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# LIGHT-AND ELECTRON-MICROSCOPIC ANALYSIS OF COLORECTAL CARCINOGENESIS INDUCED IN THE RAT BY 1, 2-DIMETHYLHYDRAZINE

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## INTRODUCTION

There is a great difference in the incidence of carcinoma of the colon in various countries.[1)2)] In fact, carcinoma of the colon has a low incidence in Japan, in other parts of Asia, in Africa and in South America, while in the United States and in Western Europe, it has a high incidence.

Japanese immigrants in the United States and American Blacks have a higher incidence of caricnoma of the colon than peoples of their original countries. These epidemiological studies indicate that carcinoma of the colon is influenced by environmental factors more than genetic factors.

Reddy, et al., [3,4)] Weisburger, et al.[5)] and Berg, et al.[6)] proposed that high intake of dietary fat as an environmental factor plays an important role in carcinogenesis in the colon. Since colonic carcinogenesis of cycacin was first investigated by Laqueur, et al.[7)] chemical induction of carcinoma of the colon in rats have been described by several authors.[4,5,8,9,10)]

Druckrey, et al.[8)] in 1967, reported that subcutaneous application of DMH, 1,2 -dimethylhydrazine, gave rise to a high incidence of tumors mainly in the large intestine of rats. In rats, DMH yielded a few tumors in the duodenum and ileum, but the great majority of tumors were produced in the colorectal portions and in the cecum. The tumor types included highly differentiated adenomas and actively proliferating carcinomas of signet ring cell type. Furthermore, they emphasized that DMH-induced carcinoma in rats was a model that might well agree with human colorectal carcinoma.

While Druckrey's experiment on DMH carcinogenesis in the colon has been reconfir-

med by many investigators mainly on a histologic basis, few systemic studies based upon the fine structure of the neoplasms thus induced have been published.

The purpose of this investigation is to analyse the effect of a high fat diet on DMH -induced carcinoma in rats and to examine, histologically for all and ultrastructurally for respresentatives, a series of these DMH-induced neoplasms as models of colorectal carcinoma of man.

## Materials and Methods

Forty-four Wistar strain male rats, 28 weeks of age, were separated into 2 groups; Group A was kept on an ordinary fat (5.1%) diet and Group B on a high-fat diet containing 20% cornoil produced by the Nihon Clea Inc.

The animals were given subcutaneous injections of DMH 20 mg/kg body weight weekly for 13 weeks. All rats were autopsied at 4 to 32 weeks after the first injection. The entire intestine was cross-cut into serial sections and, totally subjected to light microscopic analysis with several special stains including PAS, mucicarmine and alcian blue. Small pieces of tissue removed representatively for electron-microscopy were immediately immersed in 2.5% glutaraldehyde solution for 2 hours and post-fixed for the next 2 hours in S-collidine buffered 1% osmium tetroxide. The specimens were dehydrated and embedded in epoxy resion (Epon 812). Blocks were sectioned on a Porter -Blum ultramicrotome, and the sections were stained with toluidine blue and observed for orientation by light-microscopy. Ultrathin sections were doubly stained with uranyl acetate and Sato's lead solution, and observed under a JEM 100S electron microscopy.

## Observation

The first carcinoma was observed the 8th week after the initiation of the experiment and by the 22nd week and afterwards, all rats produced carcinomas in the colorectal portions (Fig. 1), though no specific changes were observed before 5 weeks. Twenty-four rats after 22 weeks or more, 13 in Group A and 11 in Group B, developed 129 tumors in the colorectal portions.

The incidence of tumors was significantly high in Group B with the tumor index (the number of tumor per animal) calculated at 22 weeks and thereafter showing 6.18, compared to 4.69 in Group A (Table 1).

Concerning histologic types, there was a definite tendency for Group B to produce mucinous and signet ring cell carcinomas with the index showing 2.55 in comparison with 0.69 in Group A, their incidence rate being 41.2% and 14.8%, respectively. On the other hand, the incidence of well differentiated adenocarcinomas was higher in Group A (77.0%) than in Group B (45.6%), the tumor index being 3.61 in Group A and 2.82 in Group B (Table 2).

Concerning the depth of neoplastic invasion, early carcinoma consisting of m (intramucosal) and sm (submucosal) lesions accounted for 63.9% of the tumors in Group A, but

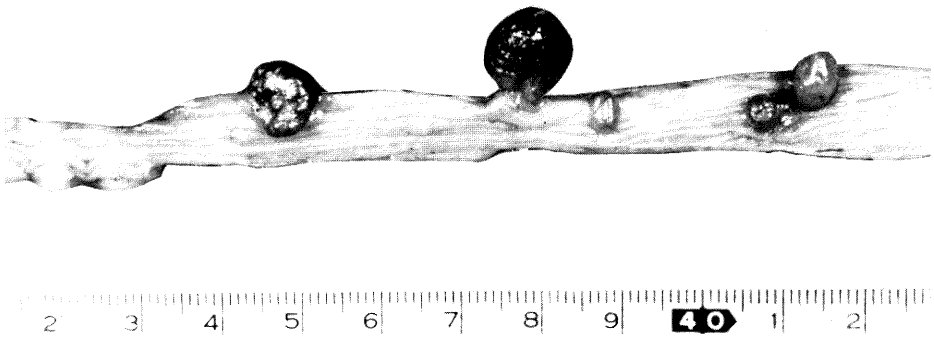


Fig. 1. Neoplastic lesions in Wister strain male rat induced with 1, 2-dimethylhydrazine.

Table 1. Tumor Index at 22 Week and Thereafter

| | Number of Rats | Number of Tumors | Tumor Index* |
|---|---|---|---|
| Group A | 13 | 61 | 4.69 |
| Group B | 11 | 68 | 6.18 |
| Total or Average | 24 | 129 | 5.38 |

* The number of tumor per animal

only 48.5% of those in Group B (Table 3).

Gross appearance of the early carcinoma indicated that Is and Ips types were dominant since Ip type and IIa+IIc type were decreased (Table 4). In advanced lesions, tumorous types were most frequently found but ulcerated types were found less frequently (Table 5).

For the investigation of tumor location, the large intestine was divided into four portions; cecum, and proximal, middle and distal one third of the colon. Table 6 reveals the location of the tumors. There was some difference in the occurrence of tumors in relation to location. In the middle portion, occurrence of tumors was the highest of the 4 locations. Interestingly, in the cecum, and proximal portion of the colon, signet ring cell and mucinous carcinomas were frequently encountered (48.8%) and, conversely, carcinomas of such type were rare (12.9%) in the distal portion.

Table 2. Histological Type and Tumor Index

| | Well | Mod | Por | Muc | Sig |
|---|---|---|---|---|---|
| Group A | 47* (3.61) | 3 (0.23) | 2 (0.15) | 5 (0.38) | 4** (0.31) |
| | | | | 9*** (0.69) | |
| Group B | 31* (2.82) | 7 (0.64) | 2 (0.18) | 8 (0.83) | 20** (1.82) |
| | | | | 28*** (2.55) | |

( ) : Tumor index
* $P<0.001$
** $P<0.01$
*** $P<0.001$
Well: Well differentiated adenocarcinoma
Mod: Moderately differentiated adenocarcinoma
Por: Poorly differentiated adenocarcinoma
Muc: Mucinous carcinoma
Sig: Signet-ring cell carcinoma

Table 3. Depth of Neoplastic Involvement

| | Early Carcinoma | | Advanced Carcinoma | |
|---|---|---|---|---|
| | m | sm | pm | tm |
| Group A | 17 | 22 | 11 | 11 |
| | 39* (63.9) | | 22 (36.1) | |
| Group B | 12 | 21 | 18 | 17 |
| | 33* (48.5) | | 35 (51.5) | |
| Total | 29 | 43 | 29 | 28 |
| | 72 (55.8) | | 57 (44.2) | |

( ): %, *$P<0.1$

m: Intramucosal involvement
sm: Submucosal involvement
pm: Intramuscular involvement
tm: Transmural involvement

Table 4. Gross Appearance of Early Carcinoma

| | Ip | Ips | Is | IIa | IIa+IIc | IIb |
|---|---|---|---|---|---|---|
| Group A | 6 | 10 | 15 | 3 | 3 | 1 |
| Group B | 2 | 5 | 18 | 2 | 5 | 1 |
| Total | 8 | 15 | 33 | 5 | 8 | 2 |

Ip : Pedunculated type
Ips : Subpedunculated type
Is : Sessile type
IIa : Flat-elevated type
IIa+IIc: Flat-elevated and depressive type
IIb : Flat type

Table 5. Gross Appearance of Advanced Carcinoma

| | I | II | III | IV | V |
|---|---|---|---|---|---|
| Group A | 17 | 5 | 1 | 0 | 0 |
| Group B | 20 | 14 | 0 | 0 | 1 |
| Total | 37 | 19 | 1 | 0 | 1 |

I : Tumorous type
II: Localized ulcerated type
III: Infiltrating ulcerated type
IV: Infiltrating type
V: Unclassified

## LIGHT MICROSCOPY

1. Early carcinogenesis

Atypical changes in the epithelium were seen at 8 weeks and thereafter, increasing in frequency in the superficial region of a small polypoid adenoma. Focal malignant transformation in the form of a carcinoma in adenoma (Fig. 2) and a *de novo* carcinoma without morphological evidence of an antecedent adenoma were frequently observed (Fig. 3).

When carcinoma became invasive and penetrated the muscularis mucosae forming a nodule in the submucosa, signet ring cells increased in number. Thus, a well differentiated adenocarcinoma tended to occupy the superficial region and signet ring cell carcinoma

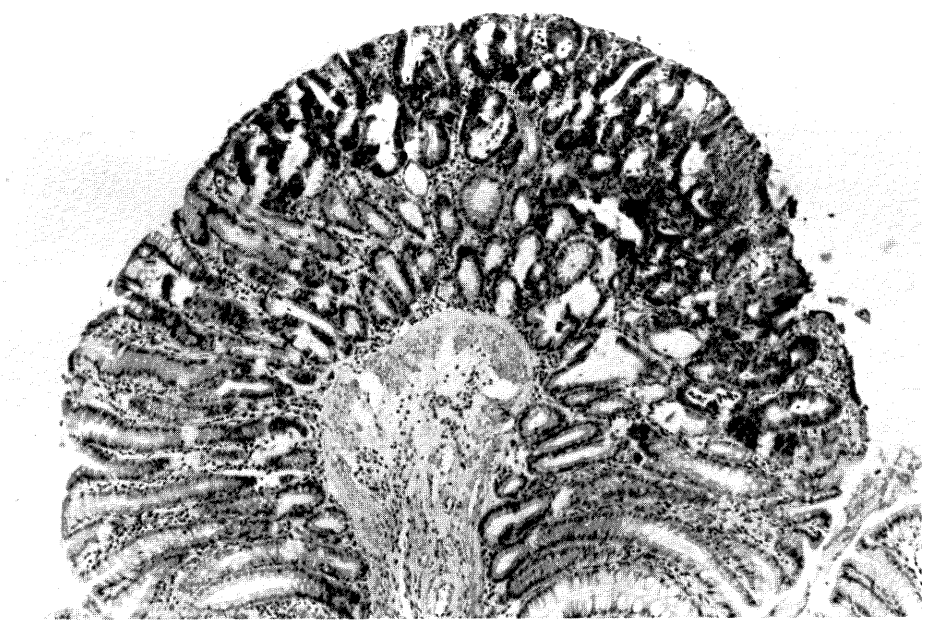
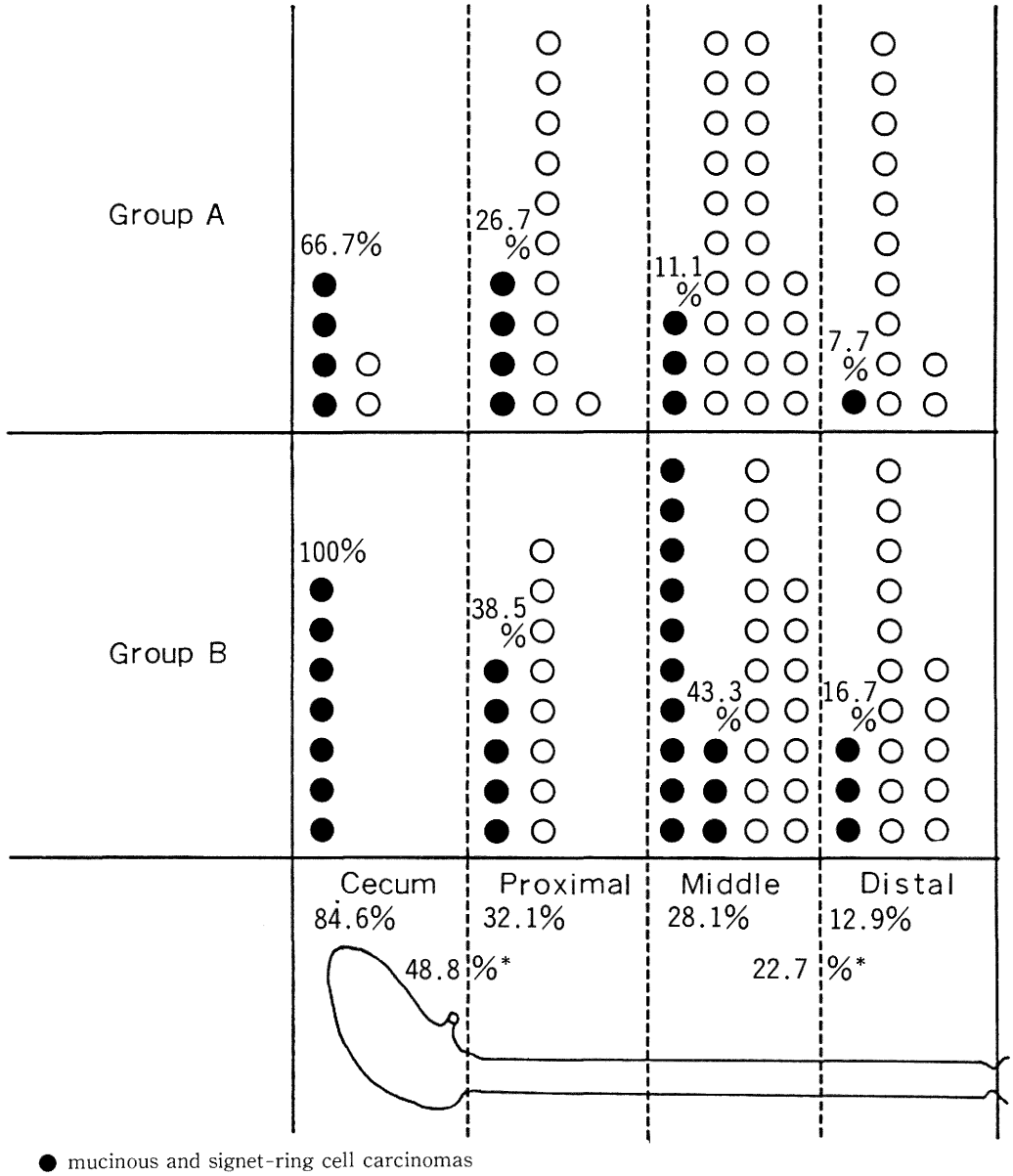


Fig. 2. Focal carcinoma in the head of a polyp induced with 1, 2-dimethlhydrazine. (H. E. ×40).

Table 6. Location of DMH-Induced Colorectal Carcinomas




● mucinous and signet-ring cell carcinomas
○ other adenocarcinomas
*$p < 0.01$

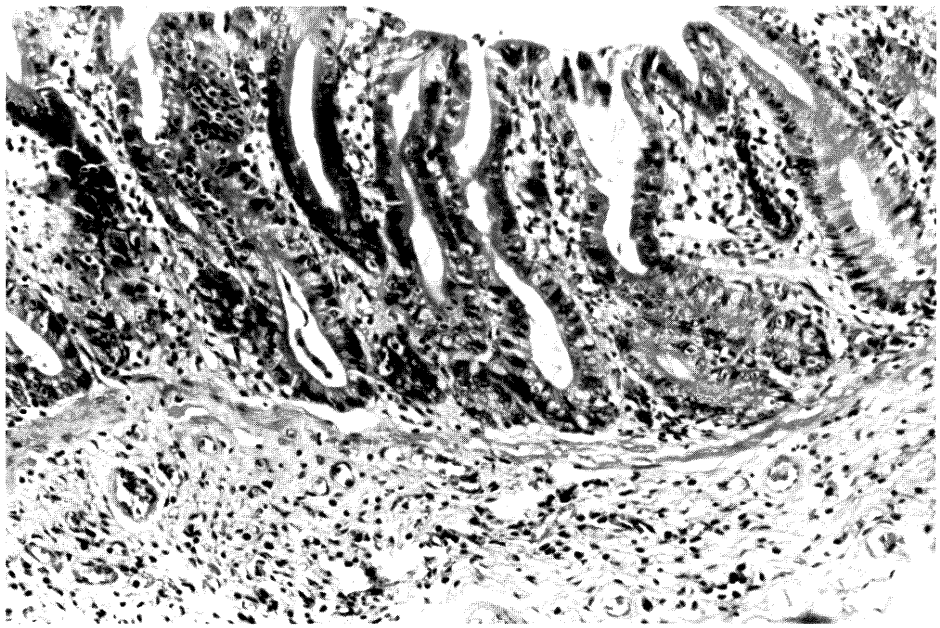


Fig. 3. Flat carcinoma, suspected to be a *de novo* carcinoma, in the colonic mucosa. (H. E. ×100).

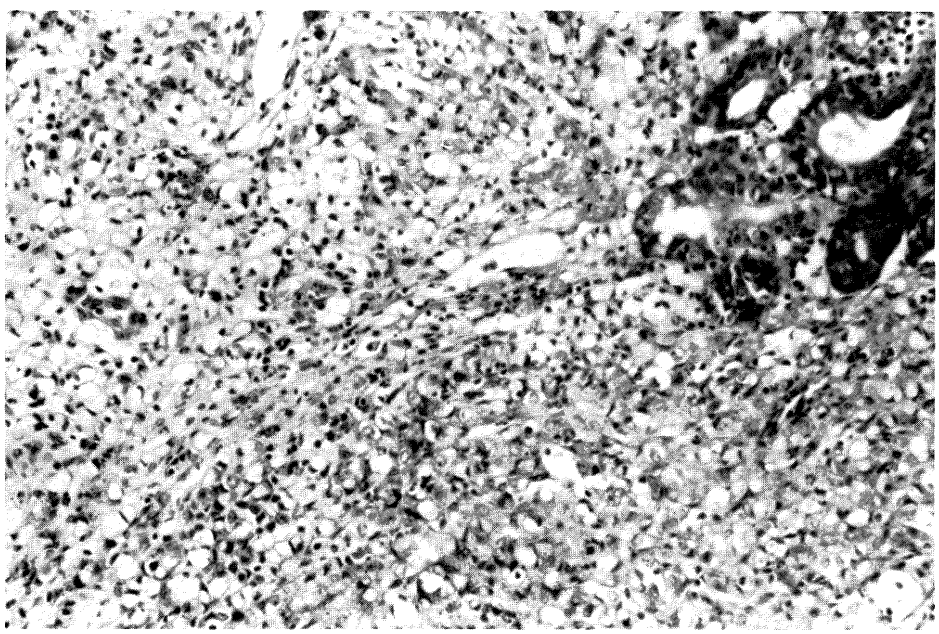


Fig. 4. Signet ring cell carcinoma is often observed in submucosal deeper layers, and more frequently seen in the proximal portion of the colon and cecum. (H. E. ×100).

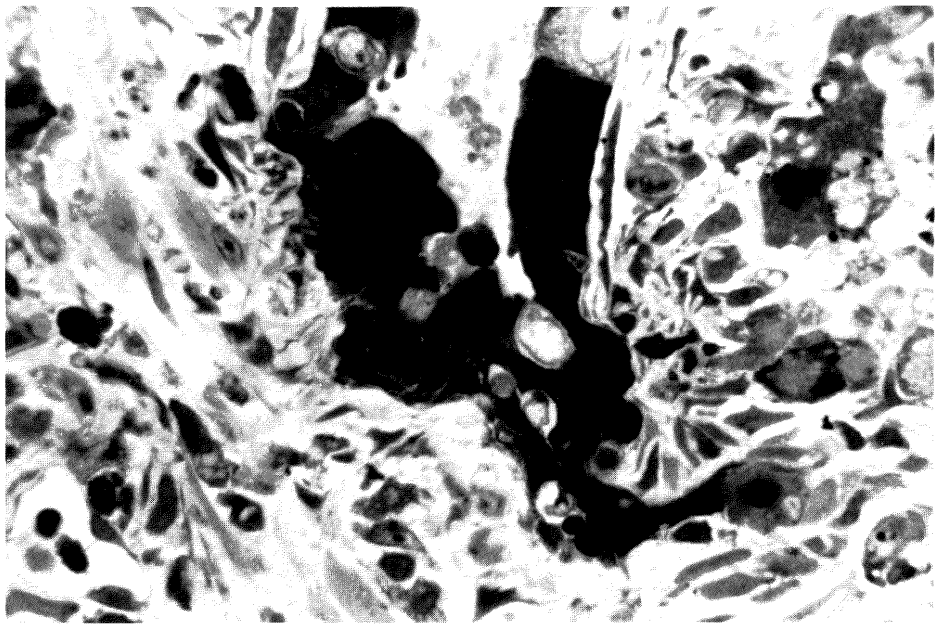


Fig. 5. Budding or microinvasion seen in neoplastic glands as the first step of massive invasion. (Toluidine blue × 400).

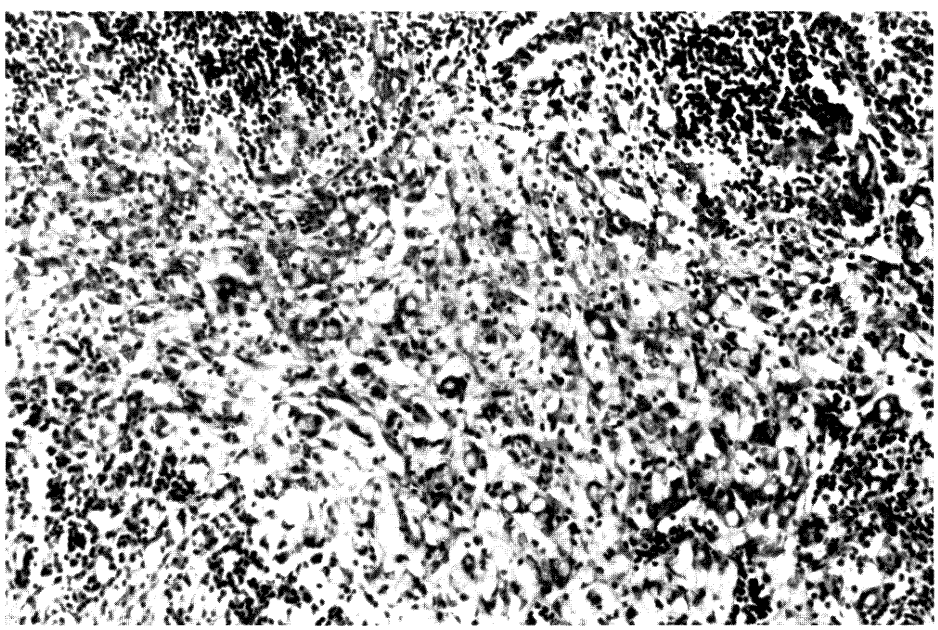


Fig. 6. Lymph node metastastasis with signet ring cell carcinoma. (H. E. ×100).

subsequently occupied the submucosa (Fig. 4). As early invasive patterns extending from carcinomatous glandular structure replaced by an *in-situ* carcinoma, budding and microinvasion were frequently observed (Fig. 5).

2. Advanced carcinoma

A variety of histologic types was present in advanced carcinomas. Signet ring cell carcinoma and mucinous carcinoma were, however, predominant - especially in Group B, which was fed high fat meal (Table 2 & 6).

There were frequent metastases to lymph nodes (Fig. 6), invasion to the pancreas (Fig. 7), peritoneal dissemination and hepatic metastases (Fig. 8). Signet ring cell carcinoma was the only histological type of carcinoma in these metastatic lesions. No lung metastases were, however, observed

## ULTRASTRUCTURE

Ultrastructurally, neoplantic cells were mainly classified into five types, undifferentiated cells, intermediate cells, absorptive cells, mucus-secreting cells and endocrine cells. Neoplastic cells forming superficial glandular structures exhibited various features in component cells, including predominant absorptive cells, undifferentiated cells and intermediate cells. Neoplastic cells of DMH-induced adenocarcinoma had relatively large nuclei of various shapes, elliptical to irregular, with frequent nuclear invaginations (Fig. 9). Mitochondria of neoplastic cells decreased in number, and occasionally had a blown -up appearance. Many mitochondria contained electron-dense inclusion bodies with a central electron-lucent core (Fig. 10). Free ribosomes were generally abundant but endoplasmic reticulum, both rough-surfaced and smooth-surfaced was scarcely observed (Fig. 10). The Golgi complex in neoplastic cells, excluding the undifferentiated variety was more prominent than in normal absorptive cells (Fig. 11). The interdigitation between neoplastic cells was uncomplicated and premature with few desmosomes. Intercellular spaces were often widened (Fig. 12). The luminal surface of neoplastic cells had scanty microvilli that were irregular and relatively short. The "fuzzy" coat associated with microvilli was also thin and scanty (Fig. 13). Intracellular canaliculi were often seen in neoplastic cells (Fig. 14).

The basement membrane of neoplastic cells was undulant and often discontinous. An especially distinctive phenomenon of neopastic cells was the cytoplasmic processes protruding into the stroma through breaks of the basement membrane; ie., an electron -microscopic microinvasion. Components of partially protruding cytoplasm of the neoplastic cell were represented almost exclusively by numerous free ribosomes. The cytoplasmic organelles such as mitochondria, rough-surfaced endoplasmic reticulum etc. were preserved in the remaining area of the cytoplasm. Fibroblastic and phagocytic cell accumulation was a peculiar finding seen around the protruding cytoplasm of neoplastic cells (Figs. 14 & 15).

Endocrine cells were seen in infiltrating neopastic glands but not in the superficial

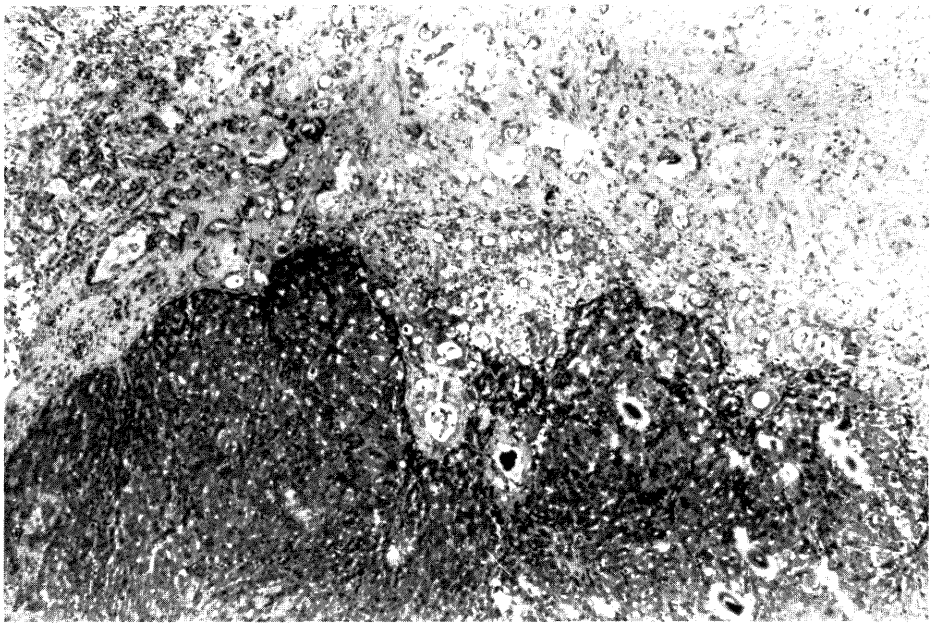


Fig. 7. Direct invasion into the pancreas by a moderately differentiated adenocarcinoma. (H. E. ×40).

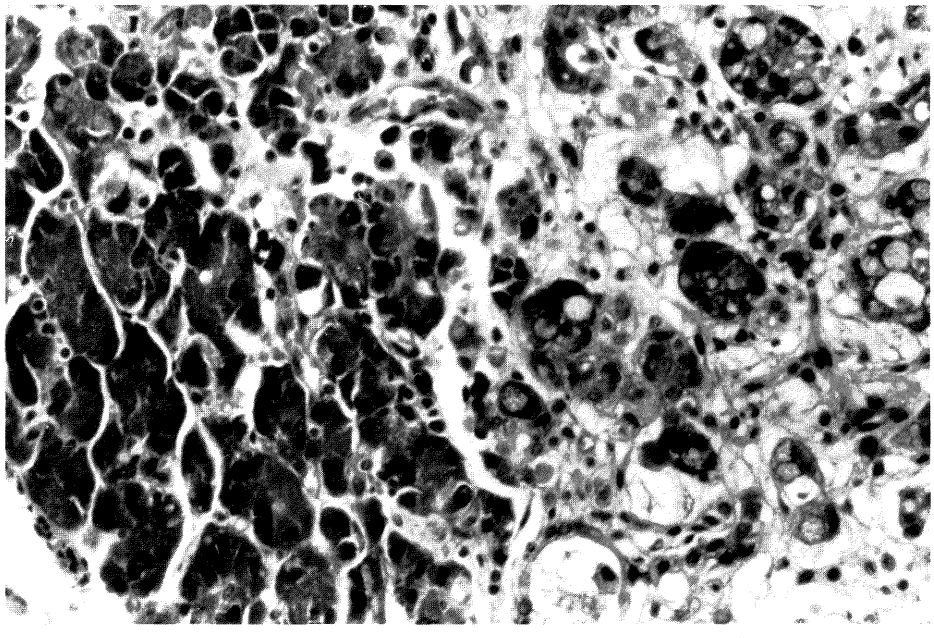


Fig. 8. Hepatic involvement by DMH-induced colorectal carcinoma possibly continuous from peritoneal dissemination. (H. E. ×200).

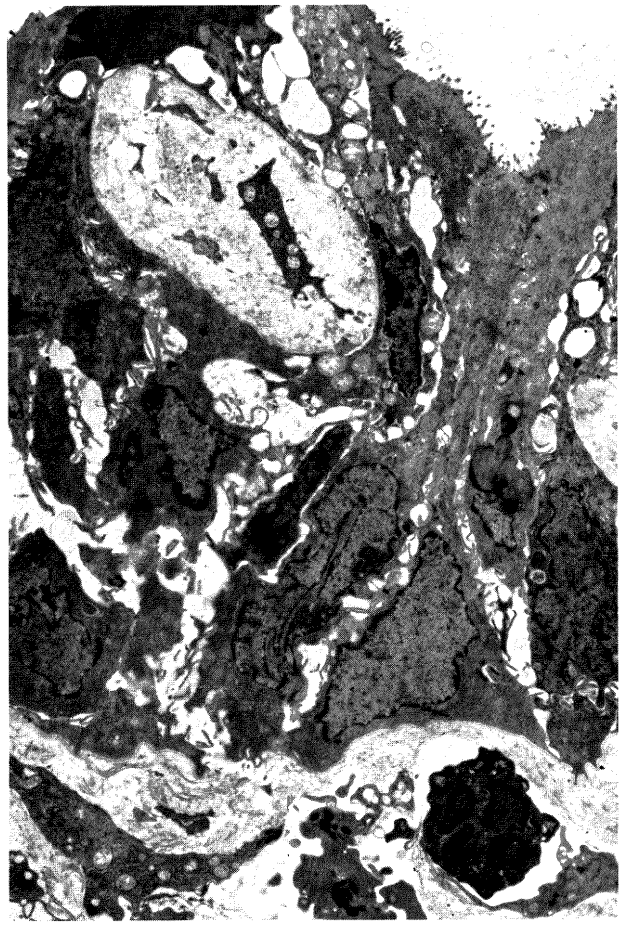


Fig. 9. Scanty and irregular microvilli in neoplastic epithelium. Neoplastic cells have relatively large nuclei of varying shape, elliptical to irregular, with frequent nuclear invaginations. ×3,300 (Original magnification ×2,000).

neopalstic structures, and showed definite desmosomes connecting neighboring neoplastic cells of other types (Figs. 16, 17, 18, 19 & 20). In the infiltrating neoplastic glands similarly immature goblet cells in various developmental stages were found.

Signet ring cells were large in size and irregular in shape with several disordered microvilli. The nucleus was depressed and impressively small. Numerous mucous granules in the cytoplasm varied greatly in size. The occasional releasing of mucous granules to the extracytoplasmic region was observed. Generally, rough-surfaced endoplasmic reticulum, Golgi complex and other intracytoplasmic organelles were poorly developed, suggesting poor biologic activity of signet ring cell carcinoma (Figs. 21, 22 & 23).

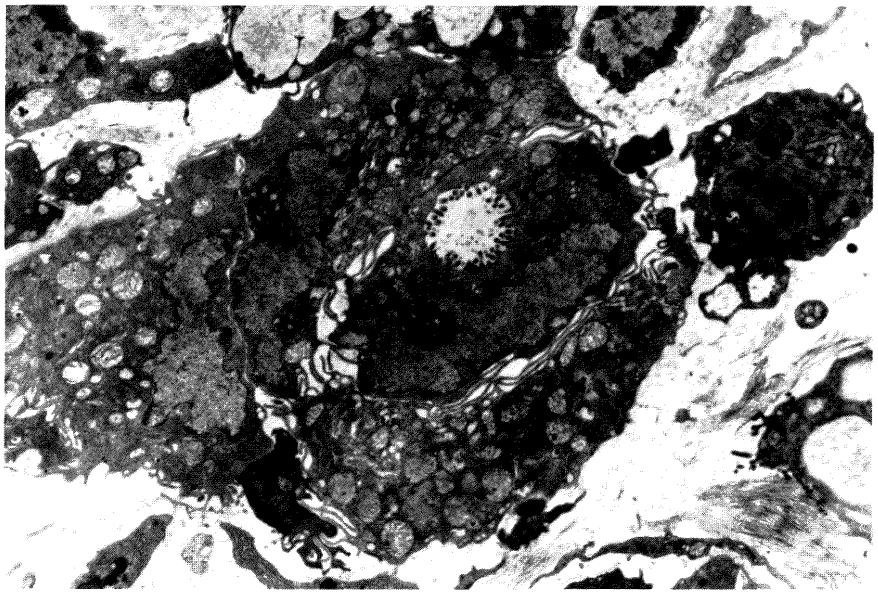


Fig. 10. Intracellular canaliculi are often observed in infiltrating neoplastic cells. ×8,300 (Original magnification ×5,000).

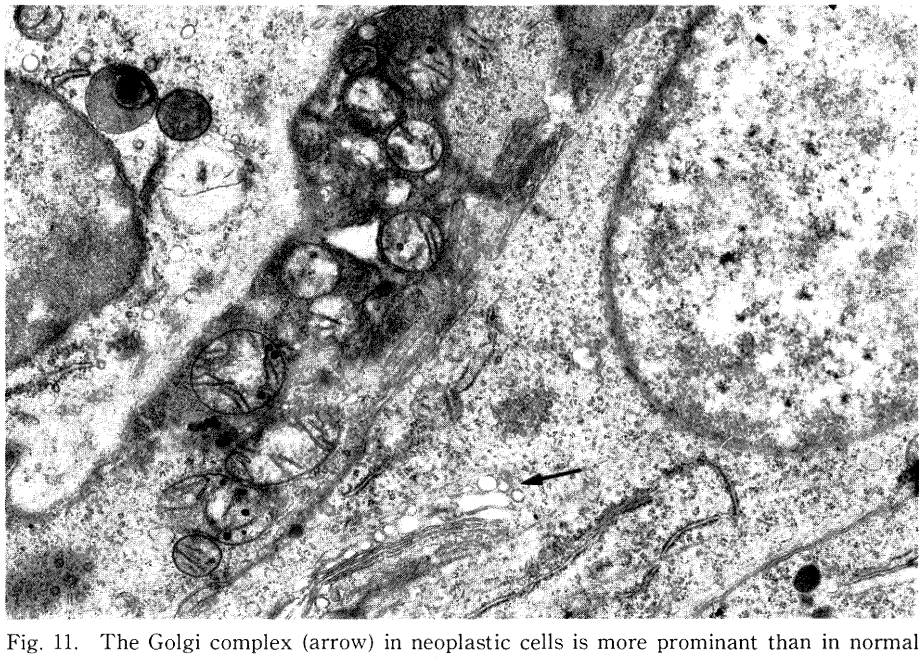


Fig. 11. The Golgi complex (arrow) in neoplastic cells is more prominant than in normal absorptive cells. ×16,600 (Original magnification ×10,000).

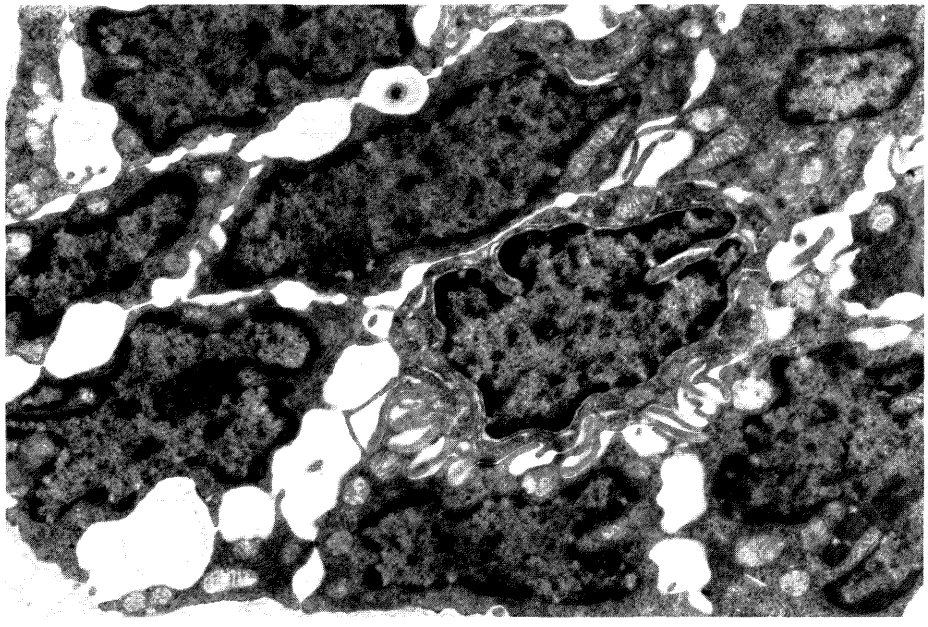


Fig. 12. Some desmosomes join the lateral surfaces of neoplastic cells with widened intercellular spaces. ×8,300 (Original magnification ×5,000).

## DISCUSSION

It is noted that the occurrence of colorectal carcinoma is mainly caused by environmental factors, particularly high dietry fat and beef to high risk, and fiber elements and cruciferous vegetables to low risk.

In this study, the high fat diet (containing 20% corn oil) group revealed a high incidence of colorectal carcinoma. It is interesting that, in the high fat diet group, mucinous and signet ring cell carcinomas appeared more frequently than in the ordinary diet group. Concerning the high fat diet, Reddy, et al. [3,4] reported that animals fed diets containing 20% lard or 20% corn oil were more susceptible to colon tumor induction by DMH compared to other groups (who were fed diet containing 5% lard or 5% corn oil). Fecal excretion of acid and neutral sterols was higher in animals fed diets containing 20% fat compared with those fed a 5% fat diet. They also pointed out that, in the animal model, two bile acids (lithocholic acid and deoxycholic acid) acted as colon tumor promoters. Furthermore, they concluded that animals fed a 20% corn oil or 20% lard and a 20% casein diet had a higher glucuronidase activity in the contents of cecum and colon than did rats fed a 5% corn oil or lard and 20% casein diet.

On the other hand, Burkitt, et al.[11] and Chen, et al.[12] indicated that a high fiber diet had the ability to significantly lower the incidence of pathologic changes in the colon of mice receiving DMH.

Concerning DMH metabolism, Fiala[13] demonstrated by using exhaled air of rats

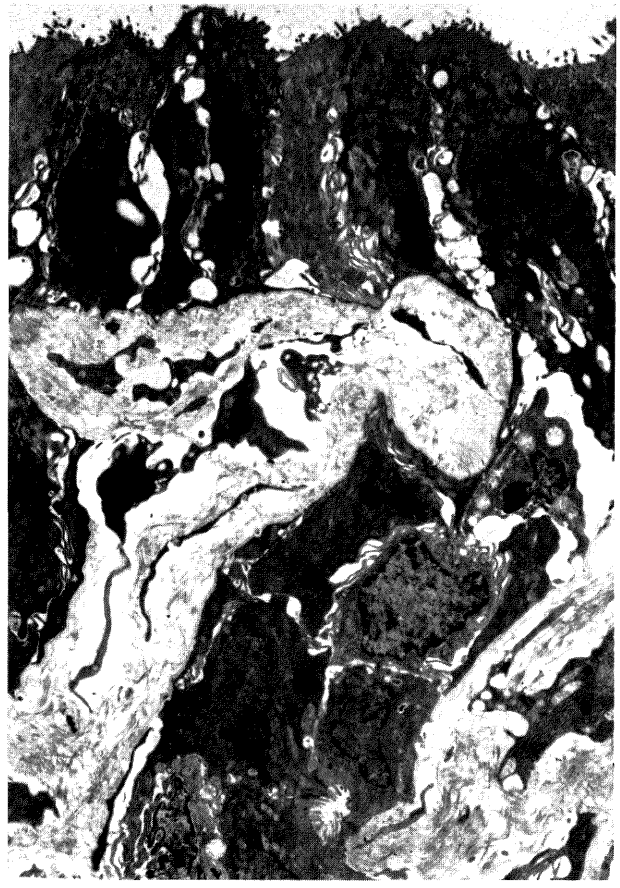


Fig. 13. The luminal surfaces of neoplastic cells have scanty microvilli. ×8,300 (Original magnification ×5,000).

treated with $^{14}C$-labeled DMH that AM, AOM, and MAM were certainly metabolites of DMH. Further information about DMH metabolism was produced by Hill[14] and Weisburger.[15] Toth[16] reported cancer-inducing effects in the colon of five hydrazine derivatives and suggested that the human population was exposed, to a considerable degree, to some of those hydrazine derivatives.

Results of the present study revealed that rats of high fat diet group are more susceptible to colorectal carcinognesis following DMH administration than those of low fat diet group, while in human beings, people in high dietry fat and beef protein environment suffer from a high incidence of carinoma of the colon.

In the present investigation, tumors were located mainly in the mid-portion of the colon, as shown in Table 6. Barthold, et al.[17] reported in their experiment on DMH carcinogenesis that neoplastic lesions occurred most frequently in the transverse and descending portions of the colon. This was confirmed by some other authors. This experiment indicates that mucinous and signet ring cell carcinomas more frequently

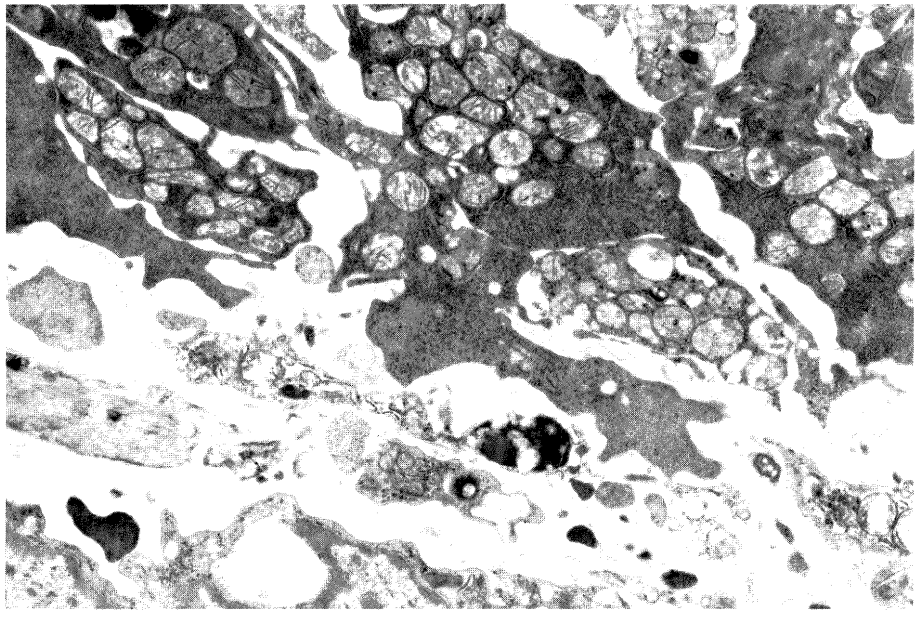


Fig. 14. The basement membrane of neoplastic cells is often discontinuous with electron-microscopic microinvasion. ×3,300 (Original magnification ×2,000).

occur in the cecum, and in the proximal and middle portions of the colon than in the distal portion of the colon.

Ward[9] concluded that mucinous adenocarcinomas originated most commonly in the ascending colon in association with a collection of lymphoid follicles and metastasized to regional lymph nodes. Identical findings were observed in the present experiment. With respect to human colon carcinoma, Sanfelippo, et al.[18] stated that greater numbers of undifferentiated neoplasms were seen in the ascending colon, but could not difinitely explain their association with lymphoid follicles. The similarity of these reports with the present investigation is that tumors occurring in the cecum and proximal and middle portions of the colon had a tendency to be poorly differentiated, and that mucus-secretion, early invasion to the serosa and lymph node metastases were also noted.

Signet ring cell carcinoma and *de novo* carcinoma without an antecedent adenoma have been frequently observed among the DMH-induced carcinomas of the colon and reported by several authors.[9,19,20,22] This is one of the points differing from the findings observed in the carcinoma of human material. In this study, various types of multifocal dysplasia were observed 8 weeks after the initiation of DMH administration, and animals sacrified after the 22nd week showed, in addition to such dysplasia, adenocarcinomas of various histologic tupes. Particularly, signet ring cell carcinoma appeared at a high frequency in peripheral parts of neoplastic invasion. This tendency was significantly distinctive in the high fat diet group. It was suspected that at light-microscopic level, signet ring cell carcinoma might be one type of the results from biologic and functional

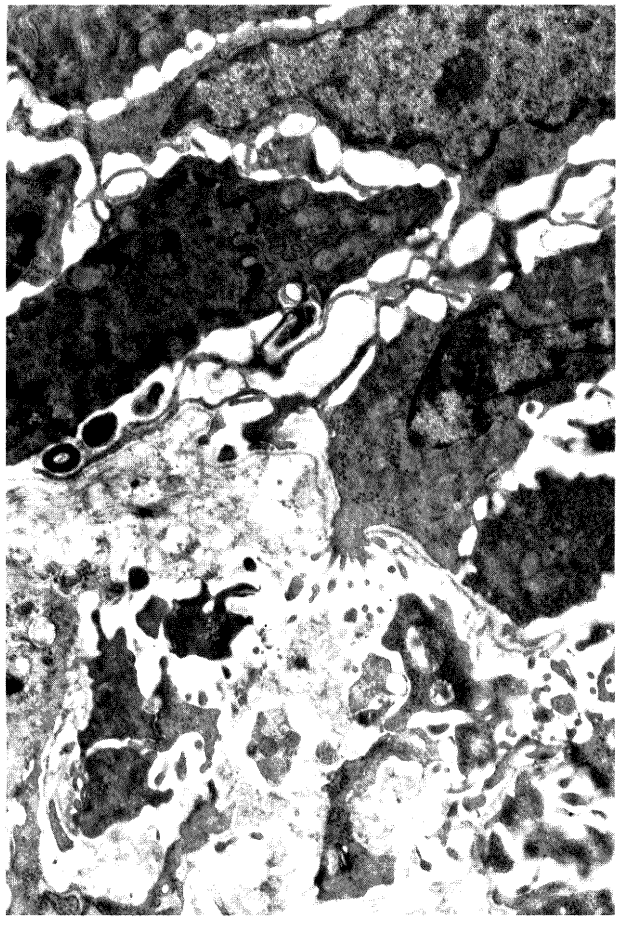


Fig. 15. Finger-like cytoplasmic projections through the basement membrane. ×8,300 (Original magnification ×5,000).

changes in accordance with neoplastic formation, and that signet ring cell element among others in an adenocacinoma might tend to perform an earlier invasion. In human colorectal carcinoma, undifferentiated cell type and signet ring cell type are rare, but its association with ulcerative colitis occurs frequently.[23,24]

It is well known that a carcinoma associated with long standing ulcerative colitis frequently occurs in the transverse colon in man and, as a precancerous change, dysplasia is antecedently encountered. It is interesting that DMH-induced signet ring cell carcinoma shows some similarity in carcinogenesis to a carcinoma associated with ulcerative colitis in man.

The electron-microscopic features of the normal colonic mucosa have been reported by many authors.[25,26,27,28] Kaye, et al.[25] classified eight types of epithelial cells. Undifferentiated cells were found at the base of the Lieberkühn's crypts and considered to be the precusors of both absorptive and goblet cells.

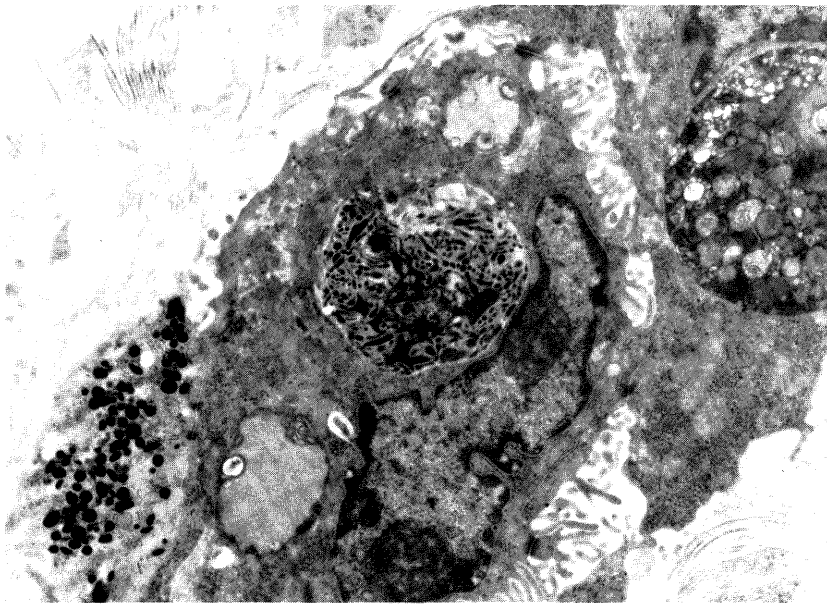


Fig. 16. An endocrine cell in a neoplastic gland having round to ovoid granules connected to a neighboring nonendocrine cell with desmosomes. ×6,600 (Original magnification ×4,000).

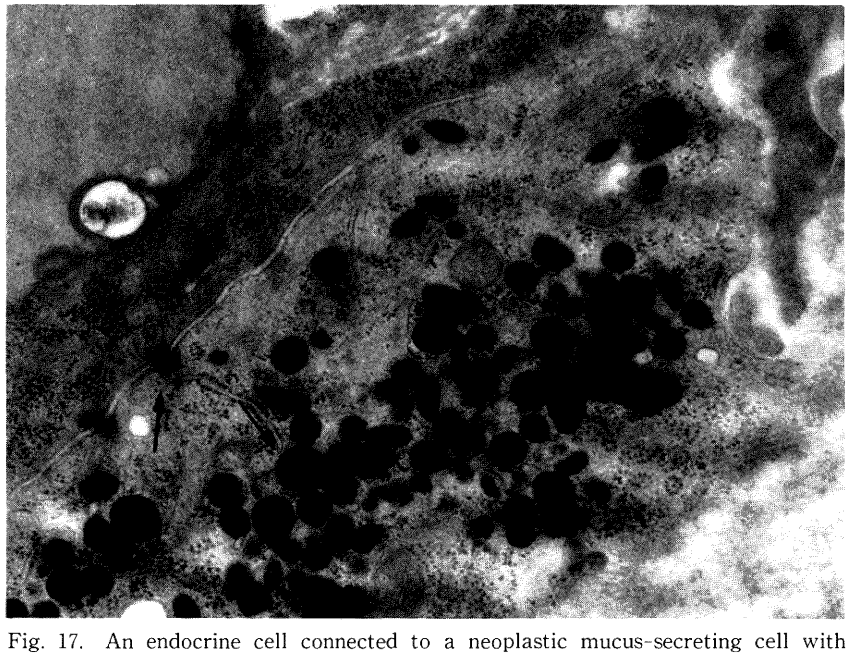


Fig. 17. An endocrine cell connected to a neoplastic mucus-secreting cell with definite desmosomes (arrow). Endocrine granules are pleomorphic measuring 200-300 nm (arrow). ×24,900 (Original magnification ×15,000).

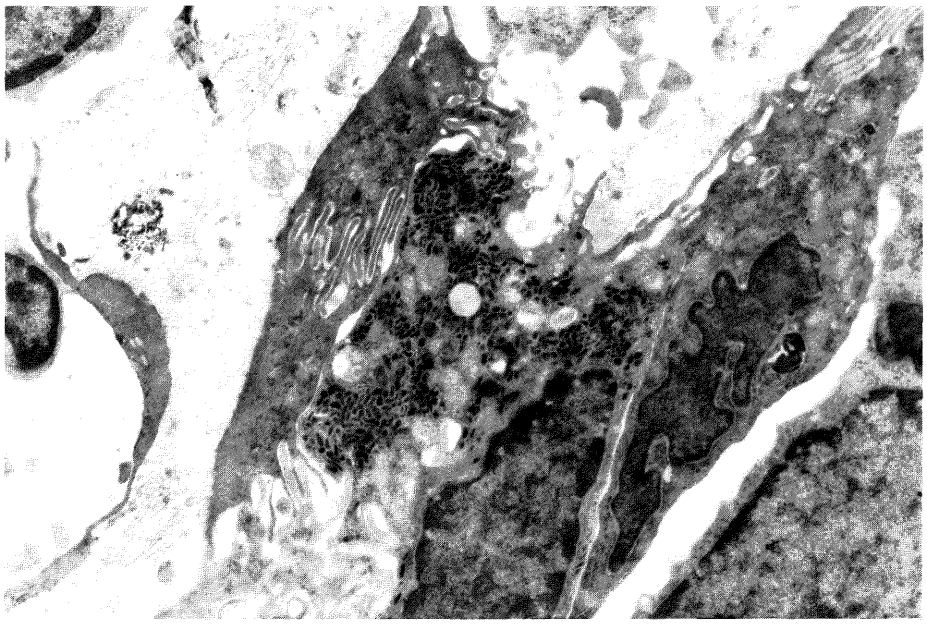


Fig. 18. A neoplastic endocrine cell with numerous round granules and a large nucleus. ×8,300 (Original magnification ×5,000).

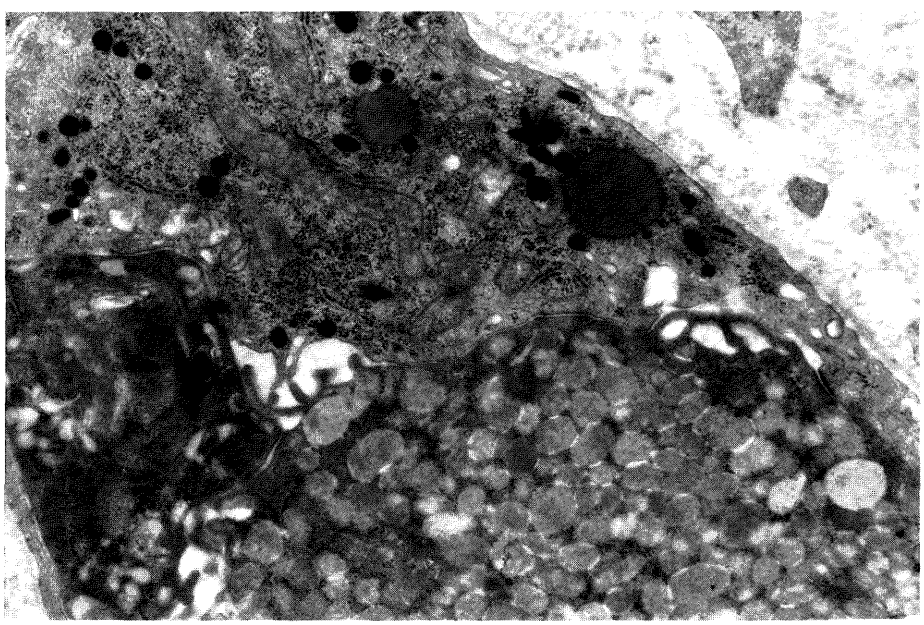


Fig. 19. A neoplastic endocrine cell with many free ribosomes and round granules. ×16,600 (Original magnification ×10,000).

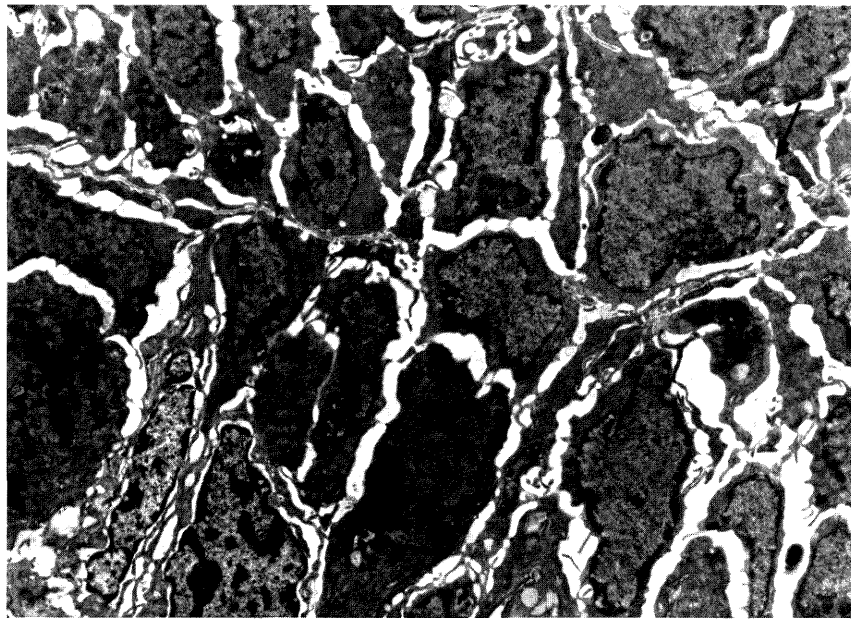


Fig. 20. A small number of endocrine granules are in what is a probable immature endocrine cell (arrow). ×5,000 (Original magnification ×3,000).

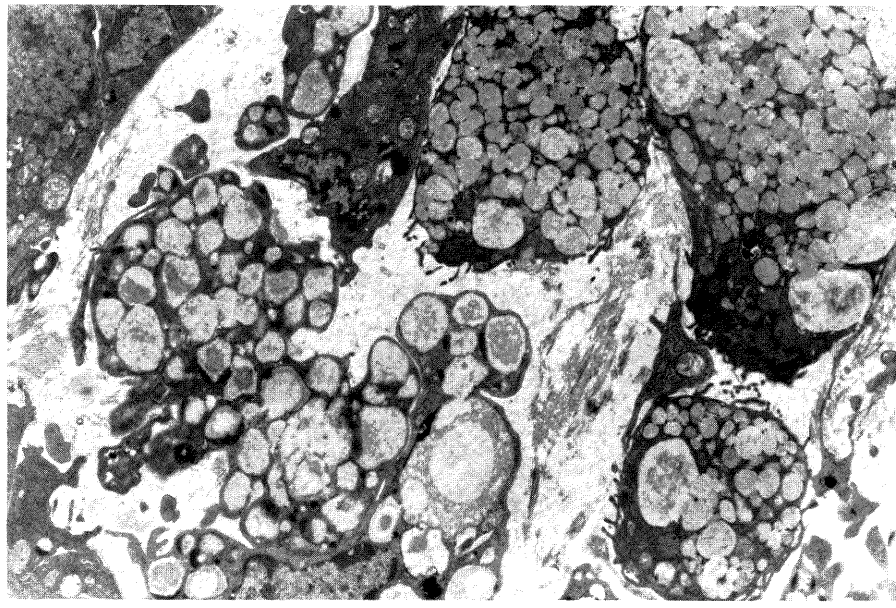


Fig. 21. Nucleus in neoplastic cells of a signet-ring cell carcinoma is depressed and small. Numerous mucous granules in the cytoplasm can be seen. ×5,000 (Original magnification ×3,000).

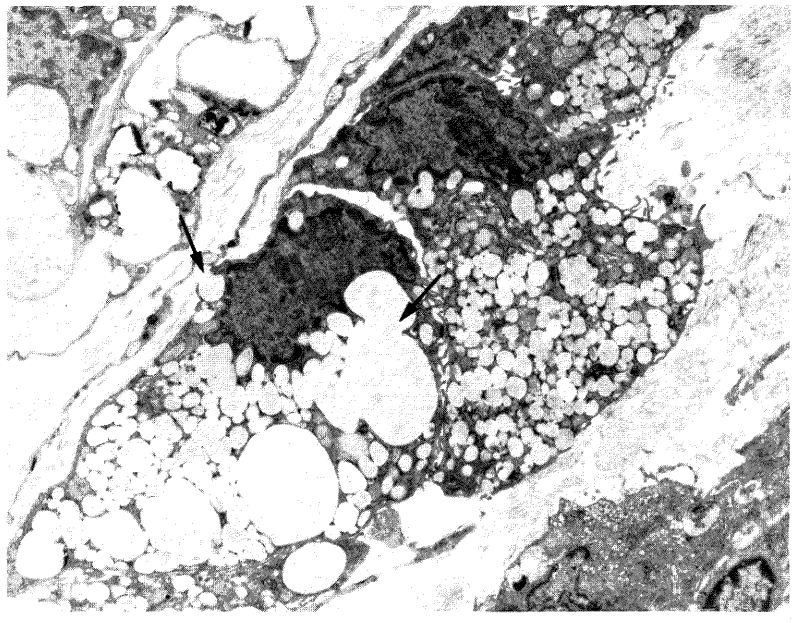


Fig. 22. Releasing phenomenon of mucous granules to the extracytoplasmic region is observed (arrow). Integration of mucous granules is seen (arrow). ×5,000 (Original magnification ×3,000).

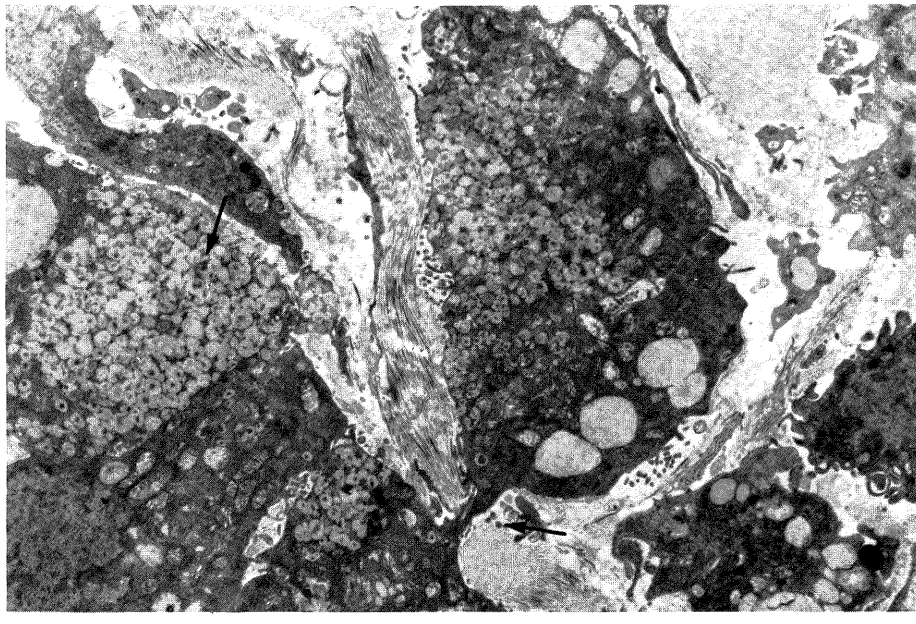


Fig. 23. Checker-board phenomenon of mucous granules is frequently observe (arrow). Neoplastic cells interrupting the collagen fibers (arrow). ×5,000 (Original magnification ×3,000).

## HISTOGENESIS

Electron-microscopy of infiltrating neoplasm disclosed various cell types composing the neoplastic mass ; immature and mature goblet cells, immature absorptive cells, intermediate cells, undifferentiated cells and endocrine cells.

It was interesting that endocrine cells and mucus-secreting cells (immature goblet cells) together composed the neoplastic mass with definite desmosmes connecting both cell types (Figs. 18 & 19). In the portion of multilayered neoplastic cells, endocrine cells were rarely found, but occasional immature cells (undifferentiated cells) were observed. It was suspected from this observation that stem cells or undifierentiated cells might have been stimulated by the carcinogen and differentiated to various cell types including mucus -secreting cells, and signet ring cells might become predominant in the deeply invaded part of the submucosa. Concerning endocrine cells in relation to colorectal experimental carcinogenesis, several reports[22,29,30,31] are available.

Pierce, et al.[29] advocated the idea that malignant stem cells might originate from normal stem cells, and normal stem cells being thought to contain all the information necessary to account for the traits of malignancy. They suspected that normal stem cells were the target in carcinogenesis, and indicated the most differentiated portions of the tumor had acini composed of vacuolated, mucous and argentaffin cells. In addition, they observed two types of undifferentiated colon cells (UCC) that differed from lymphocytes in having well-differentiated desmosomes connecting them to other epithelial cells. They declared that if this were true, there would be one common precursor cell which, if successfully cloned, should give rise to a tumor containing all of the cell types described in this adenocarcinoma. If this were to occur, it would be definitive proof of the endodermal origin of argentaffin cells. Barkla, et al.[30] described that many cells in DMH-induced adenocarcinoma had a morphology similar to that of enterochomaffin, mucous, vacuolated, and absorptive cells in normal colon epithelium. Goto, et al.[32] concluded by electron microscopic study on transplantable mucous-secreting and tubular adenocarcinomas in ACT/N rats that tumor contained mucous-producing cells at varying stages of development along with a small number of undifferentiated cells. Swartzendruber, et al.[31] suggested that, when mixed cell populations occurred in metastases, colonic tumor cells had the capacity for multidirectional differentiation, and that neoplastic mucous and argentaffin cells in the colon had a common origin.

Goldenberg and Fisher[33] examined the histologic relationship between carcinoids and mucin-secreting carcinomas of colon by heterotransplantation and reported that although no mucin-secreting cells were detected in the donor carcinoid, the cheek pouch transplants exclusively exhibited mucin-secreting tumor cells of signet ring type consistent with adenocarcinonma. They also stated that the mucin-secreting tumor cells had a selective advantage over the carcinoidal elements for unlimited propagation in an alein environment.

Shamsuddin and Trump[20,34] concluded that undifferentiated cells (UC) might have the capability to differentiate to various other cell type, including mucous cells, columnar cells and intestinal endocrine cells. They emphasized the morphologic similarity between the early EC and the UC found in their study. Pearse,[35] on the other hand, suggested the hypothesis that EC cells were derived from the neural crest. The study by Lorenzsonn and Trier[26] also supports the concept that these cells are locally differentiated from the UC. Concerning histogenesis of caricnoids, Soga[36] proposed the MU theory that neoplastic transformation is considered to occur in the primitive (anlage or stem) cell stages of the preprimordial M phase and primordial U phase of cell developments. This hypothesis has subsequently further developed to explain possible common histogenetic origin of endocrinomas and ordinary carcinomas.[37] Endocrine neoplastic cells are ultrastructually similar to carcinoid tumors composed of EC cells.

The present study and other workers' reports and speculations do not agree with Pearse's hypothesis and indicate that undifferentiated cells, a target of carcinogenesis, have multidifferentiational potential to various cell types.

On electron-microscopic examination, the basement membrane partially outlining neoplastic cells was undulated with focal areas of budding or microinvasion of the cytoplasm of neoplastic cells (Figs. 14, 15). In our observations, neoplastic cells penetrating the basement membrane had many free ribosomes and mitochondria in the cytoplasm, and a relatively small nucleous. Most of these cells were identified as immature absorptive cells and intermediate cells, and rarely had well-formed desmosomes. Although several authors[20,38,39] reported on the formation of the basement membrane outlining neoplastic cells, no definite conclusion on its mechanism and significance has been made. On the basis of the fact that infiltrating neoplastic cells are sometimes associated with the basement membrane, and that basement membrane formation is observed in neoplastic cell cultures, it is condidered one possibility that epithelial and stromal elements related to fibroblasts act reciprocally to form the basement membrane. It is strongly suspected, however, that epithelial elements play the leading part in formation of the basement membrane.

The fact that microinvasion is often associated with areas of active neoplastic cell proliferation represented by budding and mulitilayered cell mass with undulate basement membrane suggests that this phenomenon certainly occurs in accordance with the increased proliferative activity of neoplastic cells. Imai, et al. [40] on observing such a phenomenon in villous adenomas, considered the two possibilities that the absence of a stretch of the basement membrane reflected either an increased rate of growth or else a loss of maturation of epithelial cells with failure or defect in formation of the basement membrane; the latter possibility was favored because cellular features usually associated with rapid growth of tissues were not seen in segments of villous adenomas lacking the basement membrane. Ioachin, et al.[41] decribed an identical observation in villous adenemas. Shamsuddin and Trump[20] made the observation that single cells in a baso-

philic crypt of flat mucosa destroyed the basement membrane and invaded the surrounding stroma, which served as an example that individual cells can undergo malignant changes irrespective of the location. Although this phenomenon is not a particular feature of carcinoma, it is considered that neoplastic invasion to the underlying stroma is the firrst step of invasion of neoplastic cells.

## Summary and Conclusion

Forty-four Wistar strain male rats treated with DMH for analysis of experimental colorectal carcinogenesis were divided into two groups; Group A was fed on an ordinary diet (fat 5.1%) and Group B on a high-fat (20% corn oil) diet. Carcinomas thus induced were analyzed grossly, histologically and ultrastructurally.

The results obtained in this study are summarized as follows.

1) No specific changes were observed at 4 and 5 weeks after the first injection of DMH, but at 8 weeks and thereafter, dysplasia of varying degrees and neoplastic changes were observed.

2) The incidence of tumors was significantly high in Group B with the tumor index calculated at 22 weeks and thereafter showing 6.18 compared to 4.69 in Group A.

3) Neoplastic lesions were found most frequently in the midportion of the colon.

4) Concerning histologic types, there was a definite tendency for Group B to produce carcinomas of mucinous and signet ring cell types, its tumor index being 2.55 in comparison with 0.69 in Group A.

5) Mucinous and signet ring cell carcinomas were more frequently observed in the cecum and proximal colon than in the distal segments.

6) Lymph nodes were involved only by signet ring cell carcinomas.

7) Ultrastructurally, neoplastic cells exhibited microinvasion in many places.

8) As carcinoma cells invaded the submucosa and deeper, an increase of signet ring cell elements was prominent. Ultrastructurally, a coupling of mucus-secreting cells and endocrine cells was often observed, raising the suggestion that stem cells or undifferentiated cells might be affected by carcinogens and differentiate to various cell types including endocrine cells.

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