Fig. 5. Transitional mucosa of an adenocarcinoma. Crypts are much longer than in Fig. 1, and goblet cells are taller and distended with mucin. (H & E stain, × 100)

Fig. 6. Transitional mucosa of an adenocarcinoma showing marked widening of crypts. (H & E stain, × 100)
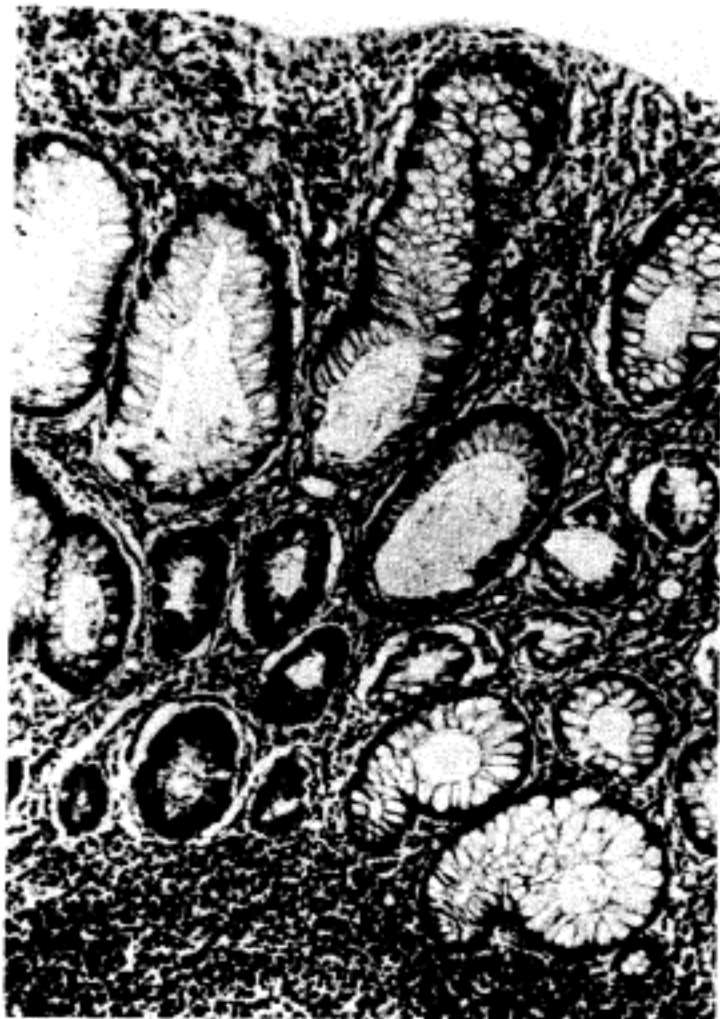

Fig. 7. Transitional mucosa of a cloacogenic carcinoma showing irregular widening and branching of crypts. (H & E stain, × 100)

Table 6. Histochemical changes of the transitional mucosa in various colonic diseases

Histological diagnosis	No. of cases	Histochemical changes	
		Mucin amount	Mucin character
Adenocarcinoma	66	Increased	Nonsulfated
Cloacogenic carcinoma	2	Increased	Nonsulfated
Squamous cell carcinoma	6	Normal	Sulfated
Metastatic carcinoma	4	Normal	Sulfated
Leiomyosarcoma	2	Normal	Sulfated
Malignant lymphoma	2	Decrease	Sulfated
Lymphoid hyperplasia	1	Normal	Sulfated
Tuberculosis	1	Focal decrease	Sulfated
Nonspecific inflammation	8	Decrease	Sulfated

DISCUSSION

The mucosa of normal colon has a histologic pattern of regular corrugation which is composed of parallel crypts. The central ones are tallest and vertical to the muscularis mucosa, whilst their flanking fellows curve slightly outward and are shorter. There are seldom branching. Most of the surface epithelium is composed of absorptive ones, whereas the crypt epithelium is mostly of goblet cells[10]. Both the goblets and absorptive cells originate in the deeper part of the crypts, where nearly all the mitoses are seen, and migrate from there toward the luminal surface. The mucin is mixed sulfated and nonsulfated acid mucopolysaccharides, although the sulfated one is predominant especially at the deeper half of the crypt[8].

Burdette proposed that the mucosa adjacent to a tumor could be histologically normal but still have undergone "premalignant" changes acquiring an increased potential for malignant transformation[19]. Filipe and his colleague[7~10, 13, 16] have reported that when a primary carcinoma develops, there are alterations in the nearby mucosa at least in the chemical composition of the mucin. Nearer the tumor, goblet cells along the crypt and of surface epithelium contain mainly nonsulfated acid mucins usually accompanied by a decrease or absence of sulfomucins, which are predominant in the normal mucosa of the human large intestine[7~10]. In the present study, it

was confirmed that sulfated mucin was decreased at the transitional mucosa adjacent to primary adenocarcinoma, being substituted by sialomucin.

In addition to the histochemical alteration, immunofluorescent studies have also shown that modifications occur in areas around the tumor in carcinoma of the colon[3~6], as have studies with ³H-thymidine[20~25].

By histology, however, widely accepted had been the opinion that a sharp line of demarcation existed between cancers and nearby mucosa without an intervening transitional zone[1~2], until Filipe and Branfoot reported increased depth of crypts with branching and larger goblet cells in the so called transitional mucosa of human colonic carcinoma[10], and experimental studies with dimethylhydrazine (DMH) a known colorectal carcinogen revealed various stages in the process of colorectal carcinogenesis[12~15, 26~30]. We have review the report by Dawson and Filipe, in which they demonstrated an increased number of goblet cells in the crypts of the transitional mucosa accompanied by a decrease in the number of absorptive cells without cytologic atypia[9]. They also noted that the goblet cells were taller and distended with mucin as compared with the normal mucosa. Saffos and Rhatigan described nonpolypoid mucosal changes adjacent to randomly selected carcinoma of the colon and discussed the possible significance[11]. We found the colonic crypts of peritumor area deeper and the number of goblet cells per crypt increased, confirming the previous

reports. Irregular widening and branching of the crypts seen in the transitional mucosa were the other configurational changes which were constantly present although variable in degree. The present study demonstrated that the number of goblet cells per unit lengh of crypt was increased accompanying over-production of mucosubstances, predominantly sialomucin, in primary adenocarcinoma and cloacogenic carcinoma, and that when the increase of crypt depth was less marked, branching or widening was more profound. These findings indicate that the increases in depth, width and/or branching of crypts reflect goblet cell hyperplasia with initial over-production of mucosubstances, which may in turn progress to dysplasia.

Still controversial is whether the histological and histochemical changes of the transitional mucosa is attributable to a response to a stimulus from which colonic carcinoma may develop, or to a response of the colonic mucosa to any irritating influence including the presence of a carcinoma. Regarding the change of mucin character, there is evidence that it may be primary rather than secondary. Filipe and Branfoot noted that similar qualitative changes of the epithelial glycoproteins had been found in human large intestine not only in the mucosa around the carcinoma but also in patches of normal mucosa at various distances from the tumor[10]. Studies on experimental animals in which colorectal carcinoma was induced by DMH further support this. The areas of mild or moderate dysplasia, which were induced by DMH administration before frank malignant lesion developed, showed marked changes in the composition of the goblet cell mucin, which were characterized by the predominance of sialomucins and accompanied by a decrease or absence of sulfated material[13, 15]. It can be concluded that DMH carcinogenesis may involve two steps: 1) an initial increase in the number of goblet cells leading to an enlarged population in which sulfomucin still predominate, 2) an eventual transformation of at least some of the cells of the enlarged population accompanied by progressive predominance of sialomucin[13, 31]. Studies with ^{35}S, in fact, showed decreased isotope uptake in the nonneoplastic mucosa of rats treated with DMH[32] and also in the mucosa adjacent to colorectal carcinoma in human[33]. The predominance of sialomucin and reduction of sulfated mucin which have been demonstrated in the goblet cells of mucosa around carcinoma, resembles the mucus secretion pattern of human fetal gut[34,35]. There have been many descriptions of tumor cells possessing features of fetal cells or capable of producing fetal proteins which can be detected in sera of cancer patients[3,4,36-9]. The production of these embryonic antigen in adult cells following malignant transformation may represent a loss of suppressor genes and a regression of the cell to a more embryonic state[38,40].

Of the various colonic lesions included in this study, all the histological and histochemical changes described above were found only in primary colorectal adenocarcinoma and anorectal cloacogenic carcinoma. This result is quite to the contrary to the opinion that colon mucosa responds to various forms of extrinsic injury in a uniform manner[41], even supporting the view that mucosal changes of transitional zone is primary and not secondary to carcinoma.

The significance that the left colonic control mucosa have deeper and wider crypts and less goblet cells per unit crypt then the right colon is yet to be detemined.

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=국문초록=

원발성 대장암의 주변점막에 관한 조직계측 및 조직 화학적 연구

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원발성 대장선암의 조직발생기원을 추구하기 위하여 지난 10여년 간 암병변 주위점막의 변화에 관한 연구가 진행되어 왔으며, 현재까지 기술된 변화들은 crypt에 구조적 변화가 초래된다는 것과 대장점액이 sulfomucin에서 sialomucin으로 바뀐다는 것으로 요약할 수 있다. 실험동물에 DMH를 투여하면 인체 대장암 주위점막에서 볼 수 있는 변화와 유사한 과정을 거쳐서 암이 발생하며, 특히 점액성분의 변화는 일정 시기의 태아에서와 같다는 보고들로 미루어 볼 때 이러한 주위점막 변화는 대장암의 전암병변일 가능성이 매우 짙으나 아직도 대장암에 의한 2차적변화일 가능성이 완전히 배제되지 않고 있다.

이에 저자들은 원발성 대장선암 66예의 주위점막에 대하여 광학현미경적 조직계측과 aldehyde fuchsin-alcian blue(pH 2.5) 및 high iron diamine(HID)-alcian blue(pH 2.5)를 이용한 조직점액성분변화를 조사하고, 이를 cloacogenic carcinoma, anorectal squamous cell carcinoma, 자궁경부로 부터의 전이암, 육종 및 장결핵을 포함한 양성병변들과 비교하였던 바 다음과 같은 결과를 얻었다.

병변으로 부터 4cm 상부의 점막을 대조점막으로 할 때 원발성 대장선암과 cloacogenic carcinoma에서는 주위점막(TM)의 crypt깊이가 대조점막(CM)보다 각각 1.71배 및 2.12배, crypt직경이 각각 2.17배 및 2.99배 증가하였으나 기타 질환에서는 TM과 CM 간에 뚜렷한 차이를 보이지 않았다. Crypt의 분지도 원발성 선암 66예 중 62예와 cloacogenic carcinoma 2예 모두에서 관찰되었으나 그 이외의 질환에서는 염증성질환 8예 중 1예에서만 볼 수 있었다. 원발성 선암의 경우 TM의 goblet cell수는 CM에 비하여 1.42배 증가되어 있었다. 원발성 선암과 cloacogenic carcinoma의 TM은 전례에서 sulfomucin의 감소와 더불어 sialomucin의 증가가 관찰되었으나 그외의 병변에서는 이러한 점액성분의 변화를 볼 수 없었다. 이상의 결과로 보아 대장선암 TM의 crypt구조 및 점액성분 변화는 원발성 대장상피암의 전암병변으로 사료되었으며 원발성 대장암의 조직기원에 있어서 가장 먼저 초래되는 변화는 sialomucin을 함유한 goblet cell 증식이고 이로 인하여 crypt의 구조적 변화가 야기되며, 세포학적 이형성 및 점액의 감소는 그후에 초래되는 암형성의 과정이라고 추측되었다.

Morphometric and Histochemical Studies on the Mucosa adjacent to Primary Carcinomas of the Colon and Rectum*

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INTRODUCTION

Studies on the peritumor area in carcinoma of the colon had been neglected until the early 1960's and it had been known that there was no singnificant or predictable change in mucosa adjacent to colon cancer[1,2]. Nevertheless several workers have continued investigations in this field with immunofluorescence[3~6] and with histochemical methods[7,8], providing that some modifications in mucin composition occur in areas around the tumor in carcinoma of the colon.

In fact, it was shown that the histologically normal mucosa nearby the tumor of large intestine is often histochemically abnormal, i.e. the transitional mucosa, as it had been named by Filipe, is characterized by an increase of sialomucins, usually accompanied by a decrease or absence of sulfomucins, which are predominant in the normal mucosa of the large intestine[9,10].

Recently Saffos and Rhatigan revealed light microscopic alterations in the number of goblet cells and the depth and shape of the crypts of the transitional mucosa[11]. The light microscopic changes found in experimental animals following administration of known carcinogen are similar to those reported by Saffos and Rhatigan[12-15], giving further morphological evidence that the alterations of the transitional mucosa might be an early stage of colonic carcinogenesis. However the possibility that the light microscopical and histochemical

changes may be secondary to the tumor growth still can not be discarded with certainty.

The present study is to clarify this issue and give aid for the early diagnosis of carcinoma of the large intestine.

MATERIALS AND METHODS

The materials consisted of 92 specimens of large intestine, which were surgically resected due to various large bowel diseases during the period from April 1978 to March 1980.

The large bowels were opened immediately, cleaned and fixed for 24 hours in 10% neutral formalin containing 2% calcium acetate. After fixation, sections were made to include part of the lesion and the wall of proximal 5cm from the lesion with a width of 0.5cm. Strips of mucosa and muscularis mucosa were coiled up in "swiss-roll" which were dehydrated with graded alcohol and embedded in paraffin. Sections were made in 5 micron thickness and were stained with hematoxylin-eosin for routine histologic and morphometric studies, and with aldehyde fuchsin-alcian blue (pH 2.5)[17] and high iron diamine-alcian blue (pH 2.5)[18], with which sulfomucins can be differentiated from sialomucins and neutral mucopolysaccharides.

The histological diagnoses of the colorectal lesions were primary adenocarcinoma in 66 cases, cloacogenic carcinoma in 2, primary anorectal squamous cell carcinoma in 6, metastatic squamous cell carcinoma from the uterine cervix in 4, sarcomas in 4 and benign conditions in 10 (Table 1).

The mucosa within 2cm from the edge of the lesion was considered as transitional mucosa, and that more than

4cm apart as control mucosa.

Examinations were performed concerning the followings:

1) Morphometric changes of the transitional mucosa

  (1) measurement of the crypt depth

  (2) measurement of the crypt width

  (3) branching of the crypts

  (4) changes of the number of goblet cells

2) Histochemical changes of the transitional mucosa

Measurement of the crypt depth was made only on those cut through their whole length and showing the lumen throughout; in most sections 5 such crypts could be measured and the mean value calculated. The same crypts were used for the goblet cell (the number of goblet cells per 100u).

RESULTS

1) Morphometric changes of the transitional mucosa

(a) Measurement of the crypt depth

In primary adenocarcinoma the transitional mucosa showed an apparent increase in depth of the crypts. It was 540-1,440 u in comparison with that of control mucosa,

Table 1. Histological diagnoses of the materials

Adenocarcinoma	66
of the left colon	50
of the right colon	16
Cloacogenic carcinoma	2
Squamous cell carcinoma	6
Metastatic carcinoma	4
Leiomyosarcoma	2
Malignant lymphoma	2
Lymphoid hyperplasia	1
Tuberculosis	1
Nonspecific inflammation	8

where it was 400-720 u. None of the control mucosa had crypts deeper than 720 u and crypts of the transitional mucosa were shorter than 720 u in only five cases. The ratio of crypt depth of transitional mucosa to that of control (TM/CM) was 1.71, which proved to be significant ($p < 0.005$) (Table 2).

In cloacogenic carcinoma arising at anorectal mucosa, the TM/CM of crypt depth was 2.12 ($p < 0.005$).

The transitional mucosa of squamous cell carcinoma primary at the anorectal area was not different from control mucosa. Mural lesions such as leiomyosarcoma,

Table 2. The crypt depth of transitional mucosa in various colonic diseases(u)

Histological diagnosis	No. of cases	Crypt depth		
		CM	TM	TM/CM
Adenocarcinoma	66	539±19	921±44	1.71±0.09*
of the left colon	50	561±18	941±46	1.68±0.07
of the right colon	16	472±17	859±22	1.82±0.05
Cloacogenic carcinoma	2	520±11	1100±99	2.12±0.21*
Squamous cell carcinoma	6	507±20	467±17	0.92±0.05
Metastatic carcinoma	4	523±16	636±56	1.22±0.12
Leiomyosarcoma	2	490±21	490±11	1.00±0.05
Malignant lymphoma	2	470± 7	505±19	1.07±0.08
Lymphoid hyperplasia	1	—	470±11	—
Tuberculosis	1	490±19	400± 6	0.82±0.04
Nonspecific inflammation	8	—	517±29	—

± standard error

* $p < 0.005$

malignant lymphoma, lymphoid hyperplasia and metastatic carcinoma, as well as nonspecific inflammatory bowel diseases did not induce significant changes in the depth of crypts. In tuberculosis the transitional mucosa was rather compressed resulting in reversed TM/CM.

The depth of crypt in control mucosa of the left colon was different from the right one. In adenocarcinoma, they were 561 and 472 u respectively (p < 0.025). In leiomyosarcoma, malignant lymphoma, lymphoid hyperplasia and tuberculosis, all of which involved the right colon, the crypt depths of control mucosae were 470-490 u, whereas in lesions involving the left colon they were 507-523 u.

(b) Measurement of the crypt width

When measured from one basement membrane of a crypt to the opposite, the crypt diameter of transitional mucosa was remarkably increased in primary adenocarcinoma and cloacogenic carcinoma (p < 0.005), sometimes as much as three folds wider as in the cases of cloacogenic carcinoma (Table 3). When the increase of crypt depth was less marked, this type of configurational change tended to be more prominent. There was no remarkable difference in the crypt width between control and transitional mucosa of various diseases other than those of primary adenocarcinoma and cloacogenic carcinoma.

Table 3. Crypt width of transitional mucosa in various colonic diseases(u)

Histological diagnosis	No. of cases	Crypt width		
		TM	CM	TM/CM
Adenocarcinoma	66	72±16	156±39	2.17±0.14*
Cloacogenic carcinoma	2	58±11	173±57	2.99±0.23*
Squamous cell carcinoma	6	61± 4	59± 4	0.94±0.05
Metastatic carcinoma	4	58± 9	73± 6	1.26±0.13
Leiomyosarcoma	2	66±12	66±11	1.00±0.06
Malignant lymphoma	2	58± 4	65±11	1.12±0.10
Lymphoid hyperplasia	1	—	55± 4	—
Tuberculosis	1	53± 2	51± 4	0.96±0.03
Nonspecific inflammation	8	—	66±16	—

± standard error

* p < 0.005

Table 4. Branching of the crypts of transitional mucosa in various colonic diseases

Histological diagnosis	No. of cases	Degree of branching	Remark
Adenocarcinoma	66	+ − + + + +	− in 4/66
Cloacogenic carcinoma	2	+ +	
Squamous cell carcinoma	6	−	
Metastatic carcinoma	4	+	focal
Leiomyosarcoma	2	−	
Malignant lymphoma	2	−	
Lymphoid hyperplasia	1	−	
Tuberculosis	1	−	
Nonspecific inflammation	8	−	+ + in 1/8

(c) Branching of the crypts

Although variable in degree, branching of the crypts was found in most of primary adenocarcinoma and cloacogenic carcinoma. At transitional mucosa the crypts frequently branched into one, two or occasionally more tributaries (Table 4). This type of crypt change was also found in metastatic carcinoma and in some nonspecific inflammatory diseases, but was focal and mild.

(d) Changes of the number of goblet cells

The total number of cells lining the crypts of transitional mucosa increased, but cytological atypia and pseudostratification were found in only a few cases, especially at the mucosa very closely approximate to the tumor. Rather universal cellular change of the transitional mucosa happened to the goblet cells. In cases of primary adenocarcinomas the goblet cell number of transitional and control mucosa was counted.

In control mucosa, the appearance of goblet cells at the deeper parts of the crypts was quite different from that of the upper parts. Those of the deeper third had basal oval nuclei and only a very small spherical collection of mucin in the apical parts of the cytoplasms. At about the mid third of the crypts, the mucin content of the cells increased markedly and they assumed the classical goblet forms, although the number of goblets tended to decrease. Nearer the opening of the crypts the goblets became further reduced in number, and in the surface epithelium they were very few. The goblet cell distribution of the transitional mucosa was roughly similar to that seen in the control mucosa. The size of goblet cells, however, seemed taller, and when compared with the control mucosa, more numerous goblets were found at mid portion of the crypts. The average number of goblet cells of the crypt was 21.2 per 100 u crypt in the transitional mucosa, which was comparable with that of control mucosa (14.9/100 u). The increase of goblet cell number was proved significant by the t-test ($p < 0.005$) (Table 5). The difference in number of goblet cells between mucosa of the left and the right colon was not statistically significant. The goblet cell number tended to increase accompanied by the widening rather than the deepending of the crypt.

2) Histochemical changes of the transitional mucosa

There were two main types of mucosubstances in the goblet cells along the crypt, the sulfomucin and the sialomucin. Their histochemical characteristics could be briefly summarized as follows.

Sulfomucin stained purple to violet with aldehyde fuchsin-alcian blue (pH 2.5), and deep brown with high iron diamine-alcian blue (pH 2.5). Nonsulfated acid mucopolysaccharide (sialomucin) had an affinity to alcian blue at pH 2.5.

In the control mucosa the goblets of the lower two thirds of crypt contained mainly sulfomucins, whereas in the upper crypt and surface epithelium, sialomucins might be present.

The mucus pattern found in the mucosa adjacent to primary adenocarcinoma and cloacogenic carcinoma of the large intestine was distinctly differ from that of control mucosa (Table 6). There was a decrease of sulfomucins with a corresponding increase of sialomucins in about 70% of the cases. The decrease of sulfomucin in transitional mucosa was nearly complete in some cases, leaving only a few crypts which were lined by goblet cells still containg sulfomucin. The pattern described was roughly similar in the transitional mucosa of carcinomas arising at the left and the right colon.

In large bowel diseases other than primary adenocarcinoma and cloacogenic carcinoma, transitional mucosa showed no conspicuous histochemical alterations of mucin.

Table 5. Changes of the number of goblet cells in adenocarcinoma (goblet cells per 100 u crypt)

Location of tumor	No. of cases	Control mucosa	Transitional mucosa	TM/CM
Left colon	60	14.5 ± 0.6	20.4 ± 0.7	1.41 ± 0.08
Right colon	16	16.1 ± 1.6	23.9 ± 1.1	1.48 ± 0.10
Total	66	14.9 ± 0.6	21.2 ± 0.6	1.42 ± 0.08*

± standard error, * $p < 0.005$

Fig. 1. Normal rectal mucosa stained deep blue by high iron diamine-alcian blue (pH 2.5) to show predominance of sulfomucin in goblet cells. (×100)

Fig. 2. Transitional mucosa of an adenocarcinoma. High iron diamine-alcian blue (pH 2.5) to show basophilia with alcian blue due to sialomucin. (×40)
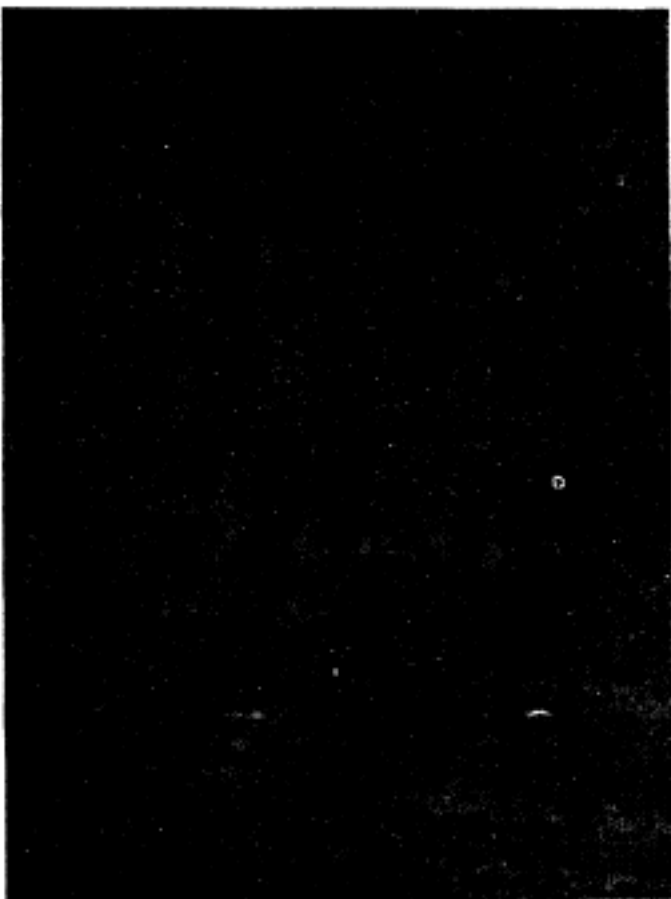

Fig. 3. Normal rectal mucosa showing predominant sulfomucin stained purple to violet by aldehyde fuchsin-alcian blue (pH 2.5) method. (×100)

Fig. 4. Transitional mucosa of a cloacogenic carcinoma showing basophilia by aldehyde fuchsin-alcian blue (pH 2.5) due to sialomucin. Note the increased number of goblet cells in elongated and branching crypts. (×100)