

Histology of Colitis: *Saguinus oedipus oedipus* and Other Marmosets

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In human oncology the association between colitis and cancer of the colon has been well known for years, yet the mechanistic relationship remains unexplained and enigmatic. Many causative hypotheses have been developed and elaborated but remain untested for want of an experimental animal to act as a surrogate for human beings. Scientific factions have arisen composed of researchers who are pro-rodent, pro-clinical patient, pro-genetic model, pro-dietary factors, pro-intestinal chemical carcinogenesis, etc; all are looking for a clue to help explain how this spontaneous colonic malignant disease and its equally unexplained inflammatory precursor arise.

There are many causes of acute colitis in marmosets, most of which are bacterial (ie, *Shigella*, *Salmonella*, *Klebsiella*, and *Pseudomonas*) and susceptible to specific antibiotic therapies. Others, found histologically, are fungal and parasitic, eg, *Candida*, *Giardia*, and *Trichomonas*. Some have been shown to have coronaviruses in the intraglandular exudates [Russell et al, (1) this workshop]. These colitides are acute but recurrent and endemic in most research colonies where space is at a premium and fecal contamination difficult to prevent completely. These enteric infections are also opportunistic, and exacerbation occurs in animals already debilitated by marmoset wasting disease, trauma, starvation, and various neoplasia, including colon cancer. Whether these acute colitides are the antecedent condition leading to chronic colitis or the two entities are of separate etiologies is not known at this point.

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A chronic colitis which ulcerates is a common, almost universal finding in the 11 species and hybrids of the 1584 *Callitrichidae* we have necropsied and studied histologically since 1966. In Table 1 the number of necropsies we have done are tabulated by calendar year by species. The variable numbers reflect largely population changes in species that have comprised the marmoset research colony over the years. For example, up until 1979, *Saguinus fuscicollis illigeri* and related subspecies (the so-called white-lipped tamarins) were the most numerous animals under study; they have been outnumbered by *Saguinus oedipus oedipus* (the cotton-top tamarin) only since 1975, when the *S. f.* subspecies were purposely reduced in numbers to permit an increase in the number of cotton-tops available for study. The table also shows the colony census of live animals by species on April 1, 1984, and the total numbers of each species necropsied. This paper, describing the histology of marmoset colitis, rests principally upon 764 *S. f. illigeri*, 290 *S. f.* subspecies, 352 *S. o. oedipus*, and 138 *Callithrix jacchus* (the common marmoset) necropsy observations.

TECHNIQUES

Complete necropsies were done as soon as possible after death. Only rarely was a dead animal not examined both grossly and microscopically. From 1966 to 1976, all tissues were fixed in Gomori's 1-2-3 fixative, and after 1976 in 10% neutral formalin. Fixed tissues were embedded in paraffin and 4 to 6 μ m sections were cut and routinely stained with PAS-hematoxylin (2). In Table 2 the principal necropsy prosectors are listed chronologically.

The histopathology of colitis in these species resembles that found in human colitis except for the absence of hyperchromatic nuclear dysplastic changes (3). The stages or phases of the marmoset

TABLE 1. NUMBER OF AUTOPSIES PERFORMED PER YEAR, PER SPECIES*

	Year																			Total	Alive after April 1
	'66	'67	'68	'69	'70	'71	'72	'73	'74	'75	'76	'77	'78	'79	'80	'81	'82	'83	'84		
<i>C. goeldii</i>	1	3	2	2	—	—	—	—	—	—	3	—	—	—	—	—	—	—	—	11	0
<i>S. f. fuscicollis</i>	—	—	—	—	1	1	—	—	—	1	—	—	—	—	—	—	1	—	—	4	0
<i>S. f. subsp. hybrid</i>	1	5	5	7	14	21	8	16	20	16	10	8	14	3	11	3	13	4	—	179	22
<i>S. f. illigeri</i>	9	11	26	28	49	63	42	49	74	58	30	14	35	20	17	7	28	25	—	585	52
<i>C. jacchus</i>	—	—	—	—	—	—	—	—	—	15	6	16	26	30	5	17	11	12	—	138	63
<i>S. f. lagonotus</i>	4	4	6	7	12	22	10	13	12	3	2	—	1	—	1	—	1	—	—	98	1
<i>S. f. leucogenys</i>	—	1	1	1	5	8	3	2	1	4	—	2	2	—	—	1	1	—	—	32	0
<i>S. mystax</i>	2	1	1	3	5	3	3	2	—	1	1	2	—	—	—	—	—	—	—	24	0
<i>S. nigricollis</i>	—	—	—	1	2	3	1	6	4	12	11	3	5	1	—	—	1	—	—	50	1
<i>S. f. nigrifrons</i>	2	2	8	7	1	—	—	9	10	9	5	3	7	12	5	3	2	1	—	86	2
<i>S. o. oedipus</i>	2	—	2	4	5	7	12	9	13	45	43	24	57	20	26	24	22	28	9	352	264
<i>C. pygmaea</i>	—	—	—	—	—	1	—	—	—	1	—	—	1	—	—	—	—	—	—	3	0
Species unknown	17	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	22	0
Total	38	32	51	60	94	129	79	106	134	165	111	72	148	86	65	55	80	70	9	1584	405

*Over one month in colony.

disease that are histologically distinguishable are: (1) simple acute colitis, (2) acute (abscessive) ulcerative colitis, (3) reparative (subsiding, or subacute) colitis, (4) chronic colitis, and (5) recurrent acute colitis with persistent chronic colitis.

Simple Acute Colitis. This entity is seen only in young, previously healthy animals and rarely in adult animals dying unexpectedly in the large, roomy, breeding facility where the clinical incidence of acute colitis is minimal. The histology resembles that of so-called, acute, self-limited colitis or acute infectious-type colitis (4). The mucosal changes are typically those of an acute infection: edema, hemorrhage, and leukocytic exudation and infiltration of the lamina propria. Recent ulcerations may be present. Signs of repair and lymphocytic infiltration are minimum. The glands are straight and "test-tube" like. When crypt abscesses are seen, they are accompanied by depletion of mucin production and decreased mitotic activity.

Apparently no animals died or were necropsied in the subacute or resolution phase of this acute process because no specimens were found with this kind

of acute colitis that were accompanied by reparative epithelial hyperplasia and crypt reconstruction.

Acute Ulcerative Colitis with Abscesses. This



Fig 1. Photomicrograph of a typical area of early chronic colitis where submucosal lymphocytic accumulations are prominent. The crypt epithelium is hyperplastic and mucin production is irregular ($\times 25$, PAS stain).

TABLE 2. NECROPSY PROSECTORS MARMOSET RESEARCH CENTER (ORAU)

Name	Service years
G. Humason, MS	1966 to date
C. Lushbaugh, MD	1962–1976
C. Richter, VMD	1976–1981
N. Clapp, DVM	1981 to date

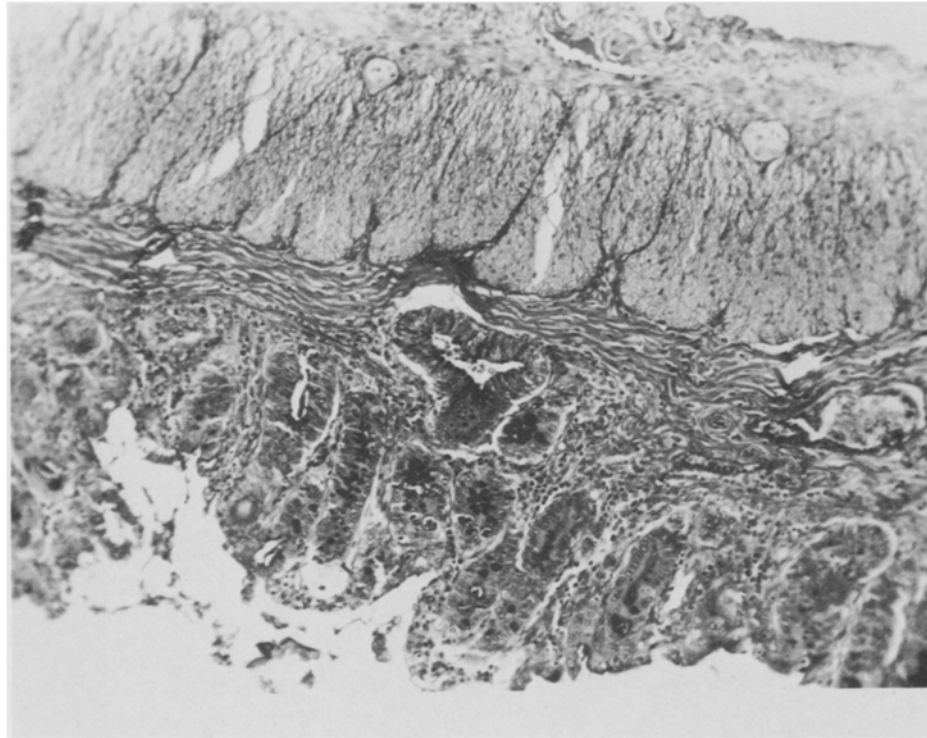


Fig 2. Photomicrograph of partially ulcerated mucosa which shows reparative hyperplasia and lateral budding of the base of a crypt ($\times 240$, PAS stain).

colitis is recognizable histologically by its great variability throughout the colon. It is present in its most florid form in the cecum and ascending colon where the mucosa is edematous, hyperemic, hemorrhagic, and hypercellular. The lamina propria is variably rich in granulocytes, lymphocytes, and plasmacytes. Acute cryptitis, crypt abscesses, and large intramucosal abscesses are present. The acute granulocytic infiltrations of the crypts are accompanied by lysis of the epithelial cells, which are usually hyperplastic and devoid of mucin production. These mucosal changes are often paralleled by the accumulation of large lymphoid masses below the muscularis mucosae in the submucosa. These often reach sufficient size to appear like Peyer's patches.

Subacute Colitis. In the next histologic type of colonic inflammation (subacute colitis), reparative changes are most prominent. The glands show greatly increased mitotic activity, tortuosity, and an increase in the height of the columnar cells. The denuded lamina propria (superficial ulcerations) is covered by new sheets of low cuboidal (squamous) epithelium. The epithelium in the necks of the glands is rich in mucin-producing cells, such that

obstruction of the glandular lumen by dystrophic goblet cells and mucin results. Although mucosal fibroplasia is minimal, it does occur in some areas where the glandular epithelium was lost in the acute phase. In the absence of fibroplasia and collagen deposition in such areas, the lamina propria becomes richly infiltrated by lymphocytes which seem to differentiate in the deeper layer of the mucosa into plasmacytes and reticular macrophages (Figure 1). In the reparative exuberance of the crypts in these areas, budding of their deepest tips occurs, producing boot-shaped deformities. These hyperplastic buds proliferate and penetrate through the muscularis mucosae at vascular clefts (Figure 2 and 3). Here nuclear crowding and pleomorphism are found.

Chronic Ulcerative Colitis. In this phase of the process, the typical histologic picture of chronic ulcerative colitis is produced (5): a greatly thickened mucosa composed of long, irregular glandular crypts that are rich in enlarged, dystrophic goblet cells separated by a densely cellular lymphocytic exudate, plasmacytes, and reticular macrophages. In some cases, this inflammatory response extends through the muscularis mucosae in such volume

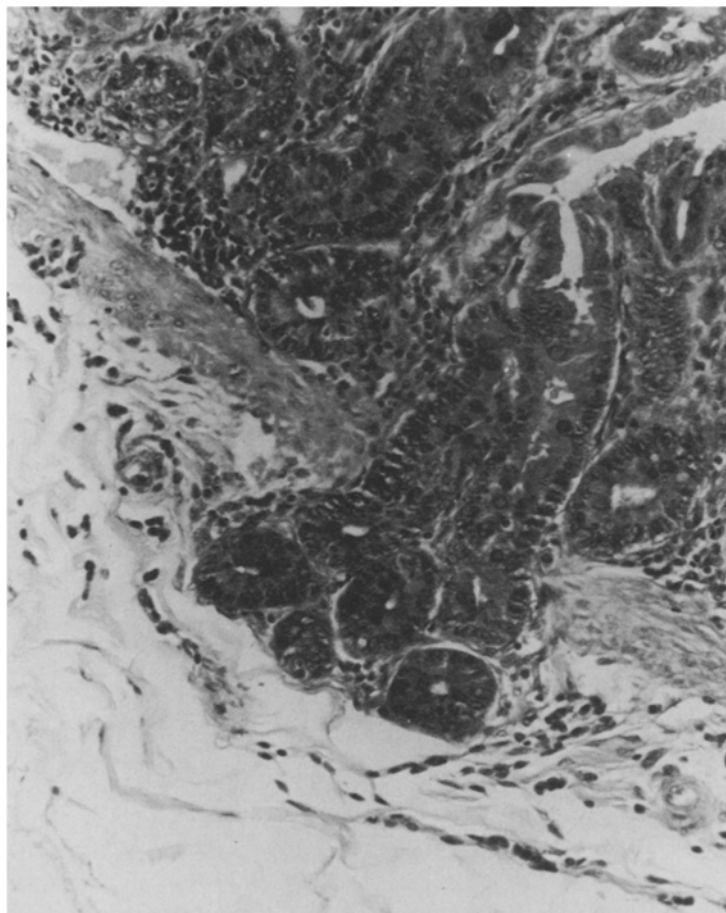


Fig 3. Photomicrograph of early branching hyperplastic glands penetrating into the submucosa through a vascular cleft in the muscularis mucosae ($\times 600$, PAS stain).

that the muscularis appears to have been pushed up into the mucosa. This illusion is heightened by hyperplastic epithelial downgrowths into the submucosa where atrophic epithelial lined spaces are formed in the center of masses of lymphocytes. These spaces can become filled with cellular detritus, inflammatory exudate, and mucin, forming large submucosal structures communicating with the intestinal lumen by wide fistulous ducts (Figure 4).

Recurrent Acute Colitis with Persistent Chronic Colitis. The chronic colitic picture is altered, apparently, by recurrent episodes of acute colitis which superimpose such changes as cryptitis, crypt abscess, mucosal abscess, and ulceration upon the residual chronic changes. At necropsy of aged animals, none have been found with completely normal colons or atrophic, cell-poor, scarred ones; this observation leads us to believe that this chronic

ulcerating colitis does not completely repair or become quiescent in these animals.

SEARCH FOR DYSPLASTIC CHANGES

A question basic to the applicability of this animal model to studies of the etiology of colon cancer is whether, in this ubiquitous chronic colitis, there are cellular changes which can be construed as being preneoplastic (6). Answering this question would seem at first glance not to be too difficult since only one (*S. o. oedipus*) of the three marmoset species examined extensively (over 20 years of observation) has developed colonic carcinoma, whereas all three species have extensive chronic colitis. Our preliminary study of this problem involved reviewing the histopathologic changes found in all animals coming to necropsy over the last 20 years. To structure our search, we leaned heavily upon the recently published

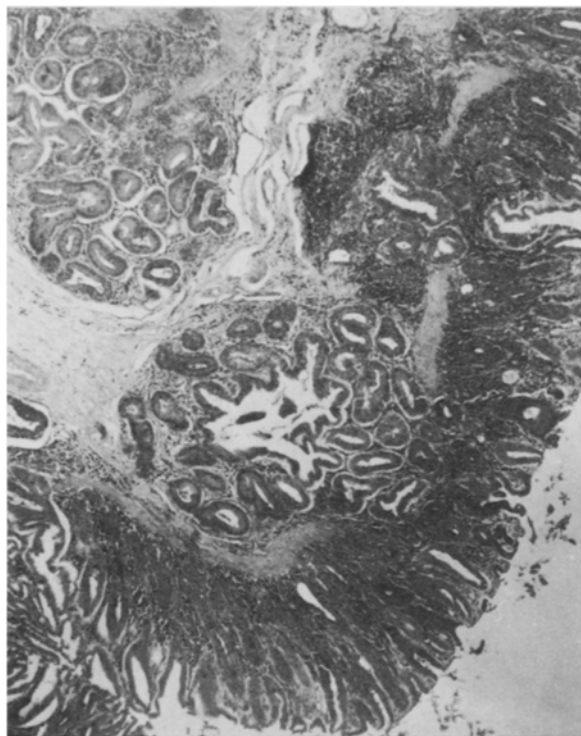


Fig 4. Photomicrograph of an extremely distorted colon wall which shows a chronic colitic mucosa in repair and two large submucosal adenomatous masses of hyperplastic glands, one with a large central excretory duct ($\times 120$, PAS stain).

study of dysplasia in inflammatory bowel disease by the Inflammatory Bowel Disease–Dysplasia Morphology Study Group (3). The biopsy classification of dysplasia in inflammatory bowel disease (Table 1, page 958 in that publication) was used as the guideline throughout our search. Rather quickly it became apparent that “dysplasia” as defined in this study of human biopsy material is not found in entire colons obtained at necropsy of marmosets with chronic colitis even when *in situ* or invasive carcinomas were present. Our modification of the IBD group’s classification schema to include a numerical grade 4 for carcinoma was not a practical solution since the “positive grades 2 and 3 for low- and high-grade dysplasia” could not be used to grade our histologic preparations. Since our material is routinely fixed in formalin which “causes nuclear chromatin to appear relatively condensed” (p. 964, ref. 2), the absence of hyperchromatism in the basal portion of the crypts in marmoset colons cannot be explained as a histologic artifact.

One explanation could be that in marmosets the dysplastic stage identified in human colonic neoplastic transformation is missing. Instead, at a stage

of reparative epithelial hyperplasia marked by cellular crowding, nuclear pleomorphism, and stratification (but no hyperchromatism), neoplastic transformation is present but not in a histologically recognizable form. It is common in marmosets to find *in situ*, poorly differentiated carcinoma cells surrounding hyperplastic crypts and with tubular glands appearing to be embedded in them. Occasionally the basal portions of such hyperplastic crypts show loss of basement membrane and spread of poorly differentiated cells into adjacent lamina propria. Such areas are considered by us to be microscopic, malignant foci or a breakout of already transformed cells.

Another form of this peculiar neoplastic cellular proliferation was recognized by Dr. Yardley (this workshop) studying the microslides of our recent cases of marmosets with colonic adenocarcinoma. This lesion (Figure 5) shows how this abrupt neoplastic transformation can occur intraglandularly even when there is no destruction of the basement membrane of the crypt. It illustrates well the absence in this animal of a dysplastic stage as described in terms of human surgical pathology (3, 6). The cancer cells are seen in this photomicrograph to be budding into the lumen from a low cuboidal epithelium which shows pleomorphic nuclei and little mucin production. The basilar lumen of this crypt contains shed neoplastic cells. The bases of two crypts to the left in the figure show the disarrangement and pleomorphism characteristic of the chronic colitic changes which are associated with this neoplastic transformation. Other glands to the right are more orderly but quite hyperplastic. The noncancerous glandular epithelium in the elongated hyperplastic glands displays extreme dystrophic mucin production. These glands are only slightly tortuous here, but they may be distorted and branching. The edematous stroma of the lamina propria contains slightly increased numbers of lymphocytes but only a few plasmacytes and reticular macrophages. This lesion is present in 10 other sites in this section. Because of this intraluminal origin, this specimen is considered histopathologic proof of the polyclonal nature of colonic carcinogenesis of this species. Unfortunately, it still leaves the pre-neoplastic change unidentified and undescribed.

DISCUSSION

The marmoset colon cancer model would not seem to be readily applicable to studies of the

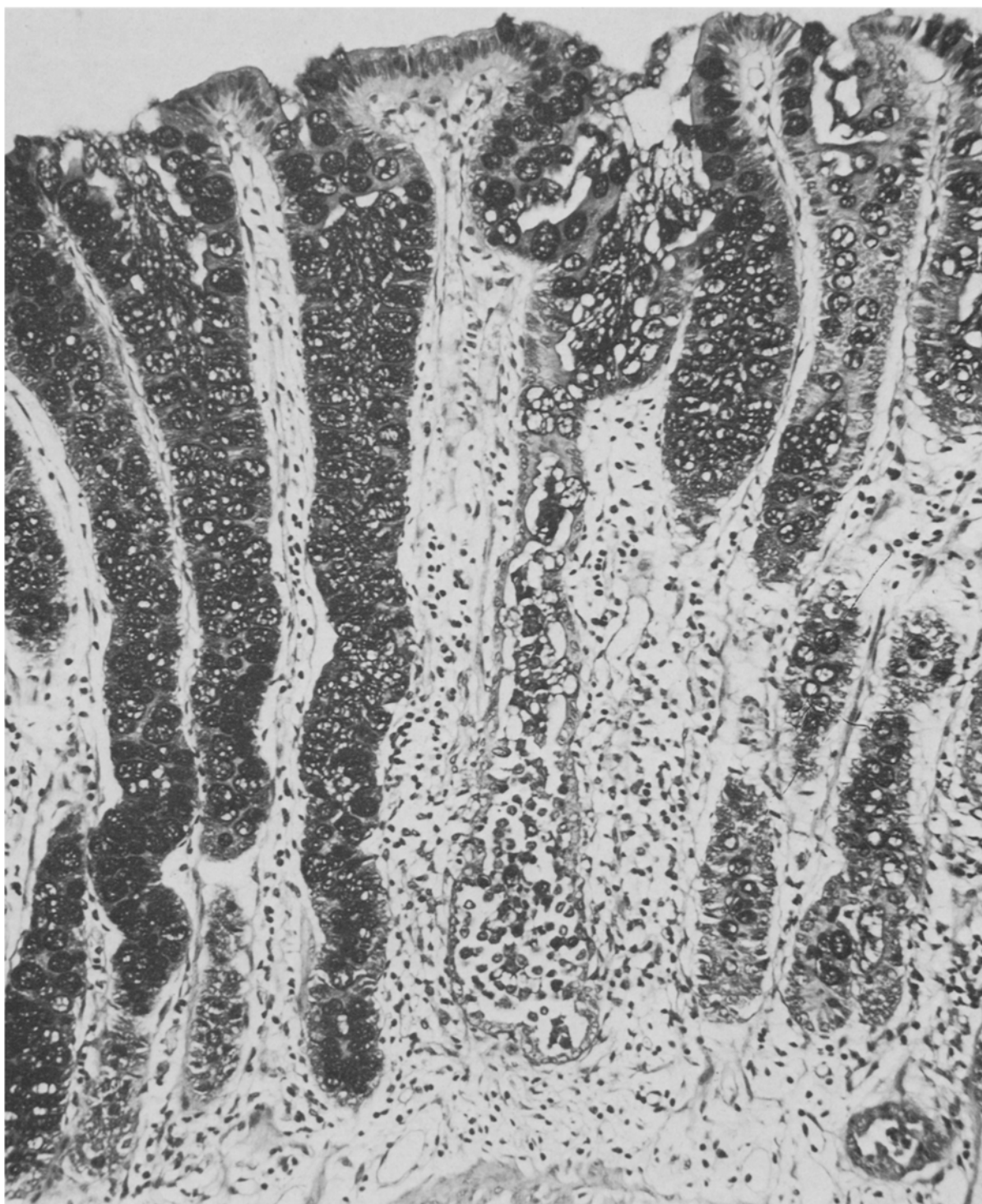


Fig 5. Photomicrograph ($\times 250$) of a PAS-hematoxylin stained histologic section of an area of chronic colitis from a necropsied cotton-topped tamarin in which a carcinoma is to be seen arising within a glandular crypt.

relationships between intestinal polyposis and hyperchromatic dysplastic colitic changes and colon cancer development. It could prove, however,

to be a simplistic model for studying chronic colitis *per se* and its precursor role, if any, in colonic carcinogenesis (5) [Chalifoux (8), this workshop].

HISTOLOGY OF COLITIS IN MARMOSETS

The absences of a precancerous polypoid stage and a histologically demonstrable dysplastic stage are provocative in themselves. Clearly, however, for the marmoset to be used as a model for studying either colonic cancer or chronic colitis, the incidence of spontaneous colitis must be reduced and the disease controlled or prevented; thus normal colon morphology and function could be made the starting point for prospective experimental designs. The increasingly proficient breeding of these animals assures successful outbreeding for genetic studies in the future. New synthetic, easily controllable, appetizing diets, designed especially for these animals, encourage our plan to control these spontaneous colonic diseases not only for the purposes of experimental research but also for the preservation of the cotton-top tamarin, an endangered species [Clapp et al (7), this workshop].

In all of these studies, competent necropsies and histopathologic studies will be necessary to provide guidelines for experimental designing and hypothesis generation.

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