Evidence for an Adenoma–Carcinoma Sequence in Dimethylhydrazine-Induced Neoplasms of Rat Intestinal Epithelium

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Carcinogen-induced primary intestinal adenocarcinomas serve as a useful animal model for human colonic adenocarcinomas. Although striking similarities between this model and the human disease state exist, there are also troublesome discrepancies—a major one being the reported lack of an adenoma–carcinoma sequence in the experimental model. However, the original morphologic descriptions of these experimental neoplasms predated the development of presently accepted morphologic criteria that have been used to describe the adenoma–carcinoma sequence in humans. Therefore, the authors reevaluated the structural evolution of dimethylhydrazine-induced rat intestinal neoplasms, using the same criteria that were recently applied to evaluate human colonic adenocarcinomas. Such an approach shows that many dimethylhydrazine-induced intestinal adenocarcinomas have peripheral foci of adenomatous epithelium associated with them. In addition, the frequency of this association correlates inversely ($P < .001$) with the depth of invasion. These findings are comparable to those which, in humans, have been used as evidence supporting the adenoma–carcinoma sequence. Thus, when assessed with equivalent criteria, dimethylhydrazine-induced intestinal adenocarcinomas appear to be similar, not dissimilar, to human colonic adenocarcinomas in their structural evolution. These data suggest that, at least in part, dimethylhydrazine-induced intestinal adenocarcinomas arise in foci of preexisting adenomatous epithelium. (Am J Pathol 1983, 110:230–235)

A VARIETY OF EVIDENCE[1–5] suggests that most, if not all, adenocarcinomas of the human colon originate from foci of preexisting benign neoplastic (ie, adenomatous or villous) epithelium. Although, based on several studies including those of familial polyposis, an association between the presence of colonic adenomatous polyps and the development of colonic adenocarcinomas has long been recognized, it was only within the last decade that the concept of an adenoma–carcinoma sequence was popularized[1,2] and widely accepted.[6–8] Since intestinal adenocarcinoma is a major cause of death from cancer in this country, animal models that provide an opportunity to study the biology of this disease have been widely used.[9–11] Although these models generate primary adenocarcinomas of the large and small bowel that have many similarities to human colonic adenocarcinomas, it has been suggested that a major deficiency of these models is their failure to duplicate the adenoma–carcinoma sequence.[11–13] However, the major histologic descriptions of tumors originating in these model systems were published prior to the classic description of the structural evolution of the adenoma–carcinoma sequence in humans by Muto, Bussey, and Morson.[2] The object of the present study was to analyze the morphologic evolution of dimethlhydrazine-induced rat intestinal adenocarcinomas with the criteria of Muto, Bussey, and Morson.

Methods

Six- to 8-week-old Wistar-Furth rats were obtained from A. R. Schmidt, Inc., Madison, Wisconsin. The animals were fed a standard pellet diet and

Supported in part by Research Grant AM27972 from the National Institutes of Health, Bethesda, Maryland, and the Brigham Surgical Group, Inc., and National Cancer Institute Grant CA22513-03.

Accepted for publication September 14, 1982.

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0002-9440/83/0207-0230$01.10

water *ad libitum*. Lard and carbohydrate supplements were used to increase the induction of intestinal neoplasms.[14] Animals were given subcutaneous injections of 20 mg/kg dimethylhydrazine (DMH) weekly for 16 weeks, beginning at 5 weeks of age. Animals were then sacrificed at various times, ranging from 8 to 36 weeks after completion of the course of DMH, and their intestinal tracts were inspected for the presence of grossly detectable neoplasms. Only animals in which grossly visible intestinal neoplasms developed were included in the study. Twenty-five percent of the animals developed no grossly detectable neoplasms.

All neoplasms were removed with adjacent 2-cm cuffs of normal intestine and fixed in 10% buffered formalin. Oriented representative tissue blocks were subsequently dehydrated and embedded in paraffin, and multiple 5-μ sections were prepared and stained with hematoxylin and eosin (H&E). We then examined all the neoplasms microscopically to determine the presence or absence of benign (adenomatous) or malignant (invasive adenocarcinoma) neoplastic epithelium. This decision was based on the same criteria used by Muto, Bussey, and Morson[2] in their evaluation of human colonic neoplasms. Foci that demonstrated tubular or papillary structures lined by dysplastic epithelium but did not show invasion through the muscularis mucosa and did not elicit a desmoplastic stromal response were considered adenomatous. Tortuous irregular glands lined by dysplastic epithelium or sheets of single dysplastic mucin-producing epithelial cells which were invading through the muscularis mucosa and eliciting a desmoplastic response were considered to represent foci of adenocarcinoma. In addition, the depth of invasion by the malignant component of the neoplasm was noted and placed at one of the following three arbitrarily defined levels: 1) minimal (limited superficial submucosal invasion); 2) intermediate (adenocarcinoma confluently filling the submucosa or invading of but not through the muscularis propria); and 3) extensive (invasion through the muscularis propria). The incidence of finding adenomatous epithelium adjacent to invasive adenocarcinoma was then separately assessed for neoplasms of each level of invasion. The frequencies of finding adenomatous epithelium adjacent to adenocarcinomas with minimal versus maximal depths of invasion were statistically compared with the chi-square test. Correlations were also sought between the level of invasion, the location of the neoplasm, and the time interval post DMH therapy.

Sections of "normal" mucosa both from animals in which neoplasms developed and from untreated control animals were also examined.

Table 1—Summary of Data

Weeks after DMH	Animals	Tumors	Adenoma only	Adenoma + carcinoma	Carcinoma only
8-12	9	9	–	1	8
13-24	40	43	3	6	34
25-36	9	9	–	3	6

Results

The number of intestinal neoplasms obtained at different time intervals after completion of DMH were as follows: 8–12 weeks—9 neoplasms from 9 animals; 13–24 weeks—43 neoplasms from 40 animals; 25–36 weeks—9 neoplasms from 9 animals (Table 1). Twelve neoplasms were from the small intestine, 23 from the proximal colon, 4 from the colonic flexure, 16 from the descending colon, and 6 from the rectum. Neoplasms exhibited widely varying gross morphologic features measuring 0.3–1.5 cm in diameter and ranging from polypoid masses to flat, plaquelike growths with heaped-up margins. Neither the gross morphologic features nor the location of the neoplasms correlated with the time interval after DMH treatment. Twelve neoplasms appeared polypoid: 5 pedunculated and 7 sessile. While all sessile lesions contained invasive carcinoma, only 2 of the 5 pedunculated lesions demonstrated the presence of invasive carcinoma.

Histologically, all lesions were composed of neoplastic (adenomatous) epithelium. Three polypoid neoplasms arising 13–24 weeks after DMH treatment contained only adenomatous epithelium and lacked foci of adenocarcinoma. Although the grades of epithelial atypia in these three lesions ranged from mild to carcinoma *in situ*,[2] in no instance did glands breach the muscularis mucosa or elicit a desmoplastic stromal response (Figure 1). These three lesions did not contain any foci of irregular glands eliciting a desmoplastic stromal response above the muscularis mucosa; if we had observed such an area, we would have considered this an instance of intramucosal adenocarcinoma with invasion of the lamina propria. Invasive adenocarcinoma was found in the remaining 58 neoplasms. However, 10 of these 58 lesions also contained foci of benign adenomatous epithelium. In 6 the adenomatous epithelium was in a polypoid form and was associated with a small focus of adenocarcinoma that breached the muscularis mucosa, superficially invaded the submucosa, and elicited a desmoplastic stromal response (Figures 2 and 3). Thus, these neoplasms gave the appearance of adeno-

matous polyps with foci of invasive adenocarcinoma.[2] The remaining 4 neoplasms, which contained both adenomatous and adenocarcinomatous elements, demonstrated more substantial degrees of invasion (Figure 4). In these lesions peripheral foci of adenomatous epithelium, which were above the muscularis mucosa and were not eliciting a desmoplastic stromal response, could be readily identified (Figure 4).

The frequency of demonstrating benign adenomatous epithelium in association with invasive adenocarcinoma significantly correlated with the depth of invasion ($\chi^2 = 13.75$, $P < 0.001$) (Table 2). Thus, benign adenomatous epithelium was found associated with 60% of adenocarcinomas showing minimal, 21% of adenocarcinomas showing intermediate,

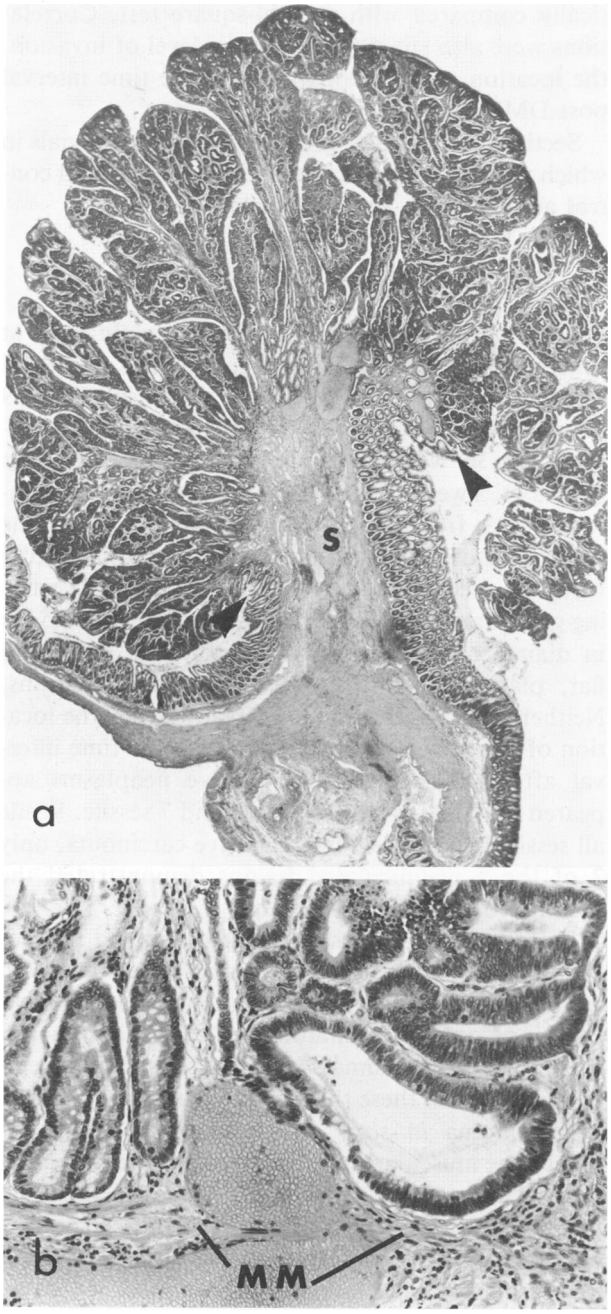

Figure 1—A benign epithelial neoplasm of rat colonic mucosa. **a**—The adenomatous epithelium creates a polypoid area of mucosal thickening that is attached to the colonic wall by a stalk(s) peripherally coated with normal colonic mucosa. This lesion is similar to adenomatous polyps seen in human colons. The *arrowheads* indicate the zone of transition from normal to adenomatous epithelium. (×15) **b**—Adenomatous epithelium (*right*) is identified as such by the cytologic appearance of its constituent cells, which show nuclear enlargement and stratification, in contrast to the epithelial cells lining the normal colonic glands (*left*). In this instance, the degree of atypia in the adenomatous epithelium is moderate. The adenomatous epithelium is confined above the delicate muscle fibers of the muscularis mucosa (*MM*) and does not elicit a desmoplastic stromal response. (×150)

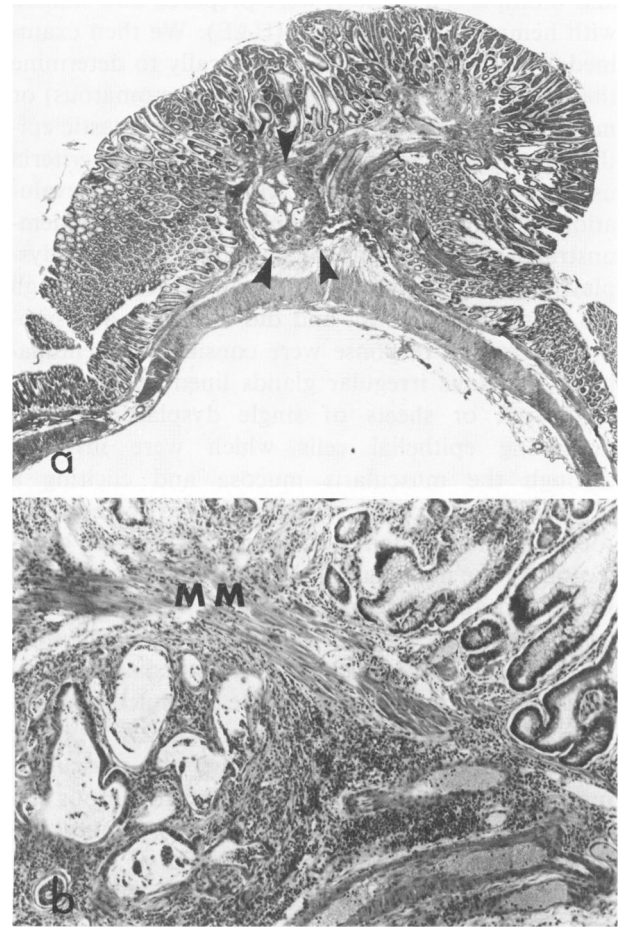

Figure 2—A predominately adenomatous neoplasm of rat colonic mucosa with associated invasive adenocarcinoma. **a**—Although the contour of this lesion is very similar to that shown in Figure 1a a pocket of glands can be seen within the stalk (*arrowheads*). (×14) **b**—Adenomatous epithelium showing a mild degree of atypia can be seen above the muscularis mucosa (*MM*). However, the pocket of glands within the stock consists of irregular tortuous adenocarcinoma glands, which are located below the muscularis mucosa and which are eliciting a desmoplastic stromal response. (×120)

and 3% of adenocarcinomas showing extensive levels of invasion (Table 2). We did not find a significant correlation between the depth of invasion and the time interval after DMH treatment. However, this may reflect the relatively narrow time frame we analyzed and the limited number of animals in our early and late groups. In addition, the level of invasion or the presence of adjacent adenomatous epithelium did not appear to correlate with the region of the intestine in which the neoplasm was located or with the size of the neoplasm.

The intervening "normal" mucosa in DMH-treated animals was similar to that of control animals.

Discussion

Our purpose was to analyze the morphologic evolution of DMH-induced intestinal adenocarcinomas

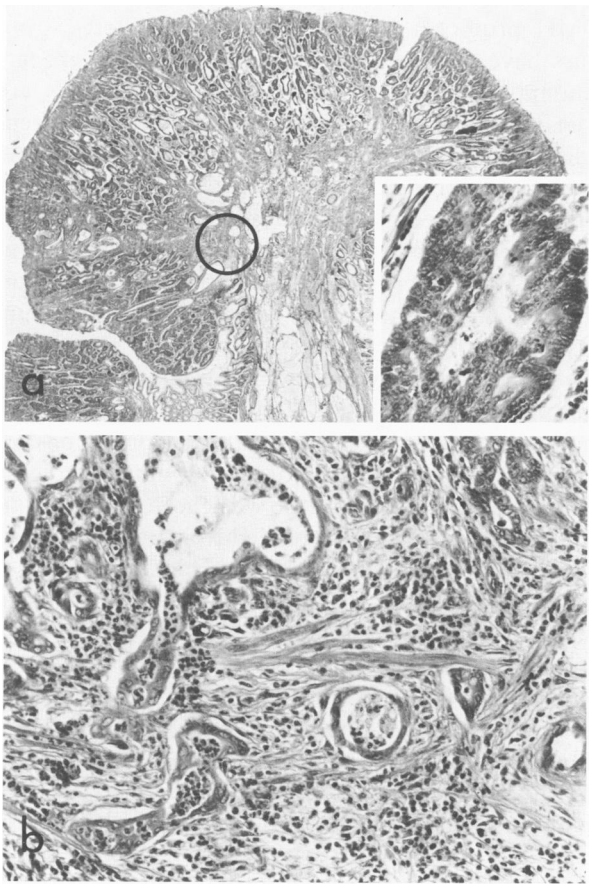

Figure 3—A predominately adenomatous neoplasm of rat colonic mucosa. **a**—Both carcinoma *in situ* (**inset**) and very early carcinoma invading into the muscularis mucosa (*circle*) are present within this polyp. (×15; **inset**, ×190) **b**—Higher magnification of the focus of the invasive carcinoma shown above. Focally individual glands are infiltrating the muscularis mucosa and eliciting a desmoplastic response. (×110)

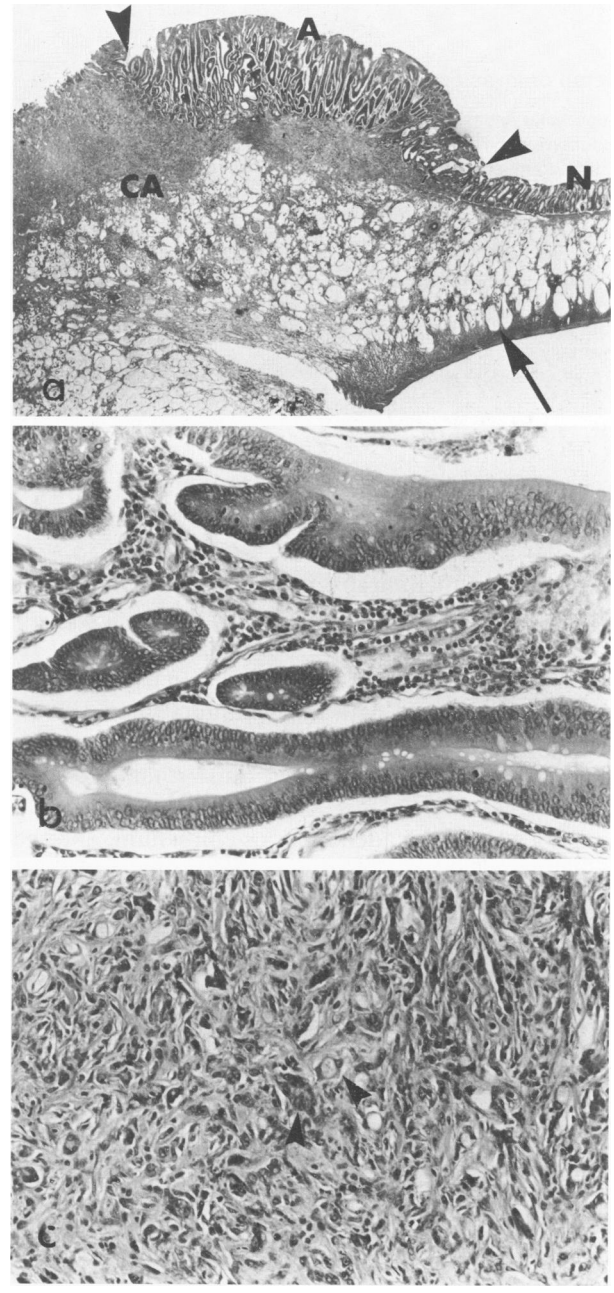

Figure 4—An invasive adenocarcinoma in the rat colon that has a remnant of peripheral adenomatous epithelium. **a**—The normal colonic mucosa (*N*) can be seen to the *right*. The submucosa is replaced by infiltrating adenocarcinoma (*CA*) with focal extracellular mucin production, and the tumor invades the inner circular layer of the muscularis propria (*arrow*). However, the mucosa adjacent to where adenocarcinoma disrupts the mucosal surface appears to be adenomatous (*A*) (between *arrowheads*). (×12) **b**—In contrast to the invasive adenocarcinoma shown below, the adenomatous element of this lesion does not cross the muscularis mucosa, and the individual glands do not elicit a desmoplastic stromal response. (×180) **c**—The invasive portion of the lesion consists of small, irregular neoplastic glands (*arrowheads*) and single cells, which are eliciting a striking desmoplastic stromal response. (×160)

Table 2—Relationship between Depth of Invasion and Frequency of Associated Adenomatous Epithelium

Depth of invasion	Number of cases	Number associated with adenomatous epithelium	%
Superficial submucosal	10	6	60*
Extensive submucosal or limited muscularis propria	14	3	21
Through muscularis propria	34	1	9*

DMH = induced adenocarcinomas in the rat intestine are sometimes associated with adenomatous epithelium. Comparable to data derived from man, the frequency of this association correlates inversely with the depth of invasion.

* $\chi^2 = 13.75$; $P < .001$.

in rats and compare the histologic evolution of these tumors with that of human colonic adenocarcinomas. To accomplish this goal, we distinguished adenomatous epithelium from adenocarcinoma, using the same criteria that have recently been applied to human colonic neoplasms.[2] Thus, our aim is not to argue the validity of these criteria but to use these criteria to accumulate data in this animal model comparable to published data derived from human beings. Using this approach, we show that intestinal adenocarcinomas in this animal model demonstrate an association with adenomatous epithelium, and the frequency of this association correlates inversely and significantly with the depth of invasion. These findings are strikingly similar to those found in human colonic epithelial neoplasms.[2] In human beings this data is widely regarded as a major pillar of evidence in support of the concept of an adenoma–carcinoma sequence.[6-8]

A variety of other data also support the adenoma–carcinoma concept for human colonic adenocarcinomas. Not only do patients with familial adenomatous polyposis syndromes uniformly develop colonic adenocarcinoma if untreated,[5,6] but the risk of colonic adenocarcinoma correlates with the number of colonic adenomas even in patients with only one, two, or three such lesions.[3] Furthermore, there is some evidence that prophylactic removal of adenomatous polyps reduces the rate of colonic adenocarcinoma.[4] Moreover, the frequency of finding coincident foci of adenocarcinoma in an adenomatous polyp directly correlates with the size of the adenoma, suggesting the possibility that the risk of carcinomatous degeneration of benign neoplastic colonic epithelium may increase with time.[2] Finally, the presence of adenomatous (dysplastic) epithelium, which may or may not be in a polypoid form, is thought to be a useful predictor of those patients at highest risk for colonic adenocarcinoma as a complication of chronic ulcerative colitis.[15-19]

In other rat species DMH alters the morphologic appearance of grossly normal intestinal mucosa. For example, in both Fischer[12] and BD[13] strains, alterations, which include pleomorphism of crypt cell nuclei, prominent nucleoli, and cytoplasmic basophilia, are noted focally throughout the mucosa. However, while DMH often produces multiple intestinal tumors in these strains, the yield in the Wistar–Furth strain is comparably very low (in Wistar–Furth animals that develop intestinal neoplasms there is usually only one neoplasm per animal). Thus, it is not surprising that the intervening grossly normal mucosa in this species does not contain multiple microscopic foci of early neoplasia.

Others who have studied the morphologic evolution of DMH-induced intestinal neoplasms using DMH protocols and rat strains that differed from ours have not found evidence for an adenoma–carcinoma sequence, although isolated adenomas are well described.[12,13,20,21] However, we feel this discrepancy is not due to protocol factors, but rather to the use of morphologic criteria that were different from those we utilized and thus different from those used to describe the adenoma–carcinoma sequence in man.[2] Photomicrographs published by other authors, which demonstrate adenomatous epithelium (using the criteria of Muto, Bussey, and Morson) at the periphery of invasive adenocarcinomas, support this view (Figures 11 and 12).[2] Thus, we feel our data is not in gross disagreement with previous published morphologic descriptions of DMH-induced colonic neoplasms in the rat. Rather, we believe that previous studies, in large measure, failed to interpret findings similar to ours as evidence for an adenoma–carcinoma sequence, since they used criteria different from ours and thus different from those applied to human colonic neoplasms. Indeed, a preliminary report of the early colonic lesions produced by the carcinogen azoxymethane in Fischer rats suggests that foci of epithelial dysplasia predate carcinomas in this model as well.[22] Therefore, it appears that the morphologic evolution of DMH-induced intestinal epithelial neoplasms in rats mimicks that of human colonic epithelial neoplasms with regard to the adenoma-carcinoma sequence, although these tumors may vary from human tumors in other biologically important ways. We propose that, at least in part, DMH-induced in-

testinal adenocarcinomas arise from preexisting foci of adenomatous epithelium.

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Acknowledgment

The authors are grateful to Drs. Harvey Goldman and Helmut Rennke for their critical review of the manuscript. We would also like to acknowledge the highly skilled technical assistance of Susan Carlson.