Title: Intestinal content, yeast cells and ulcerative colitis

Author Howard W. Steer

Institution Southampton General Hospital,
Southampton University Hospitals NHS Trust,
University of Southampton School of Medicine,
Southampton, SO16 6YD United Kingdom.

Abstract

The rectal intestinal content and large intestinal mucosa have been examined by transmission electron microscopy and immunohistochemistry in 320 patients of whom 105 have ulcerative colitis. There is a highly significant increase in the number of yeast cells with an intact cell wall in the rectal intestinal content of patients with ulcerative colitis (even those 25 newly diagnosed and untreated patients). There is a highly significant increase in the number of yeast cells lacking a cell wall in the rectal intestinal content of patients not suffering from ulcerative colitis. The persistence of the yeast cell wall is associated with ulcerative colitis. The role of the yeast cell wall, mannan, macrophage mannose receptors and mannanase in ulcerative colitis is explored.

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Ulcerative colitis is an inflammatory condition which affects the large intestine. It is associated with major mucosal inflammation of the colon and rectum. Although detailed analyses of the characteristics of the mucosal inflammation at a cellular and at a molecular level have been made, the cause of ulcerative colitis is unknown. The inability of previous investigations to establish the cause of ulcerative colitis leads to the conclusion that any investigation should start by examining the intestinal content.

1.1 What is the intestinal content?

The gastrointestinal tract is a site of great biological activity. It is the site of:

- material to provide energy;
- material to enable the body to develop, to replace tissues, to allow foetal development to take place and to facilitate the production and secretion of body fluids.

To carry out these functions food/fluids have to be ingested. The food/fluids may be in a state ready to be absorbed, be broken down to a size capable of being absorbed or be broken down and biochemically modified to produce molecules capable of being absorbed.

Many functions of the body assist in fulfilling these actions. Amongst these processes some of the more dramatic (and potentially pathological) occur in the colon and rectum. The food in the colon has already been exposed to a vast array of digestive enzymes but still there is a significant amount of undigested food. In order to assist further food break down there are significant numbers and varieties of bacteria in the colon. It has been estimated that the gastrointestinal tract contain 10^{14} microorganisms.

The intestinal content includes all the material present within the lumen of the intestine.

Investigations of this intestinal content can be challenging. It can involve a variety of scientific methods with the complexity of the ecosystem making the understanding of the findings and the interpretation of the findings difficult. The current investigations began with a morphological examination of the intestinal contents using the transmission electron microscope. The large variety of morphological forms (figure 1) seen in the luminal content made this investigation quite daunting. However, a decision was soon

made that the investigation would concentrate on specific ultrastructural observations namely:

Yeast cells	(www.howardsteer.co.uk/papers/007)
Blastocystis hominis	(www.howardsteer.co.uk/papers/008)

1.2 Material and Methods

The patients involved in this study have been referred to hospital with lower gastrointestinal symptoms and seen in an outpatient clinic or as an inpatient. Following full and informed consent from the patients the investigation of these patients has included a sigmoidoscopy of an unprepared rectum. During this examination faecal samples have been obtained. Ethical approval has been obtained for the study. In addition, mucosal biopsies have been obtained from consenting patients after colo-rectal resections for disease states. Again ethical approval has been obtained for this part of the study.

The mucosal biopsies and the samples of intestinal content have been processed for transmission electron microscopy and for immunohistochemistry.

The transmission electron microscopic study has involved fixing the faecal samples in 3% cacodylate buffered glutaraldehyde (pH 7·3) at 4°C for four to twenty four hours. The faecal samples are then rinsed in cacodylate buffered 10% sucrose (pH 7·3) at 4°C for twenty four hours. Following postfixing in veronal acetate buffered 1% osmium tetroxide (pH 7·3) at 4°C for two hours, the faecal samples are rinsed in chilled tap water at 4°C. Dehydration is carried out in a graded series of ethyl alcohol and the faecal samples are cleared in propylene oxide. The faecal samples are embedded in epoxy resin. Sections are cut 25nm thick and mounted on copper grids prior to being stained with 1% uranyl acetate and Reynolds lead citrate. The sections are examined with a Philips 7000 electron microscope.

Those faecal samples and biopsies used for immunohistochemical studies have been resin embedded by the technique of Britten, Howarth and Roche (1993). Endoscopic biopsies or faecal samples are immediately placed into ice acetone containing 2mM phenyl methyl sulphonyl fluoride and 20mM iodoacetamide and fixed overnight at -20°C. The fixative is replaced with acetone at room temperature for 15 minutes followed by methyl benzoate at room temperature for 15 minutes. The faecal samples and biopsies are then infiltrated with processing solution consisting of 5% methyl benzoate in glycol methacrylate (GMA solution A) at 4°C with three changes of GMA solution A with two hours in each change of solution. The embedding solution consists of 10 millilitres GMA solution A and 70 millilitres benzoyl peroxide. The embedding solution is freshly prepared by dissolving the benzoyl peroxide in solution A by gently shaking. When dissolved add GMA solution B (250µls). The processed biopsies are embedded in the embedding solution, polymerized at 4°C for 48 hours and stored in airtight boxes at -20°C.

The immunohistochemical studies have been performed using the following antibodies:

Antibody	**Clone**	**Source**
Macrophage mannose receptor	mouse monoclonal (19.2)	B. D. Pharmingen
Tumour necrosis factor α	mouse monoclonal (4H31)	Celltech Therapeutics

1.3 The patients

The first patient was recruited on 10th October 1985. Since that time 383 samples of intestinal content have been obtained from 320 patients.

The diagnoses of the patients studied and the numbers of patients in each group are as follows:

Diagnosis		**Number of patients**
Haemorrhoids	(diagnosis 1)	46
Pruritis ani	(diagnosis 2)	24
Chronic anal fissure	(diagnosis 3)	27
Colo-rectal cancer	(diagnosis 4)	83
Colonic adenoma	(diagnosis 5)	35
Ulcerative colitis	(diagnosis 6)	105
Total		***320***

Many fungi have been noted in the gastrointestinal tract but most of the information relating to fungi and the gastrointestinal tract has been from studies of *Candida albicans* and *Saccharomyces* species. *Candida albicans* is found in the normal flora within the mouth and the lower gastrointestinal tract. The presence of *Candida albicans* does not seem to adversely affect normal individuals but in some patients it is known to have an adverse effect which can be extremely serious. The best protection against *Candida* having an adverse effect is the "existence of a normal bacterial flora" (Bernhardt and Knoke 1997).

2.1 Ultrastructure of the colonic yeast cells

Characteristically, the yeast cells seen in the gastrointestinal tract have a cell wall which is outside the yeast cell plasma membrane. This is classically seen in *Candida albicans*. The cell wall is a dynamic structure and is responsible for the mechanical strength of the yeast cell. The constituent molecules of the cell wall are produced by the yeast cell or its plasma membrane. The final assembly of the cell wall is thought to take place extracellularly. The yeast cell wall is vital to the well being of the yeast cell. The cell wall is readily identifiable with the electron microscope (figure 2). The importance of the cell wall, its specific antigenicity (see pages 15) and its biology are such that particular attention has been given to the yeast cell wall in this part of the study. It has been decided to document the following characteristics of yeast cells.

(1) Yeast cells with an intact cell wall (2.1.1)
(2) Yeast cells with a disrupting cell wall (2.1.2)
(3) Yeast cells lacking a cell wall (2.1.3)
(4) Yeast cell wall containing bacteria with minimal/no yeast cell content (2.1.4)
(5) Phagocytosis of yeast cells (2.1.5)

2.1.1 Yeast cells with an intact cell wall

Yeast cells with an intact cell wall are easily identified with the electron microscope. They can appear oval (figure 3) or circular (figure 4) and have a diameter of 3.4µm to 4.6µm. The cell wall is 0.12µm to 0.17µm thick and appears layered. The cell wall may have one or more specialized areas (figure 5) which are considered to be the sites related to previous detachment at the "budding process".

The yeast cell content is variable, is often electron dense (figure 2, 5 and 7) and contains a number of cytoplasmic structures. Some yeast cells have a more electron lucent cell contents (figure 5 and 6).

The bacteria in the intestinal content that surround the yeast cells appear healthy, viable and are in close proximity to the cell wall of the yeast cells (figure 6). The morphology of the bacteria varies. Occasionally the bacteria are in intimate contact with the yeast cell wall (figure 7) with the bacteria sometimes appearing to be attached to the yeast cell wall (figure 8).

2.1.2 Yeast cells with a disrupting cell wall

Some yeast cells in the intestinal content have the ultrastructural characteristics of yeast cells but their cell wall is not intact. The defect in the continuity of the cell wall may be small and localized (figure 9) or there may be larger areas of discontinuity of the cell wall (figure 10). In some yeast cells only a small section of the cell wall remains (figure 11). Frequently, at the sites of disruption of the cell wall bacteria are found (figure 9 and 10). These bacteria are morphologically normal, viable and are sometimes attached to the disrupted end of the cell wall (figure 12). The bacteria related to the disrupting part of the yeast cell wall are approximately 0·4 to 0·5µm in diameter.

The contents of those yeast cells with a disrupted cell wall can be normal (figure 9), may give the appearance of dispersing (figure 10), may be reduced in quantity (figure 11) or may contain normal bacteria. The bacteria in the intestinal content surrounding those yeast cells with a disrupting cell wall are ultrastructurally normal (figure 9, 10 and 12).

2.1.3 Yeast cells lacking a cell wall

The intestinal content has structures which have the ultrastructural characteristics of yeast cells except for the fact that they lack a cell wall (figure 13 and 14). These structures are 3.4µm to 4.7µm in diameter, are frequently electron dense with an internal arrangement which resembles that of yeast cells with an intact cell wall. Some of these structures have a more diffuse appearance (figure 15), may be larger (figure 15) or may be both more diffuse in appearance and larger in size (figure 16). Some of these structures are disrupting (figure 16). These structures are surrounded by bacteria of the intestinal content which are ultrastructurally normal (figure 15 and 16). Some of the surrounding bacteria are in close proximity to these yeast cells which lack a cell wall and some of the bacteria are adherent to the surface of the yeast structure (figure 17).

2.1.4 Yeast cell wall containing bacteria with minimal/no yeast cell content

Sometimes the intestinal content contains structures which are similar to the yeast cell wall but the yeast cell content is dramatically reduced or absent. In stead of the typical yeast cell content these structures contain bacteria (figure 18). Frequently, there are numerous bacteria in these yeast cell wall structures and these bacteria are

ultrastructurally normal. These bacteria do not appear to be "attached" to the inner aspect of the yeast cell wall (figure 19).

2.1.5 Phagocytosis of yeast cells

Polymorphonuclear leucocytes, eosinophils and yeast cells with an intact cell wall can be found in the intestinal content of patients particularly those with ulcerative colitis. Under such circumstances an interaction between the yeast cells and the host cells is sometimes seen. Nonphagocytosed yeast cells can be in close proximity to the exudate cells with extracellular electron dense fibrillar material between the exudates cells and the yeast cells (figure 20).

Some of the yeast cells with an intact cell wall are in the process of being phagocytosed (figure 21) and some have been phagocytosed by polymorphonuclear leucocytes (figure 22). The polymorphonuclear leucocytes are viable and do not have any of the ultrastructural characteristics of necrosis. In addition to the phagocytosed yeast cells these polymorphonuclear leucocytes possess phagocytosed bacteria (figure 22 and 23). The phagocytosed yeast cells can appear ultrastructurally normal (figure 22) or show signs of disintegration (figure 23).

3.1 Patient data and the ultrastructural findings

Each faecal sample has been examined with the transmission electron microscope and specific attention has been given to each of the ultrastructural characteristics for yeast cells outlined on pages 4 to 6. The presence or absence of these criteria has been noted. Data has been recorded on each patient with respect to the following:

Age (4.2.1)
Sex (4.2.2)
Month sample collected (4.2.3)
Diagnosis (4.2.4)
Treatment being given to the patient (page 10)
Presence/absence of the ultrastructural characteristics.

This data has been analysed statistically and for analytical/descriptive convenience the data has been subdivided into:

(1) Yeast cells with an intact cell wall (4.1)
(2) Yeast cells lacking a cell wall (5.1).

4.1 Yeast cells with an intact cell wall

Of the 320 patients which have been involved in this study 83 patients (25.94%) have yeast cells with an intact cell wall. If the characteristics of these patients are analysed the following results are obtained.

4.2.1 Age

The patients have been grouped into 10 year age bands. No children or patients under 18 years of age have been included in the study. The results obtained are shown in Table 1.

Table 1. The number of patients (and percentages) who have yeast cells with an intact cell wall by age.

Age (Years)	Yeast cells with a cell wall		Total
	No	Yes	
≤ 29	13 (61.90%)	8 (38.10%)	21 (100.00%)
30–39	23 (60.53%)	15 (39.47%)	38 (100.00%)
40–49	27 (71.05%)	11 (28.95%)	38 (100.00%)
50–59	51 (72.86%)	19 (27.14%)	70 (100.00%)
60–69	49 (77.78%)	14 (22.22%)	63 (100.00%)
70–79	42 (77.78%)	12 (22.22%)	54 (100.00%)
≥ 80	17 (94.44%)	1 (5.56%)	18 (100.00%)
Total	222 (73.51%)	80 (26.49%)	302 (100.00%)

Output 1. Score test for trend of odds of having yeast cells with an intact cell wall against age.

age (years)	cases	controls	odds	[95% Conf.	Interval]
<=29	8	13	0.61538	0.25507	1.48471
30–39	15	23	0.65217	0.34030	1.24986
40–49	11	27	0.40741	0.20210	0.82130
50–59	19	51	0.37255	0.21999	0.63091
60–69	14	49	0.28571	0.15775	0.51747
70–79	12	42	0.28571	0.15042	0.54269
>=80	1	17	0.05882	0.00783	0.44201

Test of homogeneity (equal odds): chi2(6) = 9.99
Pr>chi2 = 0.1252
Score test for trend of odds: chi2(1) = 8.49
Pr>chi2 = 0.0036

A Chi-squared test of homogeneity of odds has been performed on the age data and it shows the odds are homogeneous ($p = 0{\cdot}13$). A test for linear trend of the log odds against the numerical code used for the age categories (the Score test) has been performed and it shows that there is a trend in the odds against age ($p = 0{\cdot}004$) and it seems that as the age category increases, the odds of having yeast cells with an intact cell wall decreases, hence it is more likely for patients to have yeast cells with an intact cell wall when at a young age than at old age.

4.2.2 Sex

The numbers of patients separated by gender who have yeast cells with an intact cell wall are shown in Table 2.

Table 2. The number of patients (and percentages) who have yeast cells with an intact cell wall by gender.

Gender	Yeast cells with a cell wall		Total
	No	Yes	
Male	144 (73.85%)	51 (26.15%)	195 (100.00%)
Female	93 (74.40%)	32 (25.60%)	125 (100.00%)
Total	237 (74.06%)	83 (25.94%)	320 (100.00%)

Output 2. Odds of having yeast cells with an intact cell wall against gender.

sex	cases	controls	odds	[95% Conf.	Interval]
Male	51	144	0.35417	0.25734	0.48743
Female	32	93	0.34409	0.23026	0.51418

Test of homogeneity (equal odds): chi2(1) = 0.01
Pr>chi2 = 0.9123
Score test for trend of odds: chi2(1) = 0.01
Pr>chi2 = 0.9123

A roughly similar proportion of male and female patients fall into each category. When the odds are analysed both odds appear similar which suggests it is approximately equally likely to have yeast cells with an intact cell wall in either gender.

4.2.3 Time of year stool sample collected – summer or winter

The number of patients who have yeast cells with an intact cell wall related to whether the sample has been collected in the summer (April to September) or in the winter (October to March) are shown in Table 3.

Table 3. The number of patients (and percentages) in the datasets by time of the year sample collected.

Time of the year of collection	**Yeast cells with a cell wall**		**Total**
	No	**Yes**	
Winter	133 (75.14%)	44 (24.86%)	177 (100.00%)
Summer	104 (72.73%)	39 (27.27%)	143 (100.00%)
Total	237 (74.06%)	83 (25.94%)	320 (100.00%)

Output 3. Odds of having yeast cells with an intact cell wall against the time of the year sample collected.

season	cases	controls	odds	[95% Conf.	Interval]
Winter	44	133	0.33083	0.23527	0.46520
Summer	39	104	0.37500	0.25954	0.54182

Test of homogeneity (equal odds): chi2(1) = 0.24
Pr>chi2 = 0.6248
Score test for trend of odds: chi2(1) = 0.24
Pr>chi2 = 0.6248

There appears to be a similar proportion of patients in summer and in winter having yeast cells with an intact cell wall. With the tabulation of the odds, both odds appear to be similar suggesting that it is approximately equally likely to have yeast cells with an intact cell wall both in winter and in summer.

4.2.4 The disease condition of the patient

The numbers of patients who have yeast cells with an intact cell wall against the disease condition of the patient are given in Table 4 and a Chi-square test has been performed to assess the relationship between the appearance of yeast cells with an intact cell wall in the samples and the diagnosis.

Table 4. The number of patients (and percentages) who have yeast cells with an intact cell wall by disease condition.

Diagnosis	Yeast cells with a cell wall		Total
	No	Yes	
1. Haemorrhoids	35 (76.09%)	11 (23.91%)	46 (100.00%)
2. Pruritis Ani	18 (75.00%)	6 (25.00%)	24 (100.00%)
3. Anal Fissure	20 (74.07%)	7 (25.93%)	27 (100.00%)
4. Colo-rectal carcinoma	75 (90.36%)	8 (9.64%)	83 (100.00%)
5. Colonic adenoma	29 (82.86%)	6 (17.14%)	35 (100.00%)
6. Ulcerative colitis	60 (57.14%)	45 (42.86%)	105 (100.00%)
Total	237 (74.06%)	83 (25.94%)	320 (100.00%)

Pearson's Chi-square test: $\chi^2(5) = 28.6440$, **$p < 0.001$**.

The Chi-square test compares the observed numbers of patients having yeast cells with an intact cell wall in table 4 with the numbers to be expected if there is no difference between each disease condition. The greater the difference between the observed and the expected numbers, the larger would be the value of χ^2. The value is 28·6440 which is greater than 11·07, the 5% point of the Chi-squared distribution with five degrees of freedom. Hence, the p value is less than 0·001, which means that the probability is less than 0·1% that such a large observed difference in the percentages of having yeast cells with an intact cell wall could have arisen by chance if there was no real difference between the disease conditions. Thus, there is strong evidence against the null hypothesis of no difference of disease condition on the probability of having yeast cells with an intact cell wall, or in other words, **there is a strong relationship between the appearance of yeast cells with the cell wall intact in the samples and the disease condition.**

A high percentage (42.9%) of patients with ulcerative colitis has yeast cells with an intact cell wall. Could this be due to the treatment given to the patients with ulcerative colitis? A significant number of patients (25) in the ulcerative colitis group have not received any treatment prior to the collection of the faecal sample. An evaluation of these patients who have not received any treatment would answer the question about the influence of treatment on the result. Of these twenty five untreated patients, twelve patients have yeast cells with an intact cell wall which gives a percentage of 48%. This result is higher than the overall percentage found in ulcerative colitis indicating that the incidence of yeast cells with the cell wall intact is higher before the patients have received any treatment. This data on the twenty five untreated patients is from samples taken at the time of diagnosis of the ulcerative colitis. The patients present to their general practitioner and are then referred to Hospital. There is therefore a variable time between the onset of the disease and the time of diagnosis. At the time of diagnosis 48% of the untreated patients have yeast cells with an intact cell wall. One is left wondering what the percentage might be at the time of onset of the disease?

A logistic regression analysis of having yeast cells with the cell wall intact versus disease condition has been performed (Output 4) with the data shown in Table 4. Ulcerative colitis (diagnosis 6) is used as the baseline level for this statistical analysis with the underlying odds ratio as 1.

Output 4. Logistic regression of having yeast cells with an intact cell wall versus disease condition.

Logistic regression			Number of observations	=	320	
			LR chi2(5)	=	29.72	
			Prob > chi2	=	**0.0000**	
Log likelihood = -168.30812			Pseudo R2	=	0.0811	
diagnosis	Odds Ratio	Std. Err.	z	P>|z|	[95% Conf.	Interval]
diag 1	.4190476	.1667627	-2.19	0.029	.1920977	.9141229
diag 2	.4444444	.2271068	-1.59	0.113	.1632532	1.209966
diag 3	.4666667	.2246534	-1.58	0.113	.1816512	1.198879
diag 4	.1422222	.0598724	-4.63	0.000	.0623205	.324567
diag 5	.2758621	.135155	-2.63	0.009	.1055983	.7206546

If the analysis is repeated with adjustment made for age, sex and month faecal sample collected, the results obtained are as shown in Output 4b.

Output 4b. Logistic regression analysis of having yeast cells with an intact cell wall versus disease condition adjusting for age, sex and time sample collected.

Logistic regression			Number of observations	=	302	
			LR chi2(13)	=	41.52	
			Prob > chi2	=	**0.0001**	
Log likelihood = -153.83387			Pseudo R2	=	0.1189	
parameter compared	Odds Ratio	Std. Err.	z	P>|z|	[95% Conf.	Interval]
diag 1	.3242092	.1398825	-2.61	0.009	.1391772	.7552358
diag 2	.3445193	.1856229	-1.98	0.048	.1198385	.9904463
diag 3	.3664179	.1841244	-2.00	0.046	.1368514	.9810793
diag 4	.1094813	.0545044	-4.44	0.000	.0412644	.2904715
diag 5	.2321397	.1222403	-2.77	0.006	.0827033	.6515919

If we consider patients with haemorrhoids (diagnosis 1), the odds ratio is 0.32 meaning that it is 0.32 times more likely (which would imply less likely as it is less than 1) to have yeast cells with an intact cell wall in patients with haemorrhoids than in patients with ulcerative colitis, the 95% confidence intervals are 0.14 and 0.76 which does not include 1, meaning that we are 95% confident that the true odds ratio lies between 0.14 and 0.76. This is statistically significant. If one takes the inverse of these odds ratios, which gives 3.125 ($^1/_{0.32}$) and the 95% confidence intervals of 1.32 ($^1/_{0.755}$), 7.19 ($^1/_{0.139}$),

meaning it is 3.125 times more likely to have yeast cells with an intact cell wall in patients with ulcerative colitis than in patients with haemorrhoids alone with 95% confidence that the true value will fall between 1.32 and 7.19.

If one considers patients with carcinoma of the colon and rectum (diagnosis 4) and again considers the inverse of the odds ratio, which gives 9·09 ($^1/_{0.1095}$) and the 95% confidence intervals become 3·4 ($^1/_{0.29}$) and 24·4 ($^1/_{0.041}$) meaning it is 9·09 times more likely to have yeast cells with an intact cell wall in ulcerative colitis than it is in colorectal cancer with 95% confidence that the true value will lie between 3·4 and 24·4.

If one compares patients with ulcerative colitis against all the non-ulcerative colitis patients, the numbers of patients who have yeast cells with an intact cell wall are shown in Table 5.

Table 5. The number of patients (and percentages) who have yeast cells with an intact cell wall by disease condition.

Diagnosis	Yeast cells with a cell wall		Total
	No	Yes	
Ulcerative colitis	60 (57.14%)	45 (42.86%)	105 (100.00%)
Others	177 (82.33%)	38 (17.67%)	215 (100.00%)
Total	237 (74.06%)	83 (25.94%)	320 (100.00%)

Pearson's Chi-square test: $\chi^2(1) = 23.2893$, **$p < 0.001$**.

The p-value ($p<0.001$) from the Chi-square test shows that there is a strong relationship between the appearance of yeast cells with an intact cell wall in the samples and the dichotomized disease groups.

The logistic regression analysis output from the data based on the dichotomized disease groups (ulcerative colitis and non-ulcerative colitis) is shown in Output 5. Ulcerative colitis is used as the baseline for this statistical analysis.

Output 5. Logistic regression of having yeast cells with an intact cell wall versus disease condition (ulcerative colitis and others)

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Logistic regression                     Number of obs   =        320
                                        LR chi2(1)      =      22.37
                                        Prob > chi2     =     0.0000
Log likelihood = -171.98575             Pseudo R2       =     0.0611
------------------------------------------------------------------------------
Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
------------------------------------------------------------------------------
 .2862524    .076196    -4.70   0.000     .1698917    .4823098
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This reveals that the odds ratio of having yeast cells with the cell wall intact in non-ulcerative colitis patients relative to ulcerative colitis patients is 0·286 with the 95% confidence interval 0·17 and 0·48 which suggests that the odds of having yeast cells with an intact cell wall in non-ulcerative colitis patients is significantly less than that of the ulcerative colitis patients. Conversely, it suggests that the odds of having yeast cells with the cell wall intact in ulcerative colitis is 3.5 ($^1/_{0.286}$) times more likely with 95% confidence interval of 2·07 and 5·89 than in non-ulcerative colitis patients. This effect is greater after

adjusting for age, sex and time of collection of the sample with **the odds being 4.1 times more likely to have yeast cells with the cell wall intact in ulcerative colitis.**

5.1 Yeast cells lacking a cell wall

Of the 320 patients which have been studied there are 211 patients (65·9%) who have yeast cells lacking a cell wall. If the characteristics of these patients are analysed the following results are obtained.

5.1.1 The disease condition of the patient

If the patients are subdivided into the six disease categories (page 4) which have been used in the study, the number of patients (and the percentages of patients) having yeast cells lacking a cell wall are shown in Table 6.

Table 6. The number of patients (and percentages) who have yeast cells lacking a cell wall by disease condition.

Diagnosis	Yeast cells lacking a cell wall		Total
	No	Yes	
1. Haemorrhoids	12 (26.09%)	34 (73.91%)	46 (100.00%)
2. Pruritis Ani	5 (20.83%)	19 (79.17%)	24 (100.00%)
3. Anal Fissure	4 (14.81%)	23 (85.19%)	27 (100.00%)
4. Colo-rectal carcinoma	16 (19.28%)	67 (80.72%)	83 (100.00%)
5. Colonic adenoma	9 (25.71%)	26 (74.29%)	35 (100.00%)
6. Ulcerative colitis	63 (60.00%)	42 (40.00%)	105 (100.00%)
Total	109 (34.06%)	211 (65.94%)	320 (100.00%)

Pearson's Chi-square test: $\chi^2(5) = 48.2423$, **p < 0.001**.

The p-value ($p<0{\cdot}001$) from the Chi-square test shows that there is a strong relationship between the appearance of yeast cells lacking a cell wall in the samples and the disease condition.

If the logistic regression analyses are performed from the data using ulcerative colitis as the baseline with the underlying odds ratio as 1, the results obtained are as follows:

Output 6. Logistic regression analysis of having yeast cells lacking a cell wall versus disease condition

Logistic regression		Number of obs	=	320	
		LR chi2(5)	=	47.90	
		Prob > chi2	=	**0.0000**	
Log likelihood = -181.316		Pseudo R2	=	0.1167	
Odds Ratio	Std. Err.	z	P>|z|	[95% Conf.	Interval]
diag 1 | 4.25	1.659284	3.71	0.000	1.977256	9.135133
diag 2 | 5.7	3.081766	3.22	0.001	1.975439	16.44698
diag 3 | 8.625	4.978203	3.73	0.000	2.782659	26.73364
diag 4 | 6.2812	2.149507	5.37	0.000	3.211858	12.28389
diag 5 | 4.3333	1.88515	3.37	0.001	1.847225	10.1654

The odds ratio indicates that it is 8·63 times more likely with the 95% confidence interval of 2·78 and 26·73, to have yeast cells lacking a cell wall in the faecal samples from patients with a chronic anal fissure (diagnosis 3) than it is relative to patients with ulcerative colitis. This effect is more pronounced with the odds ratio increased to 10·8 with the 95% confidence interval of 3·01 to 39·32 after adjusting for age, sex and time of collection of the sample.

If one compares patients with ulcerative colitis against all other patients, the numbers of patients with yeast cells lacking a cell wall are shown in Table 7.

Table 7. The number of patients (and percentages) who have yeast cells lacking a cell wall by disease condition (ulcerative colitis and non-ulcerative colitis).

Diagnosis	Yeast cells lacking a cell wall		Total
	No	Yes	
Ulcerative colitis	63 (60.00%)	42 (40.00%)	105 (100.00%)
Others	46 (21.40%)	169 (78.60%)	215 (100.00%)
Total	109 (34.06%)	211 (65.94%)	320 (100.00%)

Pearson's Chi-square test: $\chi^2(1) = 46.8110$, **$p < 0.001$**.

The p-value ($p<0·001$) from the Chi-square test above shows that there is a strong relationship between the appearance of yeast cells lacking a cell wall in the samples and the dichotomized disease groups (ulcerative colitis and non-ulcerative colitis).

The logistic regression analysis output from the data based on the dichotomized disease groups (ulcerative colitis and non-ulcerative colitis) is as shown in Output 7. Ulcerative colitis is used as the baseline with the underlying odds ratio as 1.

Output 7. Logistic regression of having yeast cells lacking a cell wall versus disease condition (Ulcerative colitis and non-ulcerative colitis).

```
Logistic regression                     Number of obs   =        320
                                        LR chi2(1)      =      45.96
                                        Prob > chi2     =     0.0000
Log likelihood = -182.28301             Pseudo R2       =     0.1120
------------------------------------------------------------------------------
|Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
------------------------------------------------------------------------------
| 5.5108     1.43005     6.58   0.000     3.313872    9.164412
```

The odds ratio of having yeast cells lacking a cell wall in patients with non-ulcerative colitis conditions relative to ulcerative colitis is 5.51 with the 95% confidence interval of 3.31 to 9.16 which suggests the odds of having yeast cells lacking a cell wall in the non-ulcerative colitis patients is greater by a factor of 5.5 than that of ulcerative colitis patients. This effect becomes stronger after adjusting for age, sex and time of faecal sample collected.

Thus, when patients with ulcerative colitis are compared with non-ulcerative colitis patients, the ulcerative colitis patients are more likely to have yeast cells with a cell wall and less likely to have yeast cells lacking a cell wall than patients without ulcerative colitis.

6.1 Conclusion from the ultrastructural study

These analyses have shown that the cell wall of yeast cells is associated with ulcerative colitis. A considerable amount of research has been carried out on the composition of the cell wall of yeast cells with much of this work related to yeast cells of the *Candida* species. The known presence of *Candida* in the faecal content and the morphological appearance of the yeast cells in the present study makes yeast cells of the *Candida* species ideal candidates for these yeast cells.

Are patients who do not have ulcerative colitis 'protected' from the possibility of developing ulcerative colitis by lacking a yeast cell wall? Does the yeast cell wall have to be removed to prevent predisposition to ulcerative colitis? If this is necessary, is it performed by bacteria producing mannanase or other enzymes? The absence of such bacteria would result in the persistence of the yeast cell wall and the susceptibility to ulcerative colitis. The size of the bacteria intimately related to the disrupting yeast cell wall is approximately 0·4 to 0·5µm in diameter.

7.1 The cell wall of yeast cells

The cell wall of yeast cells is an important as well as physically significant structure. It accounts for upto 30% of the dry weight of yeast cells and has as its main constituents mannoproteins, glucans and chitin. It has been estimated that approximately 20% of the cell wall is mannan. This accounts for about 40% of the total cell wall polysaccharides but this mannan cannot be released simply by using purified mannanases (Farkas,1979). It also requires proteases. The cell wall is necessary for yeast cell growth (Odds,1985). It is the cell wall which is the site of contact of the yeast cell with the environment and the cell wall is a major source of yeast cell antigenicity.

The analyses of the components (mannoproteins, glucans and chitin) of the yeast cell wall have been performed after extraction of these components. The extractions have been performed either chemically or enzymatically. The components of the cell wall have a role in the protection of the yeast cell and in providing structural support. The pathogenicity of *Candida* is dependent upon the external protein layer and in particular the mannoproteins (Tokunaga et al 1990; Calderone et al 1991; Chaffin et al 1998).

The mannoproteins consist of mannose polymers covalently linked to proteins. The biosynthesis of mannoproteins is thought to take place in the yeast cell with the mannoproteins secreted by the cell whereas the structural polysaccharides (glucan and chitin) are synthesized in the vicinity of the plasma membrane and passed extracellularly where the final biochemical formation occurs.

The mannose in the mannoproteins are covalently linked in a number of different ways to form oligosaccharides. These oligosaccharides are linked to the protein. The different *Candida* species show subtle differences in their mannoproteins. These differences affect size, complexity and biochemical linkages of the mannan.

The mannans are found throughout the yeast cell wall and are predominantly in the electron dense areas (Cassone et al 1978; Evron and Drewe 1984; Poulain et al 1978; Takamiya et al 1985; Trouchin et al 1981; Trouchin et al 1984). The cell wall of the yeast cell is composed of numerous layers which are readily seen with the transmission electron microscope. The outer fibrillar layer is composed of mannan or mannoprotein. This outer layer is lost during infection (Reiss et al 1986A; Reiss et al 1986B). The antigenic

potential of the yeast cell wall had been realized (Summers, Grollman and Hasenclever 1964) before the identification of the biochemical cause for the antigenicity. The mannans have been shown to be an important antigenic source (Hasenclever and Mitchell 1964). The lethal potential of mannans has been demonstrated in experiments with mice (Kind, Kaushal and Drury 1972; Nagase et al 1984).

Manno-oligosaccharides which have been released from *Candida ablicans* precipitate antibodies (Shibata et al 1985) and these manno-oligosaccharides are β-1,2 linked residues. This specific antibody response to β-1,2-oligomannosides has been demonstrated in mice (Cassone et al 1988), rats (Hopwood et al 1986), rabbits (Shibata et al 1992) and humans (Poulain et al 1993; Hernando et al 1993).

Glycolipids are by weight a relatively minor component of the yeast cell wall accounting for 1-7% of the dry weight of *Candida* species (Calderone et al 1991). However, these glycolipids are now recognised as being of major immunogenic importance. *Candida albicans* produces a glycolipid (phospholipomannan – PLM). PLM interacts with antibodies which are specific for β-1,2-oligomannosides. It has been demonstrated by nuclear magnetic resonance studies that linear chains of β-1,2-oligomannosides are the major component of PLM (Trinel, Plancke et al 1999).

The macrophage responses to the yeast cells have been considered to be significant. When considering the host response to *Candida albicans* the importance of macrophages can be appreciated from the biochemical interactions with macrophages. PLM has a pivotal role in this macrophage response (Jouault et al 1994; Jouault et al 1998). When such interaction occurs significant amounts of PLM are shed by the yeast cell.

To facilitate this interaction, the adhesion and receptor recognition processes are important. There have been a number of excellent review articles related to adherence and *Candida albicans* (see Calderone and Braun 1991).

When considering *Candida* and adherence it is known that:

(1) Different species of *Candida* have different adherence potentials
(2) *In vitro*, the adherence potential can be influenced by the growth medium being used
(3) Strains which are less adherent tend to be less pathogenic.
(4) The adherence and receptor recognition with respect to infection with *Candida* have been the subject of significant research with much of this research related to the macrophage mannose receptor.

8.1 Macrophage mannose receptor

Mammalian cells rarely have surface lectins or proteins with a terminal mannose whereas in lower organisms such a biochemical occurrence is not infrequent. This is seen in the surface glycoproteins of organisms such as yeast, mycobacteria, parasites and certain viruses (for review, see Ezekowitz et al 1990; Fraser et al 1998; Stahl 1992). In mammals, processes have evolved which enable terminal mannose residues to be recognised. These processes take two forms, namely, mannose receptors and mannose binding protein.

Mannose receptors are a family of lectin binding proteins located on the surface of macrophages. These macrophage mannose receptors engage yeasts (Sung et al 1983) and parasites (Blackwell et al 1985) and result in phagocytosis together with the release of biologically active secretory products such as protease enzymes and certain cytokines.

The ectodomain of the macrophage mannose receptors (that is, the domain protruding externally from the macrophage cell membrane) is distinctive in having a characteristic cysteine-rich N-terminal region, a fibronectin type 11 domain followed by eight carbohydrate recognition domains (CRD) (Taylor et al 1990; Lambeau et al 1994; Ishizaki et al 1994). The carbohydrate recognition domains are the carbohydrate binding domains and of the eight domains at least two bind to mannose (Taylor et al 1992). The macrophage mannose receptors are implicated in the endocytosis of glycoproteins that have a terminal mannose residue. They are thought to be involved in the phagocytosis of *Candida* and in the release of proinflammatory cytokines.

8.1.1 Are there any macrophage mannose receptor changes in the colonic mucosa and intestinal content associated with ulcerative colitis?

Immunohistochemical examination for macrophage mannose receptors in the non-ulcerative colitis mucosa demonstrates the subepithelial connective tissue macrophages (figure 24 and 25). If the intestinal content of the colonis examined in these patients there are significant numbers of bacteria (figure 26 and 27) which are macrophage mannose receptor positive. These bacteria appear to be randomly distributed in the intestinal content. They seem to be mainly coccal or diplococcal in shape.

If the colonic mucosa of patients with ulcerative colitis is examined there is an increase in the macrophage mannose receptor positive subepithelial connective tissue macrophages (figures 28, 29 and 30). These macrophages can be seen intimately related to the sites of epithelial ulceration (figure 30). The intestinal content of patients with ulcerative colitis also contains macrophage mannose receptor positive bacteria (figures 28, 31 and 32) which are apparently randomly distributed.

The ligand binding to macrophage mannose receptors and the cell signalling processes that are initiated by this binding leads to the production of proinflammatory cytokines. These cytokines are cell signalling proteins which are integral to the induction and achievement of the biochemical processes/functions involved with inflammation. Two such proinflammatory cytokines are:

Tumour necrosis factor alpha (TNFα)
Interleukin one beta (IL-1β)

8.2 What is the evidence for ligand binding to the macrophage mannose receptor initiating the release of proinflammatory cytokines?

8.2.1 Production of TNFα

Candida albicans causes human natural killer cells and peripheral blood monocytes to produce TNFα (Djeu et al 1986; Djeu et al 1988). A murine macrophage cell line (ANA-1 cells) produces TNFα in response to *Candida albicans* (Blasi et al 1992). The cause of this effect has been taken a stage further by showing that the mannan of *Candida albicans* induces TNFα production by mouse alveolar macrophages (Garner et al 1994).

The importance of the β-1,2-oligomannosides and the glycolipids of the cell wall of yeast cells has been outlined (page 15). In considering the production of

proinflammatory cytokines it has been demonstrated that phospholipomannan is a strong inducer of TNFα production (Jouault et al 1994; Jouault et al 1995) with the β-1,2-oligomannoside from *Candida albicans* inducing TNFα synthesis through a phosphotyrosine kinase – dependent pathway (Jouault et al 1995).

Tumour necrosis factor α positive bacteria are found in the intestinal content of non-ulcerative colitis patients (figures 33 and 34) as well as in the intestinal content of patients suffering from ulcerative colitis (figures 35 and 36). There is significantly more background staining for tumour necrosis factor α in the intestinal content of patients with ulcerative colitis. In addition, tumour necrosis factor α positive bacteria are found phagocytosed in leucocytes in the intestinal content of the patients with ulcerative colitis (figures 37 and 38).

8.2.2 Production of IL-1

In vitro, it has been shown that *Candida albicans* attachment to macrophages is associated with an increase in IL-1β (Yamamoto et al 1997). This response has been presummed to be the result of macrophage mannose receptor binding by the yeast derived ligand.

Bacteria are very effective and adaptable organisms. They have a profound effect in the recycling of natural products, for instance, the breakdown of plant material. In addition, bacterial action can be manipulated (genetic engineering) to, for instance, lead to insulin production. Can such adaptations occur in the gastrointestinal tract?

9.1 Is there any evidence that the various components of the "internal environment" are interacting with one another?

The answer to this question is yes. The evidence which will be presented in this discussion will be specifically confined to the problem of ulcerative colitis and its aetiology.

Hemicellulose is a significant component of plant material. The hemicellulose consist of a group of several heterogeneous polysaccharides and are the second most abundant polysaccharides in nature. They are frequently associated with cellulose and lignin in plant cell walls. The polysaccharides of hemicellulose are normally named after the main sugar residue in the backbone chain of the molecule, for example, xylans, mannans and galactans.

The breakdown of hardwoods presents a significant challenge to nature. Upto 5% of the major hemicellulose in hardwoods is galactoglucomannan which is hydrolysed by the action of a specific β mannanase enzyme. Investigations of this enzyme group has found that it is distributed in a wide variety of organisms including bacteria, fungi, germinating seeds of land based plants, marine algae and in animals (Dekker and Richards 1976). The hemicellulose group may therefore be potientally hydrolysed by mannanase from a variety of sources but this will depend upon the specificity of the hemicellulose and the specificity of the mannanase enzyme.

The yeast cell wall, in particular that of *Candida albicans*, has as its major components glucans, chitin and mannoproteins. The mannoproteins have a variety of biochemical linkages with the mannose residue chains having as their common linkages α 1,2-mannose, α 1,3-mannose and α 1,6-mannose and β-1,2-oligomannosides.

Mannanase enzymes specifically targetting these biochemical linkages and proteases enzymes (Farkas 1979) result in the breakdown of the mannoprotein and the disintegration of the cell wall. It has already been stated that the integrity of the yeast cell wall is important for yeast cell survival so that its disintegration may lead to the destruction of the yeast cell. There is an ever increasing bacteriological literature on mannanase producing bacteria (see Kataoka and Tokiwa 1998) with the enzyme also being produced by anaerobic bacteria.

In this study, there is ultrastructural evidence for an association between the yeast cell wall and intestinal bacteria (see page 5). This association may take the form of bacteria being in the vicinity of the yeast cell wall, being attached to the yeast cell wall and bacteria associated with the disrupting yeast cell wall.

The macrophage mannose receptor is an important receptor for yeast cell attachment to macrophages and to the activation of these macrophages. There may be many other receptors participating in this process but Is there any evidence for mannose receptors on bacteria? It has been noted that when examining tissue samples for macrophage mannose receptor changes associated with ulcerative colitis a proportion of the bacteria of the intestinal content related to the epithelial surface of the mucosa are also positive for the macrophage mannose receptor.

Can these bacterial mannose receptors be involved in the attachment of bacteria to yeast cells? If the macrophage mannose receptors are involved in the attachment of bacteria to yeast cells it may be anticipated that there would be an aggregation of such bacteria at the yeast cells. There is no immunohistological evidence from the present study for any localised accumulation of such bacteria. It must therefore be concluded that these bacteria have no specific predeliction for the yeast cells. Innumerable other questions come to mind such as Can the bacteria respond to ligand attachment of their mannose receptor by causing signalling events similar to those that occur in macrophages? Can the mannose receptors of bacteria be shed into the intestinal content and act as mannose binding proteins? Do these bacteria produce mannanase?

10.1 Hypothesis

The present work has demonstrated that the yeast cell wall is associated with the development of ulcerative colitis. The persistence of the yeast cell wall and the development of ulcerative colitis is due to the lack of appropriate bacteria necessary for the destruction of the yeast cell wall. It is hypothesised that this enables the events leading to the development of ulcerative colitis to take place and that this situation develops because of fundamental changes in the bacterial flora. As stated on page 4, the best protection against Candida having an adverse effect is the “existence of a normal bacterial flora” (Bernhardt and Knoke 1997).

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Figure 1.

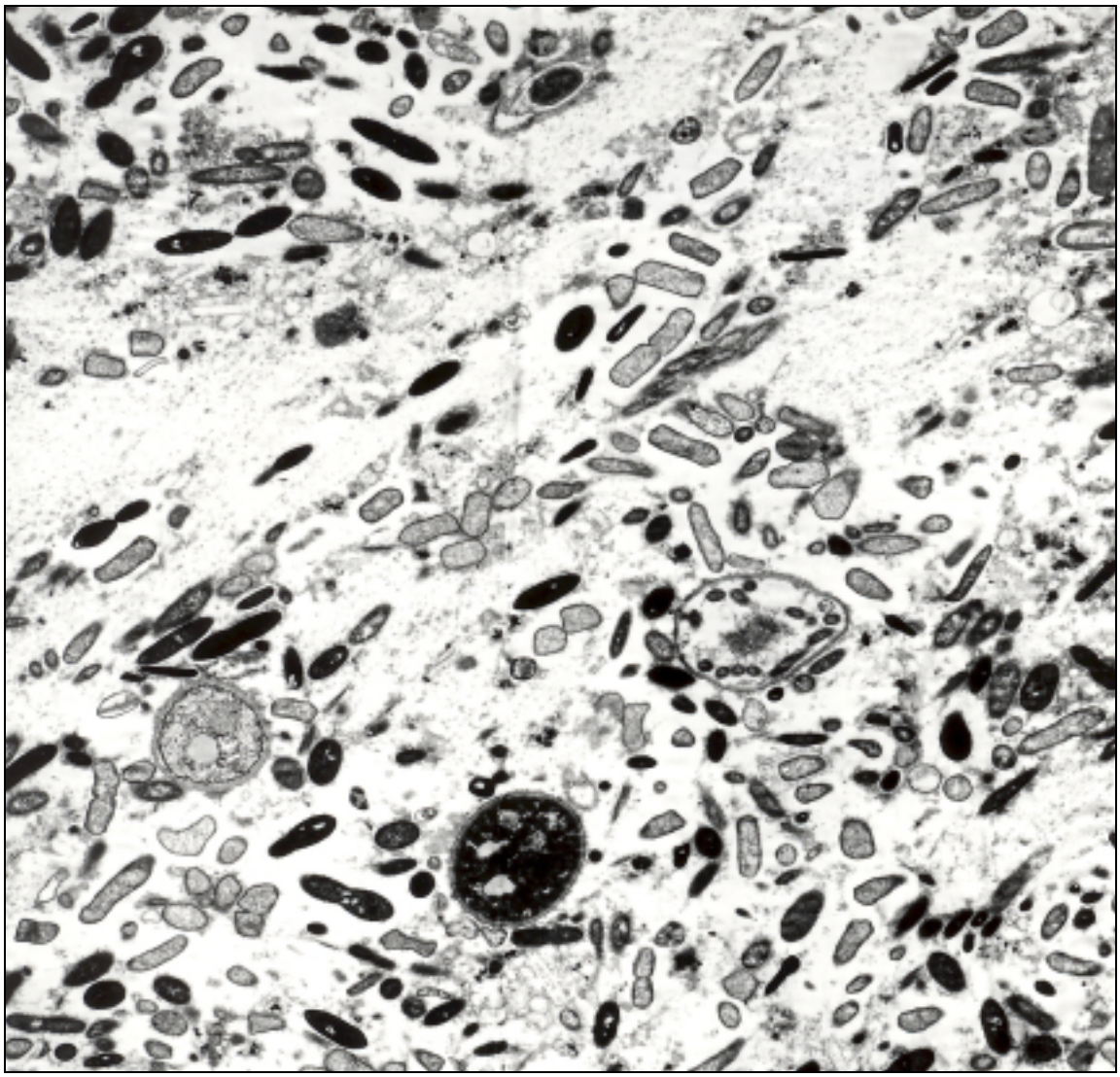

Figure 1. Transmission electron micrograph.

Intestinal contents
Male, 53 years old
Ulcerative colitis

The intestinal content showing the morphological variety of the contents.
Magnification x 4,750

Figure 2.

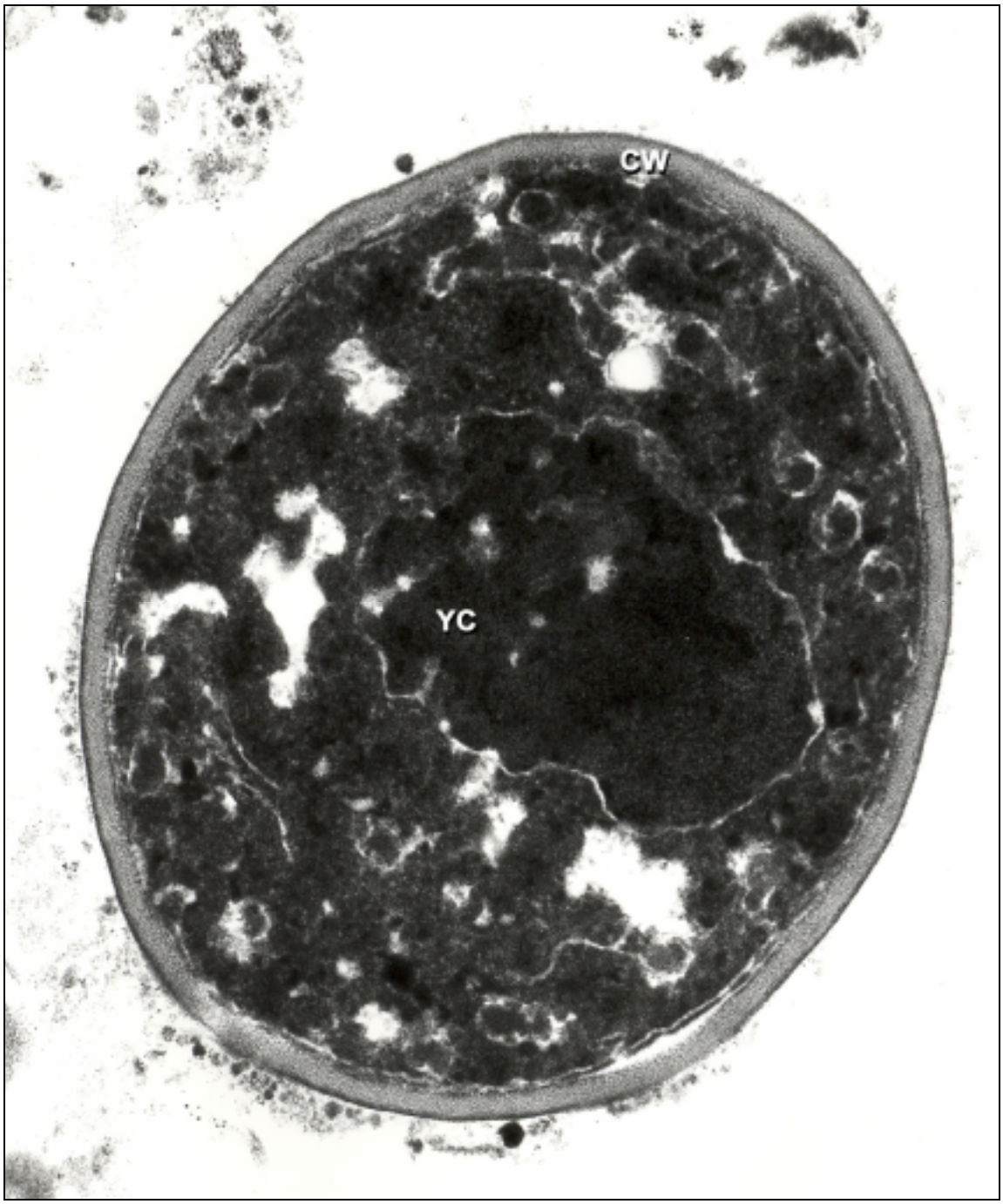

Figure 2. Transmission electron micrograph.

Intestinal contents
Female, 61 years old
Diarrhoea of unknown cause.

A yeast cell (YC) with an intact cell wall (CW).
Magnification x 28,990

Figure 3.

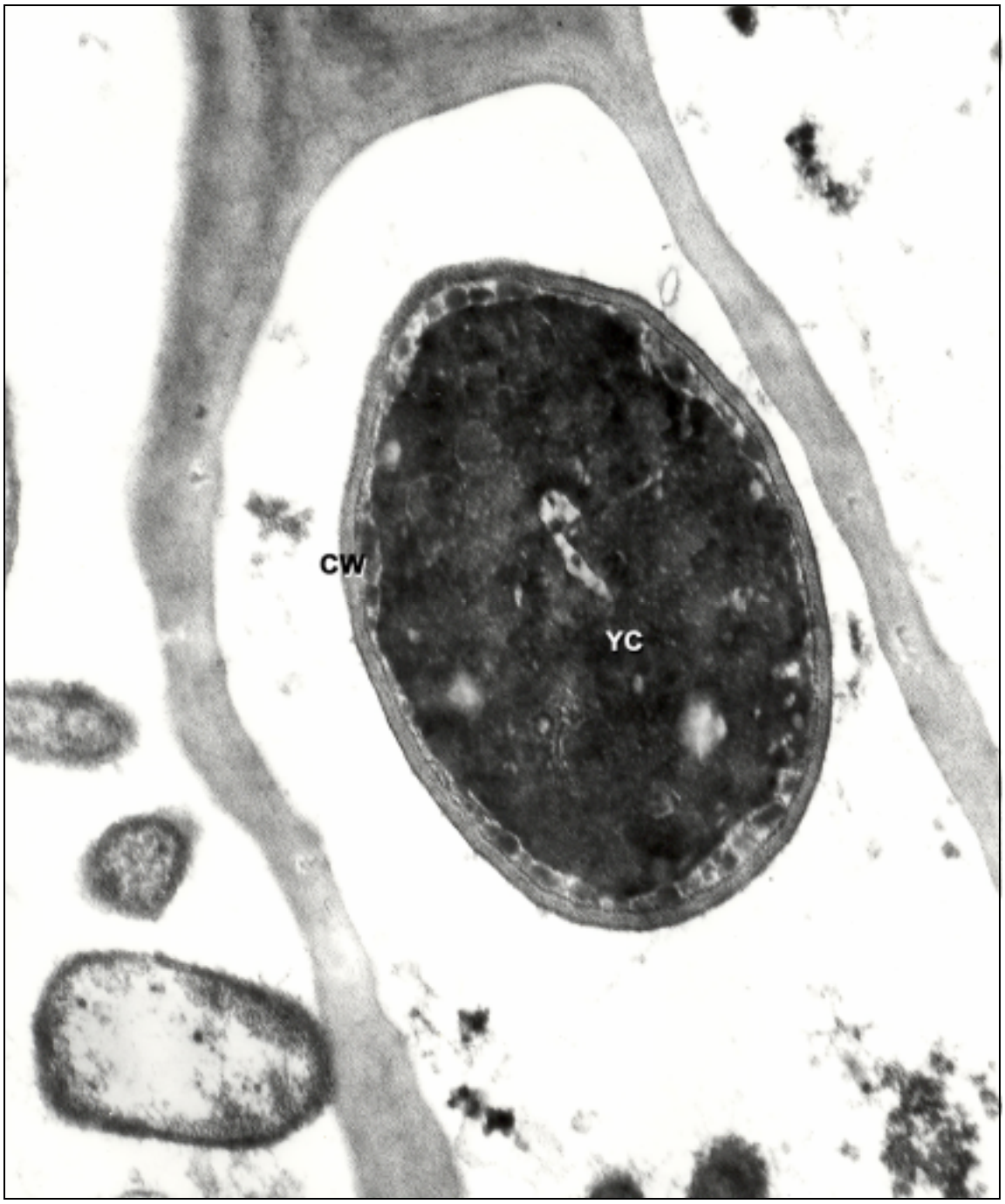

Figure 3. Transmission electron micrograph.

Intestinal contents
Female, 42 years old
Ulcerative colitis.

A yeast cell (YC) with an intact cell wall (CW). Bacteria are shown.
Magnification x 27,160

Figure 4.

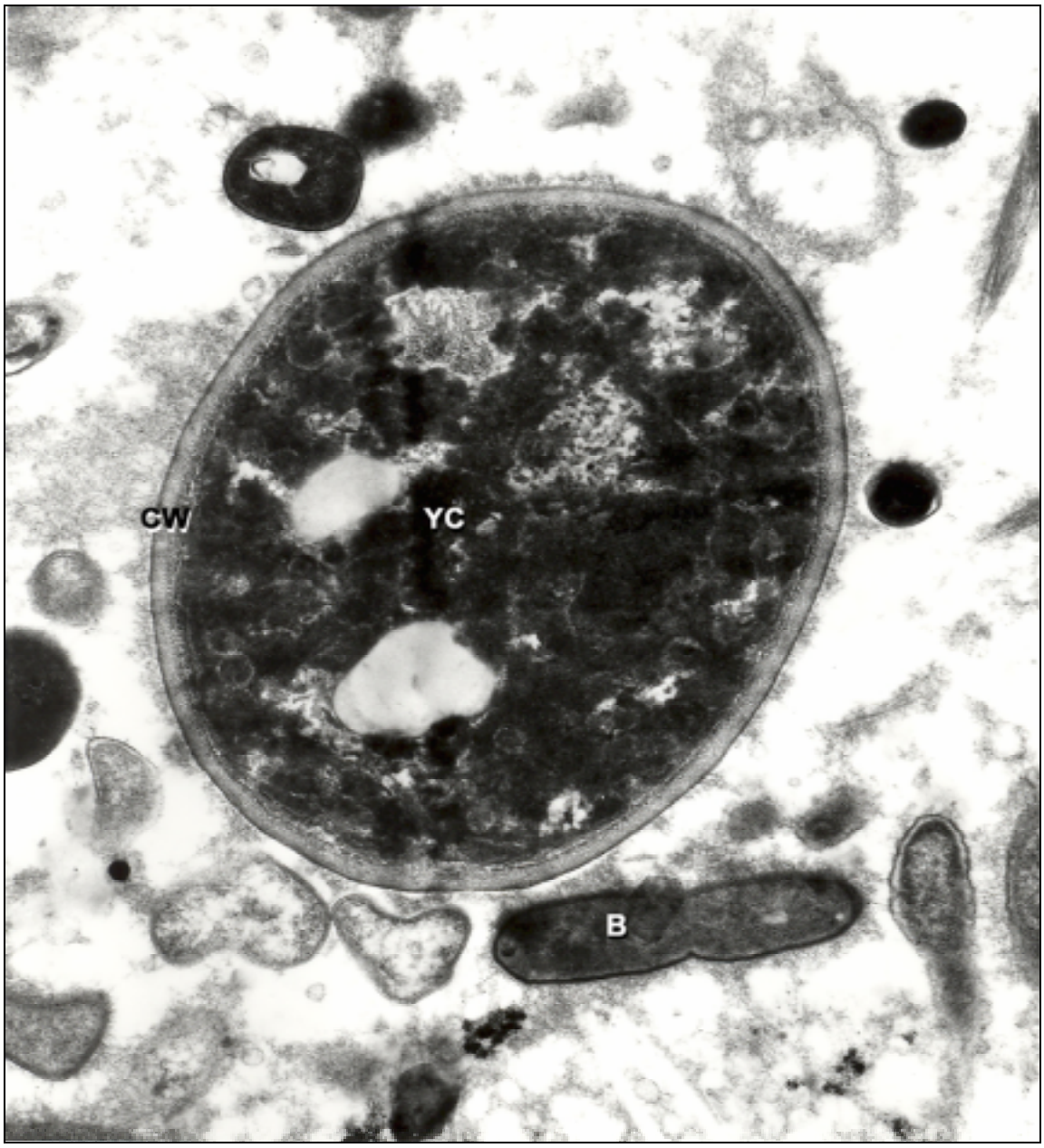

Figure 4. Transmission electron micrograph.

Intestinal contents
Male, 68 years old
Ulcerative colitis.

Yeast cell (YC) with an intact cell wall (CW). Bacteria (B) in the contents surrounding the yeast cell are shown.
Magnification x 24,000

Figure 5.

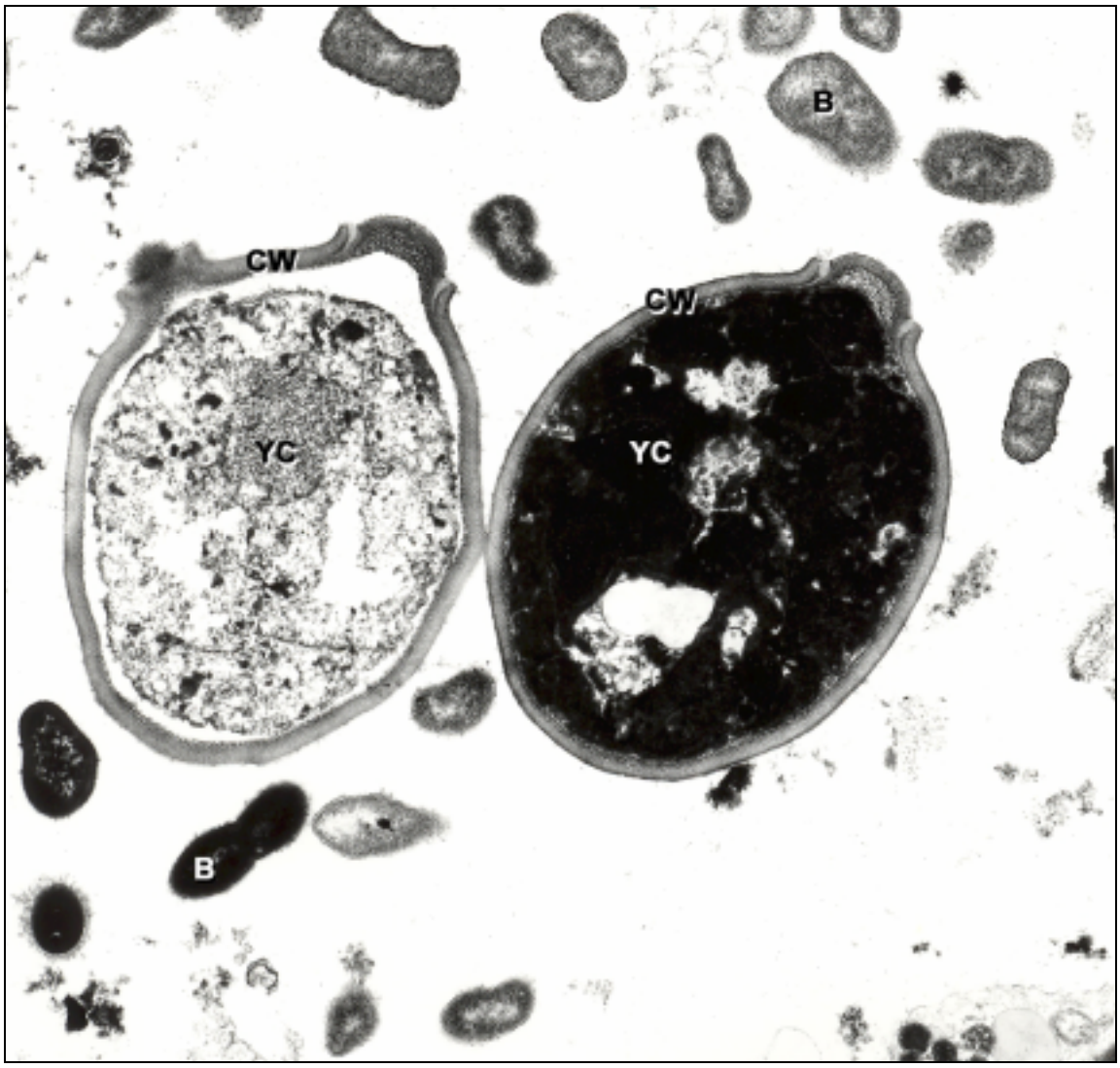

Figure 5. Transmission electron micrograph.

Intestinal contents
Male, 34 years old
Ulcerative colitis.

Yeast cells (YC) with their cell walls (CW). Bacteria (B) are present in the intestinal contents.
Magnification x 17,450

Figure 6.

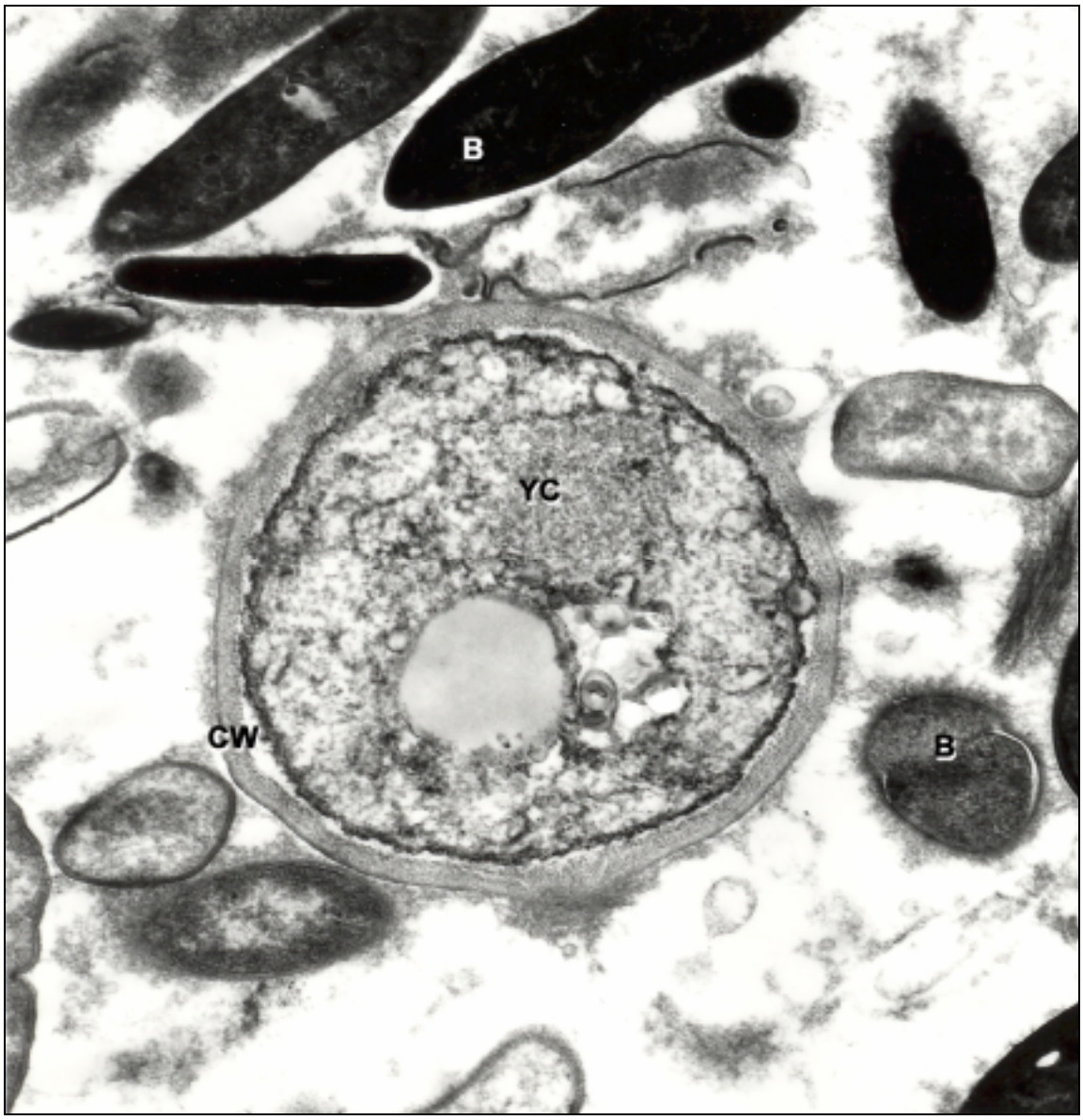

Figure 6. Transmission electron micrograph.

Intestinal contents
Male, 53 years old
Ulcerative colitis.

A yeast cell (YC) with an intact cell wall (CW). The bacteria (B) in the intestinal content surrounding the yeast cell are shown.
Magnification x 26,220

Figure 7.

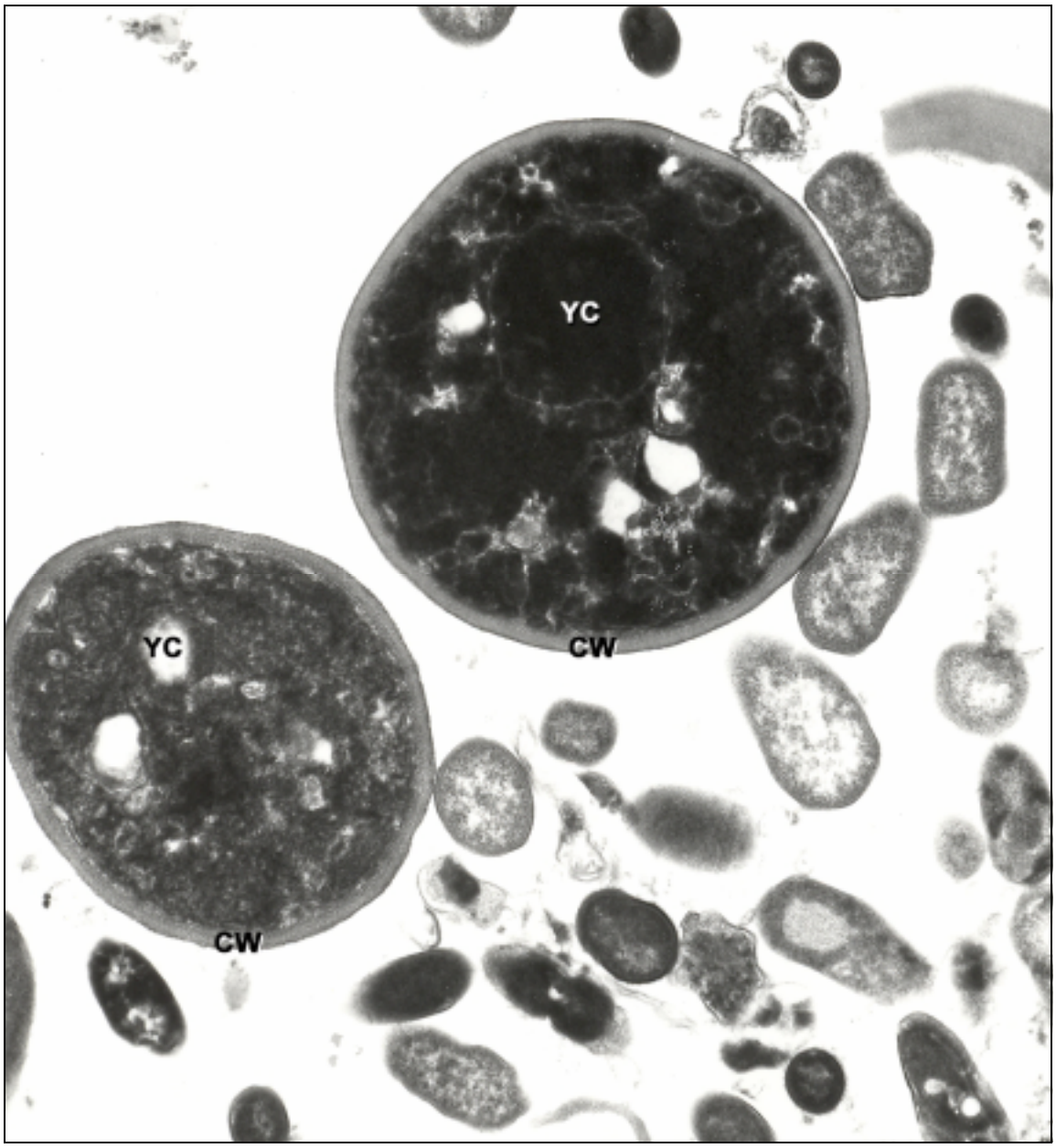

Figure 7. Transmission electron micrograph.

Intestinal contents
Male, 74 years old
Carcinoma of the rectum.

Two yeast cells (YC) with intact cell walls (CW).
Magnification x 23,145

Figure 8.

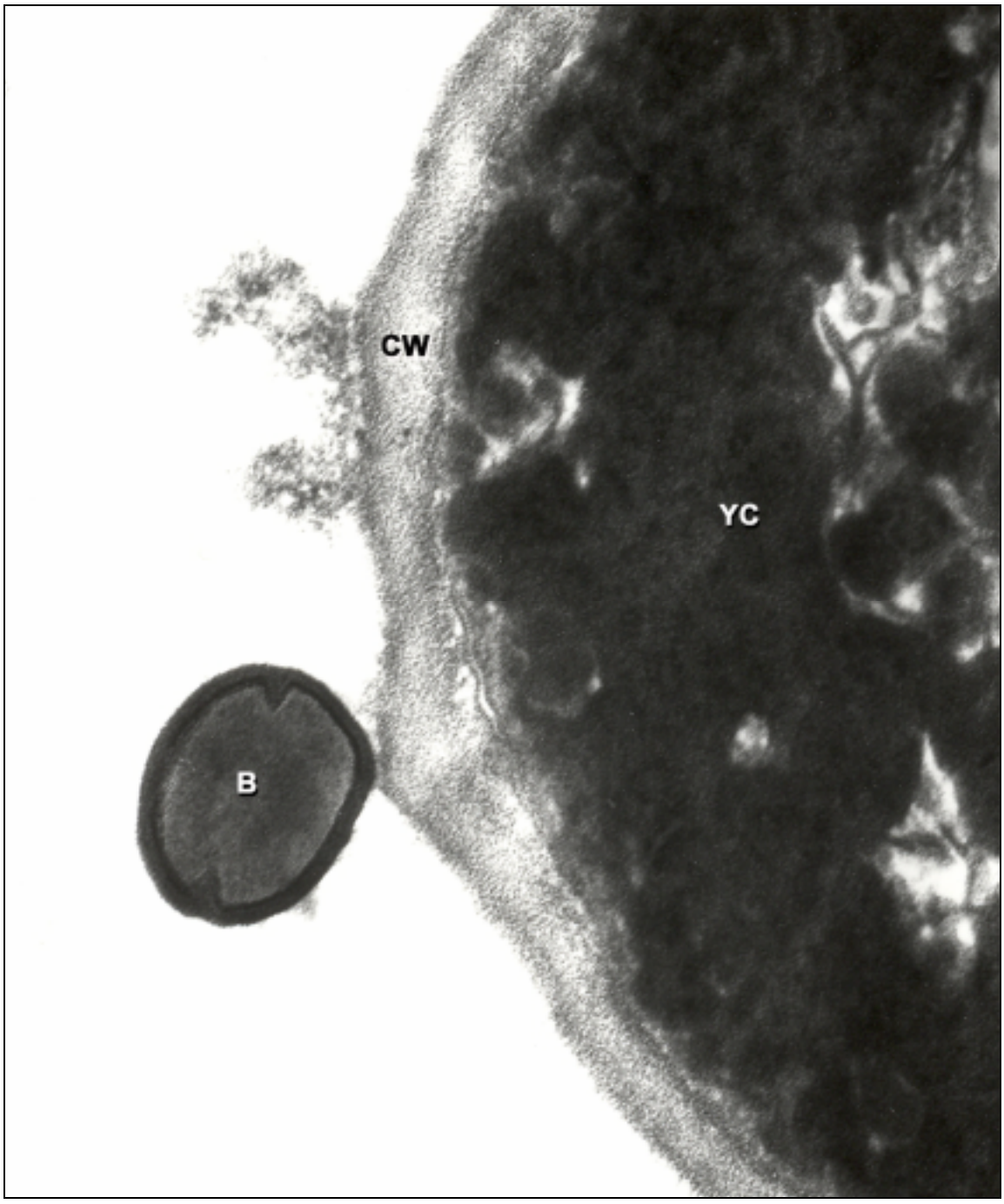

Figure 8. Transmission electron micrograph.

Intestinal contents
Male, 58 years old
Ulcerative colitis.

A bacterium (B) attached to the cell wall (CW) of a yeast cell (YC).
Magnification x 81,065

Figure 9.

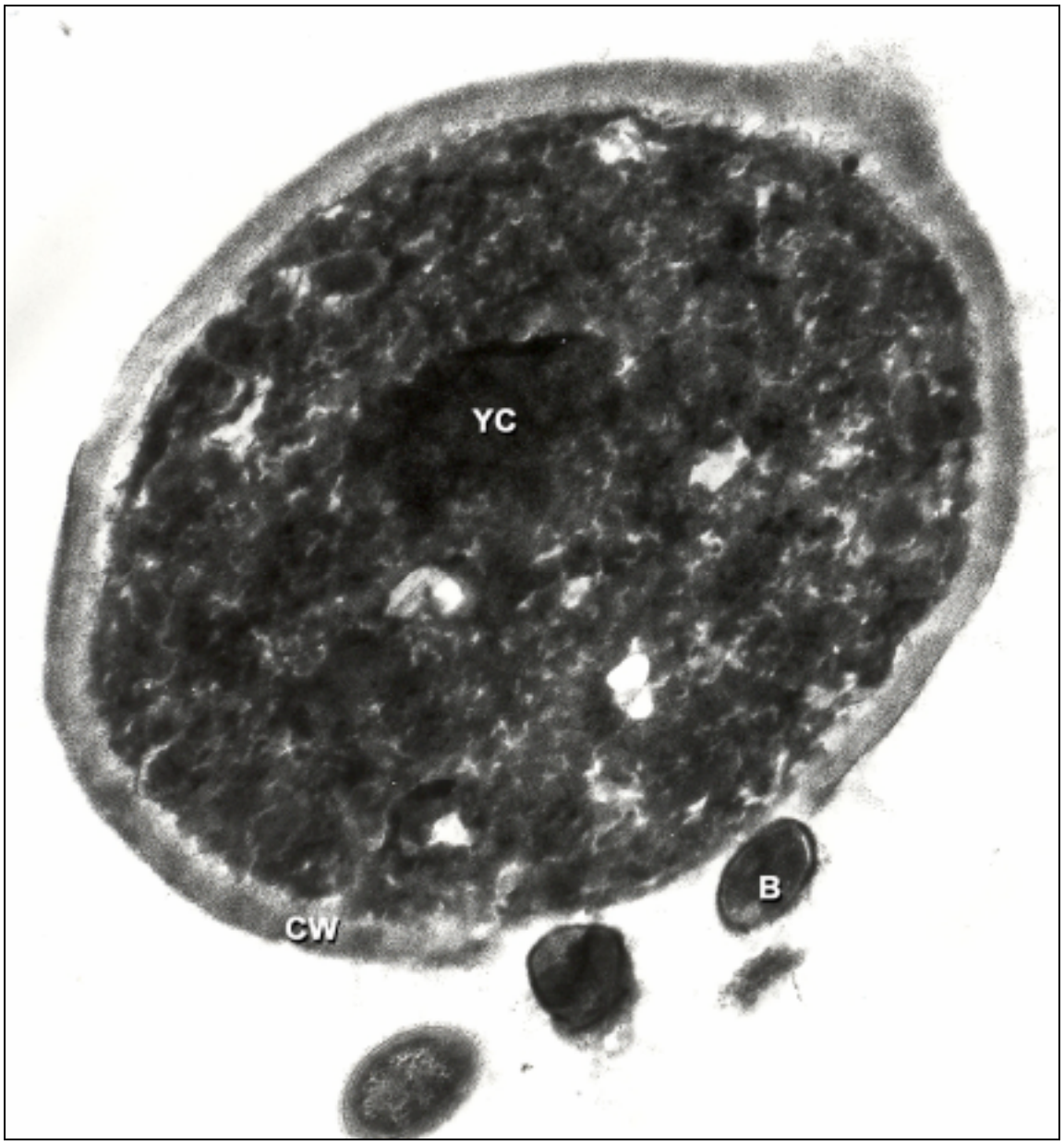

Figure 9. Transmission electron micrograph.

Intestinal contents
Male, 54 years old
Colonic adenoma.

Yeast cell (YC) with bacteria (B) located at that area where the yeast cell wall (CW) is absent.
Magnification x 37,690

Figure 10.

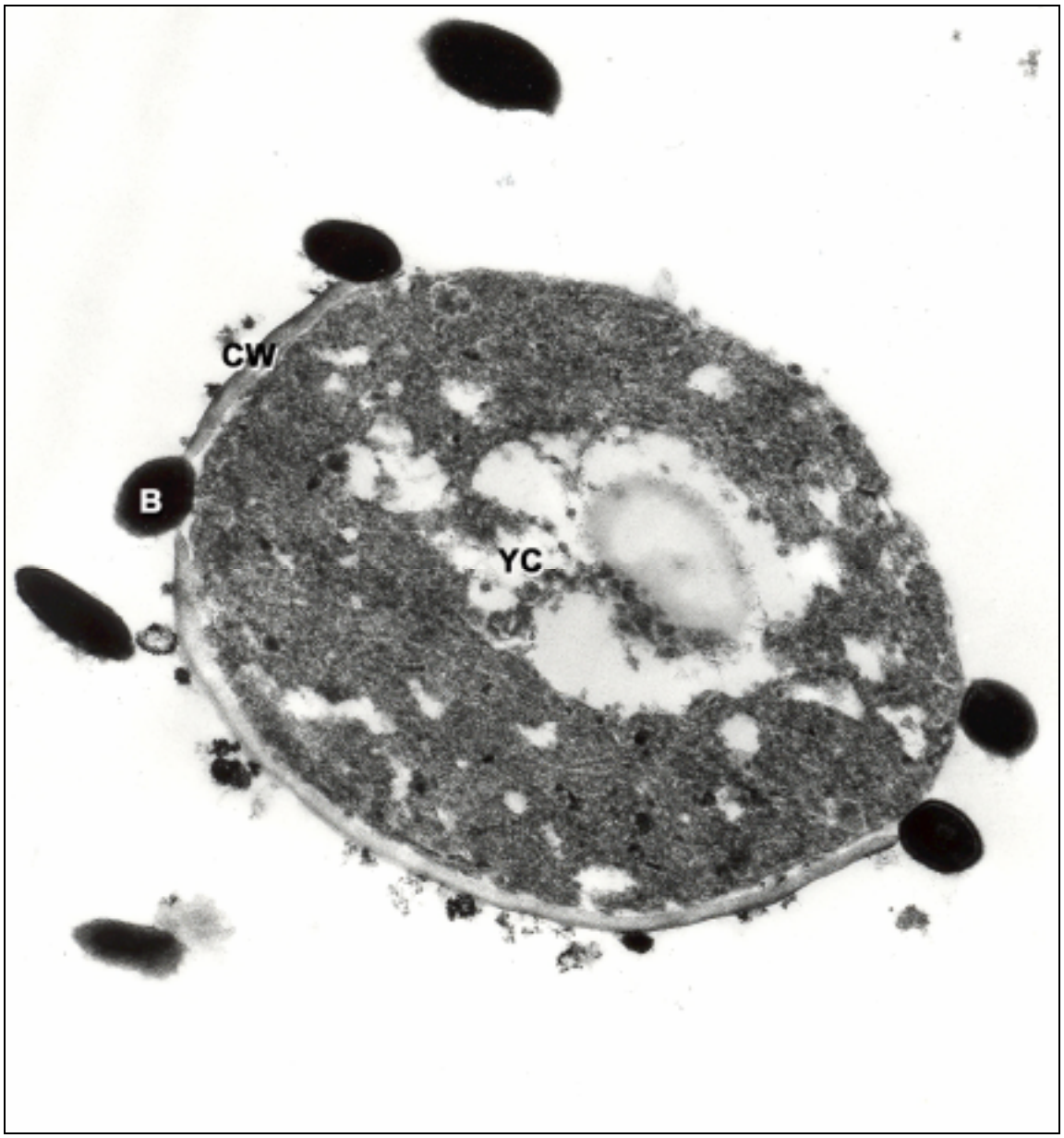

Figure 10. Transmission electron micrograph.

Intestinal contents
Female, 53 years old
Colonic adenoma.

Yeast cell (YC) with bacteria (B) related to the yeast cell wall (CW).
Magnification x 22,455

Figure 11.

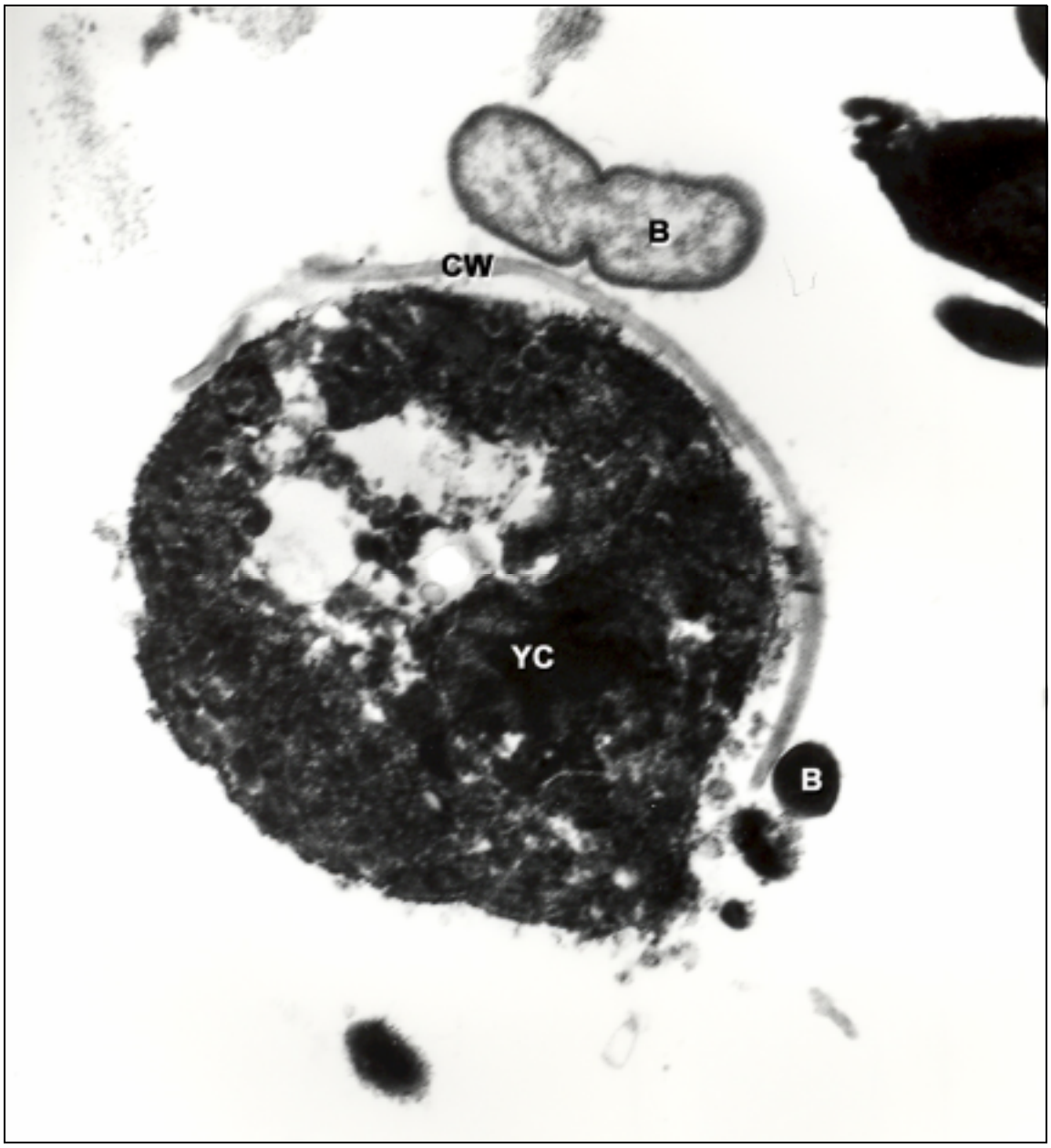

Figure 11. Transmission electron micrograph.

Intestinal contents
Female, 30 years old
Haemorrhoids and diarrhoea of unknown cause.

A yeast cell (YC) with the remnants of the cell wall (CW) and a bacterium (B) related to this cell wall.
Magnification x 26,915

Figure 12.

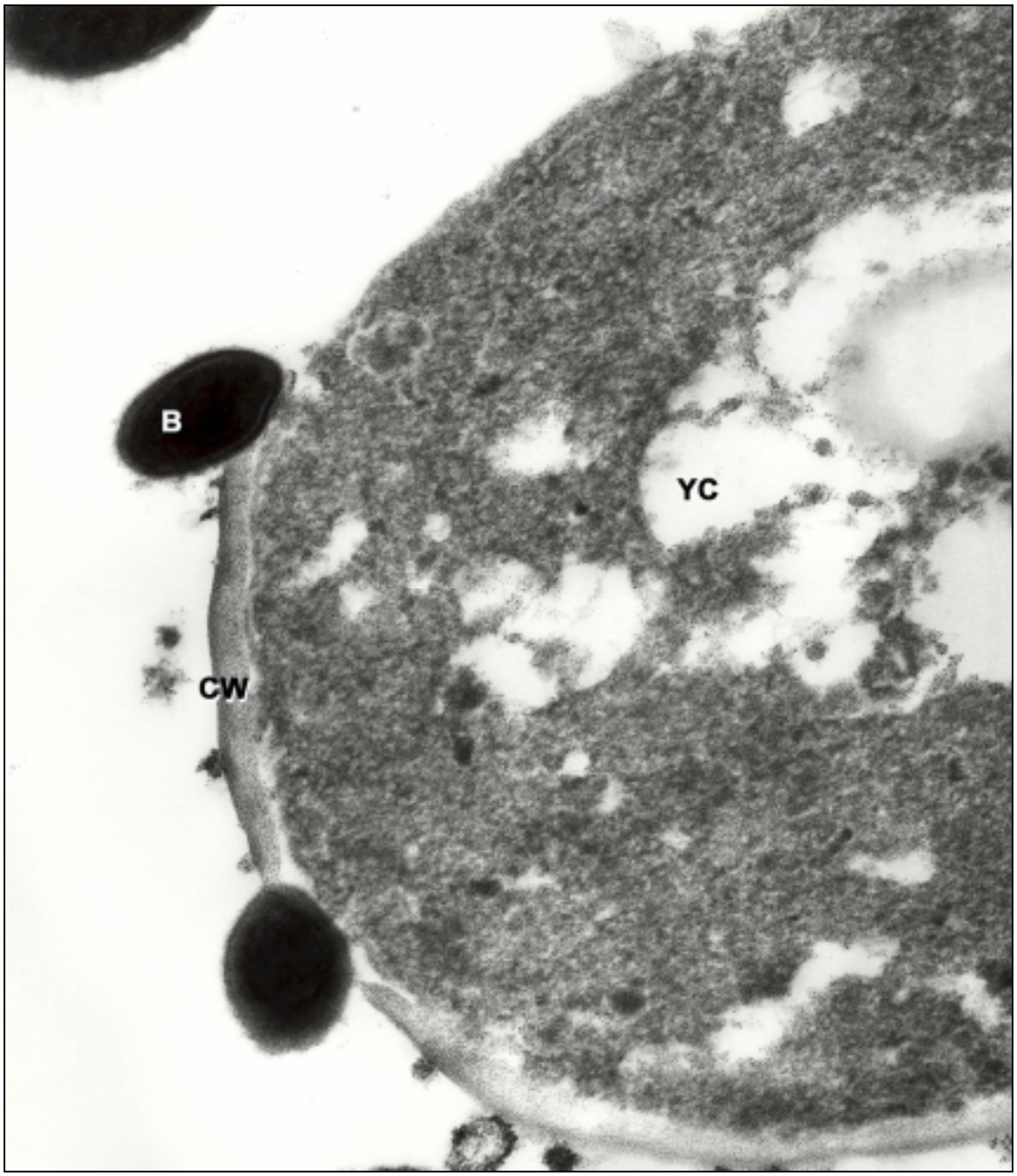

Figure 12. Transmission electron micrograph.

Intestinal contents
Female, 53 years old
Colonic adenoma.

A yeast cell (YC) with the bacteria (B) at the yeast cell wall (CW).
Magnification x 51,130

Figure 13.

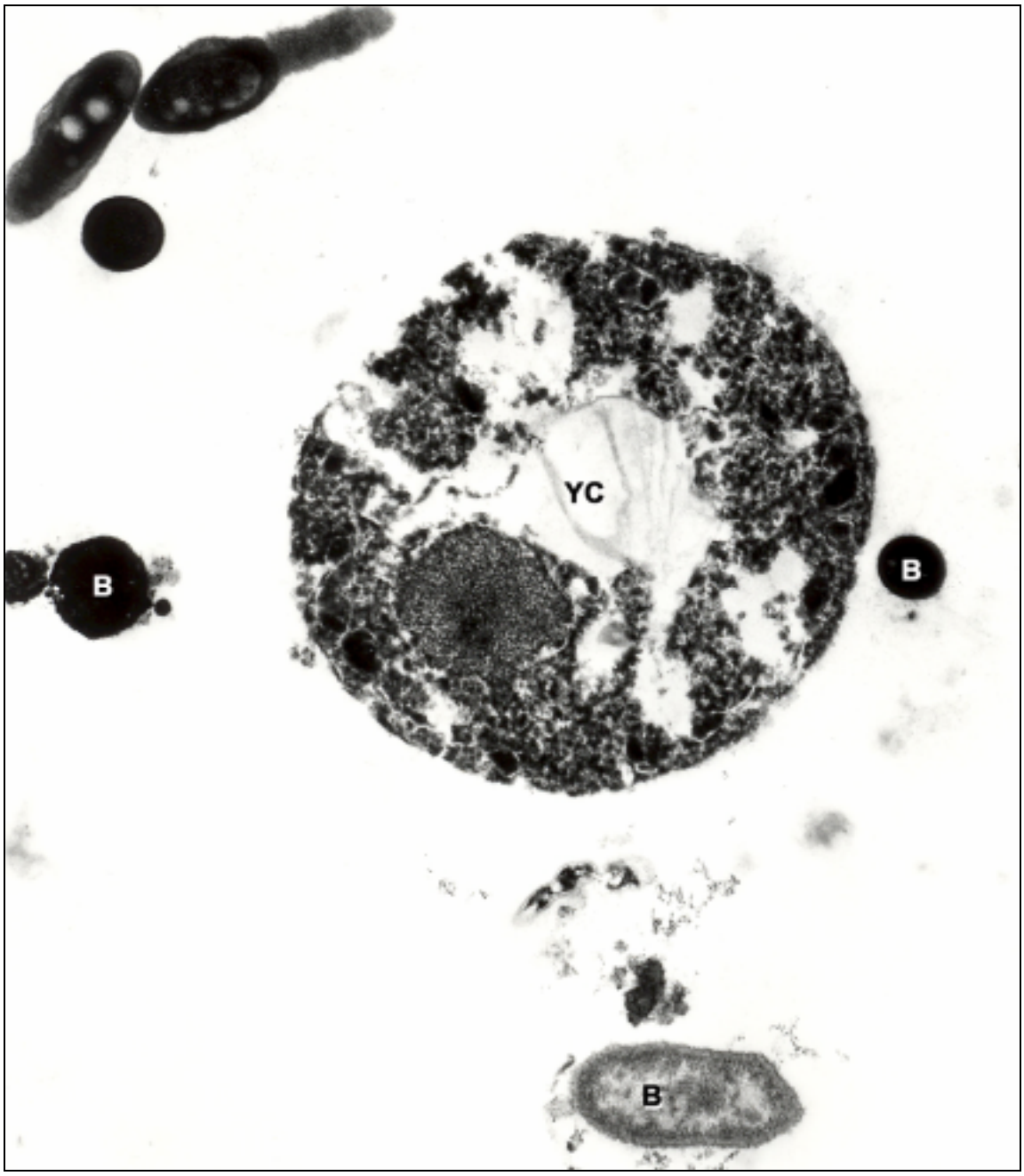

Figure 13. Transmission electron micrograph.

Intestinal contents
Male, 47 years old
Haemorrhoids.

A yeast cell (YC) lacking a cell wall. The surrounding bacteria (B) are shown.
Magnification x 23,310

Figure 14.

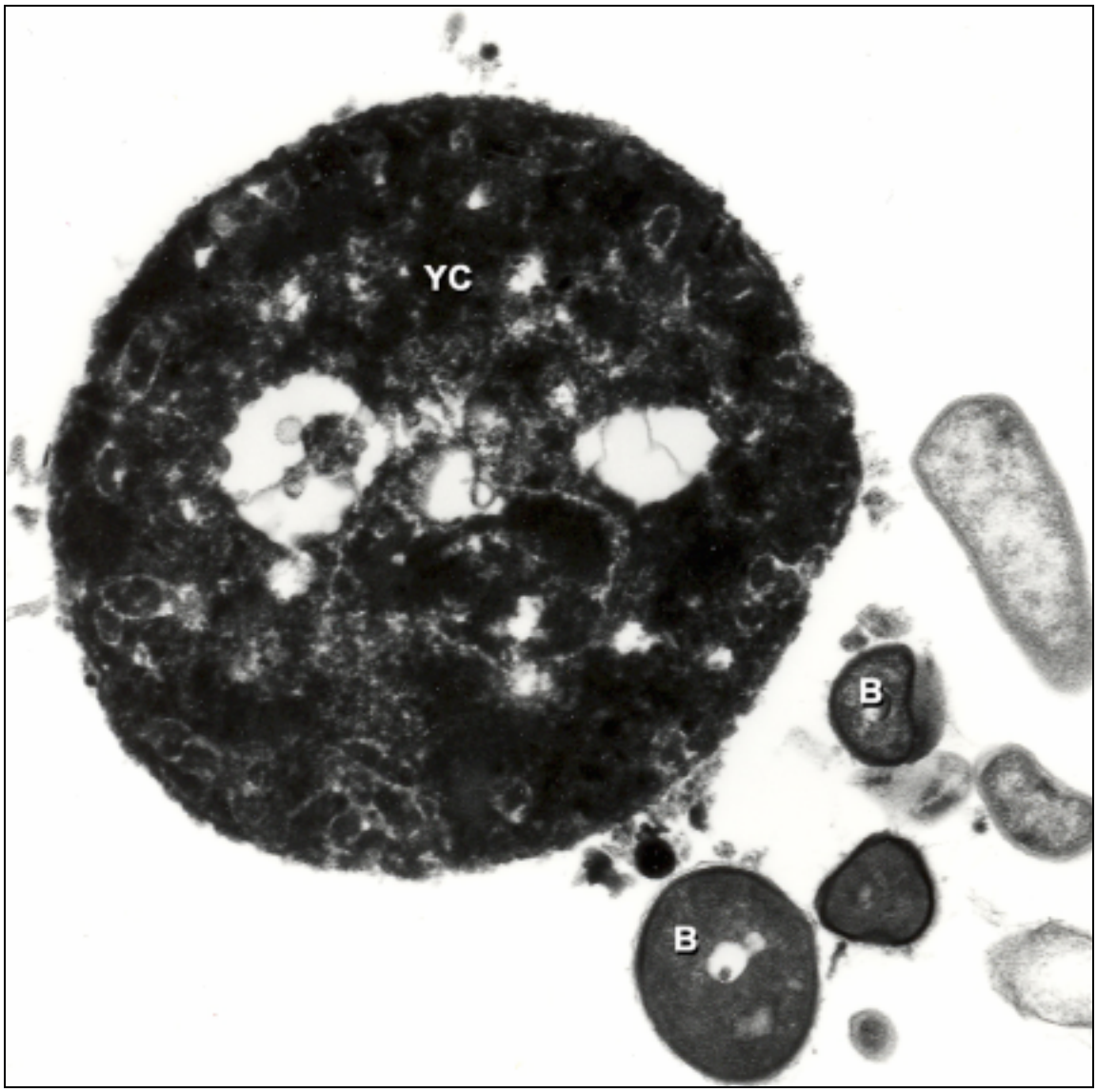

Figure 14. Transmission electron micrograph.

Intestinal contents
Female, 61 years old
Haemorrhoids and diarrhoea of unknown cause.

Yeast cell (YC) lacking a cell wall. Bacteria (B) are shown.
Magnification x 26,245

Figure 15.

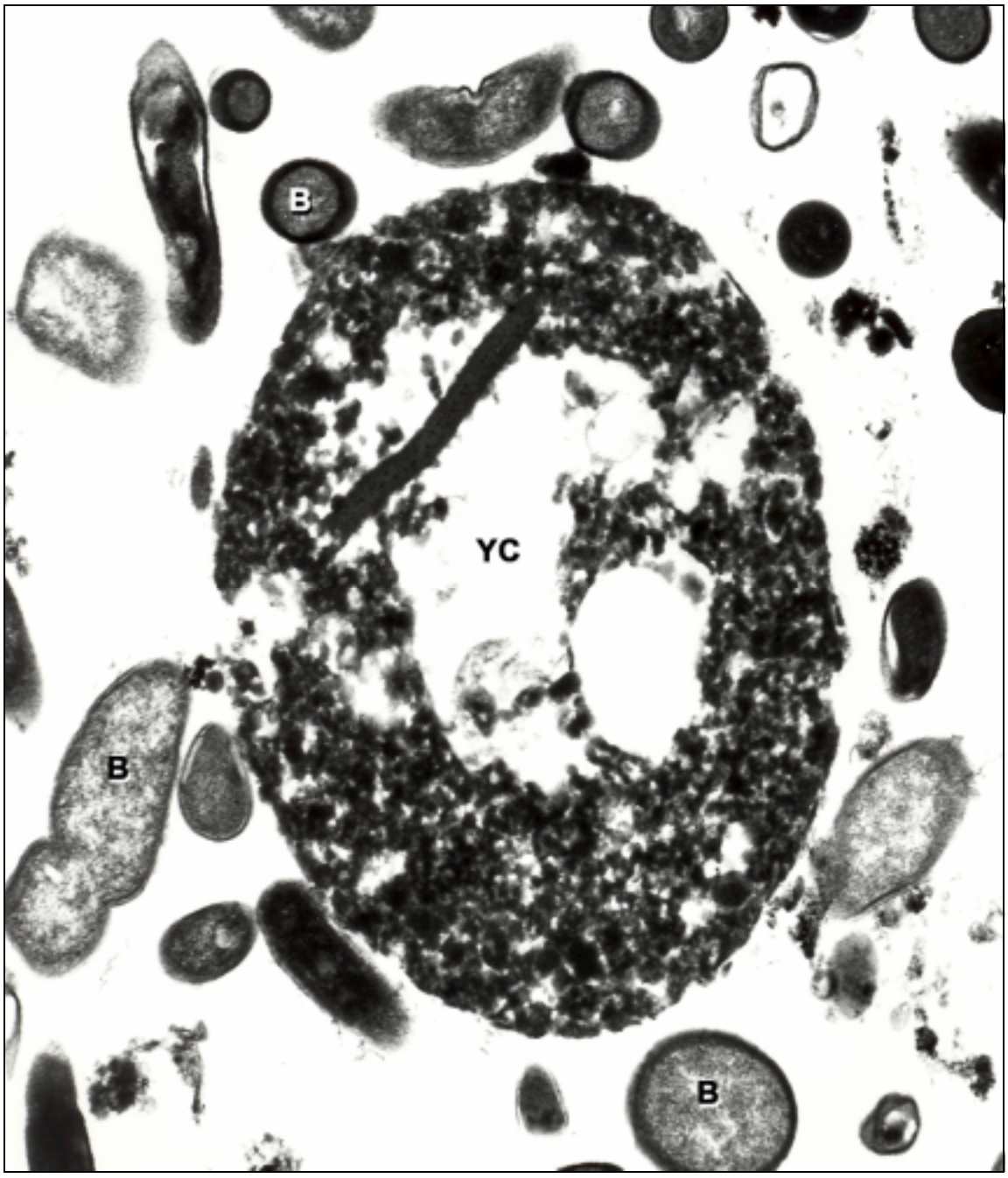

Figure 15. Transmission electron micrograph.

Intestinal contents
Female, 60 years old
Ulcerative colitis in remission.

A yeast cell (YC) lacking a cell wall. Numerous bacteria (B) are shown.
Magnification x 25,430

Figure 16.

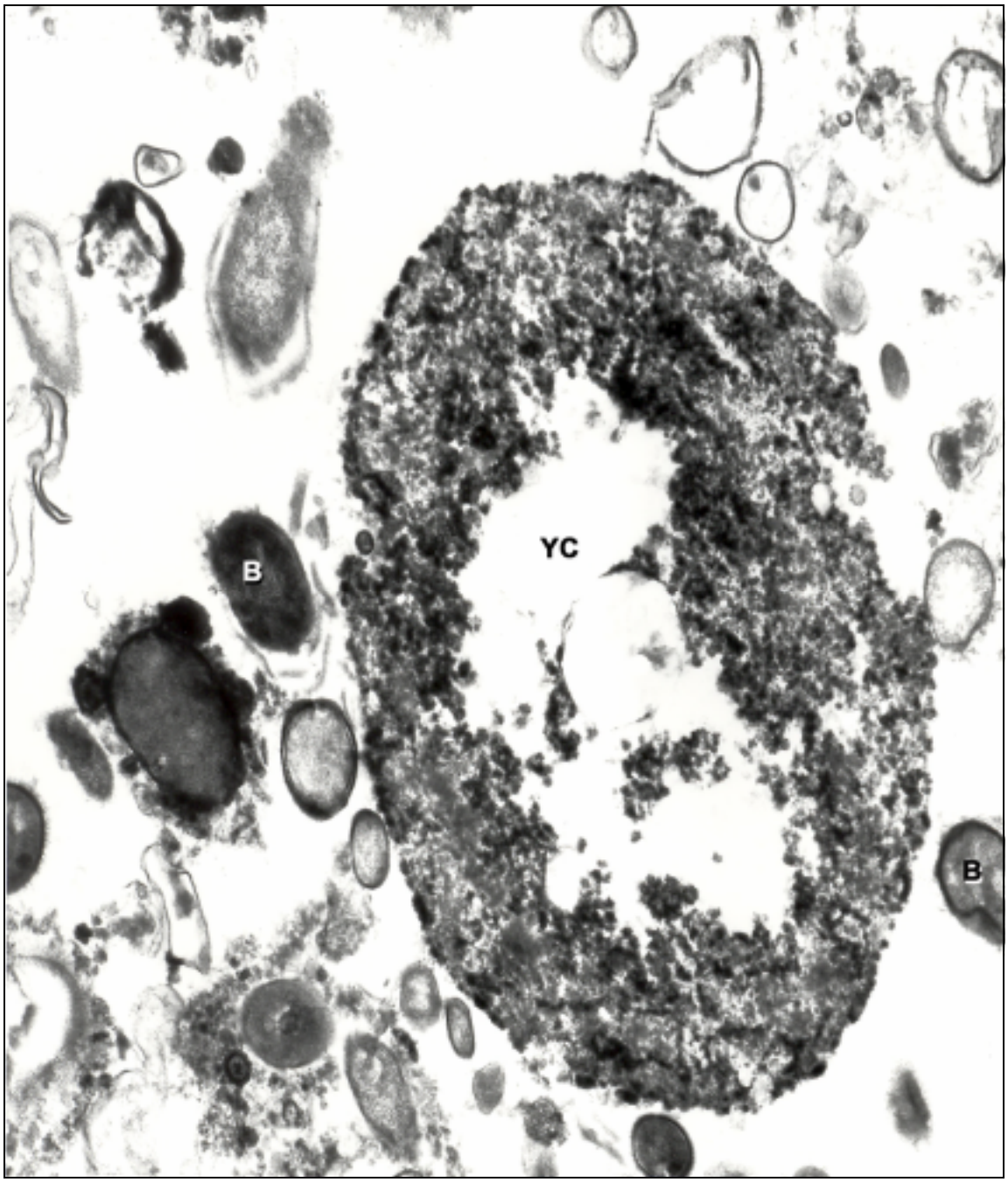

Figure 16. Transmission electron micrograph.

Intestinal contents
Female, 39 years old
Ulcerative colitis.

A disrupting yeast cell (YC) lacking a cell wall. The surrounding bacteria (B) are shown.
Magnification x 24,300

Figure 17.

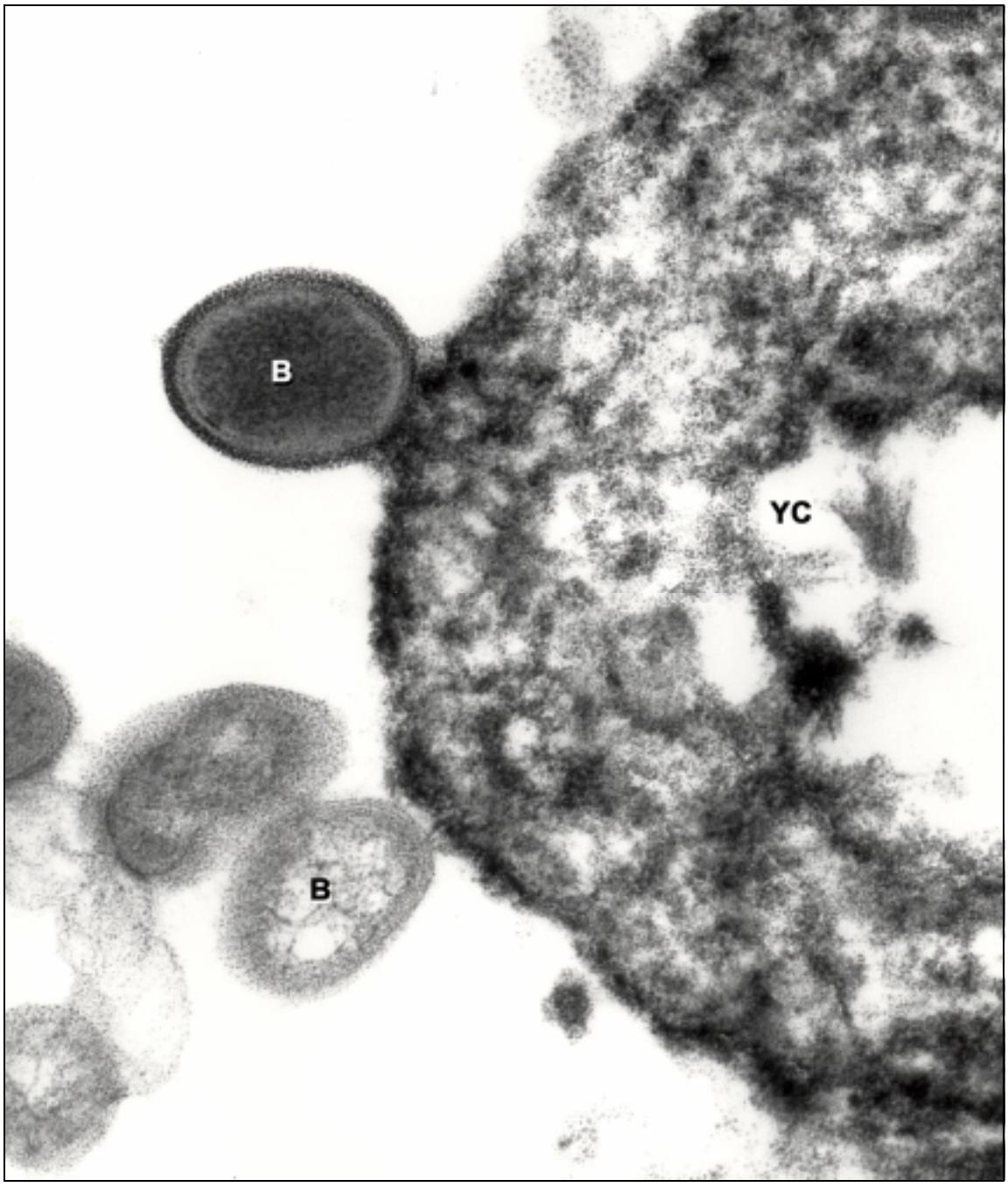

Figure 17. Transmission electron micrograph.

Intestinal contents
Male, 90 years old
Carcinoma of the rectum.

Bacteria (B) attached to a yeast cell (YC) which lacks a cell wall.
Magnification x 82,360

Figure 18.

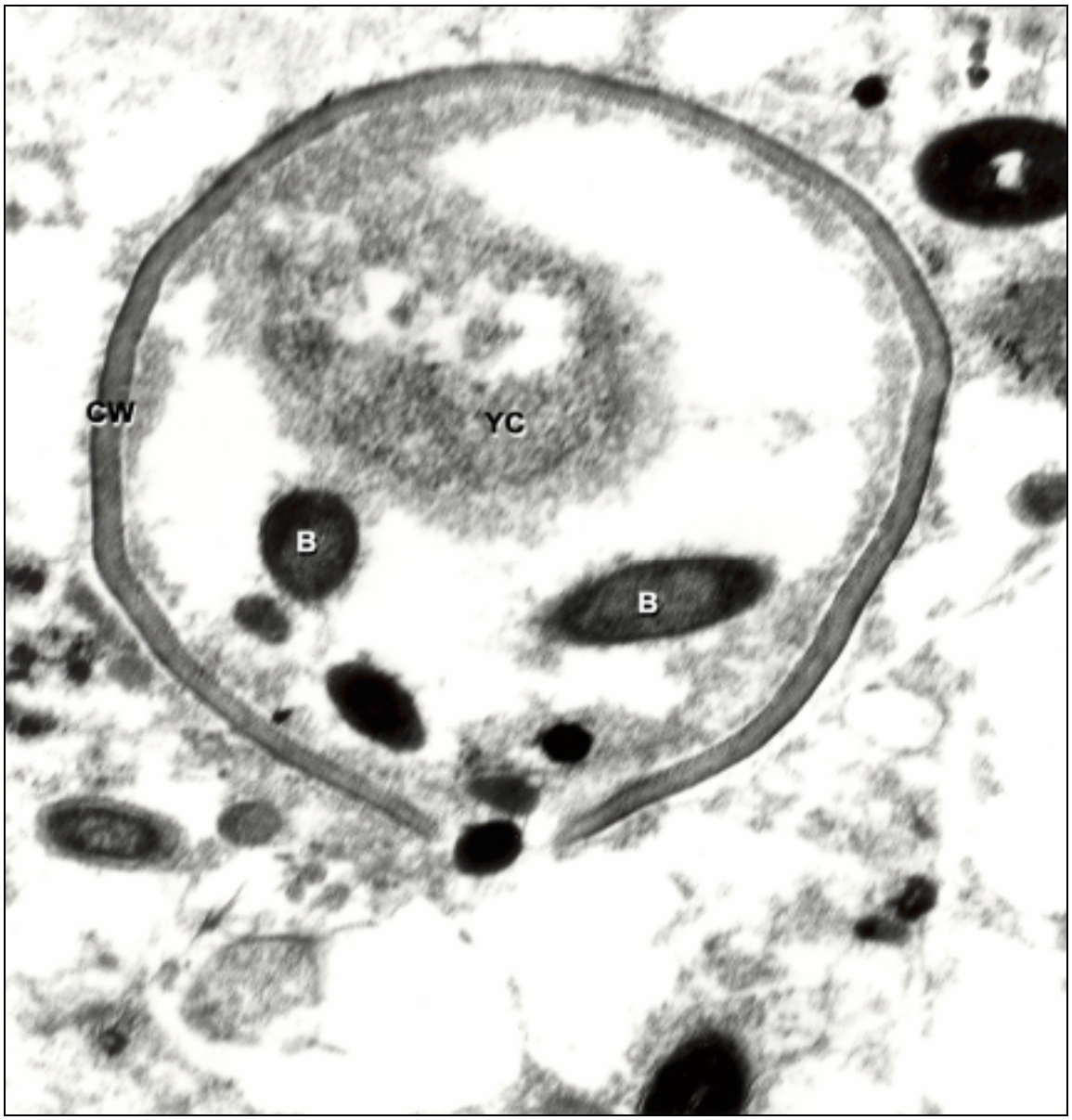

Figure 18. Transmission electron micrograph.

Intestinal contents
Male, 53 years old
Ulcerative colitis.

Yeast cell wall (CW) surrounding the remnants of a yeast cell (YC) and bacteria (B).
Magnification x 31,790

Figure 19.

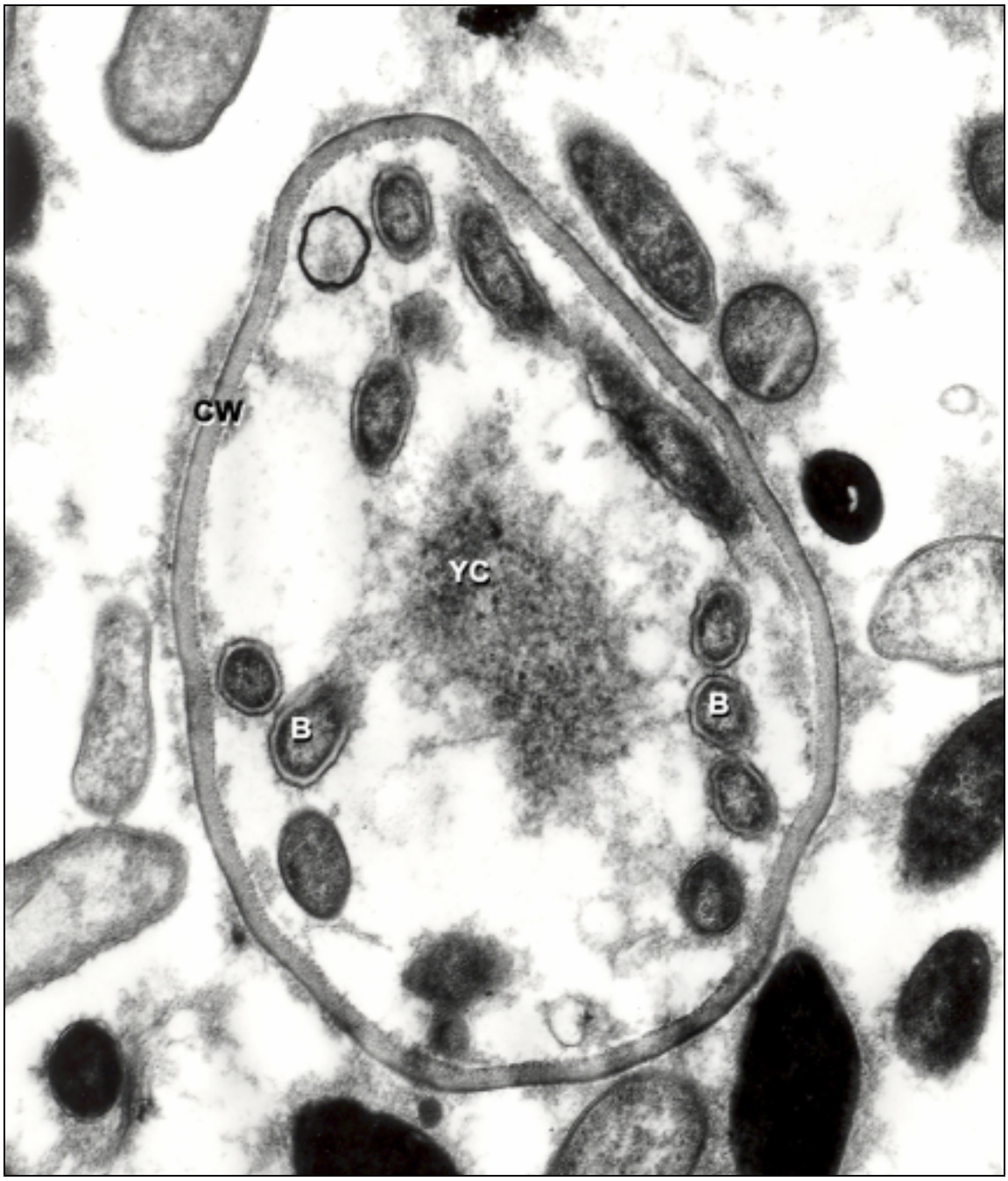

Figure 19. Transmission electron micrograph.

Intestinal contents
Male, 53 years old
Ulcerative colitis.

The remnants of a yeast cell (YC) together with bacteria (B) inside the cell wall (CW) of a yeast cell.
Magnification x 26,390

Figure 20.

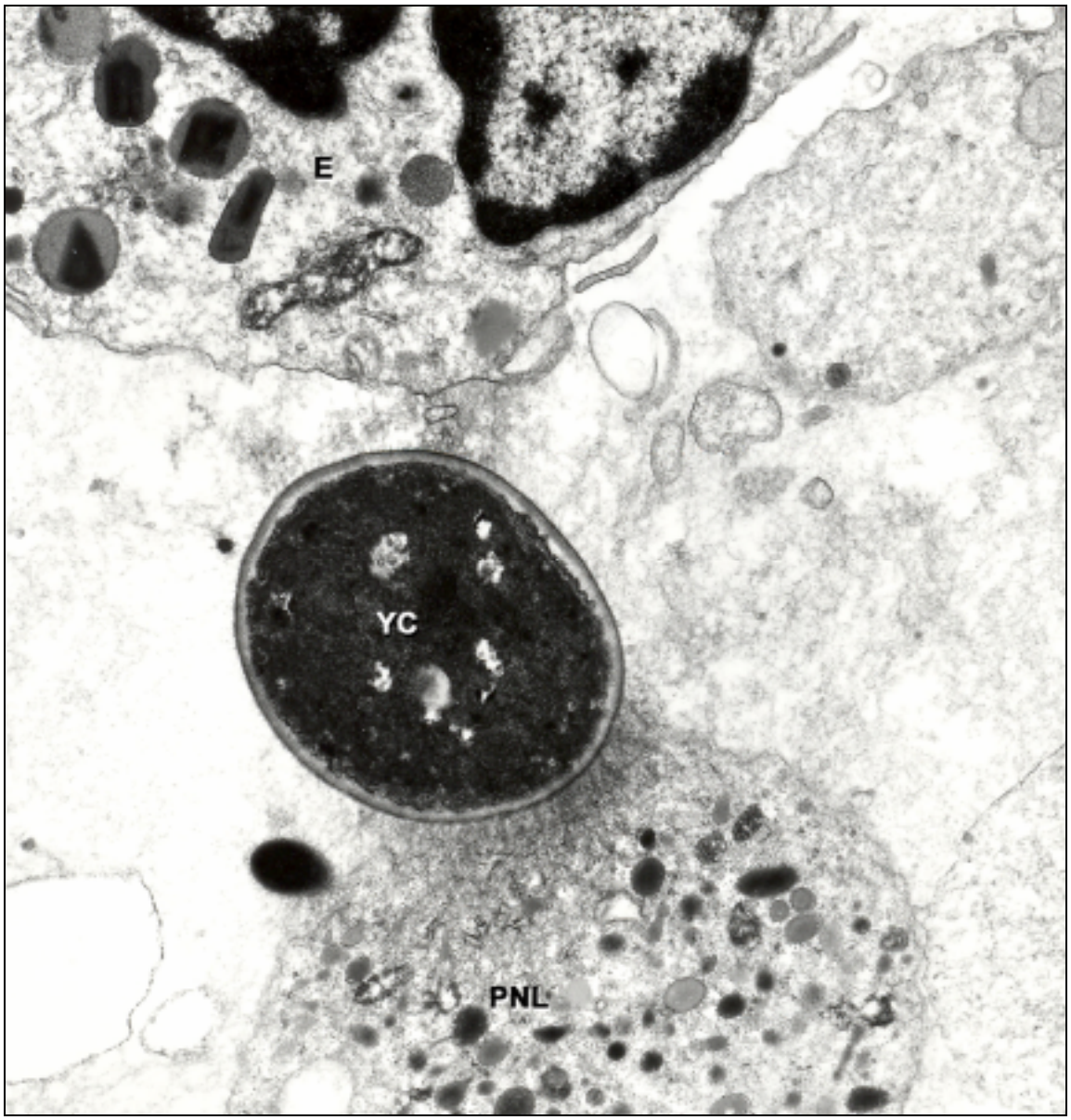

Figure 20. Transmission electron micrograph.

Intestinal contents
Female, 32 years old
Ulcerative colitis.

A yeast cell (YC) with adjacent polymorphonuclear leucocyte (PNL) and an eosinophil (E).
Magnification x 15,770

Figure 21.

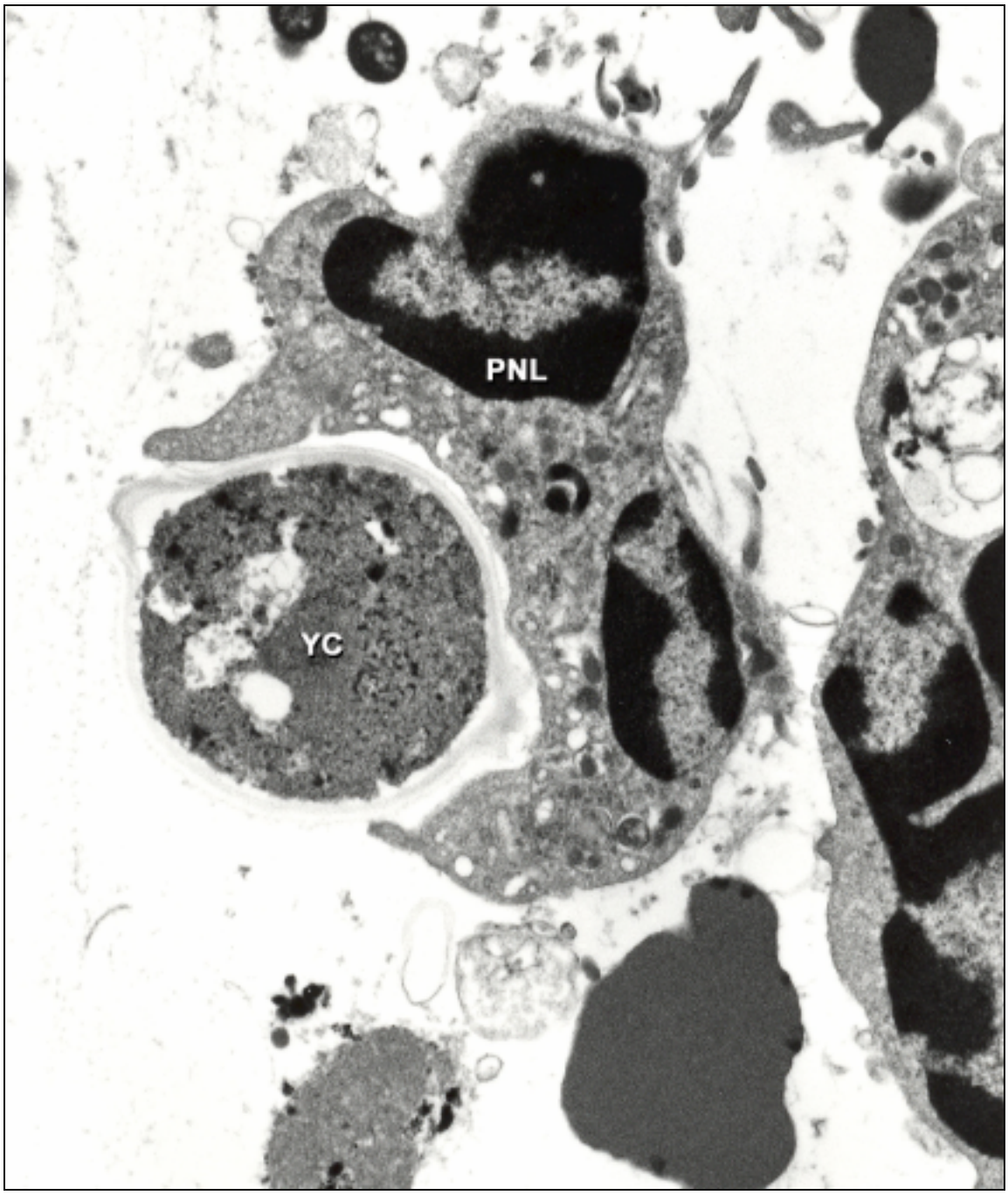

Figure 21. Transmission electron micrograph.

Intestinal contents
Male, 23 years old
Ulcerative colitis.

Polymorphonuclear leucocyte (PNL) phagocytosing a yeast cell (YC).
Magnification x 15,005

Figure 22.

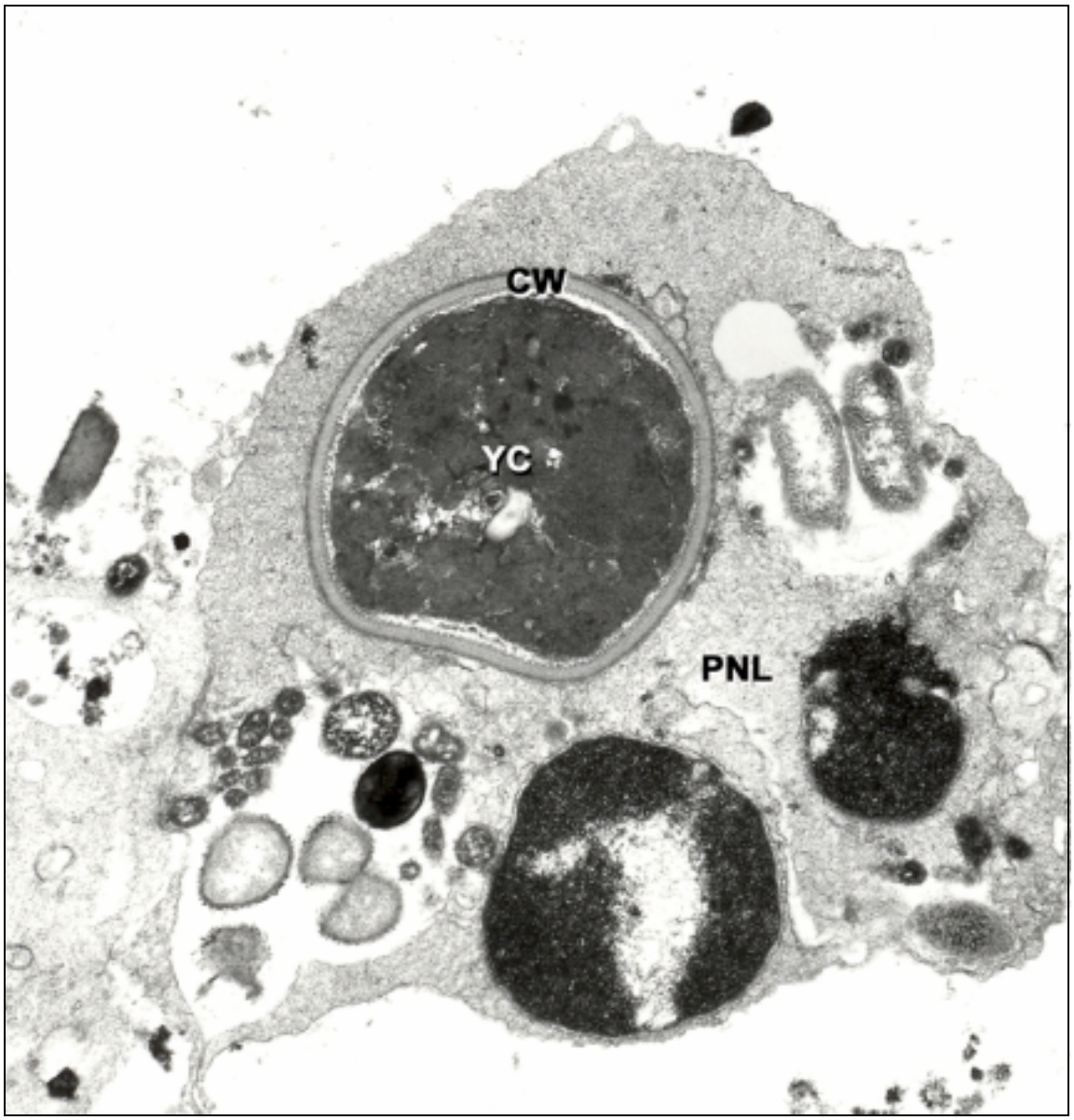

Figure 22. Transmission electron micrograph.

Intestinal contents
Male, 34 years old
Ulcerative colitis.

A yeast cell (YC) with the cell wall (CW) intact having been phagocytosed by a polymorphonuclear leucocyte (PNL).
Magnification x 16,675

Figure 23.

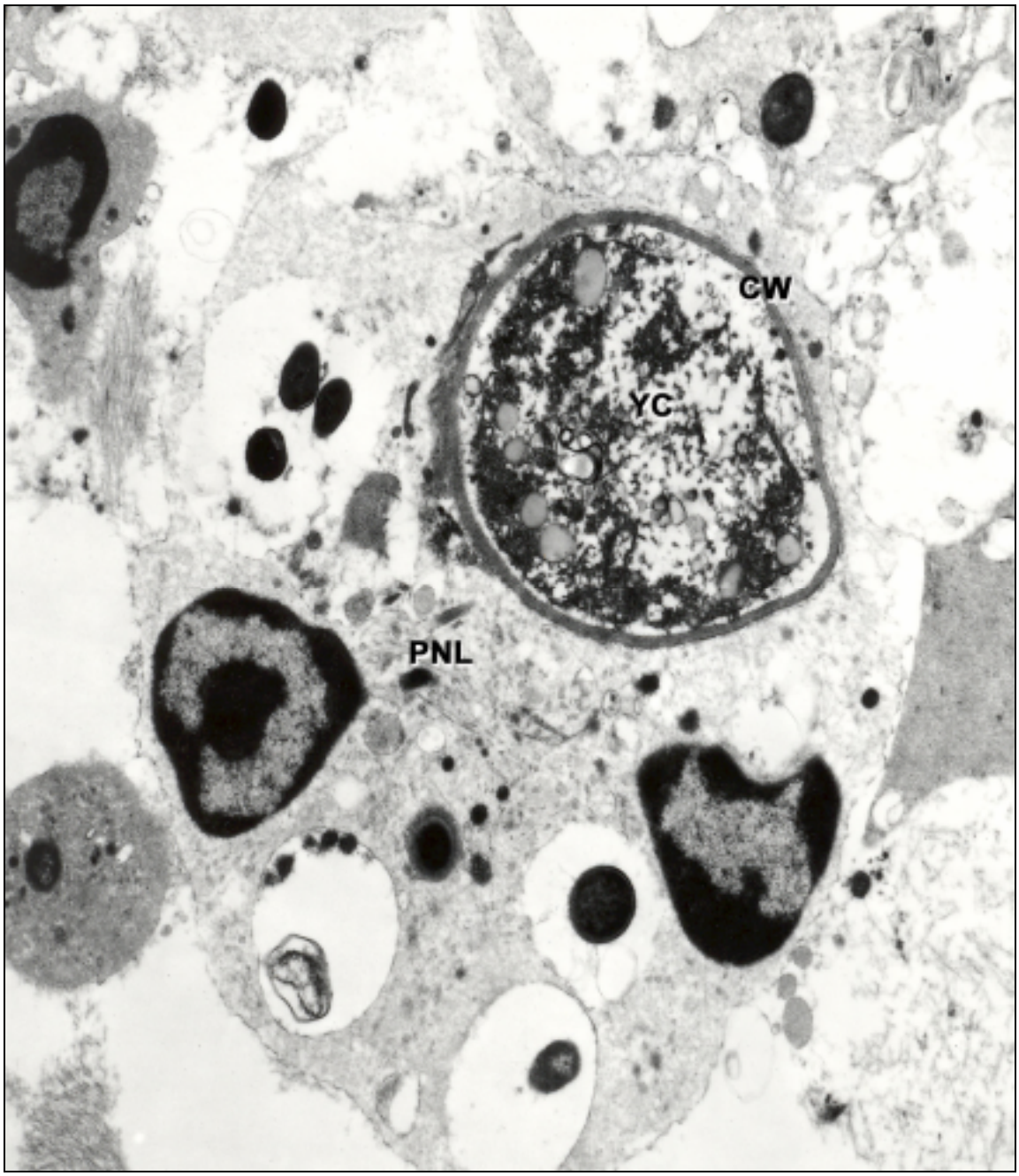

Figure 23. Transmission electron micrograph.

Intestinal contents
Male, 57 years old
Ulcerative colitis.

A yeast cell (YC) with the cell wall (CW) phagocytosed by a polymorphonuclear leucocyte (PNL).
Magnification x 13,620

Figure 24.

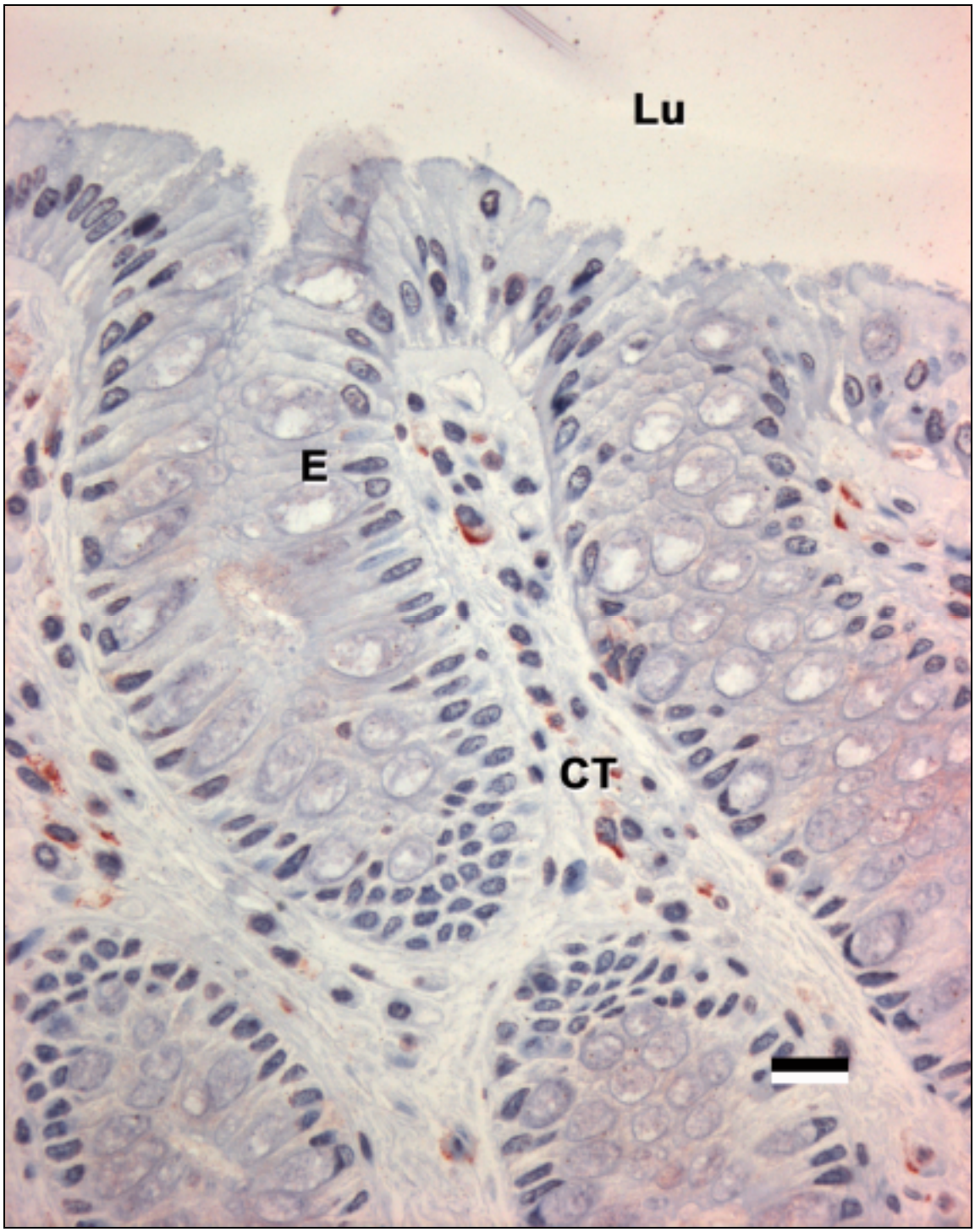

Figure 24. Macrophage mannose receptor. (Chromogen substrate).

Colonic biopsy from a 64 years old female with diverticular disease of the sigmoid colon. The colonic lumen (Lu), colonic epithelium (E) and subepithelial connective tissue (CT) are indicated. Scale bar is 20µm.

Figure 25.

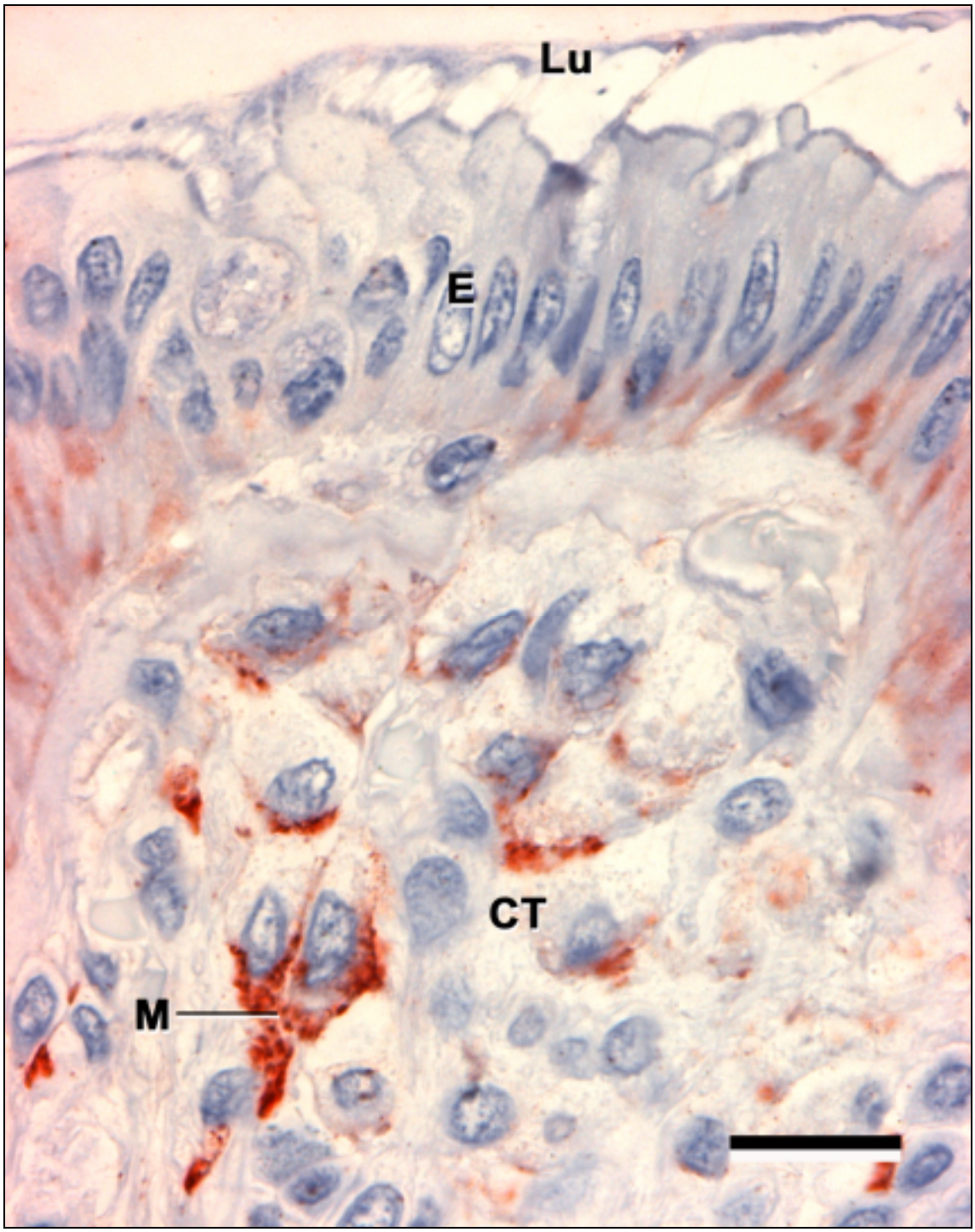

Figure 25. Macrophage mannose receptor. (Chromogen substrate).

A colonic mucosal biopsy from a 74 years old male with carcinoma of the sigmoid colon. The colonic lumen (Lu), colonic epithelium (E), subepithelial connective tissue (CT) and connective tissue macrophages (M) are shown. Scale bar is 20µm.

Figure 26.

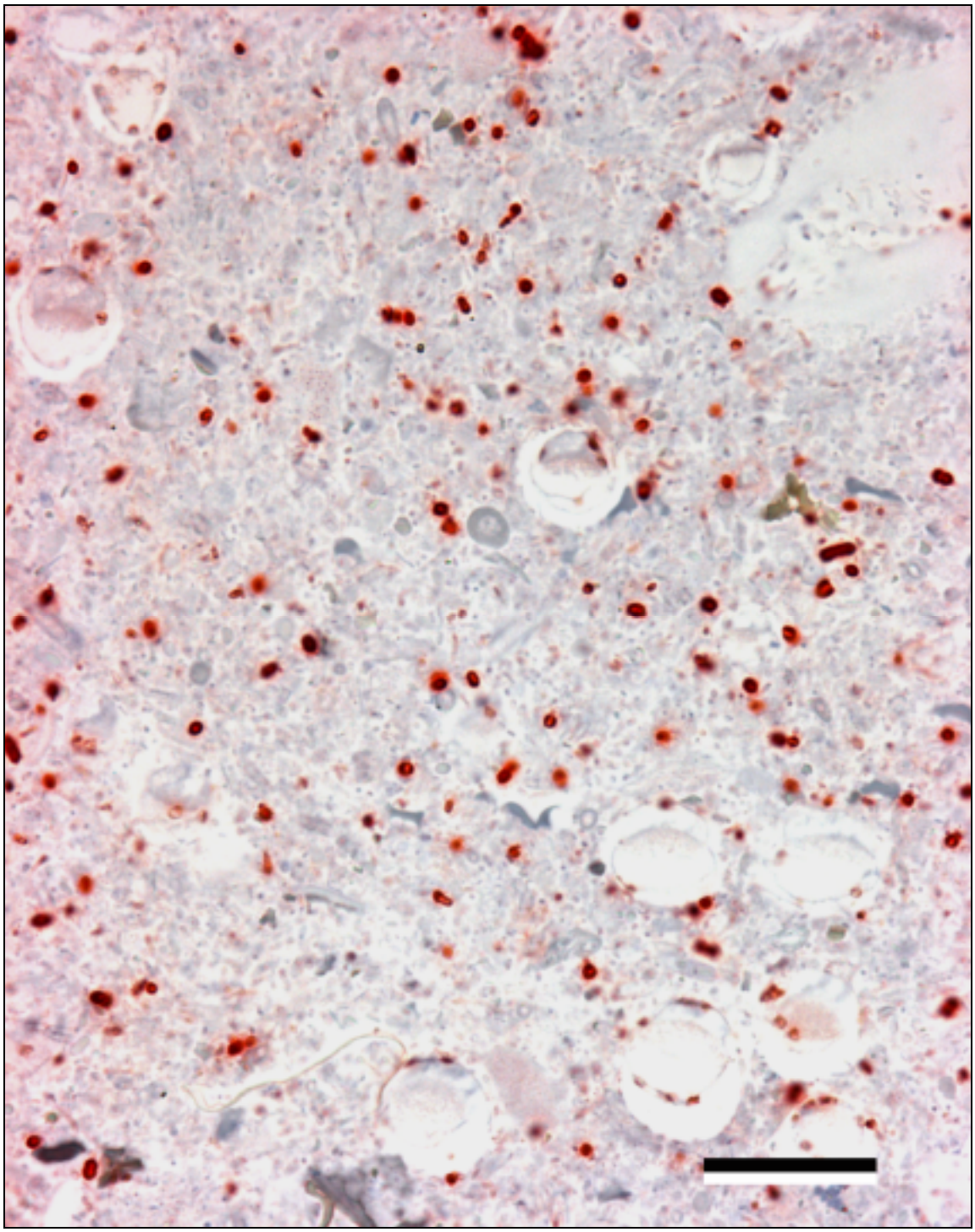

Figure 26. Macrophage mannose receptor. (Chromogen substrate).

The colonic intestinal content from a 66 years old man with carcinoma of the ascending colon. Scale bar is 20µm.

Figure 27.

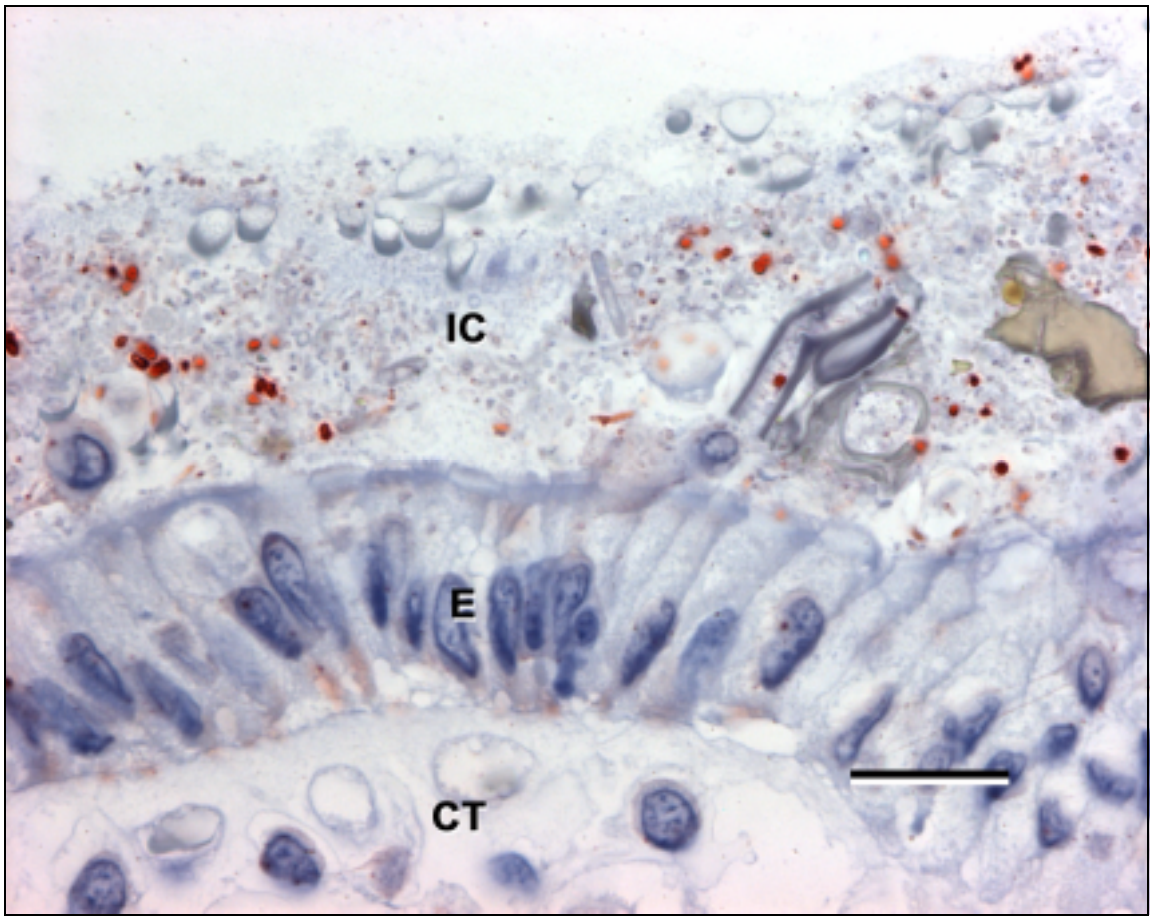

Figure 27. Macrophage mannose receptor. (Chromogen substrate).

The colonic mucosa from a 66 years old man with carcinoma of the ascending colon. The intestinal content (IC), colonic epithelium (E) and subepithelial connective tissue (CT) are shown. Scale bar is 20µm.

Figure 28.

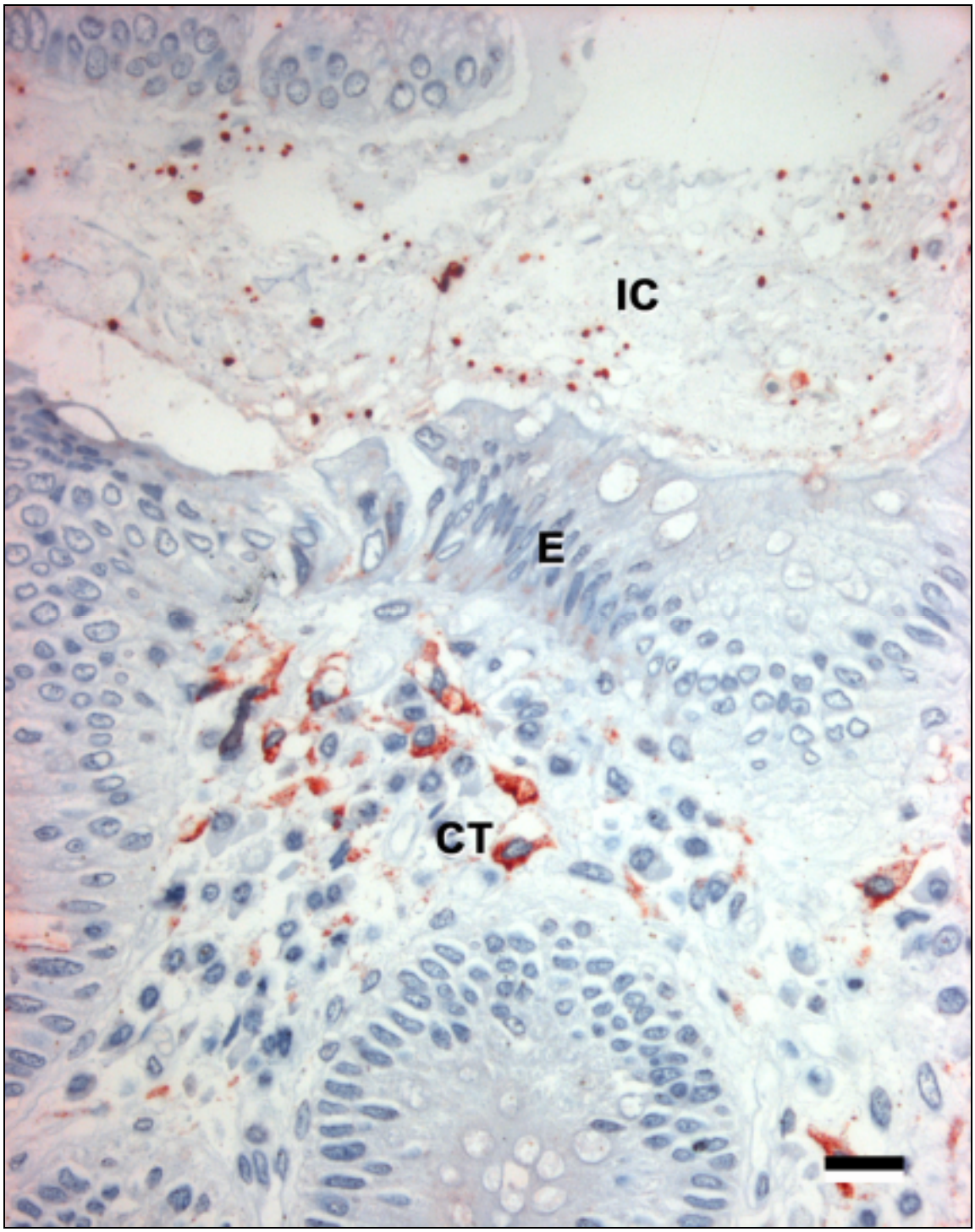

Figure 28. Macrophage mannose receptor. (Chromogen substrate).

Biopsy from the sigmoid colon of a 70 years old man with ulcerative colitis. The intestinal content (IC), colonic epithelium (E) and subepithelial connective tissue (CT) are shown. Scale bar is 20µm.

Figure 29.

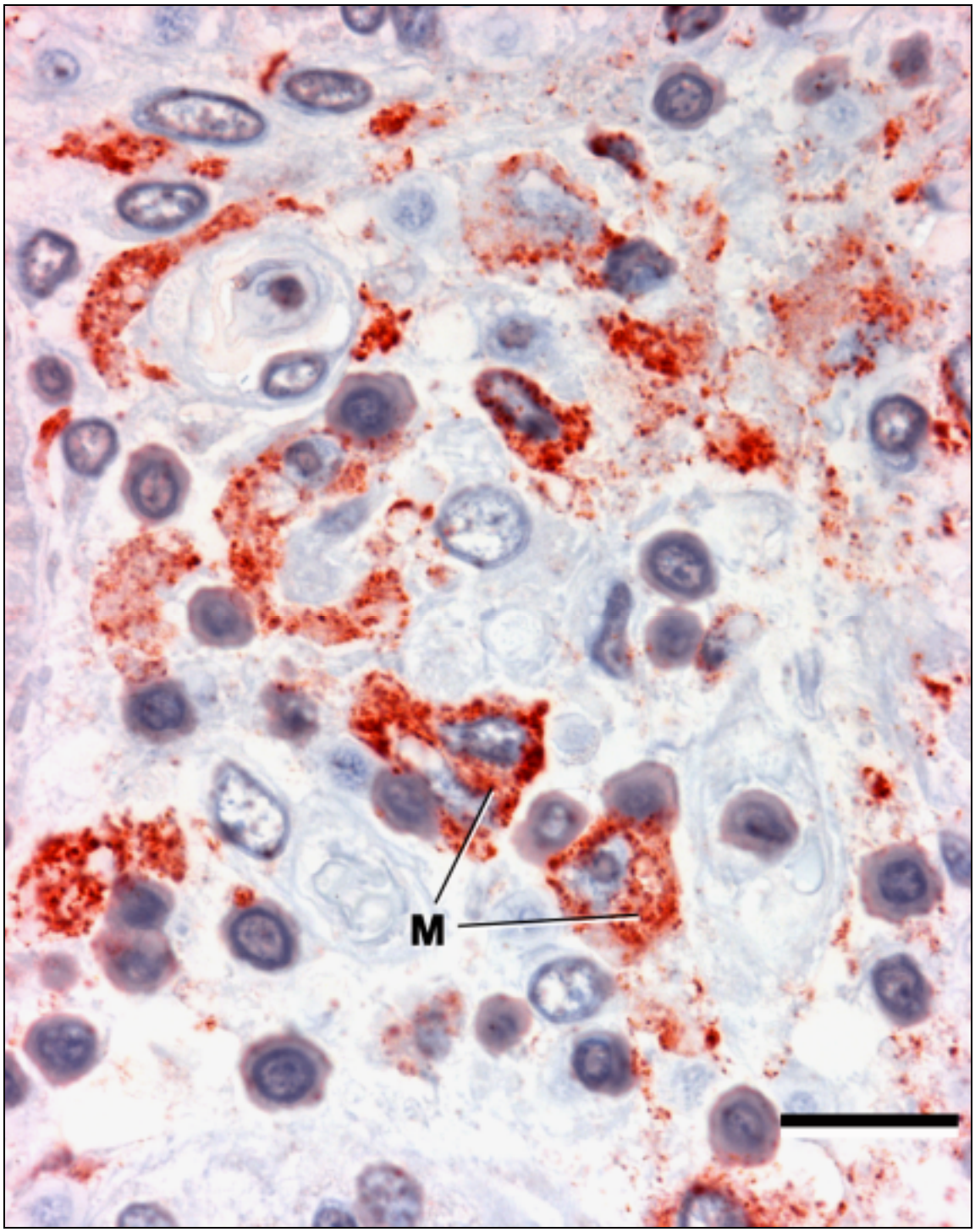

Figure 29. Macrophage mannose receptor. (Chromogen substrate).

Biopsy from the ascending colon of a 41 years old man with ulcerative colitis. Macrophages (M) in the subepithelial connective tissue are shown. Scale bar is 20µm.

Figure 30.

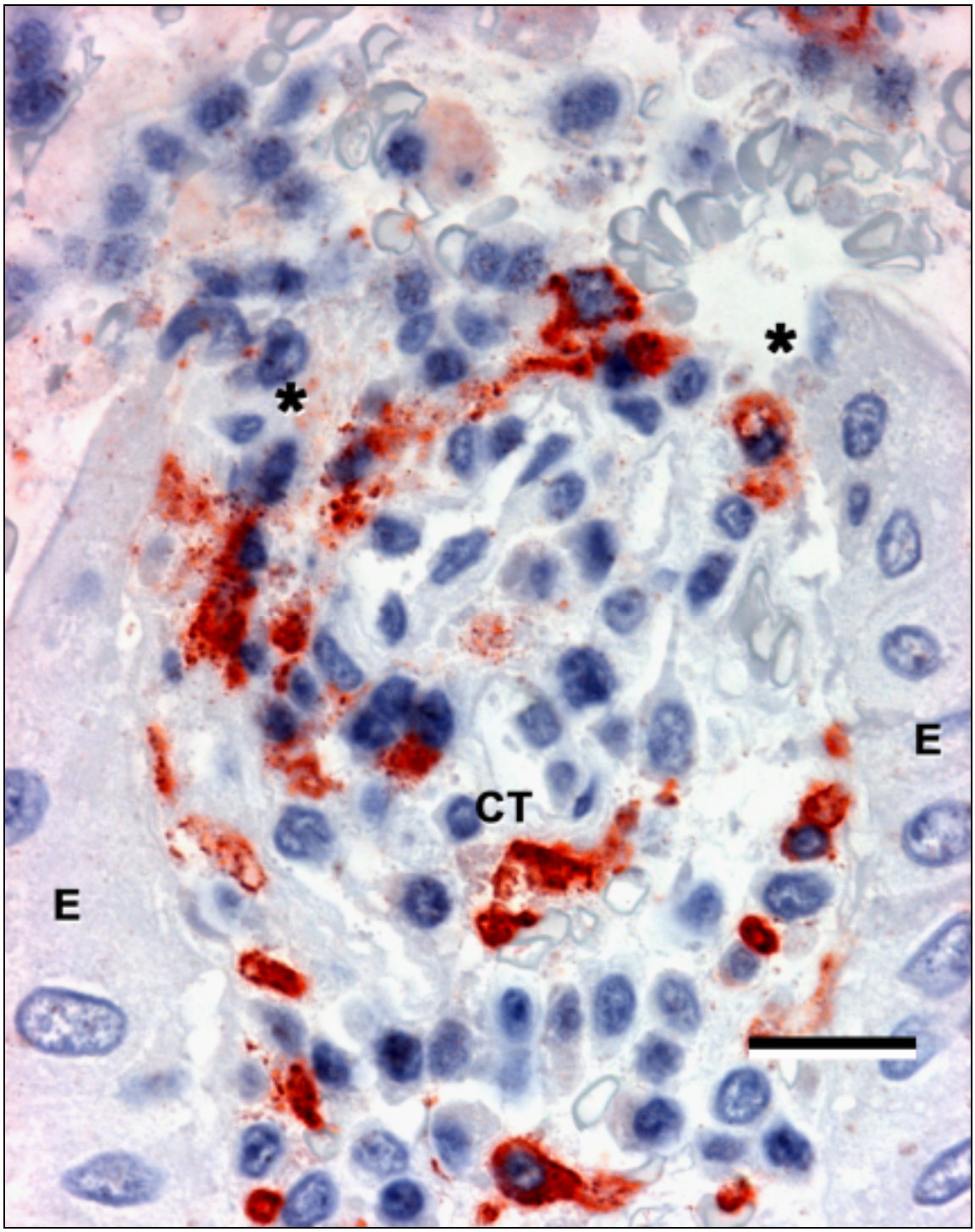

Figure 30. Macrophage mannose receptor. (Chromogen substrate).

Biopsy from the sigmoid colon of a 63 years old female with ulcerative colitis. The colonic epithelium (E) and subepithelial connective tissue (CT) are shown. The gap in the continuity of the colonic epithelium is indicated (∗). Scale bar is 20µm.

Figure 31.

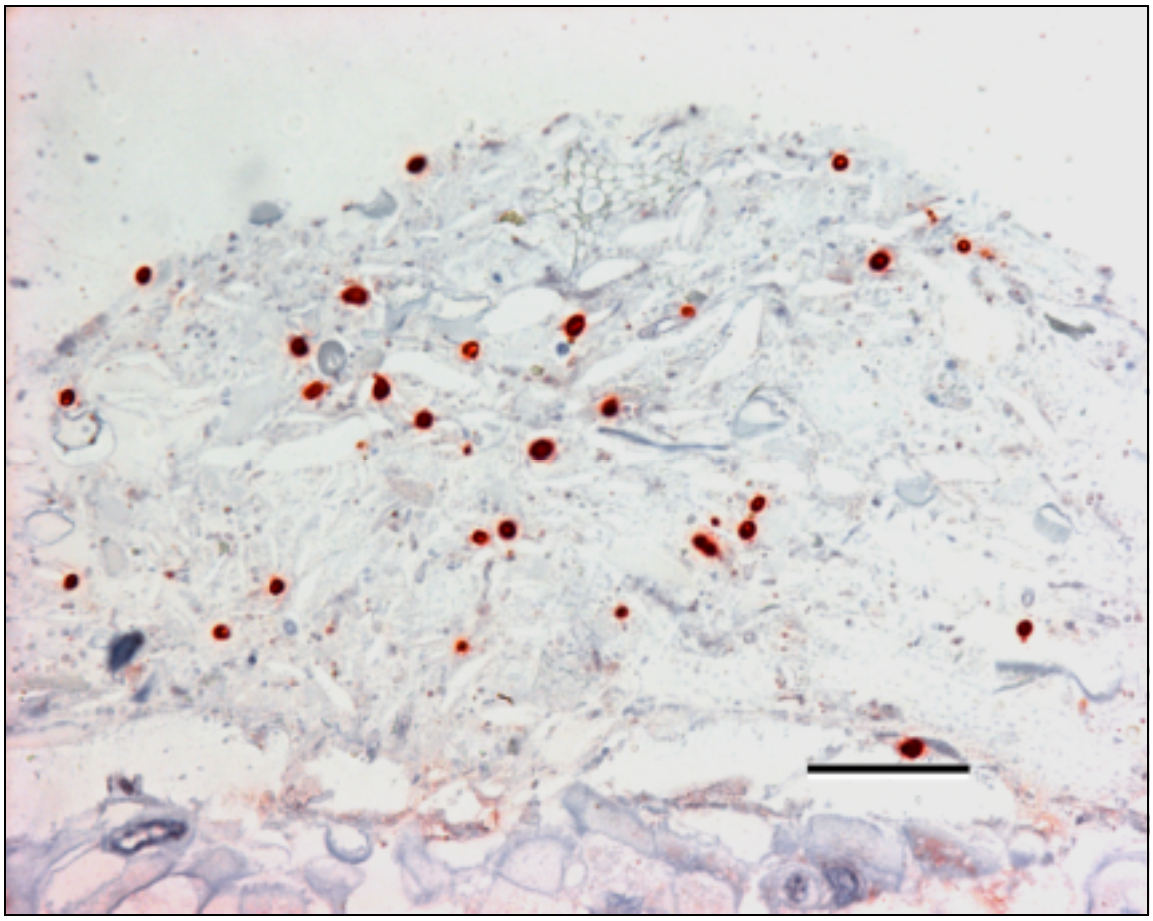

Figure 31. Macrophage mannose receptor. (Chromogen substrate).

Biopsy from the sigmoid colon of a 70 years old male with ulcerative colitis. The intestinal content is shown. Scale bar is 20µm.

Figure 32.

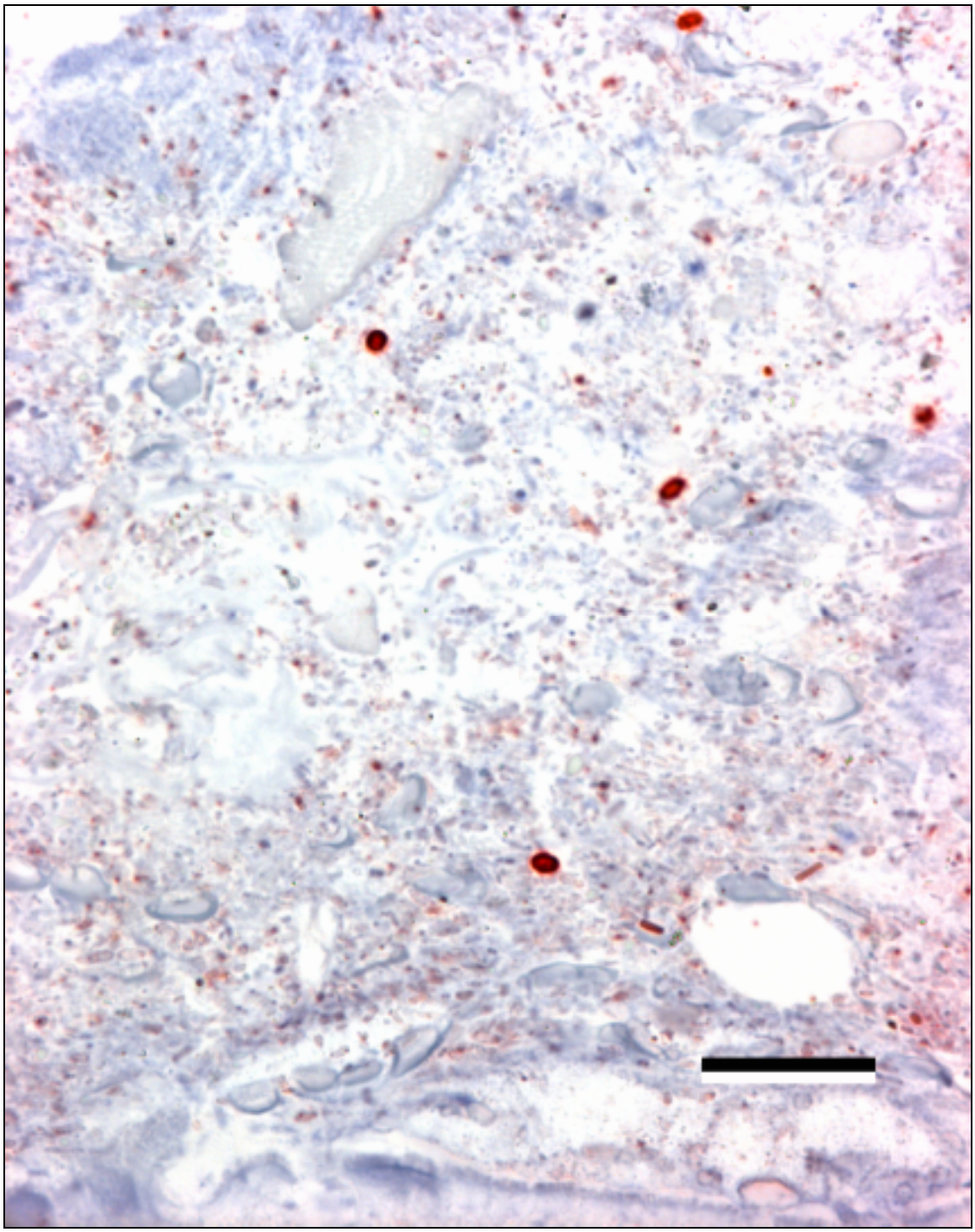

Figure 32. Macrophage mannose receptor. (Chromogen substrate).

Biopsy from the sigmoid colon of a 70 years old male with ulcerative colitis. The intestinal content is shown. Scale bar is 20µm.

Figure 33.

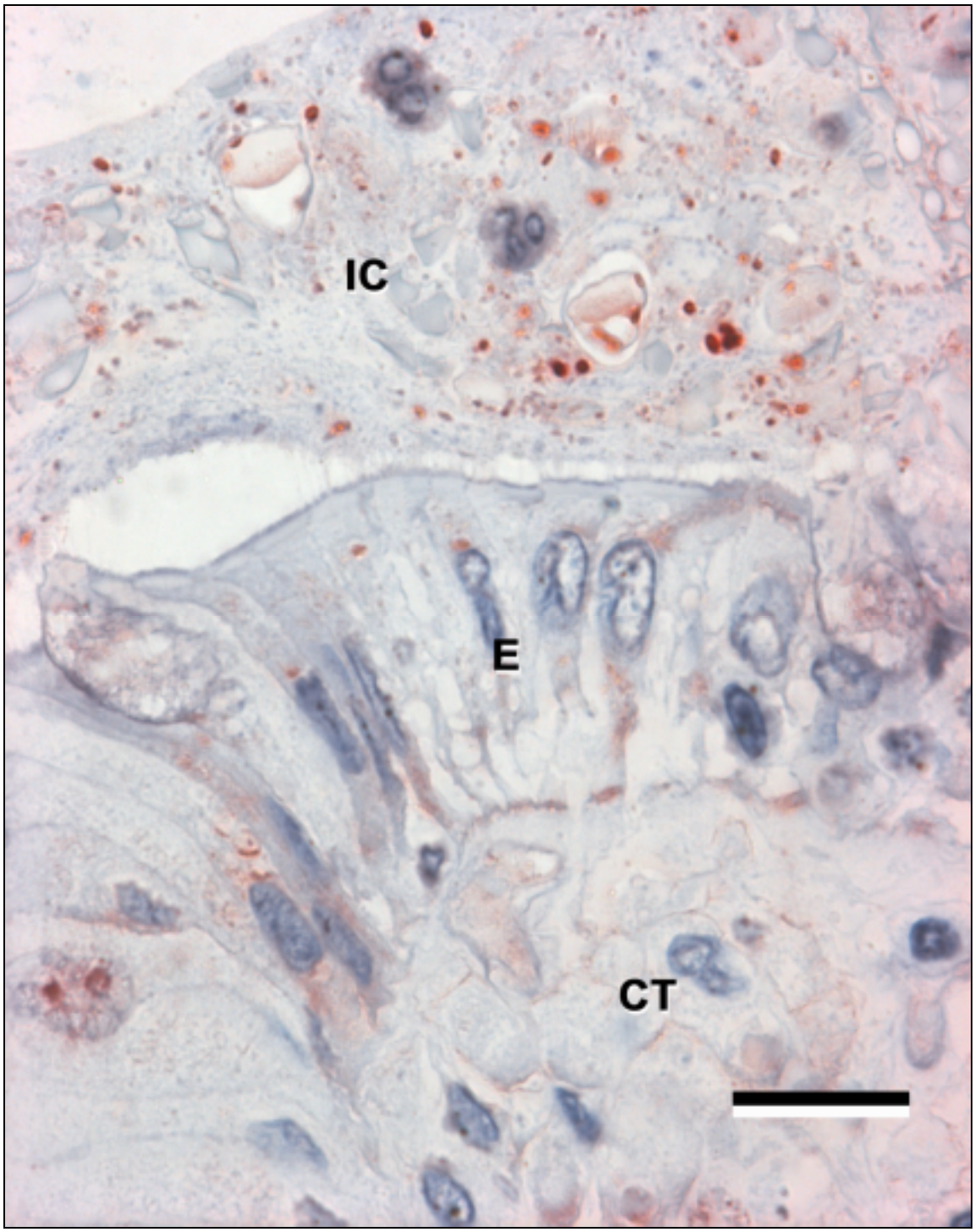

Figure 33. Tumour necrosis factor α. (Chromogen substrate).

Colonic biopsy from a 84 years old male with carcinoma of the ascending colon.
The intestinal content (IC), colonic epithelium (E) and subepithelial connective tissue (CT) are shown. Scale bar is 20µm.

Figure 34.

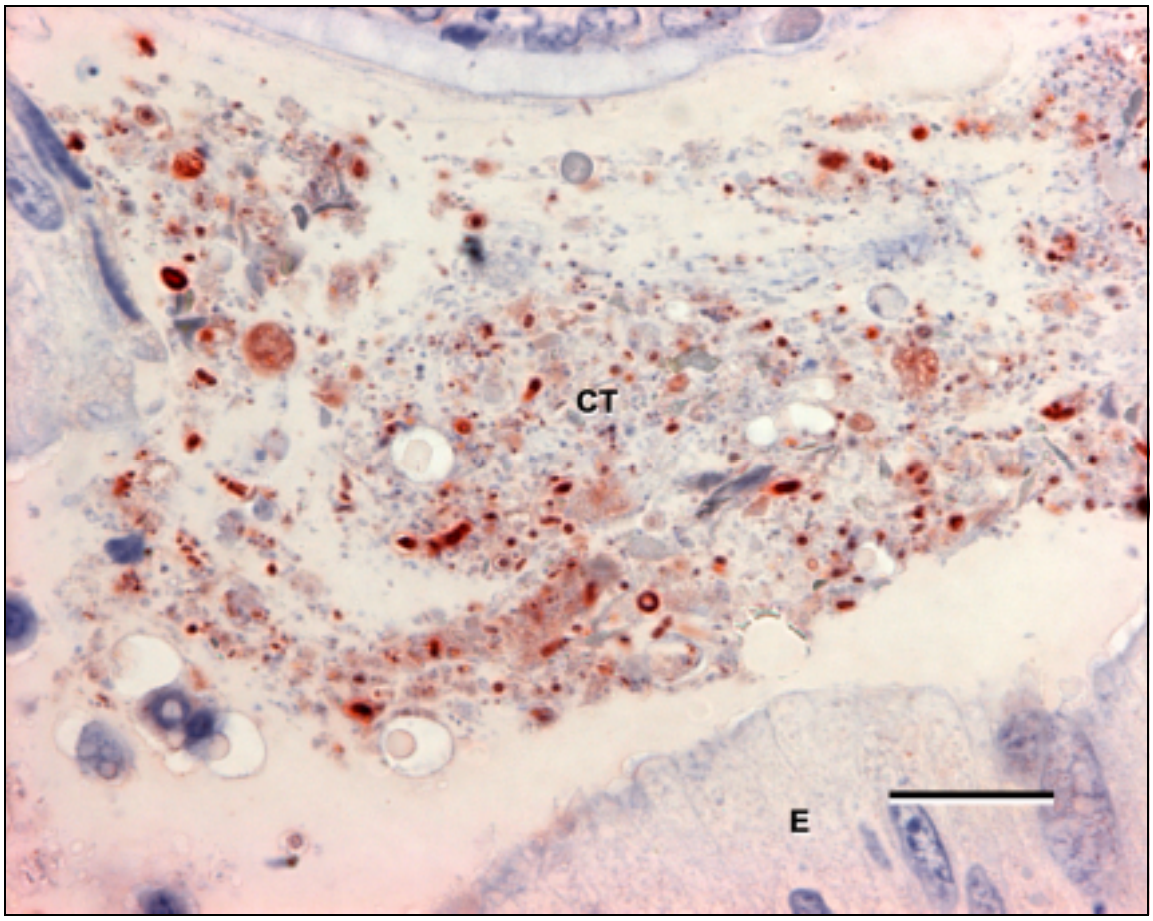

Figure 34. Tumour necrosis factor α. (Chromogen substrate).

Colonic biopsy from a 51 years old female with carcinoma of the rectum. The intestinal content (IC) and colonic epithelium (E) are shown. Scale bar is 20µm.

Figure 35.

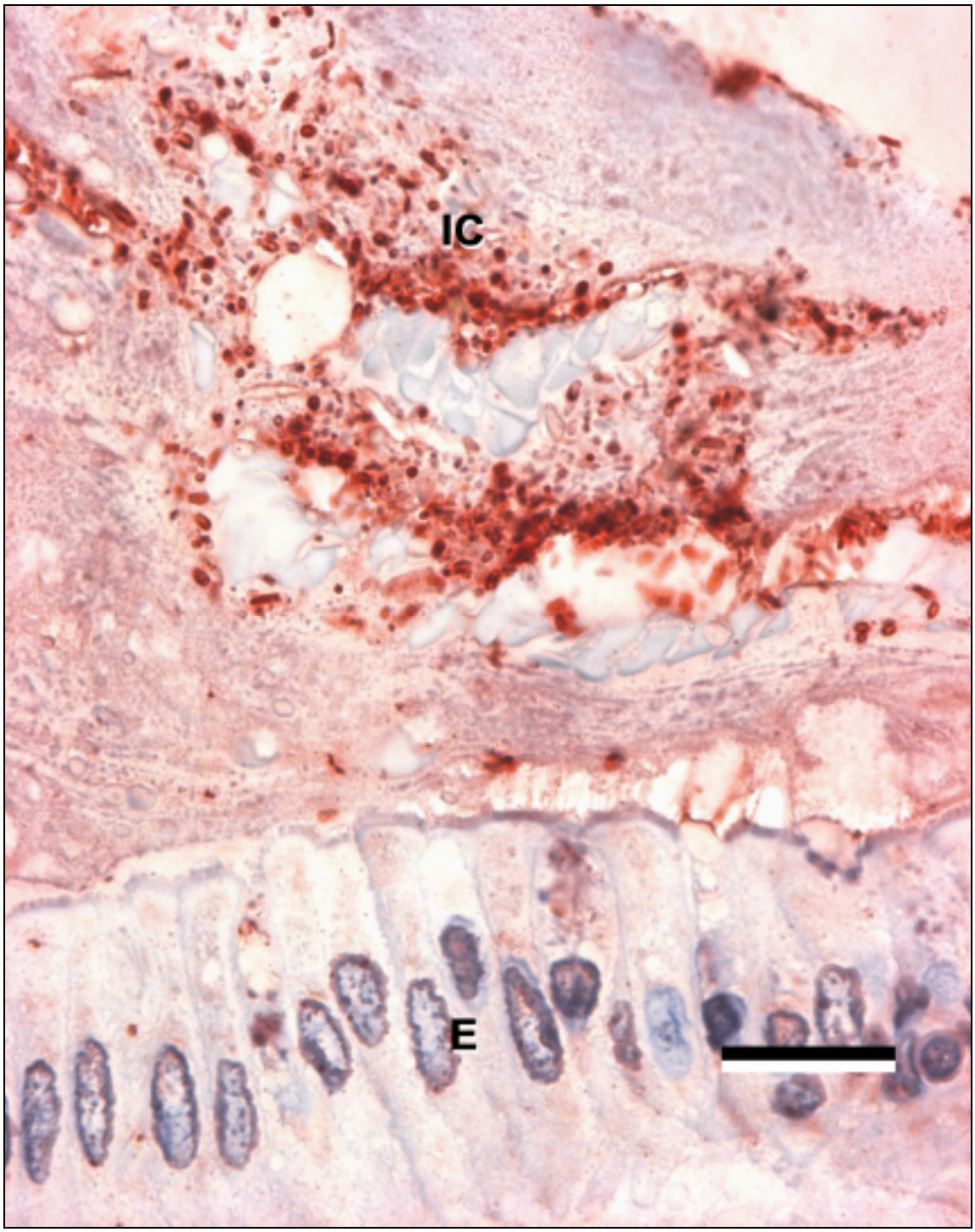

Figure 35. Tumour necrosis factor α. (Chromogen substrate).

Rectal biopsy from a 60 years old patient with ulcerative colitis. The intestinal content (IC) and colonic epithelium (E) are shown. Scale bar is 20µm.

Figure 36.

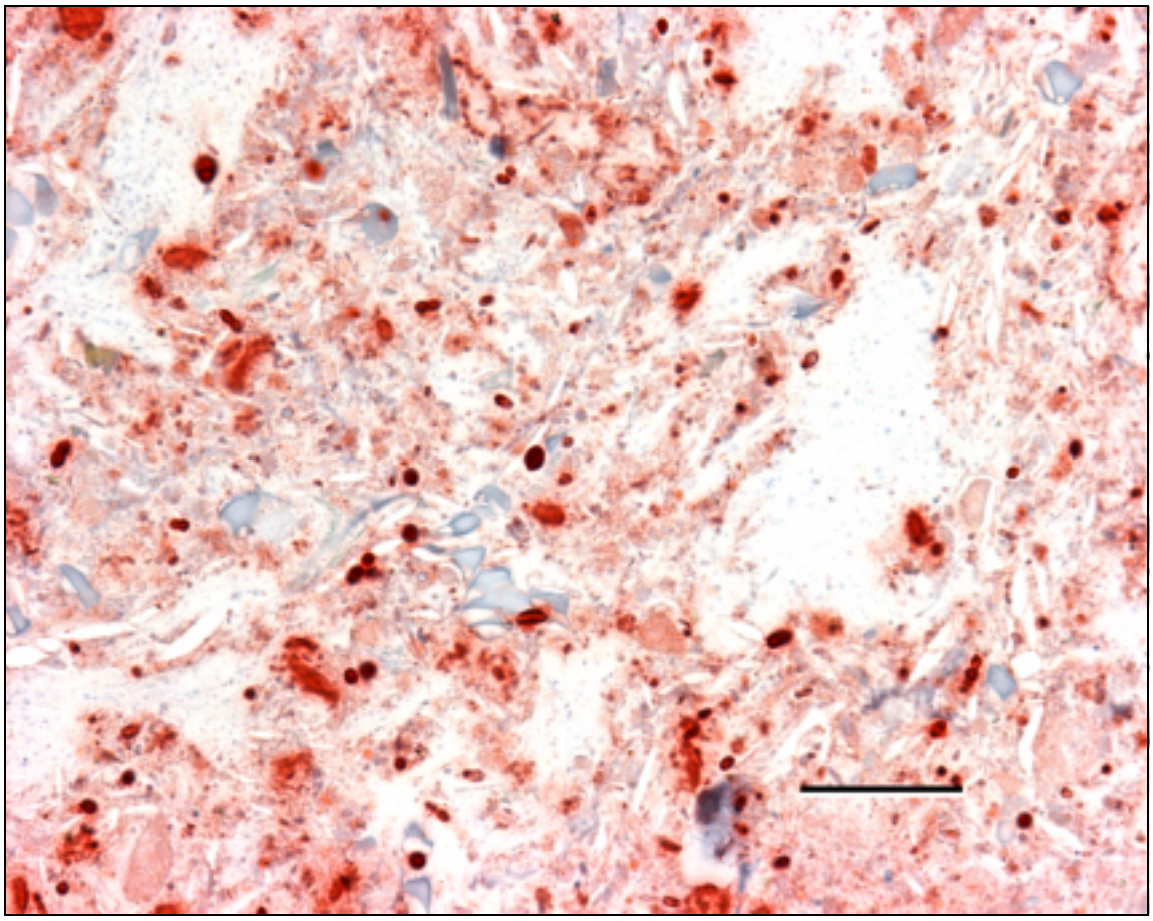

Figure 36. Tumour necrosis factor α. (Chromogen substrate).

Biopsy from the hepatic flexure of the colon of a 70 years old man with ulcerative colitis. The intestinal content is shown. Scale bar is 20µm.

Figure 37.

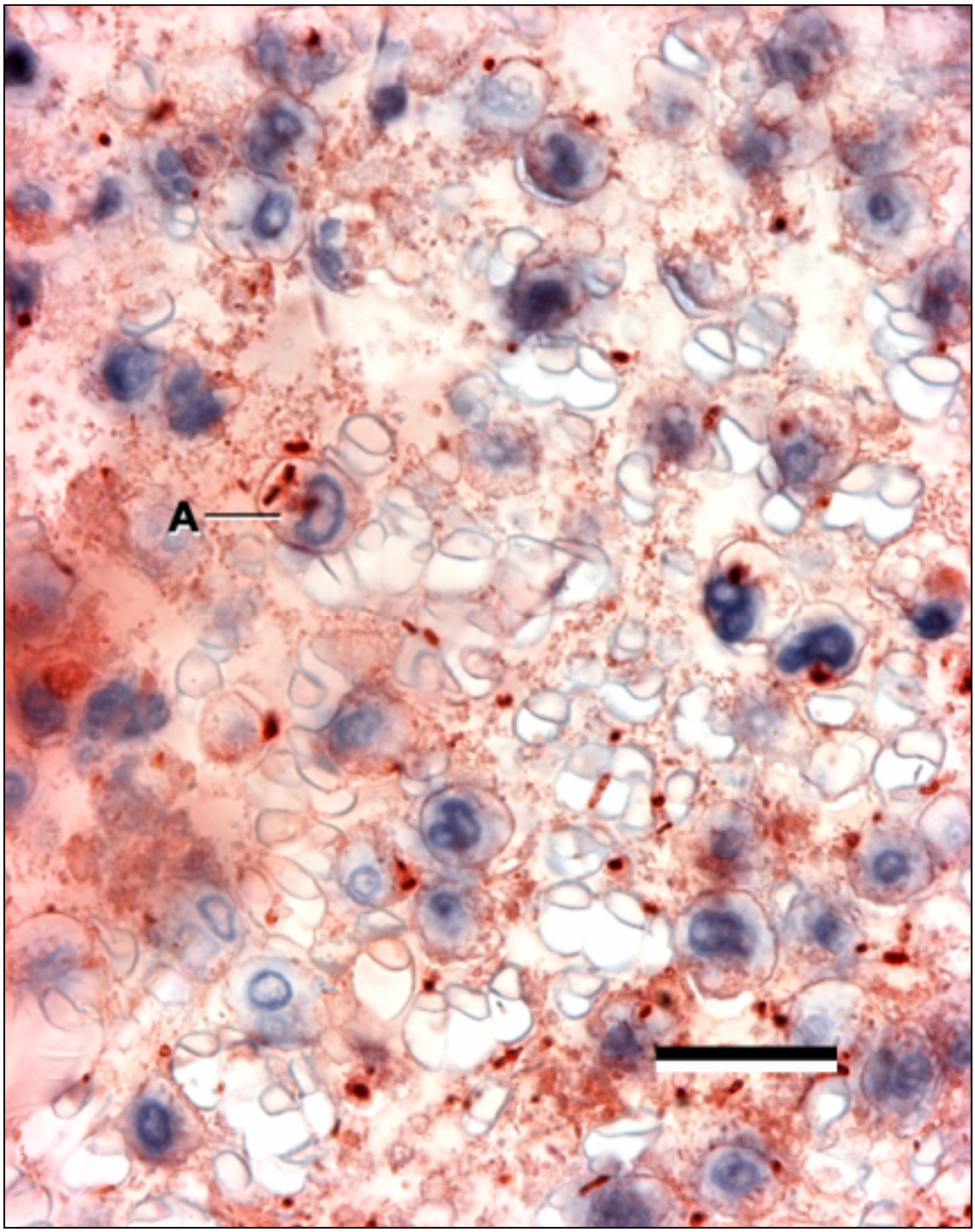

Figure 37. Tumour necrosis factor α. (Chromogen substrate).

Biopsy from the sigmoid colon of a 63 years old female with ulcerative colitis. A leucocyte in the intestinal content with phagocytosed bacteria is shown (A). Scale bar is 20µm.

Figure 38.

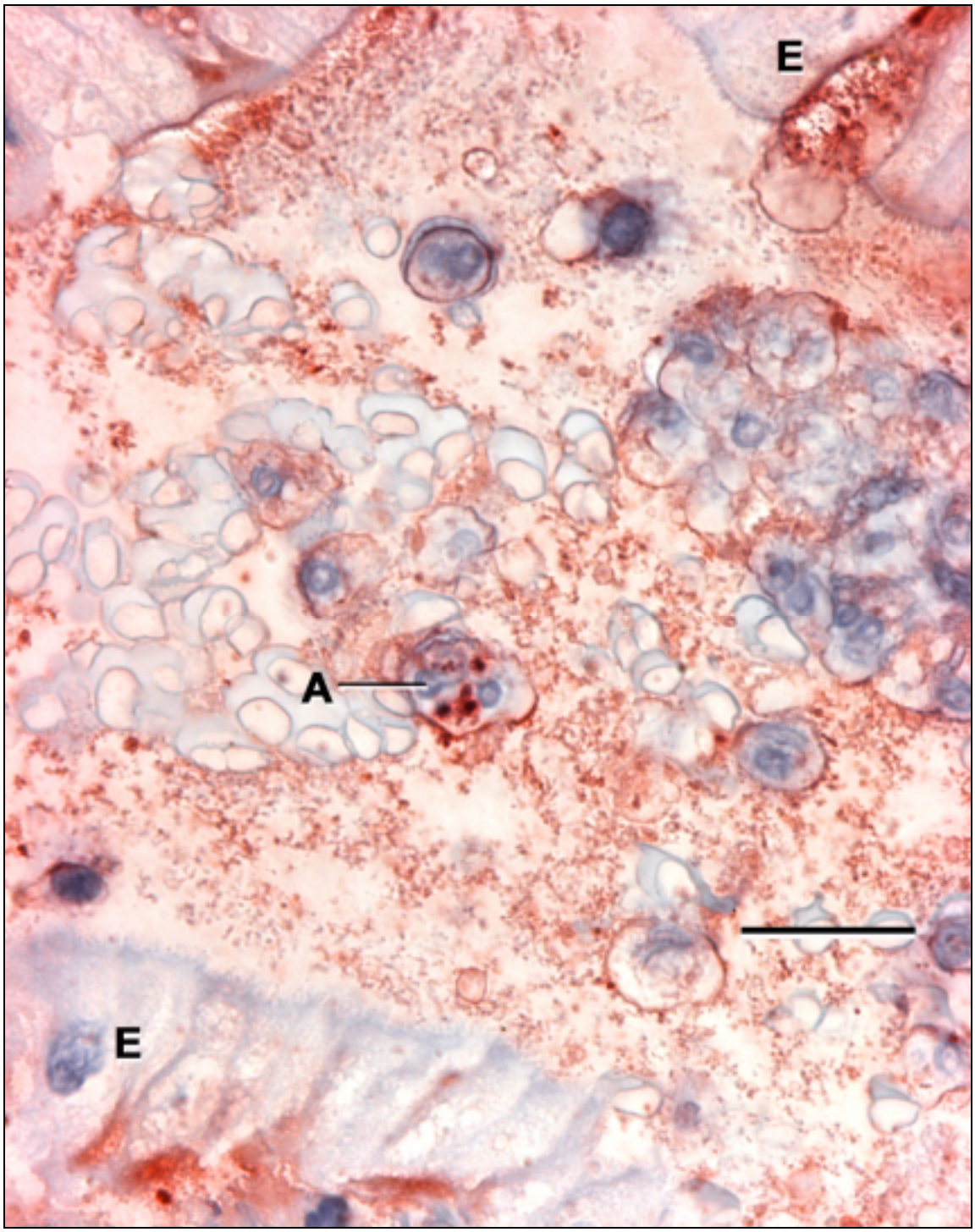

Figure 38. Tumour necrosis factor α. (Chromogen substrate).

Biopsy from the sigmoid colon of a 63 years old female with ulcerative colitis.
A polymorphonuclear leucocyte (A) with phagocytosed bacteria is present in the intestinal lumen and the colonic epithelium (E) is shown. Scale bar is 20µm.