Production of Intestinal and Other Tumors by 1,2-Dimethylhydrazine Dihydrochloride in Mice

I. A Light and Transmission Electron Microscopic Study of Colonic Neoplasms

Bela Toth, DVM, Linda Malick, PhD, and Hidesuke Shimizu, MD

Single or ten weekly subcutaneous injection(s) of 1,2-dimethylhydrazine dihydrochloride were administered separately to Swiss mice. The repeated application gave rise mainly to high incidences of tumors in the large intestine. These neoplasms occurred most frequently in the colorectal area and in cecum adjacent to ileum. Light microscopically, these lesions were classified as polypoid adenomas and adenocarcinomas. Most of the adenocarcinomas were highly invasive, although they metastasized rarely. The fine structure of the malignant cells exhibited features typical of columnar absorptive cells. A distinctive alteration was the disorderly arrangement and abnormal size and shape of the microvilli. In addition, the cells exhibited numerous free ribosomes, little RER, prominent Golgi bodies, and uniformly dispersed nuclear chromatin. Morphologically, the intestinal tumors were similar to those found in man. In addition, the repeated administration of 1,2-DMH also induced significant incidences of neoplasms in blood vessels, lungs, anus, and kidneys while the single application produced tumors in blood vessels and liver. The main hypotheses attempting to explain the selective induction of large intestinal neoplasms are discussed. (Am J Pathol 84:69–86, 1976)

CONSIDERABLE INTEREST in hydrazine tumorigenesis began with the demonstration that a symmetrical dialkylhydrazine produces mainly colorectal cancers in rats. Actually, in the original study, 1,2-dimethylhydrazine dihydrochloride (1,2-DMH) was administered by repeated subcutaneous injections and gave rise to malignant neoplasms in the colon, rectum, duodenum, small intestine, and liver.[1] Interestingly enough, in a subsequent experiment when the compound was given in the drinking water, it produced only malignant hemangioendotheliomas of blood vessels in liver which metastasized to the lungs.[2] In mice and hamsters, the subcutaneous and intramuscular administrations of this chemical essentially evoked tumors of the intestine.[3,4]

The aims of the present study were: a) to describe the morphologic characteristics of induced colonic tumors by light and transmission elec-

From the Eppley Institute for Research in Cancer, University of Nebraska Medical Center, Omaha, Nebraska, and the Department of Public Health, Jikei University School of Medicine, Tokyo, Japan.

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Address reprint requests to Dr. Bela Toth, Eppley Institute for Research in Cancer, University of Nebraska Medical Center, Omaha, NE 68105.

tron microscopy; b) to establish the types and incidences of tumors induced by ten weekly repeated treatments of 1,2-DMH; and c) to determine the possible tumorigenicity of a single dose of 1,2-DMH.

Findings of cytochemical and scanning electron microscope studies are reported in a separate paper.

Materials and Methods

Swiss albino mice from the colony randomly bred by us since 1951 were used. They were housed in plastic cages with granular cellulose bedding, separated according to sex in groups of ten, and given Wayne lab-blox diet in regular pellets (Allied Mills, Inc., Chicago, Ill.) and tap water *ad libitum*.

The chemical used was 1,2-dimethylhydrazine dihydrochloride, symmetrical (1,2-DMH); molecular weight, 133.02; melting point, 168 C; obtained from K and K Laboratories, Inc., Plainview, N.Y. The chemical was dissolved in freshly made sterile physiologic saline. The mice were given subcutaneous injection(s) in the interscapular region with a tuberculin syringe using a 24-gauge needle.

Toxicity study was carried out prior to the chronic experiment. Six dose levels of 1,2-DMH in quantities of 25, 20, 15, 10, 5, and 2.5 μg in 0.01 ml physiologic saline/g body weight were administered as a single injection, and the animals were observed for 35 days. By taking into account three parameters—survival rates, body weights, and histologic changes—a dose of 20 μg/g body weight was found to be suitable for the treatment. This toxicity technique was developed recently in this laboratory.[5]

The chronic experimental and control groups were as follows:

Group 1

1,2-DMH was given as a single injection of 20 μg/g body weight in 0.01 ml physiologic saline to 50 female and 50 male Swiss mice.

Group 2

1,2-DMH was administered as 10 weekly injections of 20 μg/g body weight in 0.01 physiologic saline to 50 female and 50 male Swiss mice.

Group 3

Untreated control of 100 female and 100 male mice. All animals were 5 weeks old at the beginning of the experiment.

The experimental and control animals were carefully checked and weighed at weekly intervals, and gross pathologic changes were recorded.

For light microscopic examination, the animals were either allowed to die or were killed with ether when found in poor condition. Complete necropsies were performed on all animals. All organs were examined macroscopically and were fixed in 10% buffered formalin. Histologic studies were done on the liver, spleen, kidney, bladder, thyroid, heart, pancreas, testis, ovaries, brain, nasal turbinale, and at least four lobes of the lungs of each mouse as well as on those organs showing gross pathologic changes. Sections from these tissues were stained routinely with hematoxylin and eosin.

For transmission electron microscopic examination, segments of colon were fixed in 2.5% phosphate-buffered glutaraldehyde, pH 7.3 for 1 hour, diced into 1-mm pieces and postfixed in buffered 1% osmium tetroxide at 4 C.[6,7] The tissue was dehydrated in ethanol and passed through propylene oxide and embedded in araldite (502).[8] One-micron sections stained with 0.2% toluidine blue [9] were examined by light microscopy. Thin sections were

stained with uranyl acetate [10] and lead citrate [11,12] and examined with a Philips 300 transmission electron microscope at 60 kV.

Results

The survival rates after weaning at 10-week intervals are summarized in Table 1. It is clear from the data that the treatments reduced the survival drastically with repeated injections but only slightly with the single injection when compared with the untreated controls.

The number and percentages of animals with tumors, and their age at death (latent periods), are presented in Table 2. The following five most important neoplasms are described in detail.

Tumors of the Large Intestine

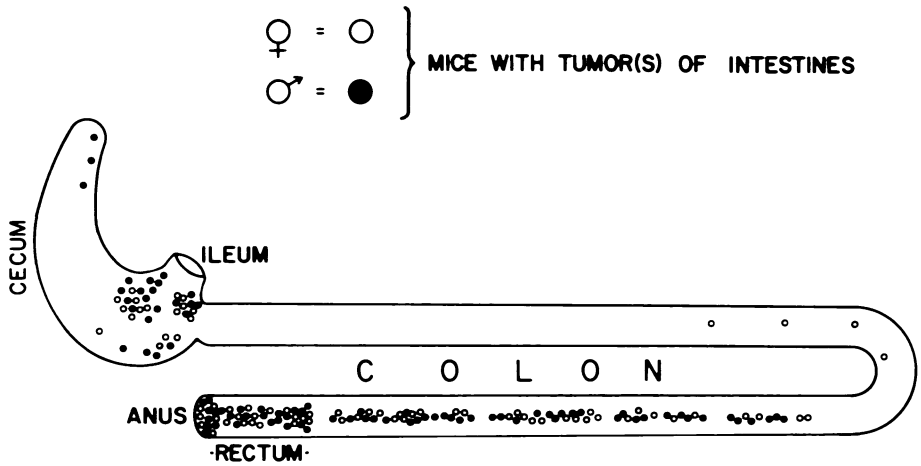
Of the animals treated with a single injection of 1,2-DMH, 1 (2%) female mouse and 1 (2%) male mouse developed an adenocarcinoma of the cecum at the 57th week and a polypoid adenoma of the cecum at the 75th week of age, respectively. Of the animals treated with repeated injections of 1,2-DMH, 41 (82%) females developed 130 tumors of the large intestines. Of these, 6 mice had 6 polypoid adenomas of the cecum and 11 mice developed 11 adenocarcinomas of this organ. In addition, 6 mice developed 17 polypoid adenomas, 12 had 18 adenocarcinomas, and 14 mice had 25 polypoid adenomas and 31 adenocarcinomas of colon. Furthermore, 5 animals developed 6 polypoid adenomas, 9 mice had 9 adenocarcinomas, and 2 mice had 5 polypoid adenomas and 2 adenocarcinomas of the rectum. Their average age at death was 54 weeks. The first tumor was observed at the 29th week and the last at the 90th week of age. In the males, 45 (90%) developed 156 tumors of the large intestine. Of these, 5 mice had 6 polypoid adenomas of cecum and 19 mice developed 23 adenocarcinomas of this organ. In addition, 8 mice had 15 polypoid adenomas, 19 had 43 adenocarcinomas, and 11 mice developed 21 polypoid adenomas and 27 adenocarcinomas of the colon. Furthermore, 11 mice had 14 polypoid adenomas and 6 mice developed 7 adenocarcinomas of the rectum. Their average age at death was 50 weeks. The first tumor was found at the 32nd week and the last at the 77th week of age.

The location and distribution of large intestinal neoplasms are illustrated in Text-figure 1. The tumors occurred most frequently in the cecum adjacent to the ileum and in the lower part of colon and in the rectum. No significant difference in tumor distribution was seen between the sexes.

Macroscopically, the lesions exhibited either sessile, pedunculated, or intermediate forms and usually had round to ovoid shapes ranging from 5 to 15 mm in diameter (Figure 1).

Table 1—Treatment and Survival Rate in 1,2-Dimethylhydrazine Dihydrochloride (1,2-DMH)-Treated and Control Swiss Mice

Group and Treatment	Initial No. and sex	No. of survivors (age in weeks)													
		10	20	30	40	50	60	70	80	90	100	110	120	130	140
1. Single subcutaneous injection of 1,2-DMH (20 μg/g)	50 F	49	49	49	48	47	42	36	27	22	12	3	1	—	
	50M	50	50	50	49	46	41	34	23	9	4	—			
2. Ten subcutaneous injections of 1,2-DMH (20 μg/g)	50 F	50	50	49	40	31	17	8	2	—					
	50M	50	50	49	41	26	10	2	—						
3. Untreated controls	100 F	100	100	97	95	91	88	81	71	57	36	14	5	1	—
	100M	100	100	96	93	88	83	74	65	48	27	12	7	1	—



TEXT-FIGURE 1—Location and distribution of large intestinal neoplasms. (Female mice with tumor(s) of intestines. *open circles:* male mice with tumor(s). *solid circles*)

Histologically, the epithelium first showed localized thickening. Along the surface of the mucosa the characteristic papillary tufting of the epithelial cells was visible (Figure 2) which was in contrast to the normal mucosa. In the polypoid adenomas, the glands were elongated, branched, and often dilated (Figure 3). On many occasions some inflammatory cellular infiltrate was also noticeable. The adenocarcinomas usually exhibited large nodular structures (Figure 4). Closely packed glands were a characteristic feature of these lesions (Figure 5). These glands were composed mainly of neoplastic tall columnar absorptive cells (Figure 6) among which were interspersed varying numbers of mucus-secreting goblet cells. The glandular structure of the malignant growth often invaded the muscularis mucosa and on numerous instances, the dilated and sometimes cystic glands of the tumor extended through the entire wall of intestine including the serosa (Figure 7). In a few occasions, the malignant lesions metastasized into the mesenteric lymph nodes (Figure 8).

Ultrastructurally, the neoplastic cells exhibited microvilli in varying numbers and sizes. In general, the microvilli were more irregularly distributed than on normal columnar absorptive cells and tended to be short and of uniform diameter or club-shaped (Figure 9). Free ribosomes were abundant and cisternae of the rough-surfaced endoplasmic reticulum (RER) rare in most cells. Mitochondria varied in number. They were scanty in cells with numerous free ribosomes and present in larger numbers in those cells with moderately developed RER. The Golgi apparatus was small in many cells but more prominent in those cells exhibiting a

Table 2—Treatment and Tumor Distribution in 1,2-Dimethylhydrazine Dihydrochloride

Group and treatment	Effective No. and sex	Animals with: Large intestine No.	%	Age at death*	Blood vessels No.	%	Age at death*	Lungs No.	%	Age at death*
1. Single subcutaneous injection of 1,2-DMH (20 μg/g body weight)	50 F	1	2	57	10	20	88 (58–128)	14	28	88 (34–111)
	50 M	1	2	75	12	24	79 (64–107)	15	30	87 (43–107)
2. Ten subcutaneous injections of 1,2-DMH (20 μg/g body weight)	50 F	41	82	54 (29–90)	23	46	61 (40–90)	24	48	62 (33–90)
	50 M	45	90	50 (32–77)	25	50	54 (34–77)	19	38	53 (35–77)
3. Untreated controls	99 F	—	—	—	5	5	113 (97–130)	21	21	95 (60–122)
	99 M	—	—	—	6	6	88 (65–105)	23	23	95 (53–125)

* Average and range. Age at death in weeks given in parentheses.

(1,2-DMH)-Treated and Control Swiss Mice

tumors of:				
Kidneys				
No.	%	Age at death*	Other organs†	
—	—	—	9 Malignant lymphomas (46, 58, 78, 79, 94, 99, 100, 105, 128) 2 Papillary adenomas of ovaries (90, 111) 1 Fibrosarcoma, subcutaneous (105)	1 Carcinoma of skin (96) 1 Hepatoma (79) 1 Adenocarcinoma of breast (88) 1 Polypoid adenoma of glandular stomach (96)
—	—	—	6 Malignant lymphomas (47, 63, 73, 86, 91, 101) 5 Hepatomas (68, 72, 81, 82, 107) 1 Fibrosarcoma, subcutaneous (99) 1 Adenoma of thyroid (75) 1 Polypoid adenoma of glandular stomach (74)	
3	6	64 (54–74)	6 Carcinomas of anus (38, 54, 58, 59, 64, 88) 4 Adenocarcinomas of glandular stomach (50, 51, 54, 88) 2 Adenocarcinomas of duodenum (38, 70) 2 Malignant lymphomas (39, 41) 1 Adenocarcinoma of jejunum (66)	1 Adenocarcinoma of ileum (33) 1 Hepatoma (64) 1 Cholangioma (73) 1 Carcinoma of salivary gland (43) 1 Adenocarcinoma of nasal cavity (66) 1 Adenocarcinoma of breast (78) 1 Carcinoma of breast (74) 1 Carcinoma of nasal cavity (70)
24	48	53 (32–73)	8 Carcinomas of anus (34, 49, 54, 56, 57, 62, 63, 64) 2 Adenocarcinomas of ileum (43, 47) 2 Adenocarcinomas of glandular stomach (53, 58)	1 Malignant lymphoma (38) 1 Adenocortical adenoma (49) 1 Fibrosarcoma of liver (63)
—	—	—	24 Malignant lymphomas (31, 36, 68, 69, 71, 79, 80, 80, 90, 94, 96, 96, 97, 101, 102, 103, 105, 106, 109, 115, 118, 122, 123, 130) 2 Adenocarcinomas of breasts (73, 93) 2 Adenocarcinomas of ovaries (104, 106) 1 Adenoma of thyroid (116) 1 Papilloma of skin (109) 1 Angioma of subcutis (114)	1 Malignant histiocytoma (58) 1 Fibrosarcoma, subcutaneous (44) 1 Papilloma of esophagus (130) 1 Leiomyosarcoma of uterus (81) 1 Adenoma of glandular stomach (115) 1 Papillary adenoma of ovary (103) 1 Angiosarcoma of liver (99) 1 Angioma of liver and ovary (97) 1 Angiosarcoma of ovary (130) 1 Angiosarcoma of liver, lung, ovary (122)
—	—	—	12 Malignant lymphomas (40, 57, 76, 88, 93, 94, 95, 97, 102, 107, 116, 126) 3 Angiomas of livers (65, 98, 105) 3 Angiosarcomas of livers (71, 87, 102) 1 Fibrosarcoma, subcutaneous (82)	1 Adenocarcinoma of duodenum (113) 1 Malignant histiocytoma (101) 1 Adrenocortical adenoma (130) 1 Carcinoma of skin (122) 1 Papilloma of esophagus (63) 1 Papilloma of forestomach (63)

greater number of mitochondria (Figure 10). Cell shape ranged from rounded contours with scanty cytoplasm to elongate forms with more abundant organelles (Figure 11). These cells frequently showed a disorderly arrangement although parallel arrays of tall columnar cells with numerous desmosomes were also observed. Complex interdigitation of lateral membranes was apparent in some instances. Nuclei were usually round or elongate, with fairly diffuse chromatin and rather large nucleoli. Goblet cells interspersed among the absorptive cells contained mucous globules and distended rough surfaced endoplasmic reticulum (Figure 12). Occasionally, cells containing small vacuoles with an empty appearance or containing finely fibrillar or flocculent material were observed (Figure 13).

Tumors of Blood Vessels

Of the animals treated with a single injection of 1,2-DMH, 10 (20%) females developed such neoplasms. Of these, 3 mice had angiomas in livers; 2 had angiosarcomas in livers; 2 had angiomas in ovaries; 1 had an angioma in subcutis; 1 had angioma in liver, uterus, and ovary; and 1 mouse had angiosarcomas in spleen and pancreas. In the males of this group, 12 (24%) mice developed blood vessel lesions. Of these, 7 mice had angiomas in livers, 4 had angiosarcomas in livers, and 1 mouse had angiosarcomas in liver and seminal vesicle.

Of the animals treated with repeated injections of 1,2-DMH, 23 (46%) females developed blood vessel tumors; of these, 8 mice had angiomas and 15 had angiosarcomas. Their tissue distribution was: livers, 18; lungs, 6; muscle, 4; fat, 3; lymph nodes, 2; uterus, 2; ovaries, 2; and kidneys, 1 case. In the males of this group, 25 (50%) mice developed vascular lesions. Of these mice, 3 had angiomas and 22 had angiosarcomas; the tissue distribution of the tumors was: liver, 11; paraepididymidal tissues, 10; muscle, 10; fat, 8; pararenal tissues, 6; subcutis, 3; lymph nodes, 3; kidneys, 1; brain, 1; lungs, 1; and spleen, 1 case.

Grossly and histologically, the observed blood vessel tumors were similar to those found and described earlier in this laboratory.[13,14]

Tumors of the Lungs

Of the animals treated with a single injection of 1,2-DMH, 14 (28%) females developed 16 tumors of this organ. Of these, 11 mice had 12 adenomas, 2 had 3 adenocarcinomas, and 1 mouse had an adenoma and an adenocarcinoma. In the males of this group, 15 (30%) mice developed 24 lung tumors. Of these, 14 had 19 adenomas, and 1 mouse had 3 adenomas and 2 adenocarcinomas.

Of the animals treated with repeated injection of 1,2-DMH, 24 (48%) females developed 62 such lesions. Of these, 21 mice had 50 adenomas and 3 had 9 adenomas and 3 adenocarcinomas. In the males of this group, 19 (38%) mice developed 26 adenomas of this organ.

Grossly and histologically, the lung tumors were similar to those described earlier in this mouse strain with other treatment in this laboratory.[15,16]

Tumors of Kidneys

Of the animals treated with repeated doses of 1,2-DMH, 3 (6%) females developed 3 adenomas of this organ. In the males of this group, 24 (48%) developed 33 kidney tumors. Of these, 22 mice had 31 adenomas and 2 mice had 2 adenocarcinomas.

Grossly and histologically, the kidney tumors appeared similar to those described by other investigators.[17]

Tumors of Liver

Of the animals treated with a single injection of 1,2-DMH, 1 (2%) female and 5 (10%) males developed benign hepatomas.

Tumors of Anus

Of the animals treated with repeated injections of 1,2-DHM, 6 (12%) females and 8 (16%) males developed squamous cell carcinomas of anus.

Other Tumors

In a few instances, other types of tumors were also observed in the various treated groups. Since they occurred in low incidences, their appearances can not be attributed to the treatment.

Tumors in Untreated Controls

The different tumors which appeared spontaneously in the untreated mice were described in detail recently.[16] Nevertheless, their incidences are included in Table 2 for comparative purposes.

Discussion

Repeated injections of 1,2-DMH in mice gave rise to high incidences of tumors in the large intestine. The neoplasms which occurred in the lower part of colon, in the rectum, and in the cecum adjacent to ileum were diagnosed as focal lesions of hyperplasia, benign polypoid adenoma, and finally malignant tumors—adenocarcinomas. The majority of tumor cells

exhibited characteristics typical of columnar absorptive cells. Ultrastructurally, the distribution, size, shape, and number of the microvilli were altered. The underlying membrane and subsurface alterations may represent a significant change. The presence of numerous free ribosomes, little rough endoplasmic reticulum, few microvilli, and uniformly dispersed nuclear chromatin suggests incomplete differentiation of the tumor cells. Observation of immature epithelial cells at the luminal surface implies a lack of orderly cell migration and maturation characteristic of normal colon epithelium. Cells with empty-appearing vacuoles or vacuoles containing finely fibrillar material resembling the vacuolated cells previously described [18] as precursors of goblet cells and columnar cells in normal mouse colon may account for the goblet cells occasionally included in the tumor cell population. It might be of interest to draw attention here to the close similarity between the present finding and the result of investigations obtained in humans. In both instances, the tumors were located mainly in the colorectal areas.[19] The similarity is striking because of dietary differences in the two species, and because the inducing agent in the mouse is a known chemical apparently not existent in nature, while the cause of human intestinal neoplasms is presently unknown. In addition, many of the light and transmission electron microscopic features of the normal mucosa, hyperplastic areas, adenomatous polyps, and adenocarcinomas in the mouse and man are similar, if not identical. Similar changes in the cellular arrangement, cell surface features, and prominence of the Golgi apparatus were seen in the tumors in each species.[20-25] Large cytoplasmic bodies and dense nuclear inclusions seen in human colonic carcinomas [26,27] were, however, absent from the mouse lesions. The repeated administration of 1,2-DMH, in addition to tumors of large intestine, also induced significant incidences of neoplasms in blood vessels, lungs, and anus in both sexes. Kidney tumors occurred only in the males. The single application of 1,2-DMH significantly enhanced the development of blood vessel tumors in both sexes, but it induced only low incidence of hepatomas in the males.

The findings corroborate the results of a previous investigation by other workers in which it was shown that the repeated applications of 1,2-DMH induced both benign and malignant tumors of the colon in mice.[3] In addition, our work shows that the compound given repeatedly also produces significant incidences of tumors in cecum, blood vessels, lungs, and kidneys which were not reported earlier. This establishes the types and incidences of tumors produced by this treatment since in previous investigations too few animals were used and the description of results was

too vague for statistical purposes. Furthermore, the current findings demonstrate the tumorigenicity of a single application of 1,2-DMH.

When 1,2-DMH was administered orally in the drinking water for life, the chemical induced the formation of tumors of blood vessels and lungs but not of the intestine.[13] From this finding and from the results obtained in rats by oral administration of 1,2-DMH,[2] two main hypotheses were proposed. One suggested that the routes of administration might be important since the chemical may be metabolized differently depending on the route of administration.[13] This might explain why only the subcutaneous route, but not the oral application, induced intestinal tumors. The other postulation suggested that the dose of the compound played a decisive role, i.e., a low amount of 1,2-DMH was required for the selective blood vessel tumorigenesis.[2] It is, of course, impossible to decide at the present time which explanation is correct; they are not necessarily mutually exclusive.

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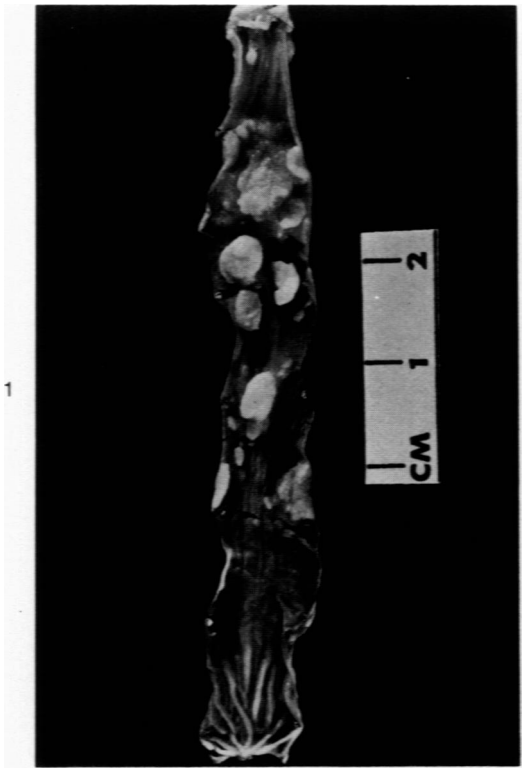

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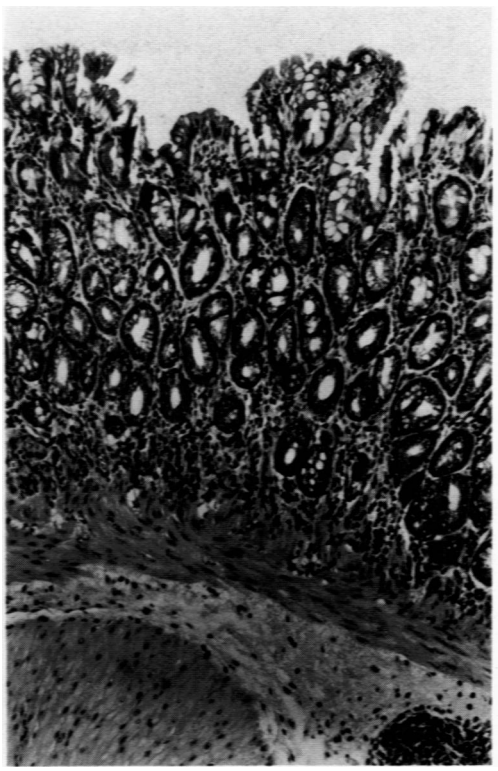

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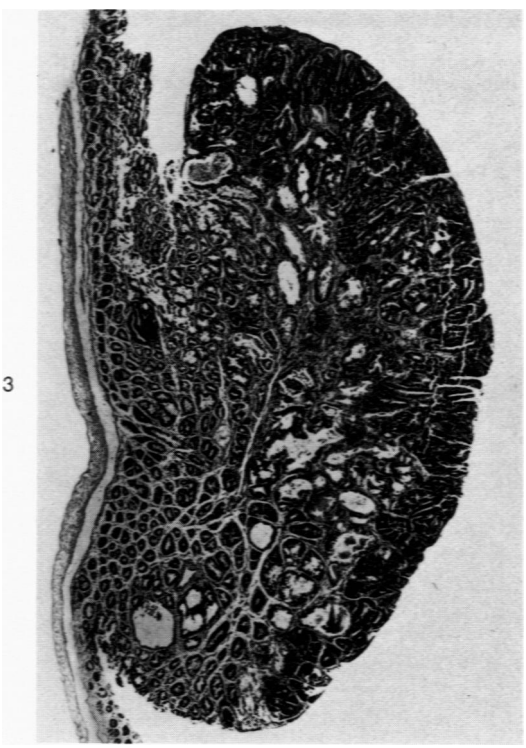

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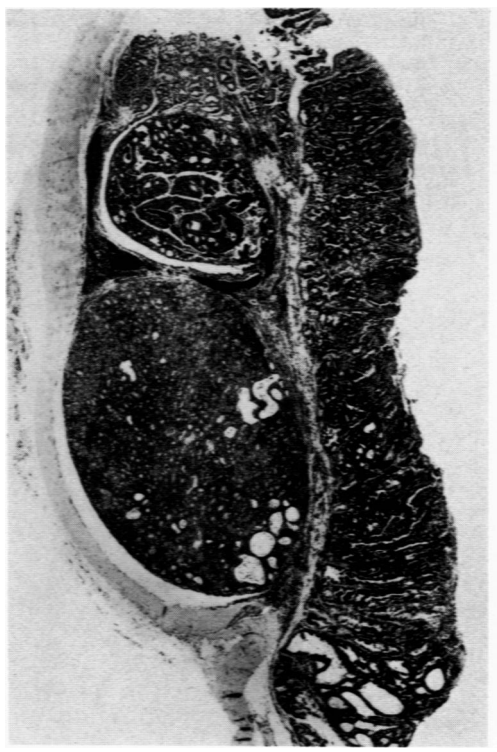

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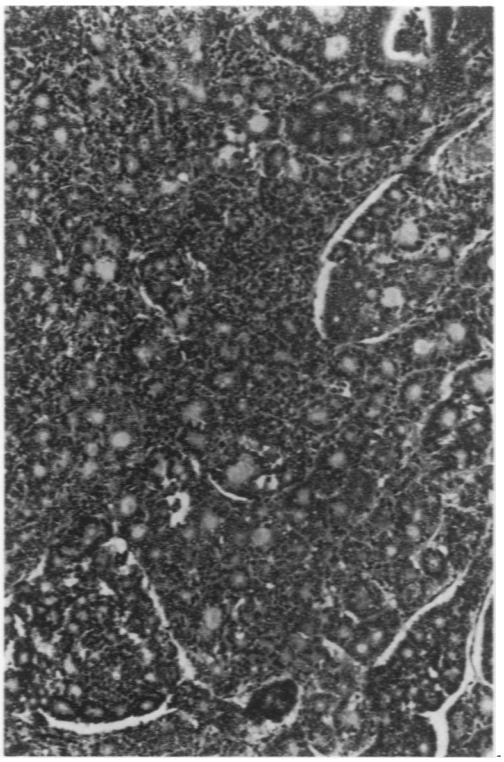
Figure 1—Polypoid adenomas and adenocarcinomas of the colon. The multiple, cauliflower-like roundish growths are abundant inside the intestine. Treated female, 58 weeks old. (Gross specimen, × 1.4) **Figure 2**—Hyperplastic epithelium, colon. The thickened epithelium exhibits atypical glands. Cells of stroma are hyperactive interspersed with inflammatory cells. Treated female, 50 weeks old. (H&E, × 85) **Figure 3**—Polypoid adenoma, colon. The growth filled the entire lumen of colon. Its glandular architecture is clearly evident. Treated female, 42 weeks old. (H&E, × 10) **Figure 4**—Adenocarcinoma, colon. Two large well-demarcated nodular tumor growths are in the muscularis mucosae and submucosa. Treated male, 47 weeks old (H&E, × 17)

Figure 5—Adenocarcinoma, colon. The acinar-like groupings of tumor cells are prevalent. In some areas, inflammatory cells are interspersed with epithelial cells. Same as Figure 4. (× 140)

Figure 6—Adenocarcinoma, colon. The glands are composed of tall columnar absorptive cells. The stroma is scanty with few inflammatory cells. Treated male, 63 weeks old. (H&E, × 350)

Figure 7—Adenocarcinoma of the colon. The tumor containing cystic ballooning of the glandular structures invaded entire muscularis layer and penetrated the serosa. Treated female, 73 weeks old. (H&E, × 40)

Figure 8—Metastatic adenocarcinoma of colon in a mesenteric lymph node. The malignant cells which retained their gland-like structures are visible under the capsula. Treated female, 51 weeks old. (H&E, × 140)



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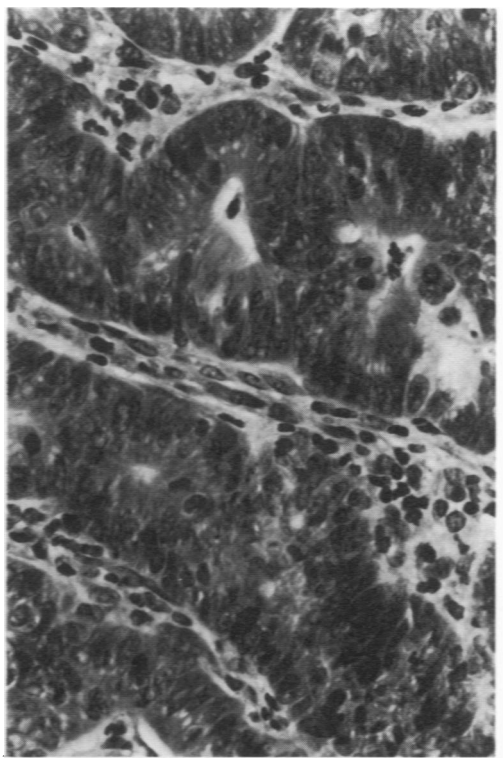

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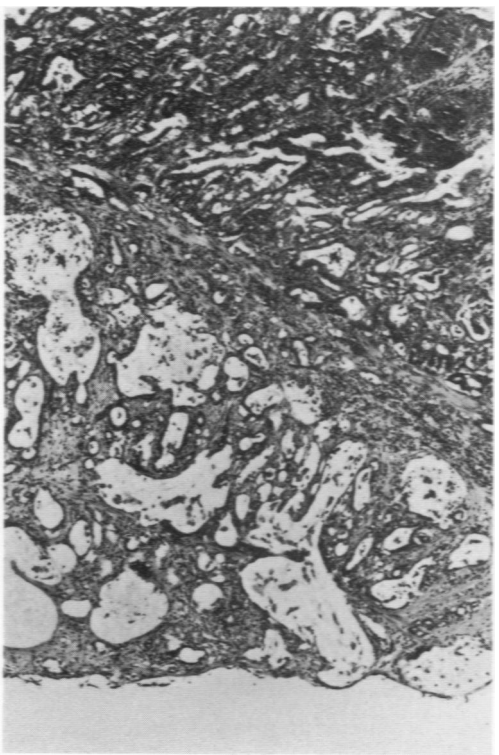

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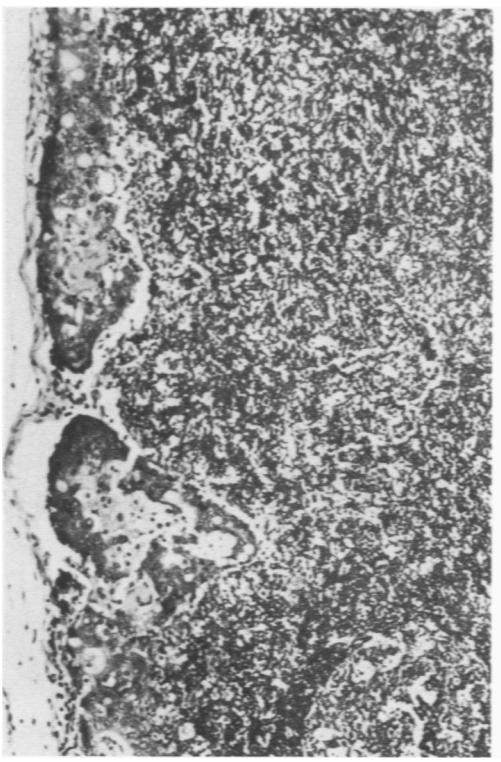

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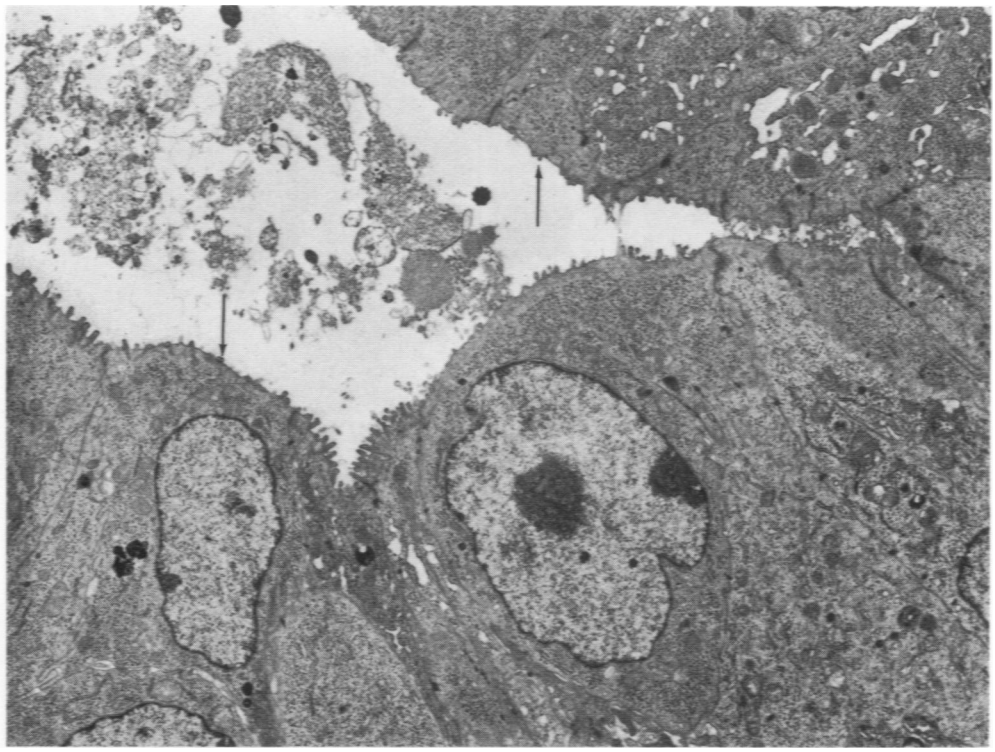

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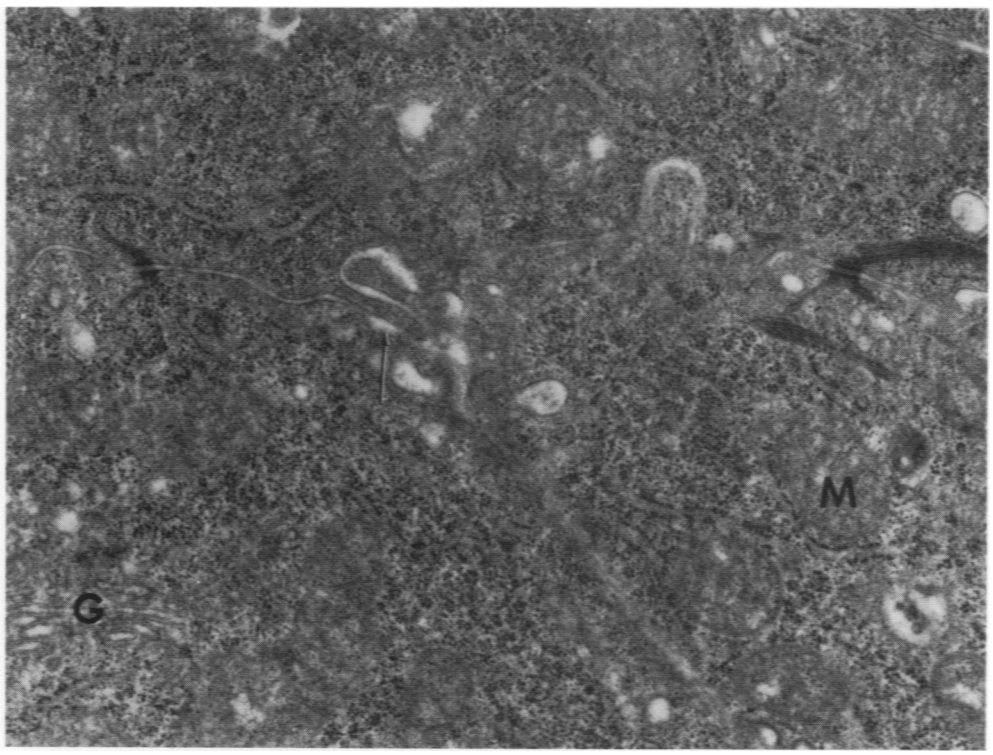


10

Figure 9—Surface areas of neoplastic absorptive cells. The irregularly arranged microvilli are more club-shaped and sparser (*arrows*) than their normal counterparts. Same as Figure 8. (× 5100) **Figure 10**—Cytoplasm of neoplastic absorptive cells of colon. Observe the prominent Golgi bodies (*G*), interdigitation of lateral cell processes (*arrow*), abundance of free ribosomes, and the electron-dense matrix of the mitochondria (*M*). Treated male, 55 weeks old. (× 25,300)

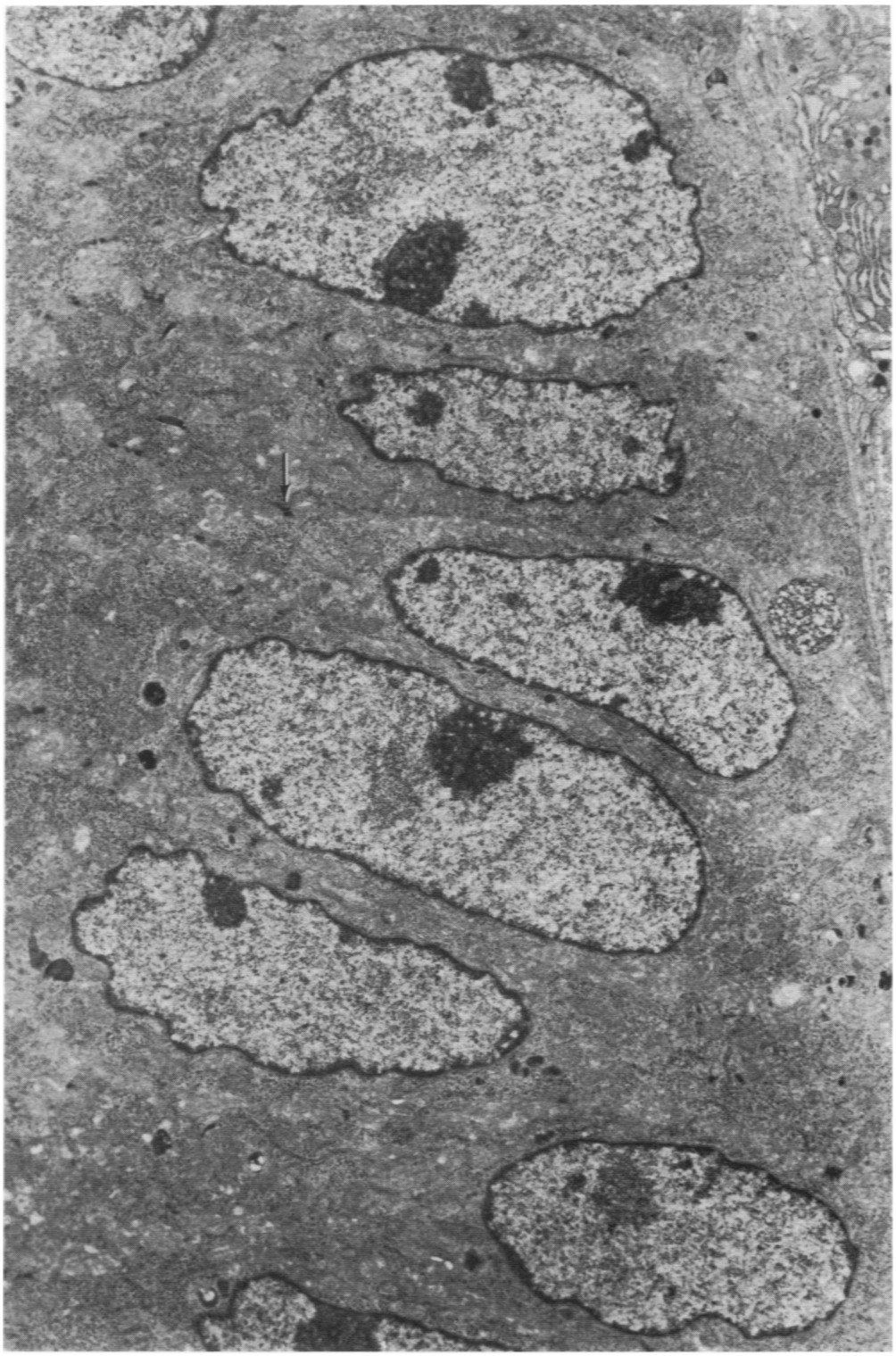
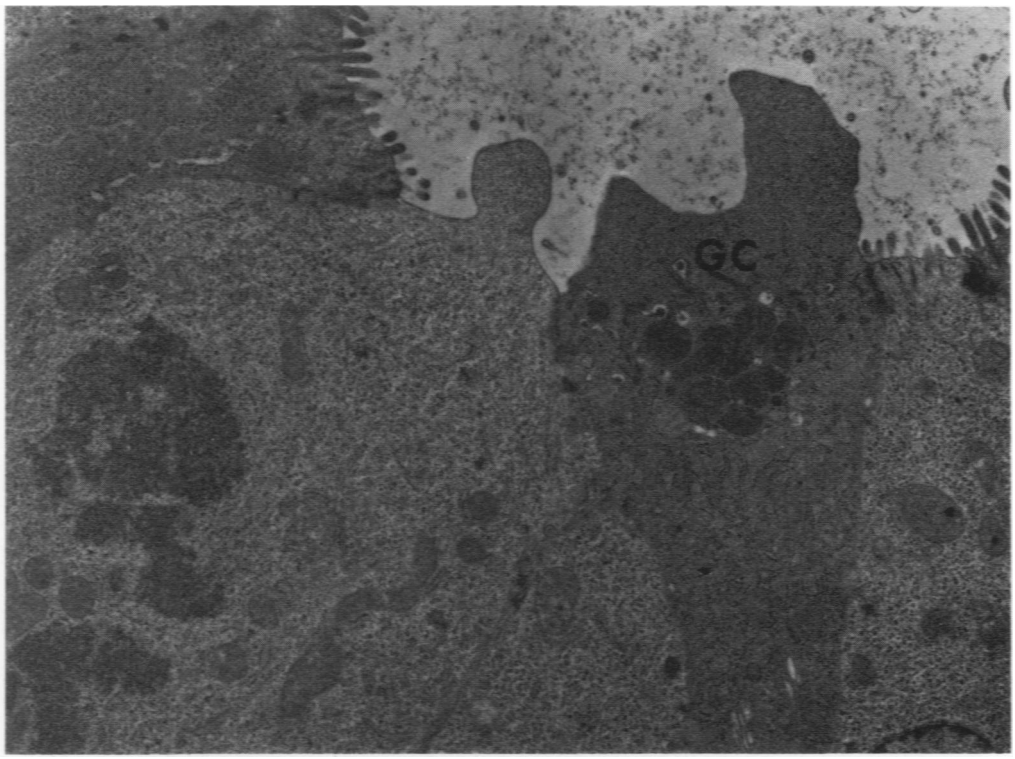


Figure 11—Columnar absorptive cells of an adenocarcinoma, colon. The cells are arranged in regular array. Note the numerous desmosomes (*arrow*). Treated male, 51 weeks old. (× 7400)

12



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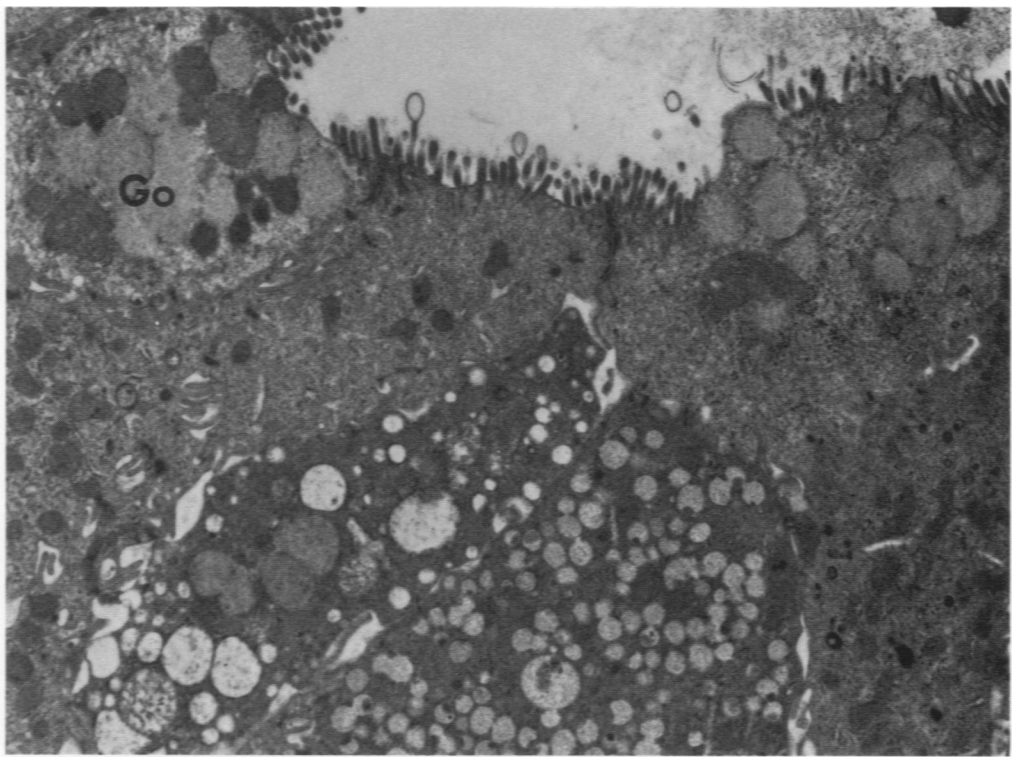

Figure 12—A goblet cell (*GC*) containing typical mucous globules, dense cytoplasm and distended rough-surfaced endoplasmic reticulum lies adjacent to neoplastic absorptive cells and a dividing cell. Treated female, 48 weeks old (× 9000) **Figure 13**—The staining intensity of mucous globules (*Go*) is variable. Two nearby cells contain numerous small and a few large vacuoles filled with lightly stained, finely flocculent to moderately dense material. The latter material appears similar to that found in mucous globules of goblet cells. Treated male, 62 weeks old. (× 9000)