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Microvascular studies in non-specific inflammatory bowel disease

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SUMMARY The microvasculature was investigated in the normal bowel and in ulcerative colitis and Crohn's disease. Tissue samples from postoperative colectomy specimens in which the microvasculature had been perfused with barium sulphate suspension were examined. Microradiography was used to study intramural vascular pattern which was abnormal in both disease states. A recently described radiograph fluorescence system was used to estimate barium concentration and hence microvascular volume in tissue samples. Highly significant negative correlations were demonstrated between barium concentration and age in normal bowel (n=44; r= −0·669; p<0·001) and in segmental Crohn's disease (n=11; r=−0·698; p=0·017). Barium concentration was significantly reduced (p<0·05) in segmental Crohn's disease (n=11) but remained normal in diffuse Crohn's disease of the colon (n=6) and ulcerative colitis (n=7). It is postulated that ischaemia may be a factor in the pathogenesis of segmental Crohn's disease in older patients.

Lesions of the small intramural vessels in the colon have been shown to be a cause of colonic ischaemia.[1] There is histological evidence of small vessel disease in some cases of ulcerative colitis and Crohn's disease,[2 3] but the functional significance of the vascular changes in these conditions is uncertain. It is possible that ischaemia may play a part in the pathogenesis of inflammatory bowel disease. The purpose of the present study was to investigate the intestinal microvasculature in non-specific inflammatory bowel disease.

Methods

SPECIMENS INVESTIGATED

Normal bowel from 44 patients who underwent resection for colorectal carcinoma was examined; as normal bowel was always removed with the pathological bowel, (mean age 65·7 years range 11–82). The specimens of seven patients with ulcerative colitis (mean age 48·8 years range 26–88), six with diffuse Crohn's disease of the colon (mean age 34·3 years range 21–55), 11 with segmental Crohn's disease of the terminal ileum or colon (mean age 50·9 years range 22–74) and normal areas of bowel from the specimens of 12 patients with Crohn's disease were also studied. The diagnoses of ulcerative colitis and Crohn's disease were confirmed histologically in material from the resected specimens using conventional criteria.[4 5]

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METHODS OF INVESTIGATION

Specimen perfusion

Fresh operative specimens were used in all cases, and were perfused in the operating suite immediately after surgical removal before postmortem clotting could prevent filling of the microvasculature.

The specimen was perfused with a 50% weight/volume solution of barium sulphate (Micropaque), through an arterial cannula which had been introduced into one of the mesenteric arteries. The barium sulphate was infused manually using a 60 ml bladder syringe at a pressure of 60–180 mmHg. Perfusion was continued until contrast medium appeared in the efferent mesenteric veins at which point the infusion was considered complete. The arterial cannula was removed and the cut edges of the bowel and mesentery oversewn to prevent leakage of the contrast. In order to check that arterial and venous filling had occurred, a survey angiogram of the specimens was taken using standard radiographic equipment (Fig. 1). The specimen was fixed in 10% formalin for 48 hours.

Microvascular studies

After fixation, multiple transverse tissue blocks of

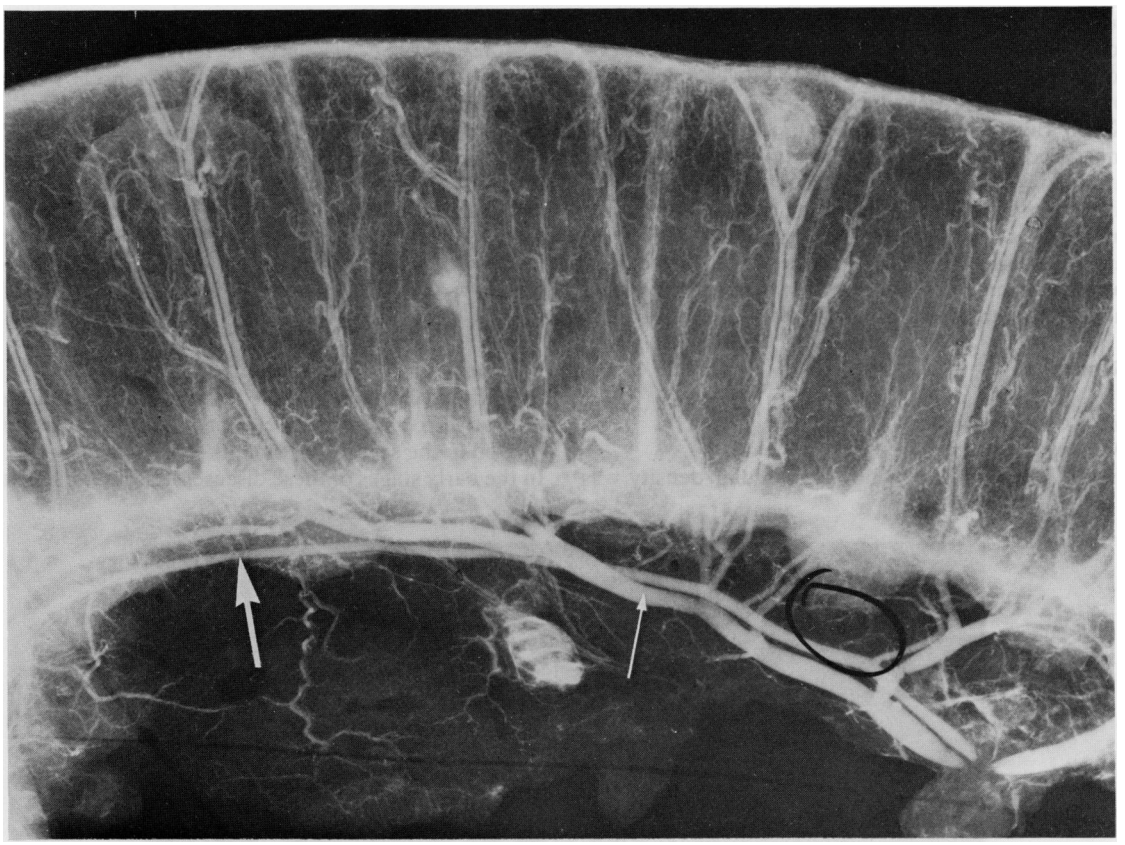

Fig. 1 *Survey angiogram showing arterial (thick arrow) and venous (thin arrow) filling with micropaque suspension. (Actual size).*

the bowel wall were taken. These were obtained from each specimen at measured intervals throughout its length so as to obtain at least five blocks from each specimen. The tissue blocks were routinely processed.

Histopathology
Five micrometre sections were cut from each block and stained with haemotoxylin and eosin and Elastic van Geison. These sections were studied histopathologically so that the appearances could be compared with the microradiographic features from the same block.

Microradiography
A 400 μm section was cut from the remainder of each block. These sections were Sellotaped to the piece of thin card which was of the same dimensions as Industrex (Kodak) radiograph film. The card was placed in direct contact with the radiograph film. Using a cabinet radiograph system (Faxitron), a radiograph of the section was taken at 10 Kvp with an exposure time of 7·5 minutes. After development, the microradiographs obtained were studied under appropriate magnification using a microfiche projector and standard microscope.

Fluorescent radiograph analysis
Small samples of tissue (2×2 cm) were obtained from areas immediately adjacent to the points at which the blocks for histopathological and microradiographic examination were taken. At least five samples from each specimen were studied. Each sample was dissected into a preparation consisting of mucosa-submucosa and a preparation comprising muscularis propria in order to estimate barium concentration and hence fractional microvascular volume in the whole sample and each bowel wall layer by fluorescent radiograph analysis.[6] The samples were stored in sealed plastic bags containing

cotton wool balls moistened with formalin in order to avoid the possibility of leaching out of barium by formalin. Each sample of whole bowel, muscularis propria and mucosa-submucosa was exposed to 140 Kev γ rays. The induced barium fluorescence was measured at 47 Kev. The 90° scatter from the sample at 140 Kev, which is a measure of tissue volume, was also recorded. The ratio of the number of counts obtained at 47 Kev to those obtained at 140 Kev for any given sample of whole bowel wall, muscularis propria or mucosa-submucosa was used as a measure of barium concentration.[6]

Results

MICRORADIOGRAPHY

Normal bowel

In the small and large bowel, vascular density was most pronounced in the mucosal and submucosal regions (Fig. 2). The large tortuous vessels in the submucosa gave rise to the smaller vessels which supplied the mucosa and muscle layer. The vessels in the muscularis propria formed a loose network of freely anastomosing vessels. In the colon, the mucosal capillaries were closely packed together and regularly arranged. Vessels appeared completely filled and uniform perfusion with contrast medium was always observed in all layers of the bowel wall. Variations in the luminal diameter of vessels were infrequent.

Ulcerative colitis and Crohn's disease

In diffuse Crohn's disease of the colon and in ulcerative colitis microvascular anatomy was closely related to the severity of the inflammatory response. In the early stages of these diseases, vascular pattern

Fig. 2 *Microradiograph of normal terminal ileum. Shows dense vascular layer in the submucosa (arrowheads). (Transverse section×5).*

was normal. The appearances of the mucosa and submucosa in severe Crohn's disease of the colon and fulminating ulcerative colitis were similar, and consisted of apparent hypervascularity and severe disorganisation of vascular architecture. These microvascular changes were associated with inflammatory disruption of the mucosal and submucosal layers of the bowel wall. Vascular pattern in the muscularis propria remained normal in ulcerative colitis but displayed localised areas of increased vascularity in Crohn's disease where deep ulceration and transmural inflammation were present (Fig. 3). Occlusive vascular changes were not a feature on histological examination of material from ulcerative colitis or diffuse Crohn's disease of the colon.

In segmental Crohn's disease, changes in microvascular anatomy were confined to the affected segments of ileum or colon and took the form of gross bowel wall thickening and a reduction in submucosal vascularity. The large submucosal vessels were replaced by narrower and irregular ones. The vascular pattern in the muscularis propria remained normal (Fig. 4). Histologically these areas showed submucosal fibrosis together with vascular changes. These consisted of medial hypertrophy and intimal fibrosis in the small intramural arteries, arterioles, venules and veins.

FLUORESCENT RADIOGRAPH ANALYSIS

Adequacy of perfusion

In order to determine whether different areas of each specimen had been uniformly perfused with contrast medium, the mean barium concentration, standard deviation and coefficient of variation (SD/mean×100%) of the five tissue samples from each specimen were calculated. In the control group, the coefficient of variation was 11·9% and

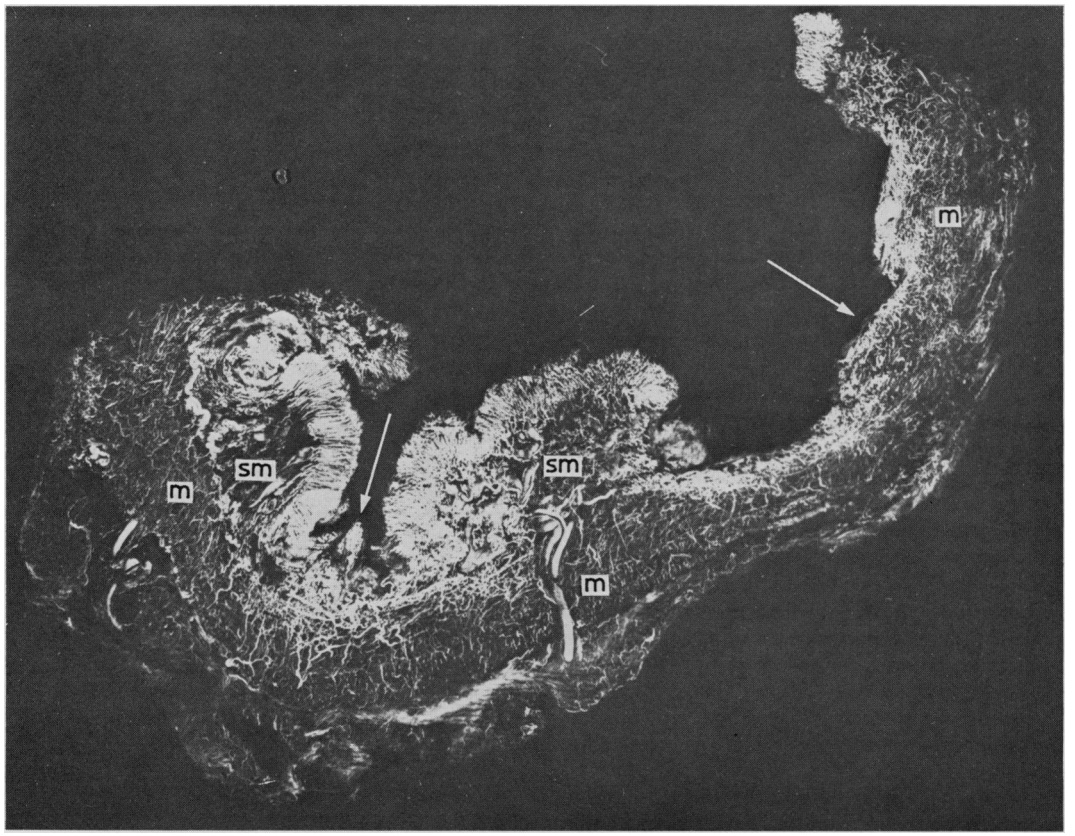

Fig. 3 *Microradiograph of severe diffuse Crohn's disease of the colon. Shows disorganisation of submucosal and mucosal (sm) vascular pattern. There is localised hypervascularity in the muscularis propria (m) where deep ulceration is present (arrows). (Transverse section ×5).*

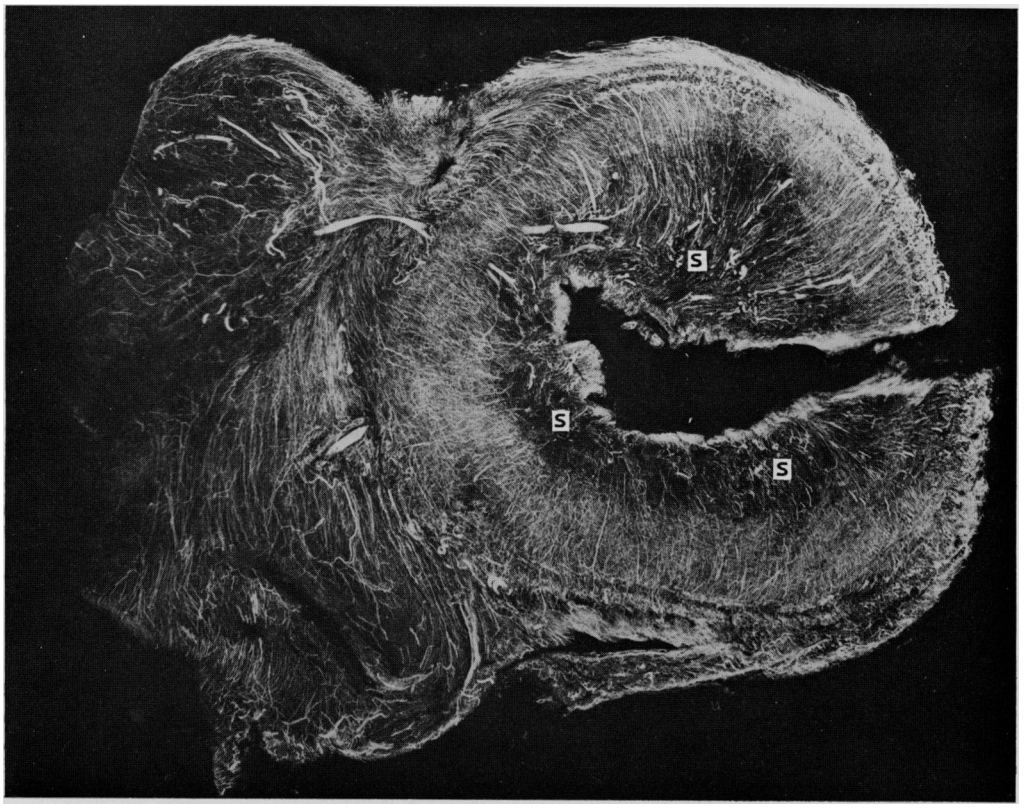

Fig. 4 *Microradiograph of segmental Crohn's disease. Shows bowel wall thickening and reduced vascularity in the submucosa (s). (Transverse section ×4).*

was greater than 20% in only two specimens. The coefficient of variation was 11·6% for ulcerative colitis, 14·6% for diffuse Crohn's disease of the colon, 14·9% for segmental Crohn's disease of the terminal ileum and colon and 11·4% for histologically normal bowel from Crohn's disease specimens. These results showed that the within specimen variation was small and that mean barium concentration for an individual patient was estimated to a high level of precision. Hence, subsequent analysis was based on the mean values which were compared statistically using analysis of variance and linear regression techniques where appropriate.

Group comparisons and correlations

Normal bowel

There were no statistically significant differences in the mean barium concentration of the whole sample, muscularis propria and mucosa-submucosa between terminal ileum and at various sites in the colon (Table 1). The mean value obtained for barium concentration in whole tissue samples from the small and large bowel was 2·031±0·546%. Mean barium concentration was 1·315±0·467% in the muscularis

Table 1 *Barium concentration in normal terminal ileum and at various sites in the normal colon.*

Site	*N*	*Barium concentration (mean value±SD)*		
		WS	MP	MS
Ileum	11	2·17	1·49	2·62
		0·59	0·53	1·02
Right colon	11	2·35	1·49	2·69
		0·62	0·43	0·77
Left colon	8	2·02	1·24	2·45
		0·62	0·57	0·78
Rectosigmoid colon	23	1·84	1·19	2·47
		0·49	0·38	0·64
p value		NS	NS	NS

WS=Whole sample
MP=muscularis propria
MS=mucosa-submucosa
NS=no significant differences.

Table 2 *Values obtained for mean barium concentration of the whole sample, muscularis propria and mucosa-submucosa in normal bowel and in non-specific inflammatory bowel disease.*

		Barium concentration (mean±SD)		
	N	*Whole sample*	*Muscularis propria*	*Mucosa-submucosa*
Normal	44	2·031±0·546	1·315±0·467	2·565±0·726
Ulcerative colitis	7	2·617±0·693	1·783±0·756	3·516±1·223
Diffuse Crohn's disease	6	2·723±0·633	1·890±0·356	3·315±1·137
Segmental Crohn's disease	11	1·258±0·659*	0·923±0·463	1·212±0·776*
Histologically normal bowel in Crohn's disease	12	2·355±0·724	1·695±0·427	2·663±0·901

* Values significantly different ($p<0·05$) from those in the normal bowel.

propria and 2·565±0·726% in the mucosa-submucosa (Table 2).

A highly significant negative correlation between barium concentration and age was present in the whole sample ($r=-0·669$;) $p<0·001$) (Fig. 5), the muscularis propria ($r=-0·608$; $p<0·001$) and the mucosa-submucosa ($r=-0·669$; $p<0·001$).

Ulcerative colitis and Crohn's disease

There were no statistically significant differences in the mean barium concentrations of the whole sample, muscularis propria or mucosa-submucosa between ulcerative colitis, diffuse Crohn's disease of the colon, the control group and histologically normal bowel from Crohn's disease specimens (Table 2). There were no statistically significant correlations between barium concentration in the whole sample, muscularis propria or mucosa-submucosa and age in ulcerative colitis, diffuse Crohn's disease of the colon and histologically normal bowel from Crohn's disease specimens.

In segmental Crohn's disease there was a statistically significant reduction in mean barium concentration of the whole sample and the mucosa-submucosa

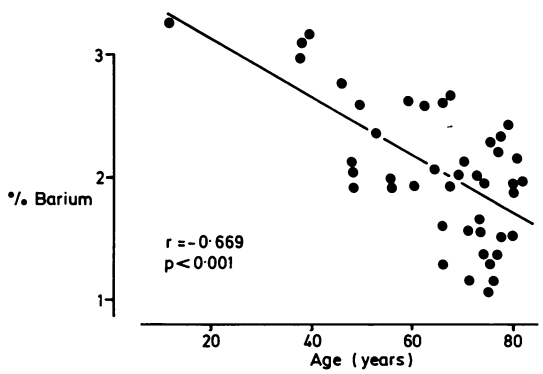

Fig. 5 *Relationship between barium concentration and age in normal bowel (n=44).*

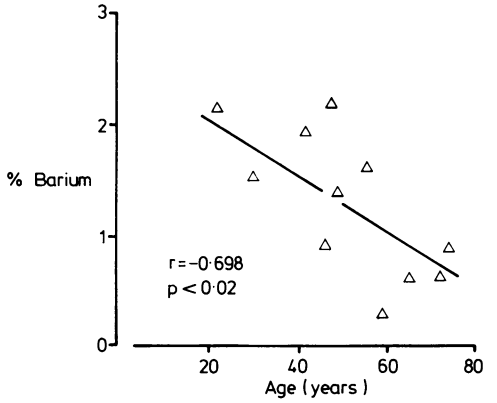

Fig. 6 *Relationship beween barium concentration and age in segmental Crohn's disease (n=11).*

when compared with any of the other groups (Table 2). By contrast, mean barium concentration in the muscularis propria did not differ significantly from that in the muscularis propria of the other groups. Statistically significant negative correlations between barium concentration in the whole sample ($r=-0·698$; $p=0·017$) (Fig. 6) the muscularis propria ($r=-0·744$; $p=0·005$), and the mucosa submucosa ($r=-0·835$; $p=0·001$) and age were observed in segmental Crohn's disease.

Discussion

It is clear from other workers[7–9] and from the present study that perfusion of the intestinal microvasculature with barium sulphate suspension achieves a high degree of vascular filling. The use of fresh postoperative specimens before postmortem clotting could prevent filling of the vessels and the injection of Micropaque of small particle size and low viscosity provided a safeguard against significant underperfusion in the present study. Some authors[7 9] have noted variations in perfusion at different points

in the same specimen but this was not seen in our study because of the immediate perfusion technique. The estimation of barium concentration in tissue samples from different areas of each specimen revealed that the within specimen standard deviation was small.

Lesions of the small intramural vessels have been reported in the bowel of elderly patients,[10 11] and the results of fluorescent radiograph analysis in the present study indicate that these lesions cause a reduction in microvascular volume. The relationship between vascular volume and blood flow is a matter of conjecture. Under physiological circumstances, normal flow within the intestinal microcirculation can be maintained in the presence of reduced vascular volume by increasing total splanchnic blood flow. If the compensatory mechanisms responsible for this increase are impaired, as for example in cardiac failure, hypovolaemia or disease of the proximal vessels, then a reduction in microvascular space may predispose the bowel to ischaemia. This hypothesis gains some support from the observation that elderly patients seem particularly prone to gut ischaemia even when their major vessels are quite patent[12] and that these ischaemic episodes are often precipitated by cardiac failure or hypotension.[13]

There is no general agreement about the appearances of the intramural vasculature in Crohn's disease. Brahme and Lindstrom[14] in a microradiographic study, observed an apparent increase in the vascularity in the bowel wall in Crohn's disease and noted rearrangement of the microvasculature secondary to inflammation. Similar changes were reported by Johansson *et al.*[15] Conversly, Thiede *et al*[8] have reported a reduction in vascularity in Crohn's disease. The present study tends to support an increase in vascularity in diffuse Crohn's disease of the colon but, our findings in segmental Crohn's disease are in direct contrast and agree with the recent conclusion by Thiede *et al.*[8] The results from the present study confirm that this attenuation in vascularity predominantly affects the larger vessel component in the submucosal layer.

The use of a radiograph fluorescence system, for the estimation of microvascular volume in tissue samples[6] was advantageous in providing quantitative information. It is of interest that in the patients with diffuse Crohn's disease of the colon, microvascular volume remained unchanged and it seems unlikely that ischaemia is a factor in the aetiology or pathogenesis of this condition. By contrast, vascular space was reduced in segmental Crohn's disease and we were further able to confirm that this decrease was confined to the mucosa and submucosa. That this reduction in microvascular volume may eventually lead to impaired blood flow is supported by the findings of Hulton *et al*[16] who showed reduced mucosal and submucosal blood flow in Crohn's disease of the terminal ileum and colon. It is interesting to note that the progressive submucosal fibrosis seen in Crohn's disease is similar to that observed in ischaemic[17] and potassium induced ulcers and strictures where small vessel occlusive changes are present.[3] Moreover, it has been suggested on the basis of histological[3] and angiographic studies[18 19] that local ischaemia can be a factor in the production of mucosal ulceration. It seems feasible that after the initial mucosal breach has occurred, superadded infection may lead to intramural abscess formation and eventual perforation. In the present study, the reduction in submucosal vascular space was more marked in older patients and we believe that this may form the pathological basis for the greater incidence of complications in older patients with localised Crohn's disease. This view gains some support from the authors previous clinical experience that elderly patients with segmental Crohn's disease are particularly at risk from complications such as perforation or stricture.[20]

Brahme *et al*[14] and Johansson *et al*[15] showed an apparent increase in mucosal and submucosal vascularity which paralleled the severity of the acute inflammatory response in ulcerative colitis. Objective methods of measurement were not used by these authors, and the result of fluorescent radiograph analysis in this study indicate that microvascular volume remains unaltered in ulcerative colitis irrespective of the degree of inflammation. Nevertheless, the present findings do support those of Reynolds[21] who showed the severe disruption of mucosal and submucosal vascular architecture which accompanies fulminating ulcerative colitis.

It has been postulated that Crohn's disease in the elderly may be a variant of ischaemic colitis. This view has some justification because of the similar clinical presentation,[22 23] distribution[24] and histological features[22] in many cases. In the present investigation the normal values obtained for microvascular volume in unaffected areas of bowel from Crohn's disease specimens, indicates that the reduction in vascular space in segmental Crohn's disease is the result rather than the cause of this disease. Nevertheless, on the basis of these data it seems that vascular insufficiency may modify the course of Crohn's disease in older patients and produce a response similar to that of progressive ischaemia.

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