

Campylobacter colitis: histological immuno-histochemical and ultrastructural findings

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SUMMARY The colonic biopsy specimens of 22 patients with colitis and positive stool cultures for *Campylobacter jejuni* were studied in order to obtain histological and immunohistochemical criteria to differentiate *Campylobacter* colitis from chronic inflammatory bowel disease. In addition we tried to identify *Campylobacter* inclusions by means of immunohistochemistry and electron microscopy as evidence for invasion of the colonic mucosa. The results show that the majority of patients with *Campylobacter* colitis have the histological picture of acute infectious colitis with increased numbers of IgA and IgM containing plasma cells in the colonic mucosa in contrast with patients with active chronic inflammatory bowel disease who show increases of IgA and IgG (ulcerative colitis) or IgA-, IgM and IgG containing plasma cells (M Crohn) in their colonic biopsies. The results of immunohistochemical stainings with *Campylobacter* antiserum show invasion of *Campylobacter* in the colonic mucosa. These findings were confirmed ultrastructurally.

In the past decade *Campylobacter* has emerged from obscurity to become an important human pathogen. Provided that proper techniques are applied *Campylobacter fetus* subspecies *jejuni* can be isolated from the stools of patients with diarrhoea as often as *Salmonella*.^{1,2} In the laboratory for microbiology of the SSDZ, Delft, *Campylobacter jejuni* was isolated as the causative organism in 41% of 732 consecutive positive stool cultures of patients with diarrhoea. *Campylobacter* may cause a spectrum of intestinal disease ranging from acute gastroenteritis to toxic megacolon, lymphadenitis mesenterialis, and even appendicitis and cholecystitis.³⁻⁵ Usually infection with this organism results in acute gastroenteritis with fever and frequent loss of often bloody stools. The disease resolves spontaneously within one week but in 20% it runs a more prolonged course or relapses resembling chronic inflammatory bowel disease.¹ Endoscopically it may

be indistinguishable from ulcerative colitis. Histological examination of rectal biopsies has ranged from normal to inflammatory changes suggestive of acute infectious colitis or ulcerative colitis or Crohn's disease.⁶⁻¹¹ In the present study we have investigated the colonic biopsies of 22 patients with diarrhoea and stool cultures positive for *Campylobacter jejuni*. The aim of our study was to determine the histological features and the number of immunoglobulin containing cells in colonic biopsies of patients with *Campylobacter* colitis and to identify *Campylobacter* inclusions immunohistochemically.

Methods

PATIENTS

One hundred and twelve colonic biopsies from 22 patients with positive stool cultures for *Campylobacter jejuni* were examined. One patient had been suffering from chronic ulcerative colitis for many years whereas one patient had previously suffered from non-specific proctitis. The others had no coexisting gastrointestinal disease. Diarrhoea with crampy abdominal pain was the presenting com-

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plaint in 15 patients, 13 of them had noticed blood in their stools, and fever was an accompanying symptom in seven of these 15 patients. Nausea was recorded frequently. Six patients merely complained of loosing blood with their stools. One patient presented with abdominal pain, nausea, and vomitus. The numbers of immunoglobulin containing cells in colonic biopsies of patients with Campylobacter colitis were compared with those of 10 healthy controls, 10 patients with active Crohn's disease of the colon and 10 patients with active ulcerative colitis. All patients with chronic inflammatory bowel disease had histologically abnormal colonic biopsies.

Stools were cultured for Salmonella, Shigella, Yersinia and Campylobacter routinely. Campylobacter was cultured on a selective medium: blood agar plates containing Campylobacter Growth Supplement and Campylobacter Selective Supplement (Oxoid LTD Basingstoke England nos. SR 84 and SR 85). Inoculated plates were incubated during 48 hours at 42°C in an atmosphere containing 5–15% O₂ and 5–8% CO₂ by using the GasPak of BBL without a catalyst in a jar.

Biopsies were fixed immediately either in a formalin sublimate mixture for four to six hours for routine histology and immunohistochemical studies or in cacodylate buffered glutaraldehyde for ultrastructural studies. Tissue samples were embedded in paraffin wax, cut in sections 4 μm thick and mounted on glass slides. Besides histological stains including haematoxylin and eosin and periodic acid Schiff, biopsies were stained specifically for IgA, IgM, and IgG heavy chains using an indirect immunoperoxidase technique.¹² Appropriate controls were done according to Sternberger.¹³ Rabbit antisera against IgA, IgM, and IgG heavy chains were purchased from Dakopatts (Denmark). Their specificity was confirmed by immunoelectrophoresis, immunofluorescence and immunoperoxidase stainings on bone marrow preparations monoclonal for IgA, IgM, or IgG. Horseradish peroxidase labelled goat antirabbit Ig was obtained from Miles (Yedah, Israel). The IgA, IgM, and IgG stained sections were used for morphometrical analysis. They were photographed with a standard magnification (100, 8 ×) and projected on a graphic tablet interfaced to a computer (Ibas I Kontron Munchen). Every photograph contained approximately 1 mm mucosa at full thickness. The lamina propria area was limited by two lines perpendicular to the muscularis mucosae and this area was measured per mm muscularis mucosae length. The number of immunoglobulin containing cells was measured in two consecutive sections in approximately the same area and expressed both per 0.1 mm² lamina propria and per

0.5 mm stretched muscularis mucosae. The latter is comparable with the 'mucosal tissue unit' described by Brandzaeg and Baklien.¹⁴ Moreover, immunohistochemistry was carried out with Campylobacter antiserum using both direct immunoperoxidase and immunofluorescence techniques. The antiserum was raised in a rabbit that was vaccinated intravenously with a Campylobacter fetus subspecies jejuni suspension containing microorganisms of serotype LAU1¹⁵ that were killed in 1.5% formalin. Antiserum titres were determined by agglutination and indirect immunofluorescence methods. After several absorption steps with *Salmonella g*, *Shigella boydii*, *Shigella sonnei*, *Proteus vulgaris* and *Escherichia coli* the antiserum showed no cross reactivity with Salmonella, Shigella, or *Escherichia coli*. The antiserum gave a positive immunofluorescence with 37 out of 38 different Campylobacter LAU serotypes; representing all known serotypes at that time in the Netherlands. Immunofluorescence was achieved by removing paraffin with xylol and alcohol, preincubation with normal swine serum 1:10, incubation with rabbit Campylobacter antiserum 1:40, once again incubation with normal swine serum 1:10 and incubation with swine antirabbit FITC. Sections stained with preimmune serum from the same rabbit served as controls. Colonic biopsies from eight patients with Crohn's disease of the colon and nine patients with ulcerative colitis stained with Campylobacter antiserum served as controls as well and were all negative. In addition in three patients in whom immunohistochemistry with Campylobacter antiserum had given positive results ultrastructural examination of the biopsies was done using a Zeiss EM 109 electron microscope.

Statistical analysis was carried out according to the Student's *t* test, *p* < 0.05 was considered significant.

Results

The results are summarised in the Table.

HISTOLOGICAL FINDINGS

In 19 patients the colonic biopsies showed a histological picture consistent with acute infectious colitis. In the patient with longstanding ulcerative colitis with superimposed Campylobacter enterocolitis the histological features of ulcerative colitis dominated with marked distortion of crypt architecture. In two patients histological examination revealed no abnormalities. Based on our findings in patients in whom follow up biopsies were obtained, and the time lapse between the onset of bloody diarrhoea and the biopsy we believe the histological changes of Campylobacter colitis can be divided in three stages.

Table Histological and immunohistochemical findings in 22 patients with positive stool cultures for *Campylobacter jejuni*

Days after onset of symptoms	Histological findings	IgA IgM IgG containing cells			Immunohistochemistry <i>Camp. antisera</i>			
					Glycocalyx	Crypt surf epithelium lamina prop	Histiocytes	
1	?	Act. chronic inflam.	↑	↑	↑	+	+	+
	?	Act. chronic inflam.	↑	↑	↑	+	+	+
2	9	Acute colitis II	↑	↑		-	-	+
	30	Normal				+	-	-
3	?	Acute colitis II	↑	↑		+	-	-
4	?	Acute colitis II	↑			+	-	-
5	17	Acute colitis III				+	-	-
6	7	Acute colitis I	↑			-	+	-
	14	Acute colitis III	↑	↑		+	-	-
	30	Normal				-	-	-
7	?	Acute colitis II	↑			+	-	-
8	6	Acute colitis I	↑	↑		+	+	-
	30	Normal	↑			+	+	+
9	?	Acute colitis II	↑			+	+	-
10	6	Acute colitis I	↑			+	-	-
11	3	Acute colitis I	↑	↑		+	-	-
12	10	Acute colitis II	↑	↑		+	-	-
13	?	Acute colitis III	↑			-	+	+
14	>7<14	Acute colitis II	↑	↑		+	+	+
15	?	Acute colitis II	↑	↑		-	-	-
16	?	Acute colitis II	↑			-	-	-
17	14	Acute colitis II	↑	↑		+	-	-
18	?	Normal	↑			-	+	+
19	>7<14	Acute colitis II	↑			+	+	+
20	?	Acute colitis II				+	-	-
21	?	Acute colitis I	↑			+	-	-
22	>14	Normal				-	-	-

I = early stage, II = late stage, III = residual stage, ↑ = increased, ? = unknown.

EARLY STAGES (FIVE PATIENTS)

In the first seven days after the onset of bloody diarrhoea histological changes are evident and diagnostic for acute infectious colitis. Epithelial changes are conspicuous. The surface epithelium is infiltrated by neutrophils and superficial ulcerations are present. Often the surface is covered with exudate. Transmigration of granulocytes in the crypt epithelium gives rise to cryptitis (incipient crypt abscesses) that may ultimately result in crypt abscesses: crypts filled with granulocytes lined by a flat degenerated epithelium. There usually is mucin depletion of the epithelium. The lamina propria shows oedema and is diffusely infiltrated with granulocytes, histiocytes, plasma cells, and lymphocytes. Granulocytes and histiocytes predominate. Occasionally the histiocytic component of the infiltrate is very pronounced and gives the infiltrate a granulomatous appearance. Granulomata with giant cells, however, were never observed. Apart from separation of crypts because of oedema and loss of crypts, crypt architecture is well preserved. The pathological changes are confined to the mucosa

and tend to be more pronounced in the distal parts of the colon (Fig. 3).

LATE STAGE (12 PATIENTS)

From seven to 14 days after the beginning of symptoms the inflammation gradually subsides and the histological findings become less characteristic. The epithelium shows features of regeneration: large pale nuclei with prominent nucleoli and there is increased mitotic activity. Transmigration of neutrophils in crypts is either absent or may be seen focally adjacent to normal crypts giving the inflammation a discontinuous appearance. The lamina propria is oedematous especially in the upper layers and contains an inflammatory infiltrate consisting mainly or solely of mononuclear cells, especially in the deeper layers (basal cryptitis). Lymphoid hyperplasia may be found and crypt architecture is not distorted.

RESIDUAL STAGE (THREE PATIENTS)

After 14 days inflammatory changes are minimal or absent. There may be a slight increase of lympho-

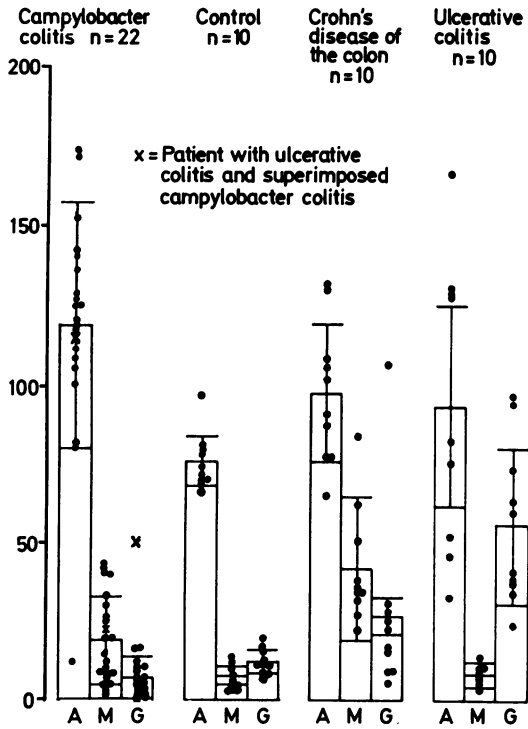


Fig. 1 Numbers of immunoglobulin containing cells per 0.1 mm² lamina propria \pm standard deviation.

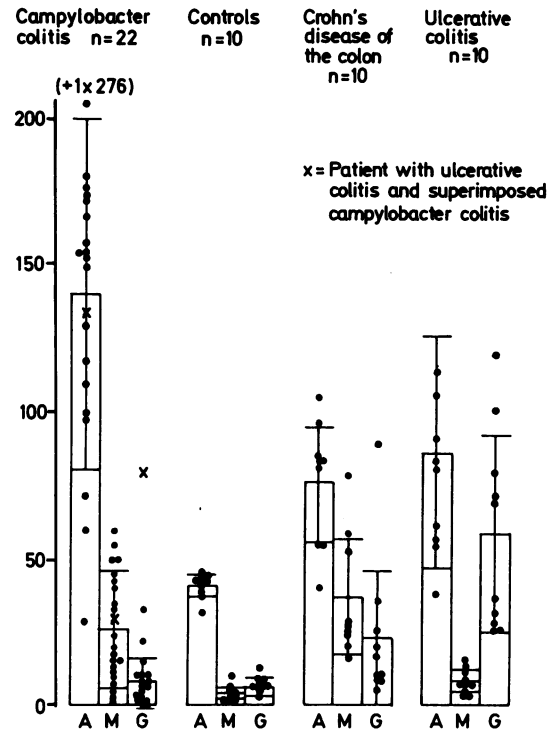


Fig. 2 Numbers of immunoglobulin containing cells per 0.5 mm muscularis mucosae \pm standard deviation.

cytes and plasma cells in the lamina propria and crypts may not reach to the muscularis mucosae. In one patient we observed branching of crypts.

IMMUNOHISTOCHEMICAL FINDINGS

The numbers of IgA, IgM and IgG containing cells are listed in Figs. 1 and 2. IgE containing cells were observed sporadically and therefore not included. The total number of immunoglobulin containing cells is increased in Campylobacter colitis compared with controls. This increase is because of IgA and IgM containing cells, both the number per 0.5 mm muscularis mucosae ($p=0.0002$ and $p=0.03$ respectively) and the number per 0.1 mm² lamina propria ($p=0.0005$ and $p=0.001$ respectively) differs significantly. The number of IgG containing cells per 0.1 mm² lamina propria was lower in Campylobacter colitis ($p=0.01$) compared with controls but this is probably due to oedema of the lamina propria as the number of IgG containing cells per 0.5 mm muscularis mucosae does not differ. Biopsies of patients with Campylobacter colitis differ significantly from biopsies of patients with Crohn's colitis and ulcera-

tive colitis with respect to the number of IgG containing cells. The number of IgG containing cells is significantly lower in Campylobacter colitis compared with Crohn's colitis and ulcerative colitis, both the number per 0.5 mm muscularis mucosae ($p=0.01$ and 0.00007 respectively) and the number per 0.1 mm² lamina propria ($p=0.002$ and 0.00003 respectively) differs significantly. Immunohistochemical staining with Campylobacter antiserum gave positive results in one or more biopsies in 19 out of 22 patients. The results with immunofluorescence were equal to that obtained by immunoperoxidase. Positive staining was found in the glycocalyx (Fig. 4), in the surface epithelium especially goblet cells and focally in the lamina propria. Occasionally positive staining was also found in crypt epithelium and in histiocytes in the lamina propria. Positive immunohistochemical results did not correlate quantitatively or qualitatively with the degree of inflammation and were also obtained in one patient that showed no histological abnormalities.

In order to confirm the immunohistochemical findings with Campylobacter antiserum serial sec-

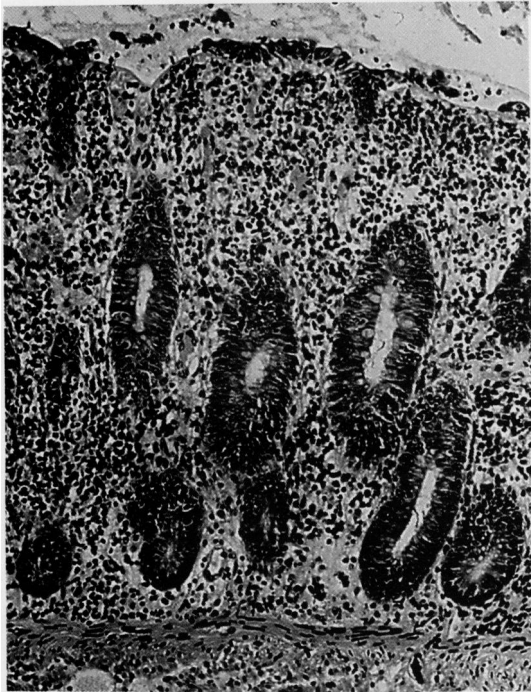


Fig. 3 Colonic biopsy (seven days after the onset of symptoms) showing diffuse infiltration of the lamina propria with granulocytes, histiocytes, lymphocytes and plasma cells, erosion and focal cryptitis.

tions of the biopsies of four patients giving positive results were examined ultrastructurally. These sections were compared with the ultrastructural picture of a *Campylobacter jejuni* culture (Fig. 5). In all these biopsies characteristic features of *Campylobacter* could be demonstrated in either the glycocalyx (Fig. 6) overlying the surface epithelium or in the cytoplasm of the surface epithelium (Fig. 7) including Goblet cells.

Discussion

In this study we have shown that *Campylobacter* infection produces the histological picture of acute infectious colitis and that the number of immunoglobulin containing cells in the colonic biopsies of these patients differs from those of patients with chronic inflammatory bowel disease with regard to the number of IgG containing cells. In addition we have been able to show *Campylobacter* immunohistochemically in colonic biopsies in 19 out of 22 patients with positive stool cultures for *Campylobacter jejuni*.

Campylobacter colitis should be distinguished from other bacillary dysenteries and from active chronic inflammatory bowel disease because it may mimic these diseases both clinically and endoscopically. Stool cultures and colonoscopy with biopsy are important tools in the differential diagnosis. Histo-

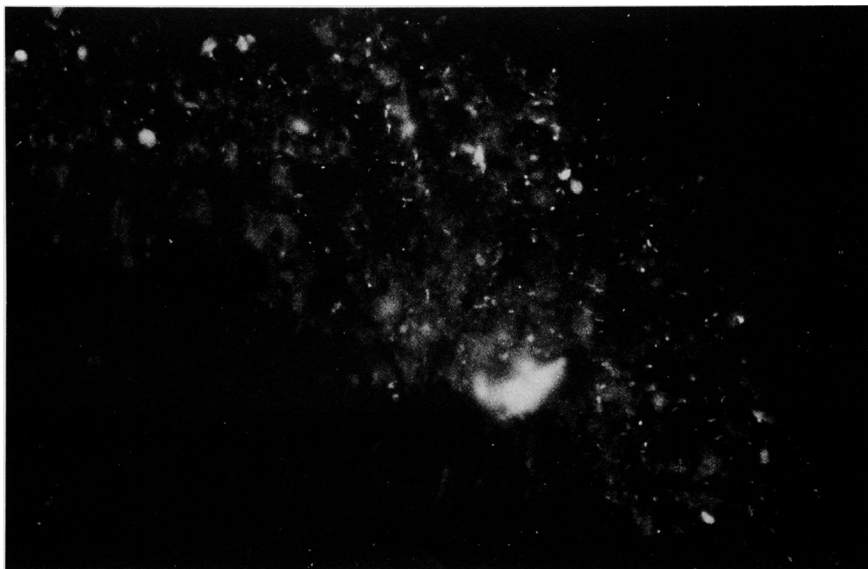


Fig. 4 Indirect immunofluorescence with *Campylobacter* antiserum showing positive staining in the glycocalyx overlying the surface epithelium $\times 400$.



Fig. 5 Electron micrograph of a *Campylobacter* culture fixed in formalin and embedded in epon $\times 10\ 000$. Showing *Campylobacter* microorganisms (arrowhead).

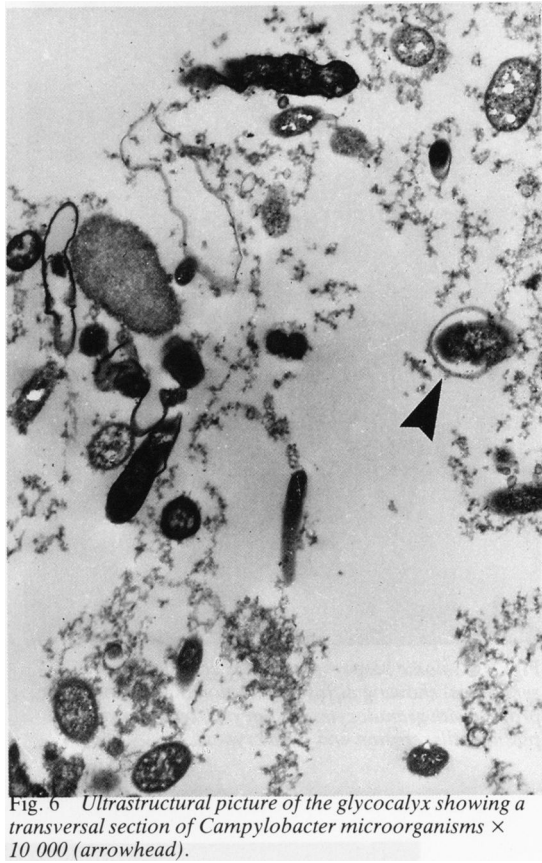


Fig. 6 Ultrastructural picture of the glycocalyx showing a transversal section of *Campylobacter* microorganisms $\times 10\ 000$ (arrowhead).

logically considerable overlap with chronic inflammatory bowel disease exists and differentiation may be difficult especially in the late stages.

Determination of the number of IgA, IgM, and IgG containing cells may be helpful in the differential diagnosis between *Campylobacter* colitis and active chronic inflammatory bowel disease because the number of IgG containing cells is normal in the former whereas the number of IgG containing cells is increased in active chronic inflammatory bowel disease.¹⁶ As these findings are non-specific the histological diagnosis of *Campylobacter* colitis ultimately relies on demonstration of *Campylobacter* microorganisms immunohistochemically. *Campylobacter* colitis cannot be distinguished histologically from other bacillary dysenteries nor is it possible to diagnose *Campylobacter* infection histologically when it is superimposed on chronic inflammatory bowel disease.¹⁷ Immunohistochemistry with *Campylobacter* antiserum may prove useful in these cases. It also makes it possible to diagnose *Campylo-*

bacter colitis retrospectively. Although most patients recover from their illness within one week the median duration of excretion of microorganisms in untreated cases is two to three weeks,¹⁸ this may explain by *Campylobacter* can still be shown immunohistochemically after the patient has recovered. Because immunohistochemistry with *Campylobacter* antiserum yields positive results in 19 out of 22 patients with stool cultures positive for *Campylobacter jejuni* it can be considered a sensitive technique.

The mechanism by which *Campylobacter* causes disease is not known.¹⁹ Although the organism was originally isolated from the blood,²⁰ positive blood cultures have only been reported occasionally.²¹ *Campylobacter jejuni* has been shown to invade chicken embryo cells *in vitro*²¹ but evidence for tissue invasion in man is lacking. Alternatively tissue damage could be produced by toxins. Our findings with *Campylobacter* antiserum that were confirmed ultrastructurally clearly show that *Campylobacter*

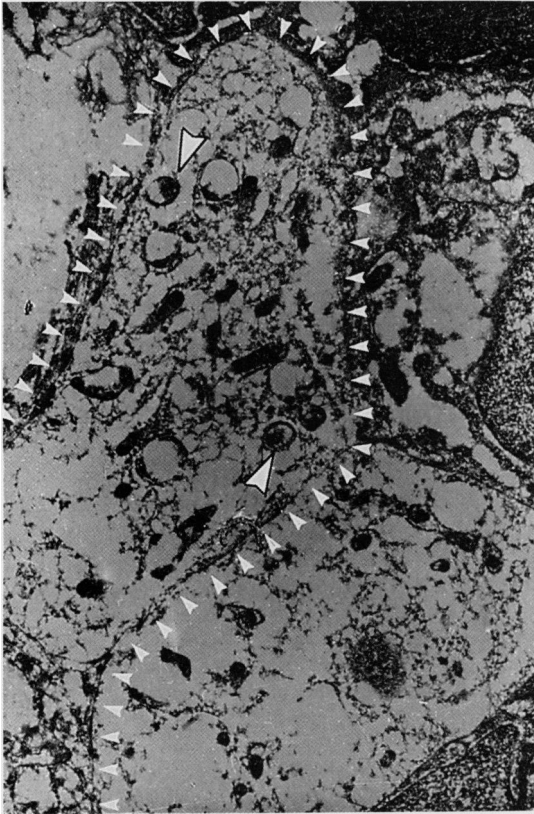


Fig. 7 Electron micrograph of moderately preserved Goblet cell (the cell wall is marked by small arrowheads) showing several transversal sections of *Campylobacter* microorganisms (large arrowheads) $\times 6000$.

invades the colonic mucosa. Recently it has been shown that *Campylobacter jejuni* also may produce a cholera like enterotoxin.²²

It is concluded that *Campylobacter* infections produce the histological picture of acute infectious colitis. The histological diagnosis of acute infectious colitis, however, is difficult especially in the later stages. Immunohistochemical demonstration of immunoglobulin containing cells and *Campylobacter* microorganisms contributes significantly to the histological diagnosis of *Campylobacter* colitis.

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