Experimental Intestinal Carcinogenesis and Polyp Development in Rats and Mice

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1,2-dimethylhydrazine (DMH), the carcinogen used in this experiment is characterized by marked organotropy and certainty of tumor production in the small and large intestine of rats (Druckrey et al., 1967). It is also active in mice (Wiebecke et al., 1969).

The present experiment was carried out with 150 adult female Wistar rats and 40 adult NMRI mice. DMH was injected subcutaneously in aqueous solution. One group of rats received 14 mg, and the other group 21 mg DMH/kg body weight/week. The mice were treated with only 14 mg DMH/kg body weight/week as the LD50 in mice is considerably lower than in rats. Animals were killed periodically from the 3rd month of the experiment onwards, in order to obtain as many precursory lesions as possible. Forty minutes before they were killed the animals received an intraperitoneal injection of tritiated thymidine at a dose of 2 µC/gram body weight.

After an induction period of 200 days and a total dose of about 300 mg DMH/kg tumors of the bowels were found in every case. On the average the tumor induction time was longer in those animals which had received the lower single dose.

Species-specific as well as dosage-dependent differences were observed in the localization of tumors. Rats often showed isolated short-stalked polyps within the large intestine, whereas mice more often tended to develop broad based and widespread tumors. In the large intestine of rats tumors were distributed uniformly, whereas in mice a preponderance in the descending colon and rectum was observed.

In rats treated with 14 mg DMH/kg tumors of the small intestine were rare compared with those of the colon. In the group treated with 21 mg DMH/kg their number rose considerably. Small bowel tumors mainly affected the duodenum, and only a few tumors occurred in the ileum.

The earliest changes in the small intestine during carcinogenesis were mucosal hyperplasias. Later there was sometimes a rather rapid transition of small areas into infiltrating carcinoma which began with a loss of epithelial differentiation in the neck of the crypts and cessation of cell migration to the villi. Soon atypical crypt cells began to infiltrate the lamina propria. In agreement with the findings of Schauer et al. (1969) alkaline phosphatase activity in the brush border did not decrease earlier than in microcarcinoma.

The majority of small bowel tumors were more or less differentiated adeno-carcinomas but mucus-producing and mixed carcinomas were also observed. Focal
differentiation of Paneth cells occurred within adenocarcinomas as well as in anaplastic carcinomas.

In a few cases benign villous adenomas developed in the ileum. They showed maximal labelling in the upper mucosal areas near the surface and thus displayed the same proliferative activity as adenomas of the large intestine. Stalked adenomatous polyps did not occur in the small intestine.

Mucosal hyperplasias were also the earliest changes in experimental cancerization of the colon and rectum. These lesions were mainly localized on mucosal folds and were characterized by marked elongation of the crypts with increased cell proliferation but well preserved differentiation towards the mucosal surface.

Careful investigation of serial sections revealed small adenomatous areas within the hyperplastic mucosa. Here the majority of DNA-synthesizing cells were found near the surface. The lesions showed a fan-like expansion to the sides until typical polyps developed. Increasing vascularisation of the growing polyp caused splitting of the muscularis mucosae and, in connection with a distension of the mucosal fold bearing the polyp, stalk formation. Polyps with more than one proliferative center became lobulated.

The change in epithelial proliferation during polyp development was investigated quantitatively by evaluation of the $^3$H-index in 5 mucosal zones between base and surface, in samples of normal and hyperplastic mucosa and in adenomatous polyps.

In normal mucosa the regeneration zone occupied about 60% of the mucosal thickness and the proliferative maximum was in the lower two zones. In hyperplasia the proliferation zone was extended to 80%, but normal conditions were maintained because the index maximum was still in the lower two zones and the superficial cells were differentiated. An essential quantitative change occurred in adenomatous polyps, where the proliferation zone was extended up to the mucosal surface and even the index maximum was transposed to a zone near the surface, i.e. at complete change from normal conditions had taken place. The results were statistically significant and confirmed our quantitative findings in human specimens (WIEBECKE, 1970; WIEBECKE et al., 1969 a and b, 1970, 1973) as well as observations in hereditary polyposis of COLE and McKALEN (1963) and DESCHNER et al. (1963).

The morphogenetic mechanisms of polyp development can be outlined as follows: An initial hyperplastic stage is succeeded by a partial loss of epithelial differentiation, so that proliferating cells shift up to the mucosal surface, where maximal epithelial proliferation is established. The result is a marked increase in tissue growth at this site which, by branching and twisting of gland tubes, causes a mainly horizontal expansion (WIEBECKE, 1970; WIEBECKE et al., 1969 a and b, 1970, 1973) until typical fungiform polyps are developed.

In villous polyps, which because of their small number were not evaluated quantitatively, the index maximum was also transposed to the upper mucosa. Here we noted a vertical trend of growth but the factors inducing this different kind of growth are unknown.

The majority of tumors produced experimentally were broad based polyps with signs of malignant degeneration and polypous carcinomas. However, microcarcinomas without polypous precursors were found on several occasions as a result of rapid malignant transformation — the so-called “de novo-carcinomas”.