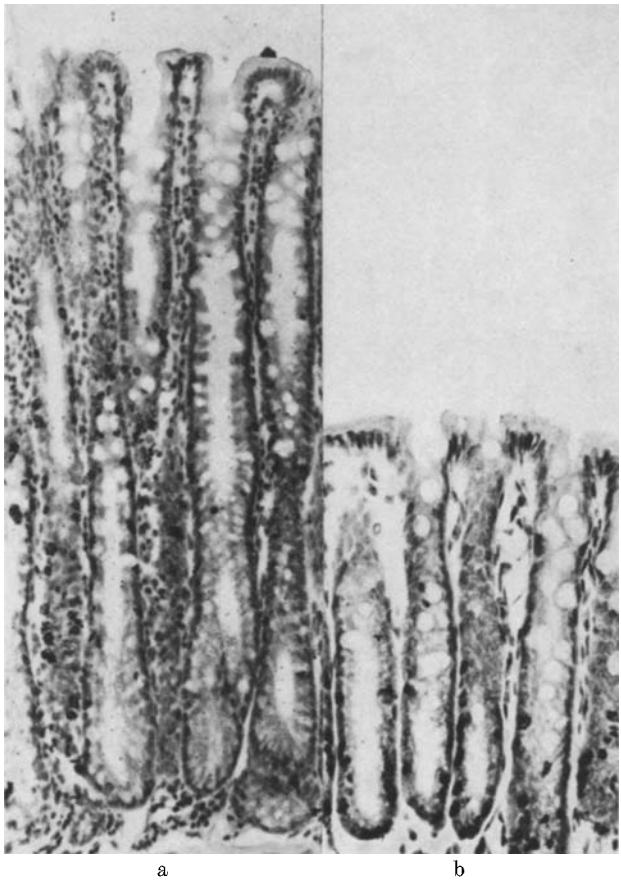
other so that the rests of the crypts are included within the stroma, while they continue to proliferate (Fig. 3). They often demonstrate their malignant potency by early infiltration into the submucosa and the muscularis propria (Fig. 4). Loss of the epithelial differentiation capacity in a larger area leads to ulceration.

The behaviour of the brush border alkaline phosphatase corresponds with the remarkably rapid transition of mucosal hyperplasia to carcinoma. Alkaline phosphatase activity was entirely preserved in the villus epithelium of hyperplastic mucosa but was negative in carcinoma.

Most of the carcinomas of the small intestine were more or less differentiated adenocarcinomas. Mucus producing carcinomas occurred in a smaller number but showed rapid infiltration. In early stages of this tumor type it could be observed, that malignant mucus producing cells in deeper layers of the mucosa perforate the basal membrane and infiltrate the stroma, whereas the enterocytes in the upper crypt regions behave normally. Thus it seemes, that malignant degeneration may attend only a certain cell line, whereas the other ones are not or not yet involved. However, we often found mixed carcinomas containing both adenomatous and mucus producing parts.

Focal differentiation of Paneth cells occurred within benign and malignant tumors of the small and large intestine. They never displayed clear 3 H-Tdr labelling and thus there were no convincing signs of their proliferative activity.

Though in the upper intestinal segments abrupt dedifferentiation and rapid malignant cell transformation were typical features of tumor development, also benign adenomas were found. They mostly occurred in distal segments of the small



a b

Fig. 6a and b. Autoradiogram of descending colon, rat: Mucosal hyperplasia with marked elongation of crypts. Almost normal relative size of the regeneration zone, visualized by labelled cells (a). As a comparison decending colon of a control animal (b)

bowel and displayed a villous structure. In these tumors a partial loss of epithelial cell differentiation has taken place so that proliferating but otherwise typical cylindrical cells can move on the villi. As demonstrated by the autoradiogram (Fig. 5) ^{3}H-labelling is most intensive near the surface of the polyp. The increased proliferation activity corresponds with an elongation and branching of the villi. Such villous polyps of the small intestine display the same proliferation behaviour as the neoplastic polyps of the large intestine.

Stalked adenomatous polyps, typical for the large intestine did not occur in the small bowel during our experiment.

Large Intestine. Similar as in the small intestine the earliest changes during experimental cancerisation of the colon and rectum were mucosal hyperplasias. They mainly were localized on mucosal folds. Compared with normal conditions hyperplastic mucosa shows marked elongation of the crypts (Fig. 6). The proliferation zone is broadened but in normal site as demonstrated by the distribution of

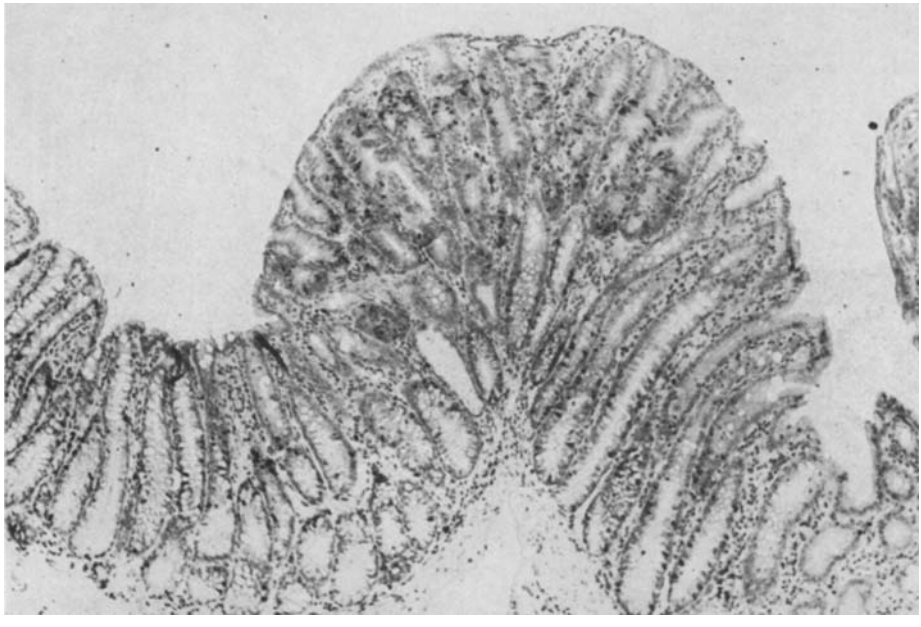


Fig. 7. Descending colon, rat: Small adenomatous area with crowding DNA-synthetizing cells (black) near the surface. Normal position of the labelled cells in the surrounding hyperplastic mucosa. Autoradiogram

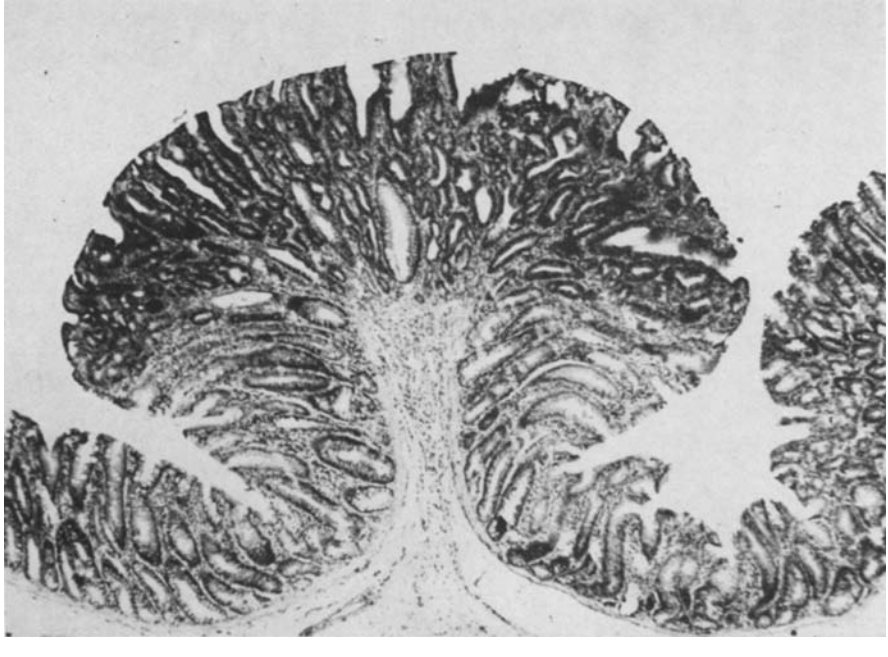


Fig. 8. Descending colon, rat: Adenomatous area on a mucosal fold with fan-like expansion to the sides

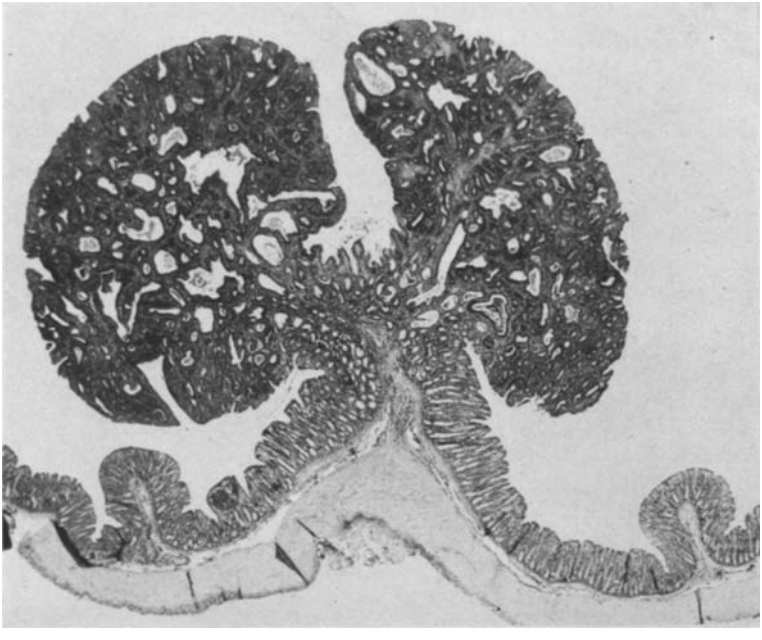


Fig. 9. Descending colon, rat: Lobulated fully developed adenomatous polyp. The lobules are separated by a small zone of normal mucosa

DNA-synthetizing cells in the autoradiogram. Cell differentiation towards the mucosal surface is maintained.

By careful exploration of numerous serial sections in such hyperplastic regions small wedge-shaped areas could be found, which were characterized by beginning tortuosity of the gland tubes, a basophilic cytoplasm of the monolayered epithelium and decrease of goblet cells (Fig. 7). These circumscript lesions lying within the mucosal level present striking autoradiographical findings. The majority of DNA-synthetizing cells is transposed to the upper mucosal layers, whereas in their normal site, the lower crypt region, their number is unchanged or even slightly decreased.

In advanced stages we find a fan-like expansion of these adenomatous areas to the sides until typical polyps are developed (Fig. 8). Adenomatous polyps as well as hyperplasias mainly arose on mucosal folds. By increasing vascularisation of the growing polyp the muscularis mucosae is splitted. Stalk formation was seen in larger polyps only and was estimated to be the result of peristaltic forces. Polyps with two or more centers of intensive cell proliferation were lobulated (Fig. 9).

Though in fully developed adenomatous polyps the most intense DNA-synthesis was in the upper mucosal layers, the superficial cells themselves were unlabelled (Fig. 10). In contrast to normal conditions, however, these cells reveal no signs of differentiation, rather those of atrophy and degeneration. Consequently superficial erosions often occurred.

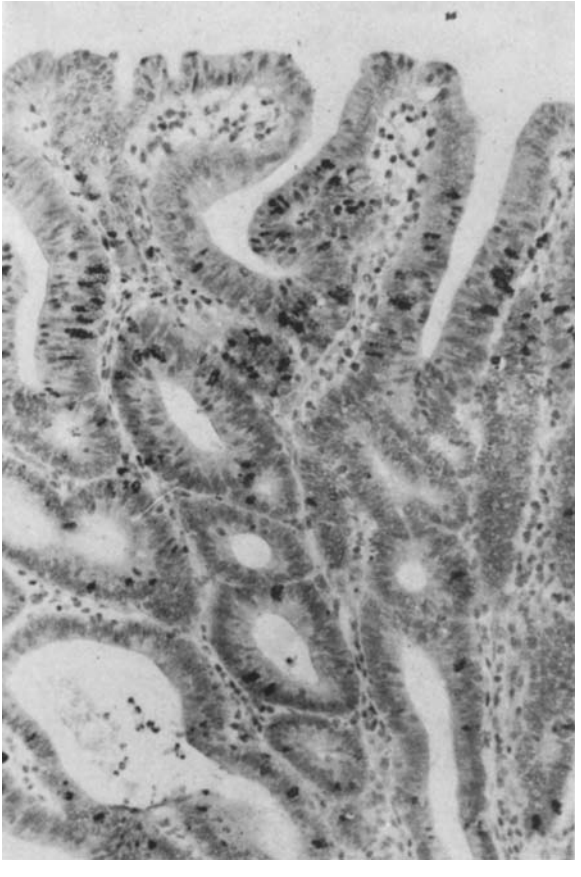


Fig. 10. Sector of an adenomatous polyp, autoradiogram: Crowding of labelled cells near the surface. No DNA-synthesis in superficial cells

The polyps frequently revealed mixed adenomatous and papillary structures, but only one pure benign villous polyp was seen in the rectum of a mouse and none in rats. Autoradiographical labelling of the villous mouse polyp was like those found in the small intestine (Fig. 5) with greatest labelling density near the villous tips. Quantitative autoradiographical evaluations could not be done in this polyp because the material was not sufficient.

Under the given experimental conditions benign polyps were by far the minor part of the tumors. Especially broad-based polyps often displayed symptoms of malignant degeneration and, probably many of these tumors were polypous carcinomas from the beginning. Besides such polypous tumors several times microcarcinomas could be found within the mucosa and therefore being undetectable macroscopically. Nevertheless, they often revealed signs of early infiltration (Fig. 11). As there is no visible precursory stage they are called "de novo carcinomas".

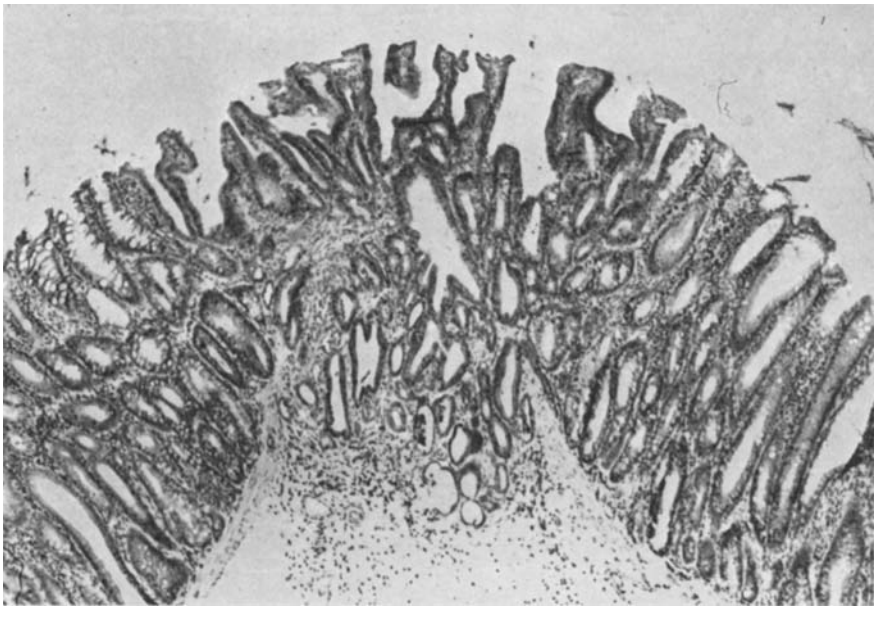


Fig. 11. Small adenocarcinoma within hyperplastic colonic mucosa infiltrating the submucosa so called "de novo carcinoma"

Large bowel carcinomas in their majority were more or less differentiated adenocarcinomas. Furthermore adenopapillary mixed types and anaplastic mucus producing carcinomas could be found rather frequently. Often adenomatous and mucus producing parts were combined in one tumor.

Differentiation of Paneth cells was found in non malignant polyps as well as in all histological types of carcinomas.

Quantitative Autoradiographical Results

Changes of the epithelial proliferation activity during polyp development were analized by zonal evaluation of the 3 H-labelling index in normal, hyperplastic and adenomatous mucosa (Fig. 12). In the mean the labelling index rises steadily from normal to hyperplastic and adenomatous mucosa reflecting an increasing cell proliferation within these lesions.

The regeneration zone of normal mucosa occupies the lower two thirds of the mucosal hight and, the maximum of ^{3}H-index is in the lower two zones.

In hyperplastic mucosa the regeneration zone is extended over four fifth of the mucosal hight and, the labelling index is generally increased. However, normal conditions are maintained inasmuch as the index maximum still remains in the lower two zones. The small shift of the index level between the first and second zone (Fig. 12) is not significant.

Whereas there are only quantitative differences of the ^{3}H-index between normal and hyperplastic mucosa an essential qualitative change takes place in adenomatous polyps. Here the proliferation zone is extended up to the surface of the

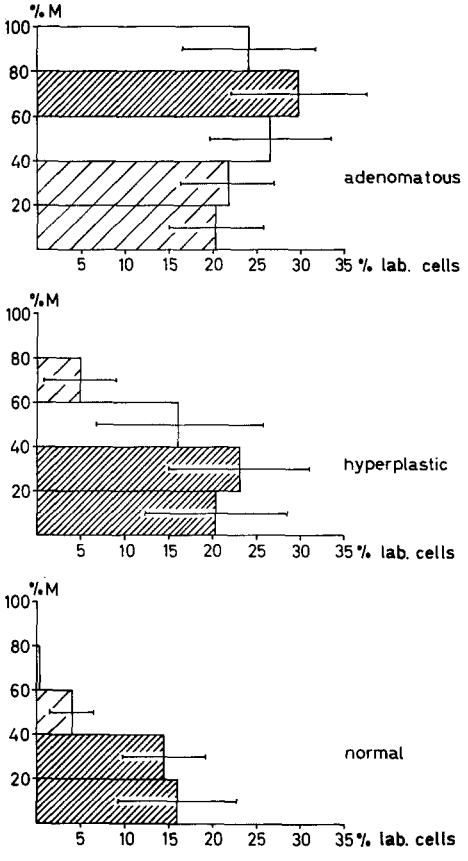


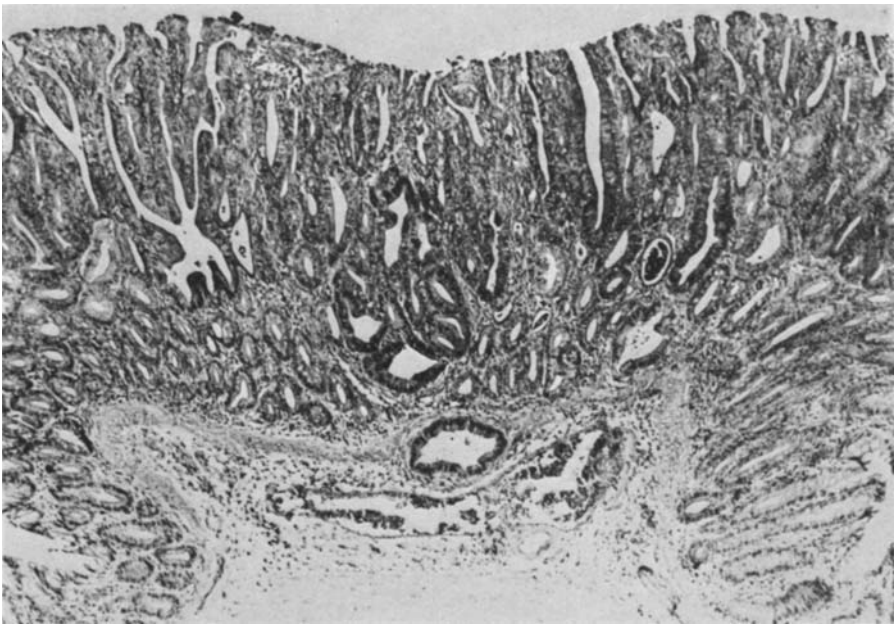
Fig. 12. ^{3}H-index in 5 equal zones (always 20% of the total mucosal hight) in normal, hyperplastic and adenomatous mucosa with standard deviation of the random samples ($p < 0.05$). Statistically significant differences between narrowly hatched (maxima) and widely hatched (minima) zones. *M* mucosal hight

mucosa and, additionally the index maximum is transposed to the fourth zone near the surface. However, the index minimum is in the lower two zones now, where in normal and hyperplastic mucosa we have the maximum. Thus in adenomatous polyps a conversion of normal conditions has taken place. The mean maximal zonal labelling index of adenomatous polyps reaches 30% and so it is nearly twice as high as in normal mucosa.

Adenomatous polyps with malignant degeneration and beginning infiltration display a proliferation behaviour different from that in benign polyps. As shown by Fig. 13, in addition to the maximum near the surface a second maximum of the labelling index has been established at the mucosal base. The basal labelling maximum is paralleled by carcinomatous infiltration into the submucosa.

Discussion

Most of the neoplastic tumors occurring in the intestinal mucosa of man could be reproduced experimentally in rats and mice by application of 1,2-dimethyl-



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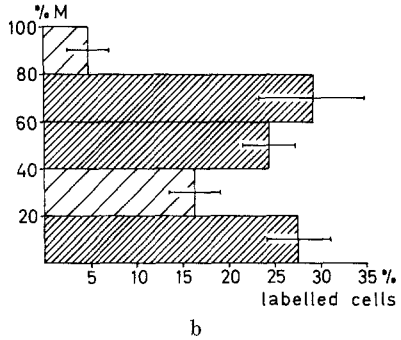

b

Fig. 13a and b. Broad based malignant degenerated polyp of rat colon with infiltration into the muscularis mucosae (a). Corresponding with this fact a second maximum of the ^{3}H-index in the basal mucosa (b). Statistically significant differences between narrowly hatched and widely hatched zones ($p < 0.01$). *M* mucosal hight

hydrazine. The tumor distribution, especially after low carcinogen dosage, also revealed similarities to human conditions as there was a strong preponderance of large bowel affection. Obviously the carcinogenetic threshold is higher in the small than in the large intestine. However, it is lower in the duodenum than in other segments of the small intestine and thus also parallels observations on the cancer distribution in the small intestine of man.

The different response of bowel segments on DMH is not fully understood. However, according to Druckrey and coworkers (1966, 1967) alkylhydrazines are

converted to biologically active agents only within the organism. Maybe that the cells of the diverse bowel segments have this functional capacity to a varying degree. But, as recently shown by Wittig and coworkers (1971), the bowel contents also play a role in tumor development.

DMH cancerisation brought forth tumors of different dignity. Benign polyps on the one hand and rapidly infiltrating non polypous carcinomas on the other hand may be regarded as the two ends of a scale of intermediate forms.

Non polypous microcarcinomas, predominantly but not exclusively found in the small intestine, are the result of rapid epithelial dedifferentiation and malignant transformation without development of macroscopically visible precursory stages. Also the alkaline phosphatase reaction did not fail earlier than in manifest neoplasma. This was also emphasized by Schauer and coworkers (1969) and Völlnagel and coworkers (1969), who additionally tested other cytoplasmic and mitochondrial enzymes.

Focal differentiation of Paneth cells, which we found in benign and anaplastic tumors of the small and large intestine was for the first time described in experimental tumors by Dunn and Kessel (1945). These cells are neoplastic without any doubt. However, the question is, whether they proliferate themselves or only are differentiated descendants of proliferating progenitors. Under normal conditions Paneth cells are considered to be irreversibly postmitotic cells (Hampton, 1968; Throughton and Trier, 1969). But also the Paneth cells found within our experimental tumors never displayed clear ^{3}H-labelling and thus there are no convincing signs of their proliferative activity.

Polyp development of the large intestine was studied for the first time with autoradiographical means by Cole and McKalen (1963) and Deschner and coworkers (1963) in patients with hereditary intestinal polyposis. The authors observed an increased ^{3}H-labelling in the upper mucosal layers of rectal biopsies. These previous findings are confirmed by our quantitative results in benign experimental polyps which provide better understanding of the morphogenetic mechanisms in polyp development. They can be outlined as follows: An initial hyperplastic stage is succeeded by a partial loss of the differentiation potency of the crypt cells, so that proliferating cells shift up to the mucosal surface. In addition, the maximum of epithelial proliferation is transposed to the upper mucosal layers. This generally is distinctive of benign neoplastic polyps as confirmed by our quantitative findings in human specimens (Wiebecke and coworkers, 1969).

The increased cell proliferation within the upper mucosal zones causes a mainly horizontal tissue expansion by branching and twisting of the gland tubules. The fan-like expansion of the adenomatous areas finally results in typical fungiform polyps.

On principle, villous adenomas have their origin in the same change of epithelial proliferation but, here we note a mainly vertical trend of growth with formation of villi. The factors inducing this different kind of growth in the two polyp types are unknown.

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