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BIOPSY STUDIES IN ULCERATIVE COLITIS

BY

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[WITH SPECIAL PLATE]

In a previous paper (Truelove, Horler, and Richards, 1955) we described an instrument for making serial biopsy studies of the colonic mucosa. We have continued such studies in ulcerative colitis and in addition have made isolated examinations of a number of other patients, including some not suffering from ulcerative colitis, who constitute a control group. Our main object in the present paper is to present the relationships between the clinical state, sigmoidoscopic appearance, and histological findings.

The method has so far proved entirely harmless. There have been no local complications such as bleeding or spread of infection through the bowel wall; nor has there been any evidence that biopsy examinations, even when repeated several times, have had adverse effects on the course of ulcerative colitis. Biopsy specimens were obtained at 6–8 in. (15–20 cm.) from the anal margin. The specimen consisted of a round piece of colonic mucosa measuring up to 0.4 cm. in diameter. In most cases part of the muscularis mucosae was included in the sample, and sometimes the whole thickness of this layer together with a small portion of submucosa. The tissue was fixed in 10% formol saline. Paraffin-wax sections were stained routinely with haematoxylin and eosin.

Results in Control Group

The control group consisted of 24 patients, as follows: 9 in whom nervous diarrhoea was confidently diagnosed, but without, of course, reference to the histological findings; 9 suffering from anaemia (chiefly iron-deficiency anaemia); and 6 who had undergone sigmoidoscopy to exclude organic disease such as carcinoma of the rectum or lower colon.

In all but two instances the mucosa was normal. The surface epithelium consisted of a single layer of columnar cells among which were a variable number of goblet cells. The glands were straight tubules arranged perpendicularly to the mucosal surface. Most of the cells lining the tubules were goblet cells, but the lower portions of the glands contained undifferentiated cells in mitosis and argentaffin cells. The lamina propria consisted of a fine reticulin network in which there were a few lymphocytes and plasma cells, together with occasional eosinophil leucocytes and macrophages. Neutrophil leucocytes were seldom seen and were never numerous. In a few specimens small aggregates of lymphocytes were present in the lamina propria, and these sometimes extended through the muscularis mucosae. A typical example of a specimen from the control group is shown in Fig. 1 on the Special Plate.

Two specimens showed evidence of increased proliferative activity in the epithelium, together with some increase of lymphocytes in the lamina propria, but the changes were not marked.

Results in Ulcerative Colitis

The biopsy specimens obtained from patients with ulcerative colitis fall into two main classes. A large proportion came from 13 patients on whom we have been making serial studies and all of whom had a minimum of four biopsy examinations. The remaining specimens were obtained by more or less isolated biopsy examinations of a further 29 patients with ulcerative colitis in every stage of severity, from complete remission to grave illness; some of these patients were at the beginning of serial studies when the present series closed. Of the total group of 42 patients with ulcerative colitis, 111 biopsy examinations were made.

In order to study the correlation between the clinical state, sigmoidoscopic appearances, and histological findings we have classified each of these factors in three stages of severity according to the following criteria.

Clinical State

Remission.—One or two bowel actions a day with the passage of normally formed stools and without blood present. No evidence of constitutional disturbance, such as anaemia, low body weight, or raised erythrocyte sedimentation rate (E.S.R.), unless the patient had recently recovered from an acute attack, in which case these factors were all returning towards normal values.

Mild and Moderate Symptoms.—This group included some patients with very slight symptoms, such as the occasional passage of blood per rectum or occasional mild attacks of diarrhoea. At the other extreme were patients with a frank bloody diarrhoea but without severe constitutional disturbances.

Severe Symptoms.—At least six bowel actions a day with gross blood in the stools and with evidence of severe constitutional disturbance such as fever, tachycardia, anaemia, much-raised E.S.R., and falling body weight.

Sigmoidoscopic Appearances

Normal Appearances.

Mild and Moderate Activity.—In its mildest form this included slight changes from the normal, such as mild hyperaemia and granularity or the presence of a few petechiae. More advanced disease showed well-marked hyperaemia and increased fragility of the mucosa, but without these features being as marked as in the severe group. Ulceration was sometimes seen in this group.

Severe Activity.—With the mucosa presenting a picture of acute inflammation. Intense hyperaemia and marked

fragility of the mucosa, with oozing of blood spontaneously or with very slight trauma. Exudate and mucopus commonly seen. Gross ulceration a common feature.

Histological Appearances

No Significant Inflammation.—The mucosa was free from active inflammation and there were no erosions or crypt abscesses. The surface and glandular epithelial cells were intact, but the general architecture of the mucosa was often disturbed, the glands appearing reduced in number and somewhat stunted and atrophic. Oedema and fibrosis of the lamina propria with occasional foci of lymphocytes were found in a few specimens (Special Plate, Fig. 2).

Mild to Moderate Inflammation.—The glandular tubules were irregularly arranged and often showed increased proliferative activity, but the epithelium was usually intact. Oedema, vascular congestion, and interstitial haemorrhage were present in the lamina propria. Lymphocytes, plasma cells, and eosinophil leucocytes were increased in number. Neutrophil leucocytes were often present, but were less numerous than in the more severely affected specimens. Variation in intensity of inflammatory change in the individual specimens gave a range of appearances from relatively quiescent to active inflammation (Plate, Fig. 3).

Severe Inflammation.—The mucosa showed heavy interstitial infiltration by lymphocytes, plasma cells, and eosinophil and neutrophil leucocytes (Plate, Fig. 4). The surface of the mucosa was often irregular owing to oedema, interstitial haemorrhage, or inflammatory exudate in the lamina propria. Small breaches in the epithelium were common, and in some specimens there were frank erosions from which purulent exudate was escaping. Neutrophil and eosinophil leucocytes were seen passing through the damaged epithelium, and areas of flattened and cuboidal cells were found especially in the neighbourhood of erosions. Glandular abnormalities included invasion of the tubules by neutrophil leucocytes, focal degeneration of the epithelium, and shedding of both necrotic and viable cells into the glandular lumina. Crypt abscesses composed of neutrophils, eosinophils, and epithelial debris were found in one-third of the samples (Plate, Fig. 5). In some of the crypt abscesses the wall of the tubule had broken down, usually over a small area, and inflammatory exudate was passing from the tubule into the lamina propria.

(It will be obvious that this system of grading depends to a large extent on subjective judgments. It is therefore worth mentioning that patients have been categorized in respect of clinical state and sigmoidoscopic appearances by one of us (S. C. T.), while the biopsy specimens have been classified by the other (W. C. D. R.) without reference to the clinical notes. In our subsequent joint examination of the data we have been careful to leave our original gradings unaltered.)

Relationships Between the Three Factors

Tables I, II, and III show the relationships between the three factors we have chosen to study. These are also shown in the accompanying graph, where they are expressed as proportions and can therefore be grasped more readily. The sigmoidoscopic findings fail to correlate with the clinical state in one major respect—namely, that more than half the patients in clinical remission show sigmoidoscopic evidence of disease. Clinicians familiar with the course of ulcerative colitis are well aware that sigmoidoscopic improvement may lag behind clinical improvement, but the extent to which sigmoidoscopic evidence of disease may persist, often for many months, is probably not generally realized.

A closely similar picture emerges when the histological findings are compared with the clinical state. Once again the major discrepancy is the finding that more than half the patients in remission show histological evidence of disease on biopsy. In some cases the changes are so marked that they fall into our "severe" group. It is not merely that the histological response falls a little behind clinical improvement when a patient goes into remission, for some of the patients in this group have been in remission for many months and still show unequivocal evidence of a diseased mucosa. Nor is this finding due to the use of cortisone and allied hormones in therapy—a possibility which exists in view of the likelihood that in some diseases cortisone may suppress symptoms while permitting the underlying disease to continue unabated. Table IV, which

TABLE I.—*Relationship Between Clinical State and Sigmoidoscopic Appearances in Ulcerative Colitis*

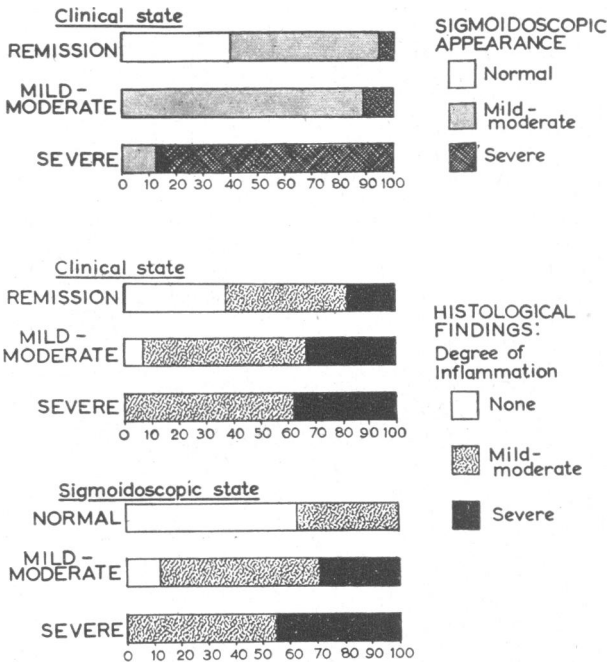
Clinical State	Sigmoidoscopic Appearances			Total
	Normal	Mild–Moderate Inflammation	Severe Inflammation	
Remission ..	16	22	2	40
Mild–Moderate ..	—	49	6	55
Severe	—	2	14	16
Total ..	16	73	22	111

TABLE II.—*Relationship Between Clinical State and Histological Appearances of a Biopsy Specimen in Ulcerative Colitis*

Clinical State	Histological Appearances			Total
	No Inflammation	Mild–Moderate Inflammation	Severe Inflammation	
Remission ..	15	18	7	40
Mild–Moderate ..	4	33	18	55
Severe	—	10	6	16
Total ..	19	61	31	111

TABLE III.—*Relationship Between Sigmoidoscopic Appearances and Histological Appearances of a Biopsy Specimen in Ulcerative Colitis*

Sigmoidoscopic Appearances	Histological Appearances			Total
	No Inflammation	Mild–Moderate Inflammation	Severe Inflammation	
Normal	10	6	—	16
Mild–Moderate ..	9	43	21	73
Severe	—	12	10	22
Total ..	19	61	31	111



Graph showing relationships between clinical state, sigmoidoscopic appearance, and histological appearance in ulcerative colitis.

TABLE IV.—*Histological Findings in Patients in Clinical Remission According to Whether they were Receiving Cortisone Therapy or Not*

	Histological Appearances		
	No Inflammation	Mild–Moderate Inflammation	Severe Inflammation
Receiving cortisone therapy	7	10	4
Not receiving cortisone therapy	8	8	3

sets out the results of biopsy examination according to whether the patient was receiving cortisone therapy or not, shows that this is not a major element in the general picture which we have found.

Among the 71 examinations made when the patients had symptoms of the disease, all but four of the biopsy specimens showed definite mucosal abnormality. The four specimens showing no significant inflammation all came from patients with very mild symptoms and only slight sigmoidoscopic evidence of disease. We do not yet have sufficient evidence to decide whether these findings are due to a patchy distribution of mucosal inflammation in certain mild cases, or whether mild symptoms and sigmoidoscopic evidence of mild activity can persist when the mucosa has become substantially normal.

The best general agreement is found between the sigmoidoscopic picture and the histological findings (Table III and Graph). However, it is interesting that among the 16 specimens taken when the sigmoidoscopic findings were judged to be entirely normal (and in all these cases the patient was also in clinical remission) six showed microscopic evidence of disease. There is nothing surprising in this finding if it is remembered that the mucosa is normally about 0.5 mm. thick, for changes can obviously occur in it which are not visible to the naked eye but evident on microscopical study. The opposite situation occurs in nine specimens showing no significant inflammation, though there was sigmoidoscopic evidence of abnormality. As we have already said, we do not yet know whether this is to be interpreted as evidence of a patchy mucosal change in mild cases of ulcerative colitis, or whether abnormalities may persist in the submucosa (for example, hyperaemia or scarring) and give rise to abnormal sigmoidoscopic appearances even though the mucosa itself is no longer inflamed.

Some light is thrown on these issues when the patients who have been studied by serial biopsy are considered. For example, there have been two patients where a histological relapse has been the prelude to clinical relapse, and we give brief details of the findings below.

Two Examples

Case 1.—A woman with mild but definite symptoms and with severe histological changes was treated with cortisone and went into remission. The histological changes became mild, and remained so on four further examinations at approximately monthly intervals, in the middle of which time the cortisone was stopped. At the next examination, approximately four months after stopping cortisone and six months since she had had any symptoms, there was sigmoidoscopic evidence of mild to moderate activity and the biopsy specimen showed marked inflammation and was classed in our "severe" group. She was in complete remission with normal bowel habits, and with a haemoglobin level of 105% (Haldane) and an E.S.R. of 11 mm. in 1 hour (Westergren). Two weeks later the situation was unaltered. In another two weeks she was passing blood in her stools.

Case 2.—A woman of 20 was treated for her first attack of ulcerative colitis with cortisone and went into complete remission with normal sigmoidoscopic findings and normal histology on three occasions towards the end of treatment. After cortisone was stopped she remained normal in all respects for the next four months. Then, although she was still in complete remission and with normal sigmoidoscopic appearances, the biopsy specimen showed definite inflammatory changes. The next month a mild relapse began, marked by the passage of mucus and a feeling of general malaise and with sigmoidoscopic evidence of mild activity. The following month the symptoms had become worse; she had lost weight and there was more definite sigmoidoscopic evidence of disease.

Discussion

The present study has shown that a small biopsy specimen obtained from just above the recto-sigmoid junction will almost always show active inflammatory changes in patients with ulcerative colitis in a stage of active symptoms. In a control group inflammatory changes were unusual and slight, and notably were not present in ten specimens obtained from nine patients with nervous diarrhoea, although in some of these cases the diarrhoea was pronounced. Moreover, among patients with ulcerative colitis, inflammatory changes were found in the mucosa in more than half of the specimens obtained while the patient was in complete clinical remission, in some instances when the sigmoidoscopic appearances were normal. Although we are primarily interested in the use of colonic biopsy by this particular technique as a research method for studying the mucosal changes by serial examination, it is possible that the special biopsy instrument will find some use in the differential diagnosis of early ulcerative colitis from nervous diarrhoea, as it provides a very safe method of taking a small biopsy. This diagnostic issue is far from being a purely academic problem. In more than 50% of patients with ulcerative colitis the disease begins with the gradual onset of mild symptoms, and these patients have nearly as bad a prognosis as those in whom there is an abrupt onset with severe symptoms (Rice-Oxley and Truelove, 1950). It has also been shown that the ideal patient for cortisone therapy is one in his first attack of the disease while the symptoms are still mild (Truelove and Witts, 1955). Any method which contributes to the earliest possible diagnosis of this dangerous disease is therefore worth consideration.

However, the special use of the method is in the field of serial biopsy, when the ebb and flow of inflammatory changes in the mucosa can be correlated with the clinical course of the disease. We have given two examples of patients in complete remission from ulcerative colitis in whom histological relapse preceded clinical relapse by a period of weeks, but experience is at present too small to judge whether this is a general occurrence. We also do not know how representative a biopsy specimen from the neighbourhood of the recto-sigmoid junction is of the state of the colonic mucosa generally. Although on occasion we have taken a second specimen from higher in the sigmoid colon, up to 12 in. (30 cm.) from the anal margin, we have not made any systematic study along these lines. We have had the opportunity to study a patient with active ulcerative colitis who also had a colostomy, so that specimens were obtained from close to the splenic flexure; they agreed with specimens taken from the recto-sigmoid junction in other patients with the disease.

The biopsy specimens from patients with ulcerative colitis show inflammatory infiltration of the lamina propria with plasma cells and lymphocytes. Eosinophil leucocytes are frequently numerous. In the presence of crypt abscesses and erosions, neutrophil leucocytes are plentiful. It is common to find evidence of epithelial damage and repair in the same specimen. We believe that the crypt abscess plays an important role in ulceration—a view in which we are in agreement with Warren and Sommers (1949), Dukes (1954), and Lumb and Protheroe (1955). In their important study of rectal biopsy specimens Lumb and Protheroe traced the origin of the crypt abscess to a degeneration of the young epithelial cells in the base of the crypt, which is followed by leucocytic infiltration. We have seen lesions similar to those described by these authors, but we are unable to decide whether they represent a primary disturbance or are merely secondary to the inflammatory reaction within the lamina propria.

Summary

Small biopsy specimens of the colonic mucosa from just above the recto-sigmoid junction have been obtained in 111 instances from 42 patients with ulcerative colitis. A group of 24 patients not suffering from ulcerative colitis and with apparently normal mucosa at sigmoidoscopy have been similarly studied as a control group.

Specimens obtained from the control group were all normal apart from two which showed slight histological changes.

Among 71 specimens from patients with ulcerative colitis in the stage of symptoms, 67 showed inflammation. More than half of the 40 specimens taken from ulcerative colitis patients in clinical remission showed inflammation.

Brief details are given of two patients being studied by serial biopsy in whom histological relapse preceded clinical relapse by some weeks.

We are grateful to Miss Shirley Thomas, who assisted at most of the biopsy examinations; to Mr. Richard Salt, who made the biopsy instrument for our use; to Miss M. Rowe for technical assistance; to Mr. M. Morris for the photomicrographs; and to Sister Houle for her active co-operation.

REFERENCES

Dukes, C. E. (1954). *Ann. roy. Coll. Surg. Engl.*, **14**, 389.
Lumb, G., and Protheroe, R. H. B. (1955). *Lancet*, **2**, 1208
Rice-Oxley, J. M., and Truelove, S. C. (1950). Ibid., **1**, 663.
Truelove, S. C., Horler, A. R., and Richards, W. C. D. (1955). *British Medical Journal*, **2**, 1590.
—— and Witts, L. J. (1955). Ibid., **2**, 1041.
Warren, S., and Sommers, S. C. (1949). *Amer. J. Path.*, **25**, 657.

ABNORMAL EPITHELIAL CELLS IN ULCERATIVE COLITIS

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[WITH SPECIAL PLATE]

Cytology is playing an increasing part in the early diagnosis of carcinoma, but has not been much used for the study of non-malignant diseases. We have recently made cytological studies on patients with ulcerative colitis and on other patients not suffering from this disease, who form a useful comparison group. In many instances we can relate the cytological findings to the histological appearances met with in a small biopsy specimen, for the present work was proceeding simultaneously with a biopsy study of ulcerative colitis, although it began a few months later.

Methods

The specimen for study has been obtained under direct vision through a sigmoidoscope, using a special tool for wiping the colonic mucosa which has been made for our use by Mr. Richard Salt, chief technician in the Nuffield Department of Anaesthetics. The tool consists of a long metal rod, with a "perspex" head screwed on to it. The perspex head is rectangular, but with smoothed edges. The two flat faces are grooved in a criss-cross manner, so that the face has a slightly abrasive action when it is drawn across a mucosal surface (Fig. A). The tail end of the instrument is fitted with a metal handle heavy enough to act as a counterbalance when the instrument is in use through a sigmoidoscope, so that it can be used with ease and delicacy.

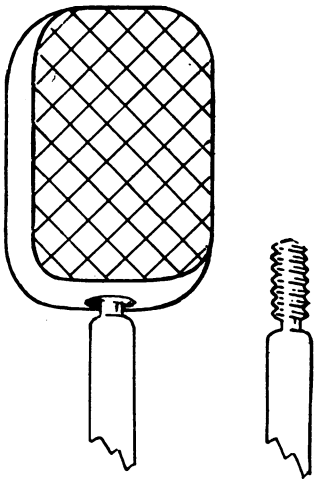


FIG. A.—The "perspex" head of special tool used for taking specimens of colonic epithelium.

When the sigmoidoscope has been inserted, a site for wiping is selected. Whenever possible we have chosen a relatively clean area of mucosa and have wiped it without preparation. When faecal material has prevented this we have first cleaned the bowel by means of the suction irrigator described in a previous paper (Truelove, Horler, and Richards, 1955). If the mucosa is merely coated with exudate, as is frequently the case in patients with ulcerative colitis, we do not irrigate it. We have found that after washing a less satisfactory film is obtained, chiefly because the presence of saline appears to hinder the formation of a good smear. This difficulty can be partly overcome by the use of albumen-coated slides.

When the mucosal surface has been lightly rubbed with the perspex head the instrument is removed from the sigmoidoscope and smears are made on clean glass slides. The flat face of the perspex head makes the spreading of these smears an easy matter. Some of the slides thus smeared are immediately "wet-fixed" in a mixture of ether and absolute alcohol in equal proportions for later staining by Papanicolaou's (1942, 1954) method, which demonstrates the epithelial cells. Other films are rapidly "air-dried" for staining with Romanowsky stains. These slides are useful for the study of any exudate, and particularly for the rapid recognition of eosinophil leucocytes.

Results in Control Group

Twenty-two examinations have been made of 18 patients not suffering from ulcerative colitis or any other organic disease of the gastro-intestinal tract. The specimen obtained in these patients consists of a small amount of mucus, sometimes with faecal debris present, and usually containing scattered clumps of columnar epithelial cells. Goblet cells are sometimes recognized among them. Bacteria are scanty. Occasional squamous epithelial cells are encountered which we think may have been carried up by the sigmoidoscope or may work their own way up the rectum and lower colon from the anal canal.

Results in Ulcerative Colitis

Ninety-four examinations have been made on 31 patients with ulcerative colitis in every stage, from complete remission to grave illness. Those few specimens from patients in complete remission and with normal sigmoidoscopic findings show features similar in every way to those found in the control group. In all other patients the specimen

S. C. TRUELOVE AND W. C. D. RICHARDS: BIOPSY IN ULCERATIVE COLITIS

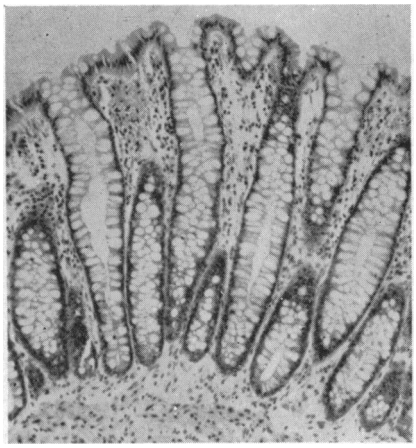


FIG. 1.—Normal colonic mucosa. (H. and E. ×120.)

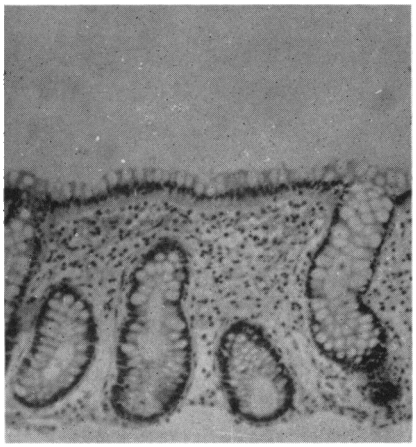


FIG. 2.—Ulcerative colitis: no significant inflammation. (H. and E. ×150.)

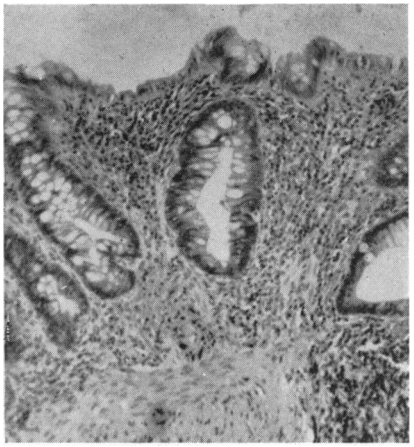


FIG. 3.—Ulcerative colitis: moderate inflammation. (H. and E. ×120.)

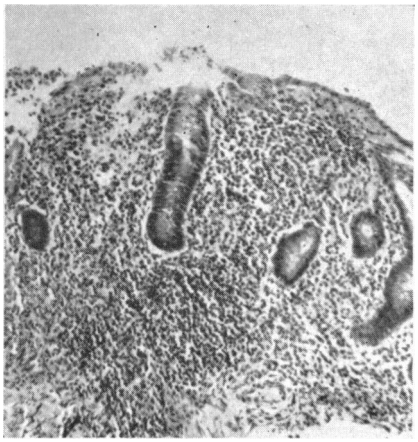


FIG. 4.—Ulcerative colitis: severe inflammation. (H. and E. ×120.)

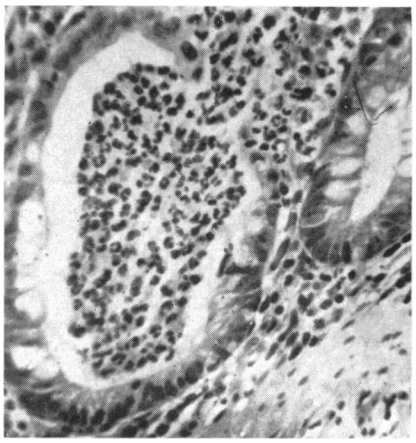


FIG. 5.—Ulcerative colitis: typical crypt abscess. (H. and E ×250.)

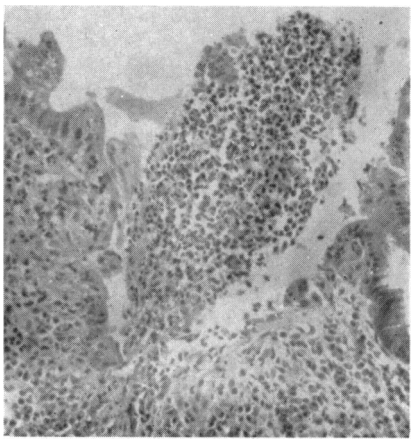
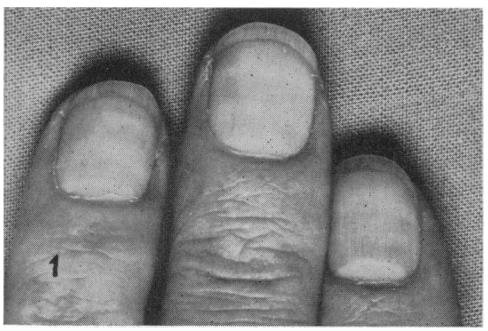


FIG. 6.—Erosion in ulcerative colitis. (H. and E. ×150.)

R. C. MUEHRCKE: FINGER-NAILS IN CHRONIC HYPOALBUMINAEMIA



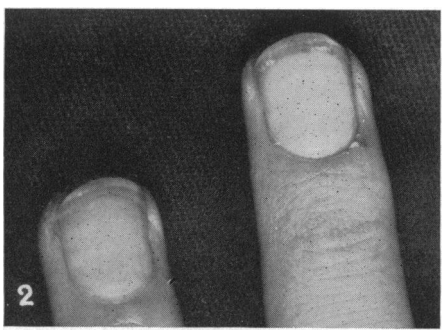


FIG. 1.—Transverse, paired white bands in abnormal finger-nails; 43-year-old male with nephrotic syndrome due to membranous glomerulonephritis. Serum albumin below 1.8 g. per 100 ml. for 26 months.

FIG. 2.—Wide distal white band and narrow proximal band in finger-nails of young adult with chronic hypoalbuminaemia and glomerulonephritis.

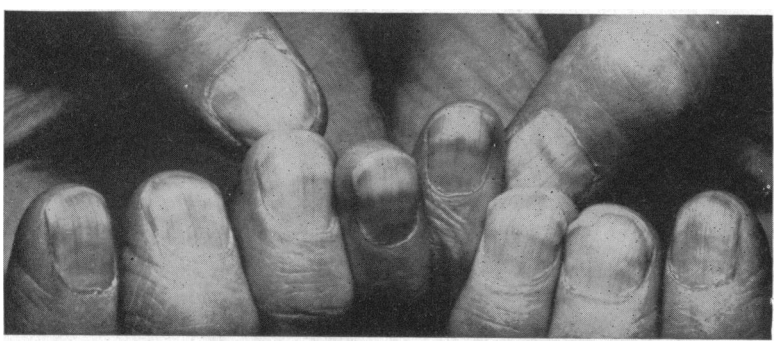


FIG. 3.—Proximal white band separated by line of normal erythema: 62-year-old male with severe hypoalbuminaemia due to membranous glomerulonephritis.

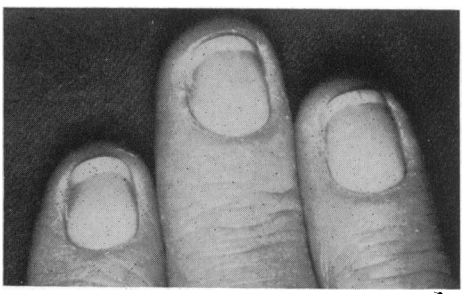


FIG. 4.—Diffusely white opaque nails which replaced paired white bands in a 56-year-old woman who had entered the chronic phase of her illness.