MORPHOMETRY AND CONNECTIVE TISSUE COMPOSITION OF THE SIGMOID COLON IN ADULT KENYANS

Dissertation in partial fulfillment of the requirements of the intercalated Bachelor of Science in Anatomy of the University of Nairobi.

By

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This work is dedicated to the late Professor James Kirumbi Kimani,
A dedicated scholar and a committed instructor of Anatomy.

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DECLARATION

I confirm that this dissertation is my original work and that it has not been presented elsewhere.

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Date: 26/9/2006

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SUMMARY

Background

The sigmoid colon in Africans is commonly affected by volvulus formation. Anatomical characteristics of this part of the colon are thought to provide some of the contributory explanation for this predisposition. Published study results for Indians, South Americans, Middle East and some African populations indicate a higher prevalence of sigmoid volvulus in males. Epidemiological evidence shows a low incidence of diverticulosis, another sigmoid colon pathology, in Africans as compared to Caucasians. The exact pathogenesis of diverticular disease of the colon remains debatable. Suggestions have been made that differences in the histomorphology of the sigmoid colon wall may explain the higher and lower incidences in Caucasian and African populations respectively. In Caucasians, the arrangement and amount of collagen and elastic fibres show regional differences. Colonic submucosal collagen fibres crosslink and become tightly packed in small bundles whereas the elastic fibre content increases proximo-distally especially in the taenia coli. These regional changes are associated with loss of wall tensile strength and predisposition to diverticular disease, common in distal sigmoid colon. Physiologically, this distal segment of the sigmoid colon is also known to present a high intraluminal pressure zone. Recent studies have demonstrated an anatomical basis for this phenomenon- an increase in the thickness of the circular muscle forming the "rectosigmoid sphincter". There is limited information on the histomorphology of the African sigmoid colon to investigate structural factors that may protect against diverticulosis.

Objective

To study the morphometric features of the sigmoid colon and mesocolon and to describe the histomorphology of the sigmoid colon wall in adult Kenyans.

Study design

A descriptive cross sectional study.

Materials and methods

Ninety six sigmoid colons (fifty eight male subjects) were harvested at autopsy. Measurements of length of the sigmoid colons and root length and height of mesocolon were taken. From these measurements the sigmoid length: mesocolic root length ratio and sigmoid length: mesocolic height ratio were calculated. and compared for gender. Sections from the proximal, middle and distal segments were processed for light microscopy and stained with Weigert's resorcin fuchsin and Masson's trichrome stains to demonstrate collagen and elastic fibres. Point counting was done to quantify the relative thickness of the submucosa and the inner circular muscle layer.

Data management and presentation

Means and frequencies of the lengths of the sigmoid colon and the height and root lengths of the sigmoid mesocolon were generated using SPSS program. The sigmoid length: mesocolic root length ratio and sigmoid length: mesocolic height ratios were compared for gender using the Student t-test. $P < 0.005$ was considered statistically significant. The ratio of the circular muscle to the submucosa was compared for the proximal, mid and distal sigmoid colon segments using analysis of variance (ANOVA). Data on sigmoid colon and mesocolon morphometry was represented in form of tables and graphs while that on histomorphology of the sigmoid colonic wall was presented as photomicrographs.

Results

The mean sigmoid colon length was higher in males (36.9cm) than in females (32.6cm) ($p=0.007$). Most (41.7%) of the sigmoid colons measured 30-34cm long. Males had shorter mesocolon roots and longer mesocolon heights. Elastic fiber bundles were demonstrated in the interphase between mucosa and the submucosa, the submucosa and the circular muscle layer and that between the circular and longitudinal muscular layers of the sigmoid colon wall. The density of elastic fibers increased proximodistally in the external muscular layers. The circular muscle layer showed a relative increase in thickness proximodistally.

Conclusion

The greater mean lengths of the sigmoid colon and smaller mesocolic root lengths in males may be part of the anatomical basis for the higher incidence of sigmoid volvulus in males. The elastic bundles between layers of the colon wall may be helpful in transmission of stress and improve the capacity of the wall to withstand intracolic pressure – which may partly be a probable protective mechanism against diverticulosis in most Africans.

The thickness of the circular muscle layer in the distal sigmoid colonic wall is consistent with the existence of an anatomical rectosigmoid sphincter.

INTRODUCTION & LITERATURE REVIEW

MORPHOMETRY OF THE SIGMOID COLON AND MESOCOLON

The sigmoid colon begins at the pelvic brim, as a continuation of the descending colon, to form a variable loop in the lesser pelvis. The loop is suspended from the pelvic wall by a fold of mesentery, the sigmoid mesocolon. Variations in the morphometry of the sigmoid colon and mesocolon have been reported[1]. A number of these anatomical variants may be related to the incidence of volvulus formation[2].

The incidence of sigmoid volvulus shows both gender and population disparities. It is common in males and in several countries in the developing world. Higher incidence of sigmoid volvulus in males has been correlated with gender disparity in sigmoid colon morphometry in India[1], Turkey[3] and in Brazil[4]. Sigmoid loops with tall mesocolons (dolichomesocolic) have a male predominance while loops with short mesocolons (brachymesocolic) have a female predominance among Indians. This correlates closely with a higher prevalence of sigmoid volvulus in males than in females[1].

In Africa, epidemiological studies indicate a higher incidence of sigmoid volvulus in males than females with a male to female ratio ranging from 9:1 in South Africa[5] to 13.5:1 in Ethiopia[6, 7]. However there is limited literature on the morphometric basis of these observations[8]. Information on the morphometry of the sigmoid colon and mesocolon may be of predictive value in determining susceptibility to volvulus formation and is also valuable in surgery of the sigmoid colon, for instance colonoscopic evaluation of the sigmoid colon[1].

Although sigmoid volvulus is the leading cause of large gut obstruction in Kenya[9], relatively less attention has been paid to its morphometry and that of the mesocolon. Information on sigmoid morphometry may also be useful in understanding the anatomical considerations during endoscopic assessment. Sigmoidoscopy is more difficult in female patients with the rate of completion of procedure significantly lower than for male patients[10]. The suggested explanation for these findings is mainly due to gender differences in sigmoid anatomy. Local data on sigmoid morphology with emphasis of gender variations may provide useful information to local endoscopists.

HISTOLOGICAL STRUCTURE OF THE SIGMOID COLON WALL

This consists of a mucosa, submucosa, muscularis externa and serosa from inside outwards. The mucosa contains a lamina propria with loosely arranged collagen fibre bundles. The submucosa is composed of obliquely crossing bundles of collagen and elastic fibres together with a rich plexus of blood vessels, lymphatics and nerves. The muscularis externa consists of an outer longitudinal and inner circular layer. The outer layer is continuous but has three longitudinal aggregations called taenia coli. The taenia increase in width distally with the widest breadths found in the distal part of the sigmoid colon[11]. The serosa is adhered to the muscularis externa by subserosal connective tissue.

The circular muscle layer of the sigmoid colon has been reported to increase in thickness at its distal end towards the rectum[12]. This has been associated with a high pressure zone at the rectosigmoid junction. It is likely that this junction may regulate the emptying of fecal matter from the sigmoid colon to the rectum and thus act as a sphincter, the "rectosigmoid sphincter of O'Beirne"[12] with an important role in involuntary fecal continence[13].

The connective tissue composition of the sigmoid colonic wall exhibits both collagen and elastic fibers. The elastic fibers are predominant in taenia coli of muscularis externa while both fibers populate the sigmoid colon mucosa and submucosa [14, 15]. The configuration of the submucosal collagen has been variously described. Lords et al describe a honeycomb pattern with the fibers mainly type III[16]. In the account by Eastwood (2003), collagen fibers in the submucosa assume a tightly packed configuration, which increases proximodistally[17]. This change in collagen configuration has been associated with reduction of tensile strength of the colon wall, weakening the wall and predisposing to diverticular disease.

Elastin fibres on the other hand, are laid down in the fascicles in between the muscle cells of the taenia coli[14]. They also increase in amount proximodistally within the taenia coli with little change in the circular muscle layer, the submucosa or the mucosa[18]. This increased deposition of elastic fibres in the taenia coli is thought to contract the colon wall and cause folding of the mucosa. This mucosal folding and a relative decrease in the tensile strength of the colon wall as earlier mentioned, increases the tendency of the mucosa to herniate through weak points of the colon wall, the key event in diverticulosis which mainly occurs through vascular entry points[17]. Since diverticulosis is relatively rare in Africans compared to Caucasians, it has been suggested there may be an ethnic variation in structure that may be of protective value in Africans[8].

JUSTIFICATION

The sigmoid colon is the commonest site for volvulus formation[5]. Knowledge on the structural basis of age and gender differences in prevalence of sigmoid volvulus may improve the understanding of the pathogenesis of this disorder. Information on morphometry of the sigmoid colon and mesocolon could also be valuable in colonoscopy. Studies on dimensions of the sigmoid colon and mesocolon have been conducted in Asian and European populations[1, 3] but limited literature exists of such studies in Africans although sigmoid volvulus is a common occurrence in African populations.

Collagen and elastic fibre composition of the colon wall determine its structural integrity and tensile strength. Low tensile strength of the colon wall predisposes to diverticula formation which is common in Caucasians but rare in Africans. Diverticula are commonest in the sigmoid colon [17]. Despite these observations literature on connective tissue characteristics of the sigmoid colon in Africans is scarce[8]. A detailed description of the connective fibre composition of the sigmoid colon in African adults may provide insight into the possible structural factors that protect against diverticula.

HYPOTHESIS

The morphometry of the sigmoid colon and mesocolon is similar for male and female adult Kenyans while the connective tissue organization is consistent for the wall of the proximal, mid and distal sigmoid colon.

MAIN OBJECTIVE

- To study the morphometry of the sigmoid colon and mesocolon and the connective composition of its wall in an adult Kenyan population.

SPECIFIC OBJECTIVES

To study the following in an adult Kenyan population;

- The sigmoid colon length.
- The vertical height and the root length of the sigmoid mesocolon.
- The arrangement of elastic and collagen fibres in sigmoid colonic wall.
- The relative thickness submucosa and circular muscle components of the sigmoid colon wall.

MATERIALS AND METHODS

Sources of material

The specimens were obtained during autopsy examinations performed at the Chiromo and Nairobi city mortuaries with ethical approval of the Kenyatta National Hospital Ethical Review Committee (KNH-ERC).

Subjects

Ninety six sigmoid colon specimens were collected from fifty adult males, a one year old male and forty five adult females.

Exclusion criteria:

Three sigmoid colon specimens had observable pathologic conditions and were excluded from the study.

Methods

The sigmoid colons were accessed through a midline abdominal incision used at autopsy. After reflection of the small intestines, the bladder (uterus in females) the sigmoid colons were identified and the root length and vertical length sigmoid mesocolon were measured in situ. The length of the sigmoid colon at the antimesenteric border was measured using a tape measure. Morphometric parameters of the sigmoid colon and mesocolon were measured as follows (figure 1):

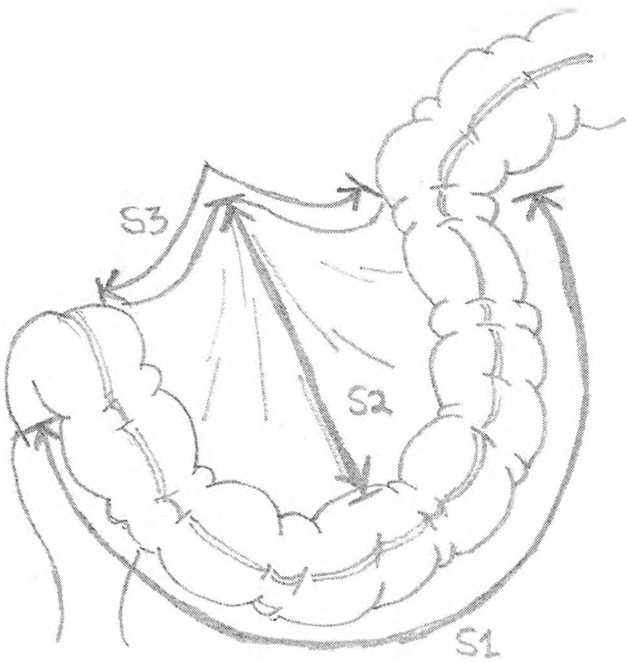


Figure 1; Morphometric parameters of the sigmoid loop and mesocolon.

S1 – Sigmoid colon length, S2 – Mesocolon vertical height, S3 – Mesocolon root length.

Two indices relating the total length of the sigmoid colon and the vertical height of the mesocolon to the length of the mesocolon root were calculated as shown;

Index 1 = sigmoid colon length/mesocolon root length (S1/S3)

Index 2= mesocolon vertical height/ mesocolon root length (S2/S3)

These ratios were compared between males and females.

Sections (1cm×1cm) for histological processing and staining were carefully dissected from three equidistant segments of the sigmoid colon, proximal, middle and distal segments from areas including tinea coli and full wall thickness (figure 2).

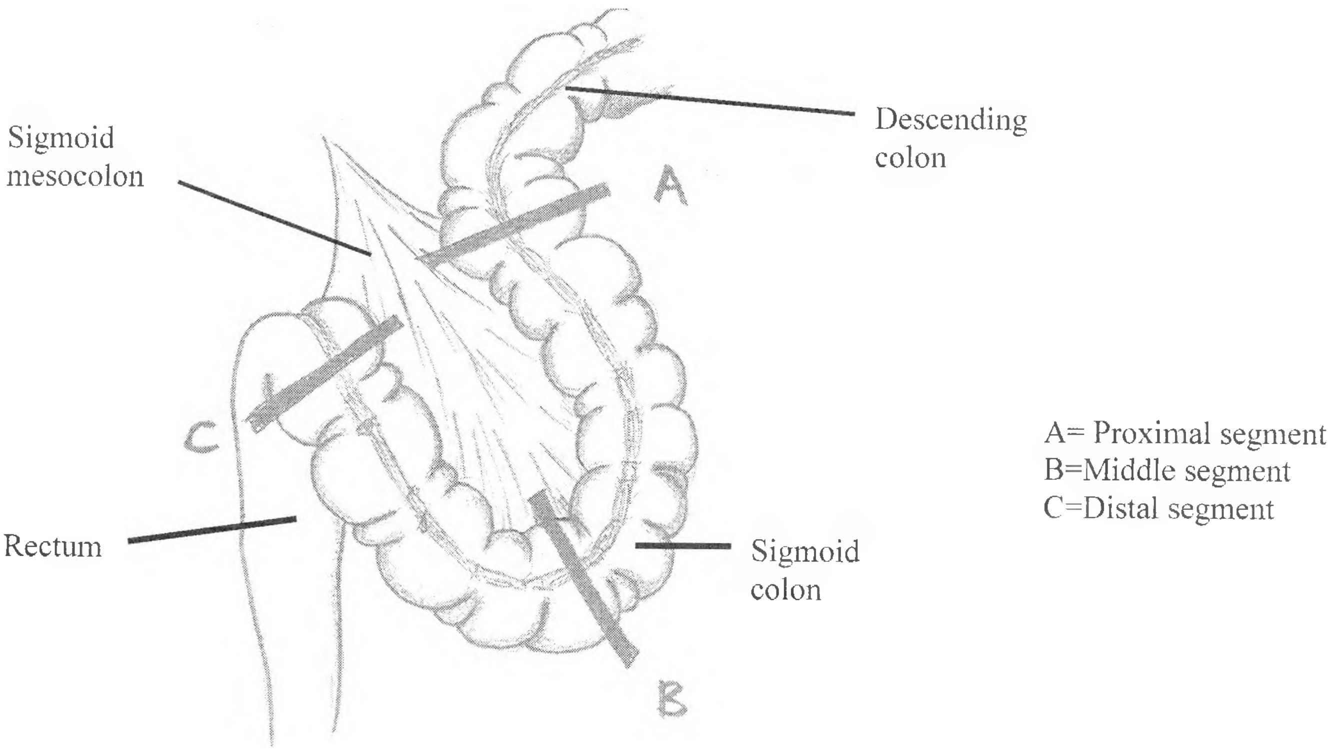


FIGURE 2: Segments of the sigmoid colon from which serial sections were taken.

Light microscopy

Materials for light microscopy were obtained from 12 specimens (6 males) that randomly selected by picking every third specimen, provided death occurred within 48 hours. Fixation of the specimen was done by immersion in 10% formal saline for 24 hours. These segments were then dehydrated in ethyl alcohol of increasing concentrations, commencing with 70% ethanol to absolute alcohol each for 1 hour. They were cleared in toluene (S.G. 0.866, B.P. 110°C) then infiltrated in molten wax for 12 hours at 60°C. After solidification 7 micrometer thin sections were cut by a Lezlar® microtome, Germany. The sections were floated in water at 60°C and thereafter mounted.

The sections were then stained using Weigert's resorcin and fuchsin stain and counter stain with van Gieson to demonstrate the elastic fibre component of the sigmoid colon wall. Other sections were stained using Mason's trichrome to show the general arrangement of collagen fibres. Slides were examined at ×40, ×100 and ×400 magnifications using a Leica® light microscope, Germany. The elastic and collagen fibre arrangement and orientation of the proximal, middle and distal portions of the sigmoid colon were described and compared.

Stereology

The stained slides were photographed and used for stereological analysis of the relative width of the submucosa. After systematic random sampling of three specimens for each segment at X40 magnification, the photomicrographs were projected to a television screen and a point grid superimposed. The number of points falling on submucosa were counted and compared to those falling on the circular muscle layer for assessment of relative thickness. The ratio of the circular muscle to the submucosa was compared for the proximal, mid and distal sigmoid colon segments using analysis of variance (ANOVA).

Data management and presentation

The results of sigmoid length: mesocolic root length/height ratios were tabulated and analyzed using computer software and statistical package of social sciences (SPSS) for windows version 11.5.0 Chicago Illinois, 2002. The means and standard deviations were calculated. The means in the two genders was correlated using student T test for statistical significance.

Sigmoid colon and mesocolon measurements are presented in the form of tables and graphs. Photomicrographs of colonic wall slides were obtained from stained sections and regional comparisons were made for collagen/elastin fiber arrangement and orientation.

RESULTS

GROSS MORPHOMETRY RESULTS

A total of ninety six sigmoid colons from fifty adult males and fourty five adult females were used for the study. (A sigmoid colon from one infant male was only used for histology.) The age of the subjects used for morphometry ranged from twenty one to fifty six. The length of the sigmoid colon in the studied sample ranged from 27.6 cm to 46.7 cm in males and 27.1cm to 40.2cm in females. The mean was calculated to be **36.9** ±5cm in males and **32.6** ±3.8cm in females, this difference was statistically significant (p=0.007) (Table1). The range of the mesocolic root length was 9.6 cm to 20.7 cm in males and 10.2cm to 25.1cm in females. The mean root length was **14.2** ±3.4cm in males and **17.6 ±**3.4cm in females. The range of the mesocolic vertical height was 3.1cm to 26.4cm in males and 9.5cm to 18.1cm in females. The vertical height of the mesocolon had a mean value of **15.1 ±**4.3 cm in males and **13.4 ±**2.6cm in females (Table1).

Two indices relating the total length of the sigmoid colon to the vertical height of the mesocolon and the total length of the sigmoid colon to the length of the mesocolon root were calculated as thus;

Index 1 = sigmoid colon length/mesocolon root length (S1/S3)

Index 2 = mesocolon vertical height/ mesocolon root length (S2/S3)

The mean value of index1 was 2.68 in males and 1.76 in females (p=0.004) and index2 was 1.22 in males and 0.74 in females (p=0.024).

Table 1;Morphometry of the sigmoid colon and mesocolon in adult males and females

Gender	**Males**			**Females**			P value
	Mean (cm)	Standard deviation	Range (cm)	Mean (cm)	Standard deviation	Range (cm)	
Sigmoid colon length	**36.9**	5.0	27.6 - 46.7	**32.6**	3.8	27.1 - 40.2	0.007
Mes vertical height	**15.1**	4.3	3.1 - 26.4	**13.4**	2.6	9.5 -18.1	0.067
Mes root length	**14.2**	3.4	9.6 - 20.7	**17.6**	3.4	10.2 - 25.1	0.012
Index1	**2.68**		p=	**1.76**			0.004
Index2	**1.22**			**0.74**			0.024

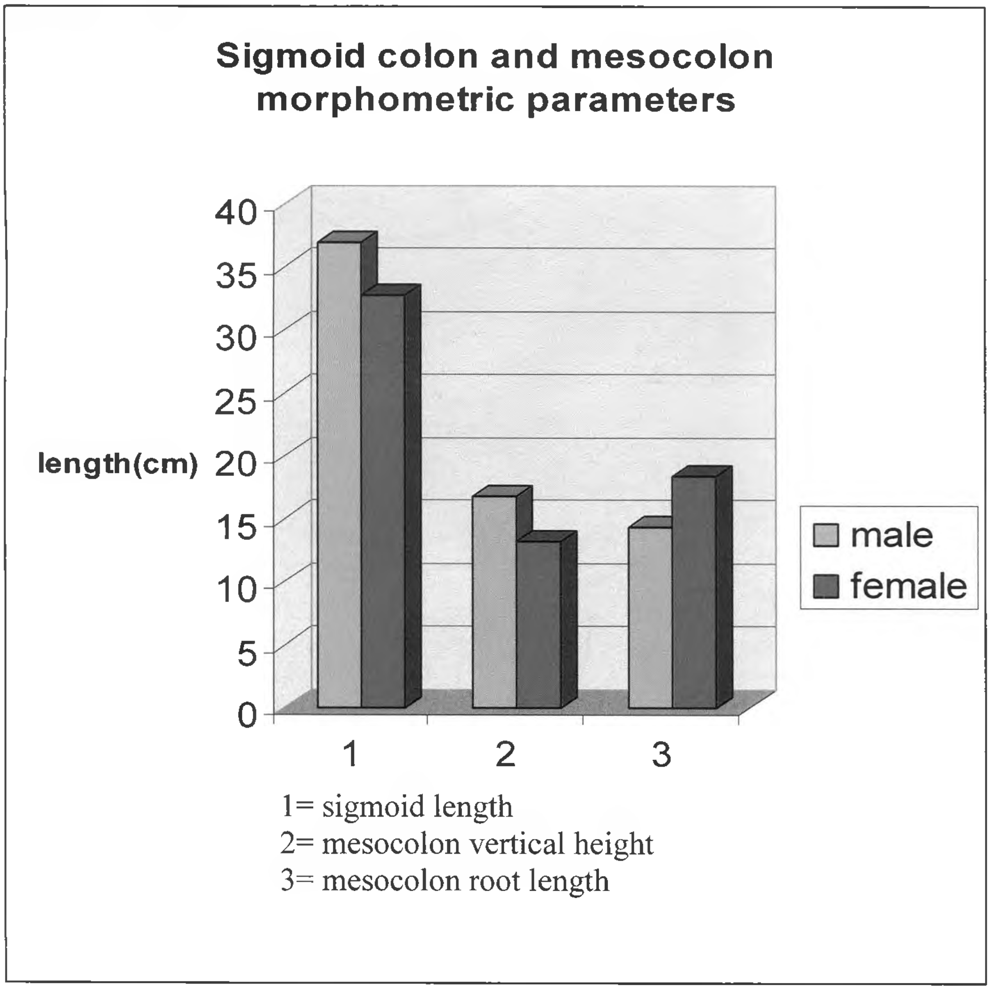
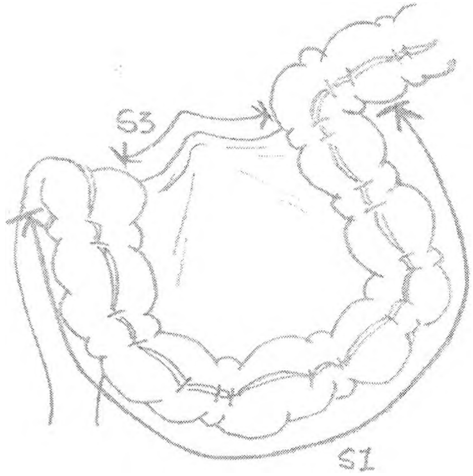
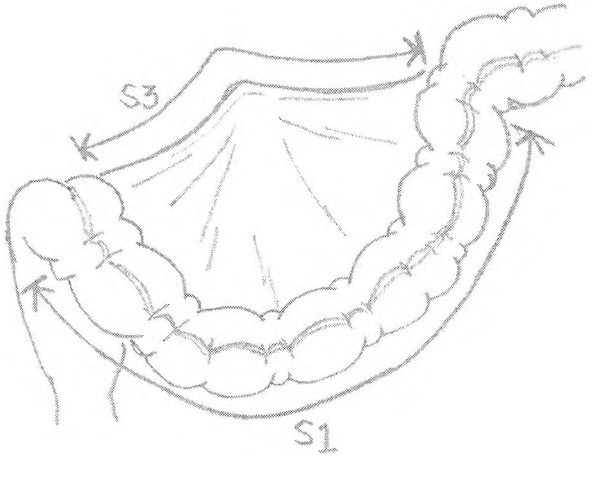


Figure 3; Bar graph comparing the morphometric parameters of sigmoid colon and mesocolon between adult males and females.

Male cases had a generally a long sigmoid loop length with a short mesocolic root length but a long mesocolic height. Females had a relatively short sigmoid loop length with a long mesocolic root length but a short mesocolic height (figure 3).



Predominant male sigmoid colon/mesocolon morphometry



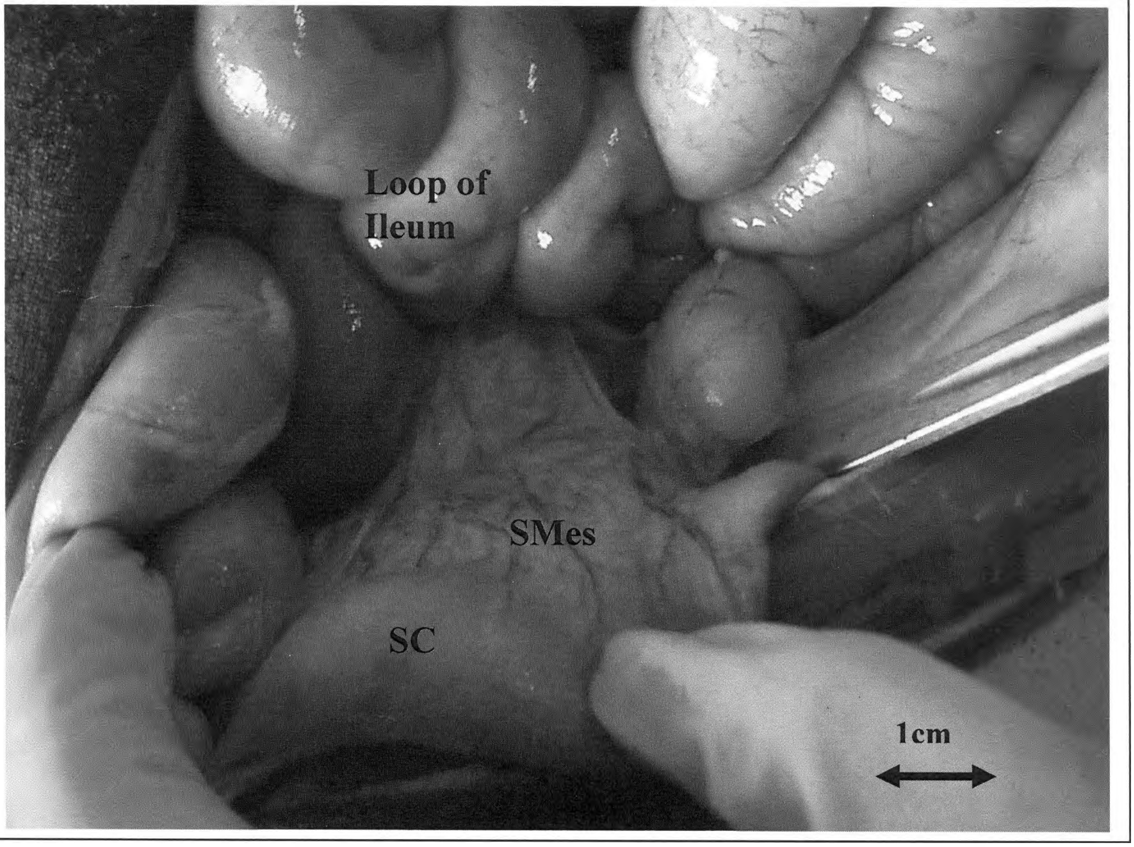
Predominant female sigmoid colon/mesocolon morphometry

Figure4; Characteristic sigmoid colon and mesocolon morphometric pattern of the male and female cases

Figure 5 below shows an image of a sigmoid colon and mesocolon of a thirty year old female subject. The mesocolic height was 3cm, the lowest recorded mesocolic parameter.

KEY
SC= Sigmoid colon
SMes= Sigmoid mesocolon
TC= Taenia coli

Figure 5;



The most frequent incidence of the length of the sigmoid colon in the studied sample fell in the range 30cm – 34.9cm with a frequency of 41.7%. The least frequented ranges fell in the ranges 20-29.9cm and 40-49.9cm (figure 6)

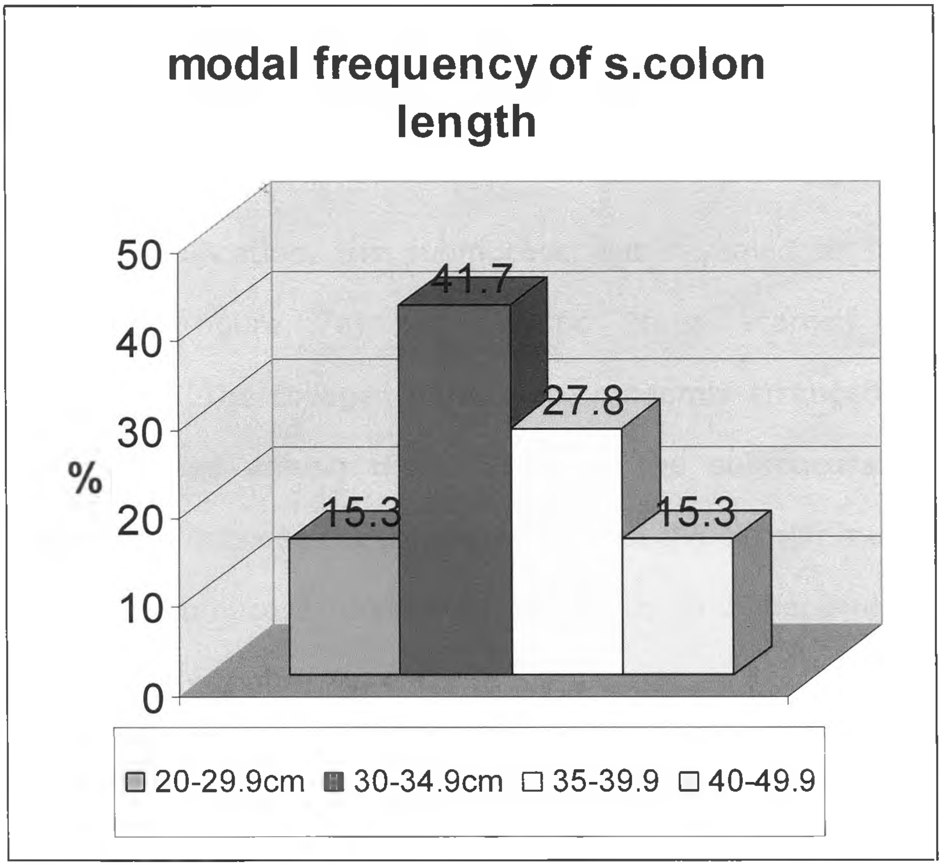


Figure 6; Frequency modal group of the length of the sigmoid colon in the studied sample

HISTOLOGY RESULTS

The sigmoid colonic wall comprises of four distinct layers namely mucosa, submucosa muscularis and serosa. Connective tissue is concentrated in the lamina propria of the mucosa, submucosa, between the muscle bundles, especially the taenia coli and in the serosa. The arrangement and composition of the connective tissue varied from one layer to another and displayed proximodistal differences.

At low magnification, the submucosa was revealed to be predominantly collagenous (figure 7a) with elastic fibres scarcely visible at low magnification. The collagen fibres were randomly arranged with few elastic fibres dispersed among them. Some of the submucosal collagen fibres permeated in between the muscle bundles of the circular muscle layer (figure 7a). The submucosa decreased in width and became more compact proximodistally (figure 7b, c, d).

Figure 7a; Photomicrograph of a transverse section of the wall of the mid sigmoid colon. Weigert resorcin and fuchsin stain. Magnification×40. Note the arrangement of the components of the sigmoid colon wall.

Figure 7b, c, d; Photomicrographs of a transverse section of the sigmoid colonic wall at proximal, middle and distal segments. Weigert's resorcin and fuchsin stain. Magnification ×40. Note the gradual thickening of the muscularis layer and increasing compactness of submucosa proximodistally.

Figure 7a

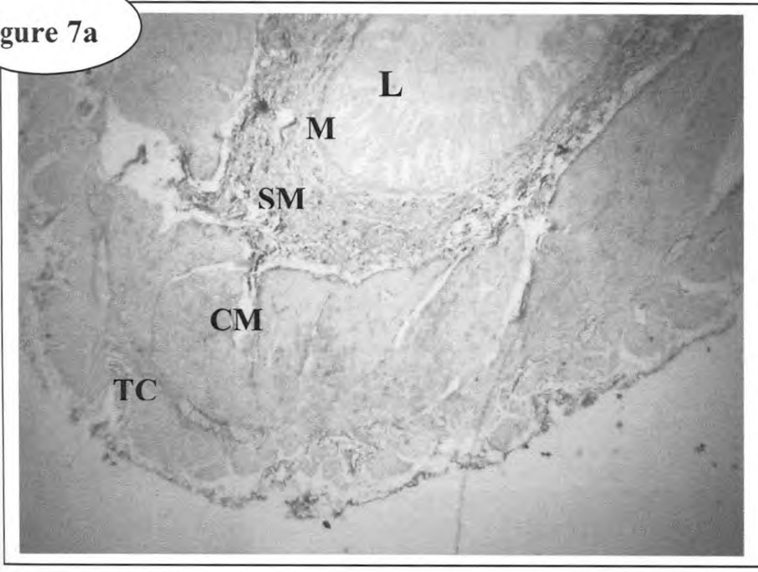
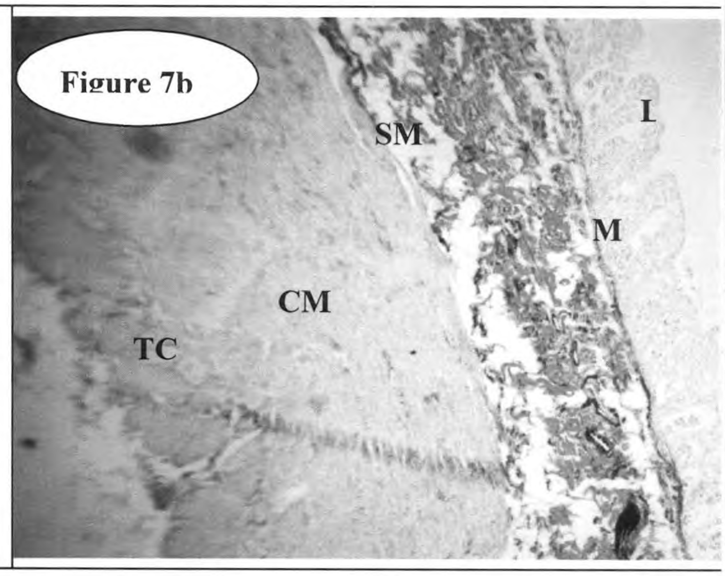


Figure 7b



Proximal sigmoid colon

Figure 7c

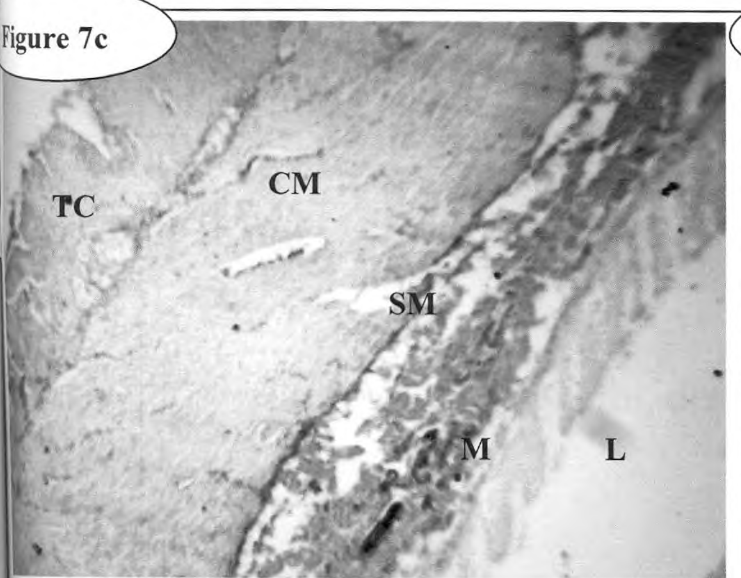
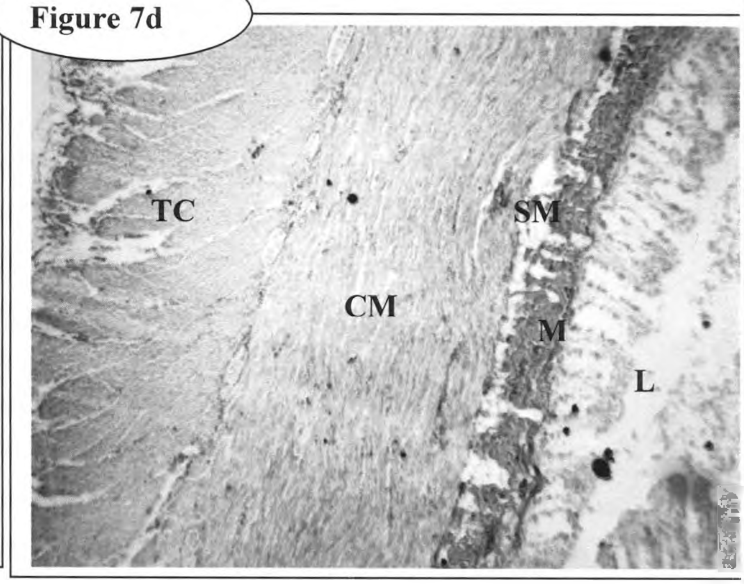


Figure 7d



Mid sigmoid colon

Distal sigmoid colon

KEY
L= Lumen
M= Mucosa
SM= Submucosa
CM= Circular muscle layer
TC= Taenia coli

The ratio of the submucosa to the circular muscle layer was calculated by point counting for the proximal, middle and distal sections of the sigmoid colon. This showed a mean decrease in the ratio of the submucosa to the circular muscle layer. In comparison to the whole wall thickness the submucosa showed a gradual decrease and the circular muscle layer showed a gradual decrease proximodistally (p=0.028) (Figure 8).

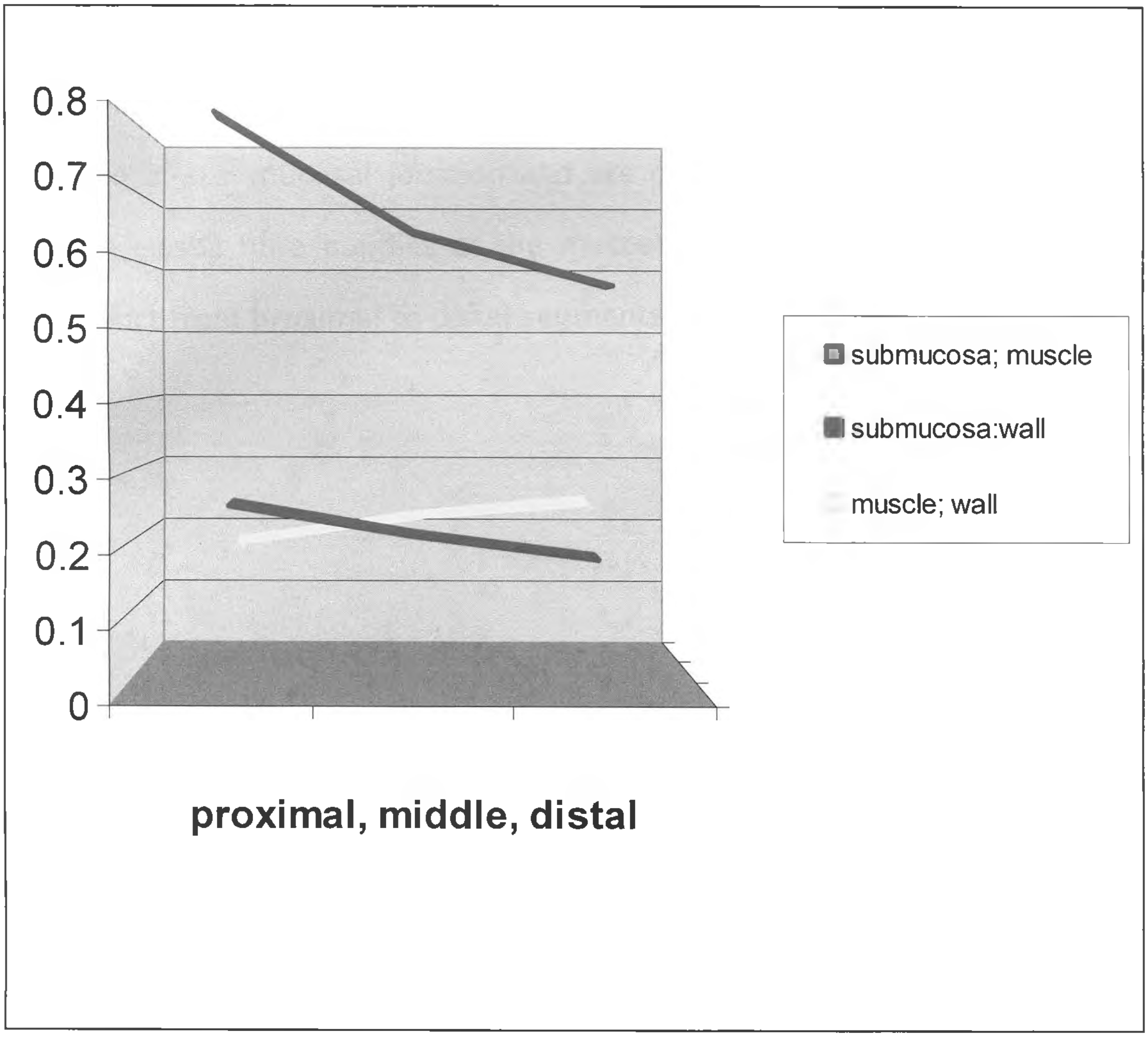


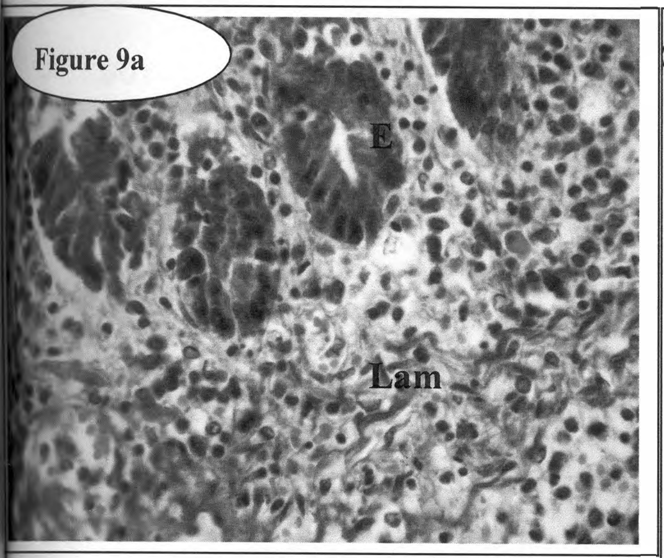
Figure 8; Line graph of the relative thickness of the submucosa and the circular muscle layer to each other and to the whole wall thickness.

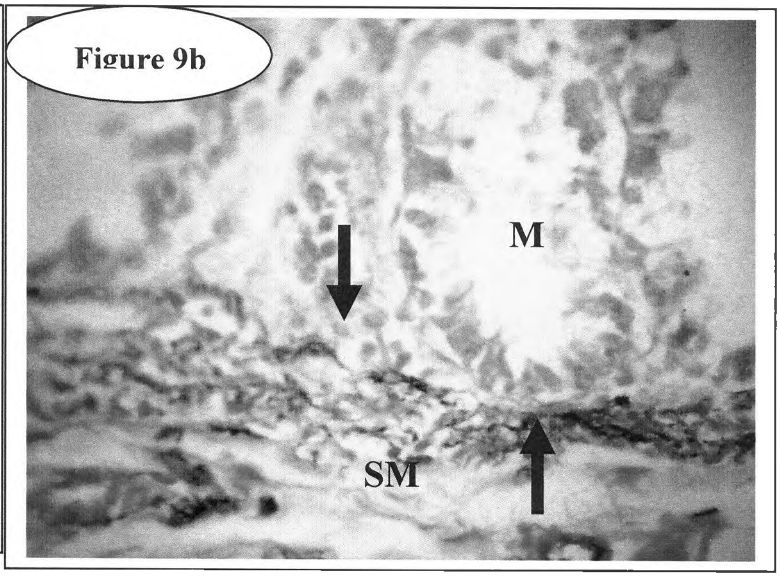
MUCOSA

The surface epithelium is thrown into crypts. The lamina propria at the base of the crypts next to the mucosal-sub mucosal junction displays a bundle of elastic fibres oriented circumferentially (figure 9b, c). The lamina propria is seen to have a loose matrix of collagenous fibres arranged around the crypts (figure 9a). Collagen fibres become condensed at the base of the crypts, near the mucosal-sub mucosal junction and are oriented circumferentially (figure 9a). The elastic fibre bundles at the mucosal-sub mucosal junction become less distinct from proximal to distal segments (figure9b, c, d).

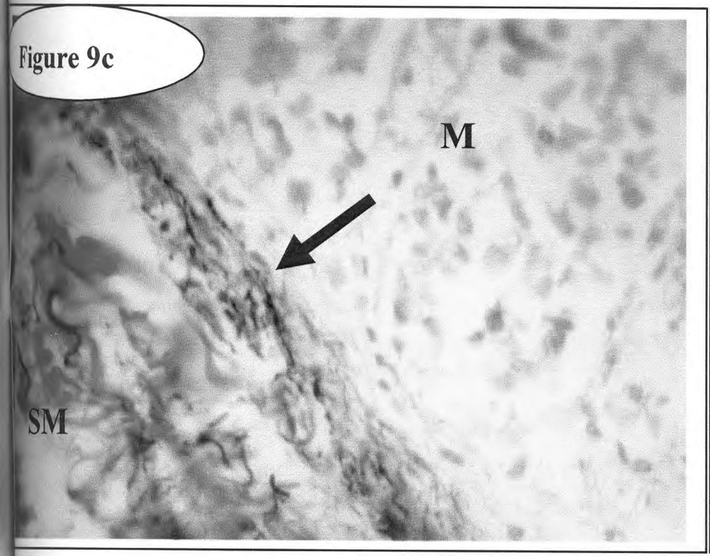
Figure 9a; Photomicrograph of the transverse section of the mucosa of the mid segment of the sigmoid colon. Masson's trichrome stain. Magnification×400. Note the predominance of collagen fibres in the lamina propria of the mucosa with muscularis mucosa.

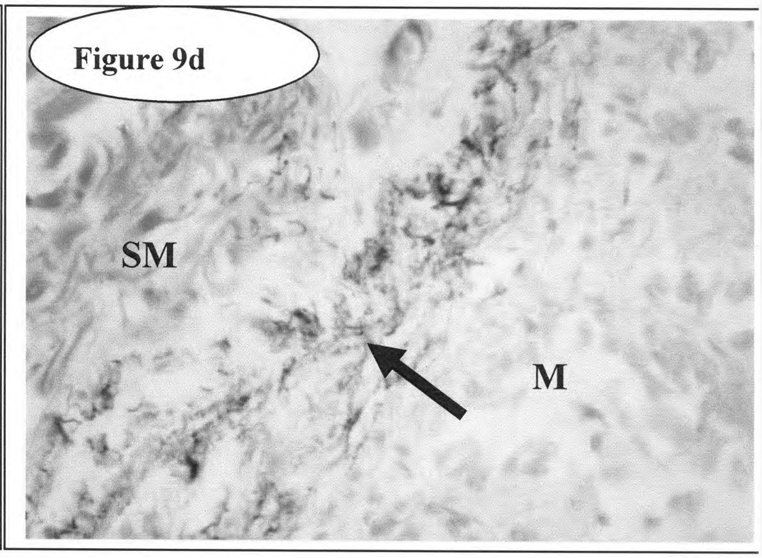
Figure 9b, c, d; Photomicrographs of the transverse section of the mucosal-sub mucosal junction. Weigert's resorcin and fuchsin stain. Magnification ×400. Note the distinct elastic fibre clusters at the mucosal-sub mucosal junction (arrow). Note the gradual loss of density of the elastic fibre bundles proximodistally at the junction (arrow).





Proximal sigmoid colon





Mid sigmoid colon

Distal sigmoid colon

KEY

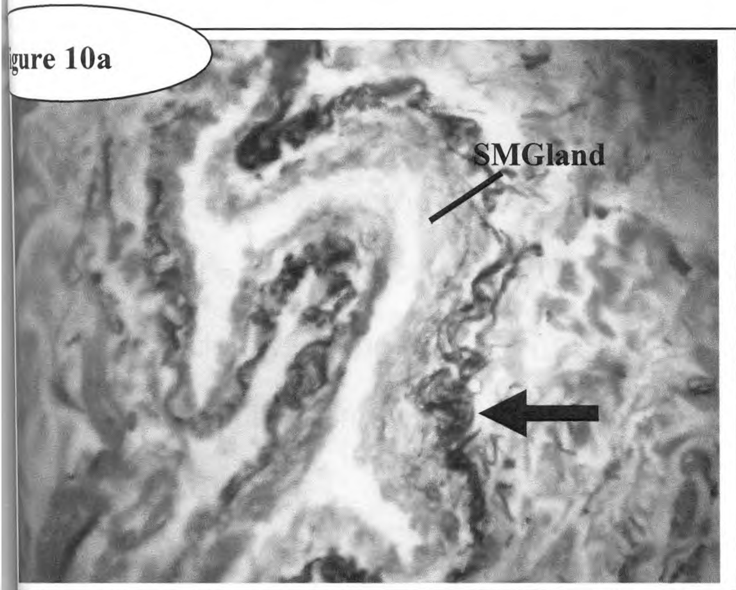
E= Epithelium
M= Mucosa
SM= Submucosa
Lam= Lamina propria

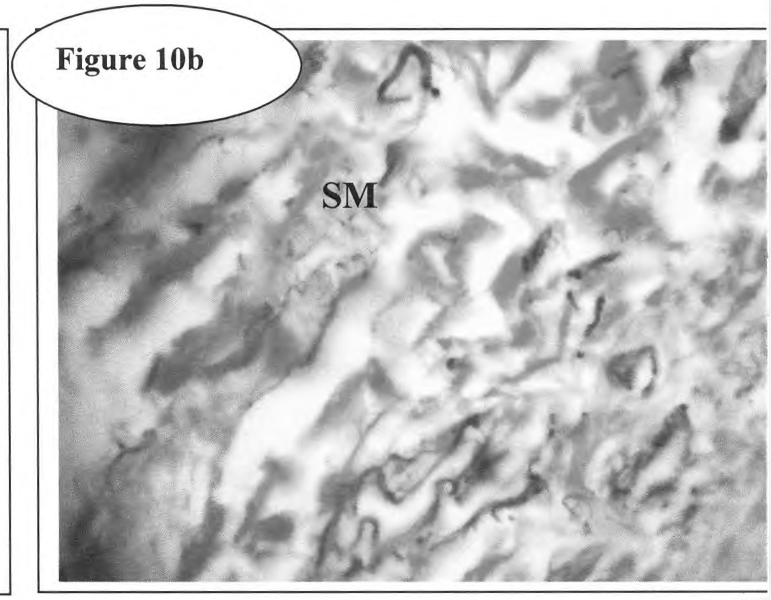
SUBMUCOSA

Elastic fibres are arranged in a loose and random manner in the submucosa. They are widely dispersed among the collagen fibres. Elastic fibres become condensed around sub mucosal glands to form distinct capsules (figure 10a). The submucosa is seen to be predominantly collagenous with the fibres arranged randomly and densely packed. There is no noticeable change in the density and orientation of the elastic and collagen fibres in the submucosa of the representative segments (figure 10b, c, d).

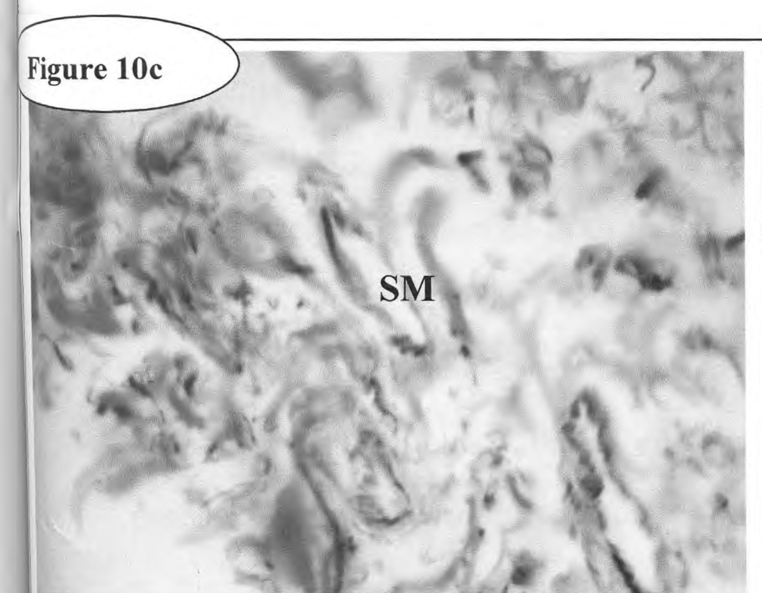
Figure 10a; Photomicrograph of the transverse section of the sub mucosa of the mid segment of the sigmoid colon wall. Weigert's resorcin and fuchsin stain. Magnification×400. Note the elastic fibres capsule surrounding the sub mucosal gland (arrow).

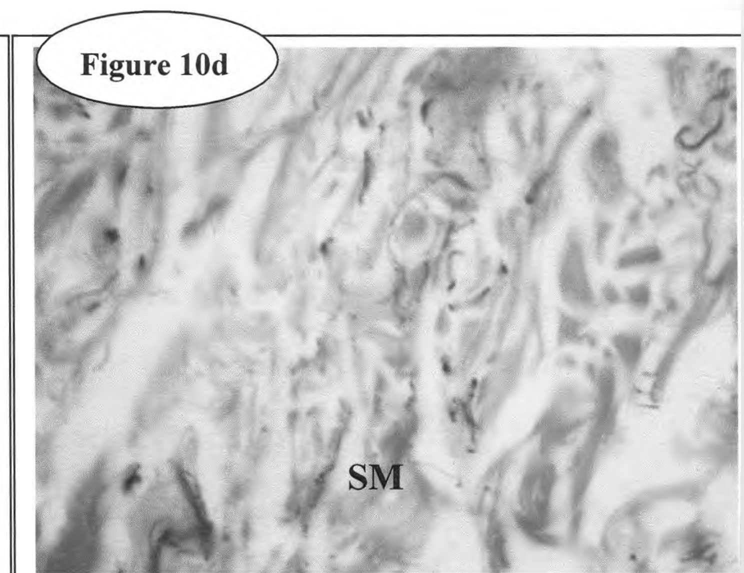
Figure 10b, c, d; Photomicrographs of the transverse section of the sub mucosa of the proximal, mid and distal colon. Weigert's resorcin and fuchsin stain. Magnification ×400. Note the randomness of arrangement of collagen and elastic fibres at all segments.





Proximal sigmoid colon





Mid sigmoid colon

Distal sigmoid colon

KEY
SM= Submucosa
SMGland= Submucosal gland

CIRCULAR MUSCLE LAYER

Elastic fibres are scanty and have a wavy in appearance. They are arranged along the axis of the muscle fibres i.e. circumferentially (figure 11a). There is a distinct lamina of elastic fibres at the sub mucosal surface of the circular muscle layer. These fibres run in a wavy pattern (figure 11b). There were no noticeable change in elastic fibre content and arrangement within the circular muscle layer in all the segments of the sigmoid colon.

TAENIA COLI

The sigmoid colon wall displays a distinct cluster of elastic fibres arranged both longitudinally and transversely at the junction between the taenia coli and the circular muscle layer (figure11c, d, e). Nerve fibre bundle between the taenia coli and the circular muscle layer are surrounded by a distinct layer of elastic fibres (figure11e). The elastic fibres between these two muscle layers are oriented both transversely and longitudinally. There is no noticeable difference in the orientation of the elastic fibres at the muscular junction between the representative segments of the sigmoid colon. Within the taenia coli, the elastic fibres are densely packed and run longitudinally in parallel alignment with muscle fibres (figure11g, h). The elastic fibre density in the taenia coli seems to increase proximodistally (figure 11f, g, h). The collagen fibres are seen clustered between the taenia coli and the circular muscle layer. Within the taenia coli, the collagen fibres appear to run longitudinally in parallel alignment to the muscle fibres.

Figure 11a; Photomicrograph of the transverse section of the circular muscle layer in the mid segment of the sigmoid colon. Weigert's resorcin and fuchsin stain. Magnification×400. Note the scarcity of elastic fibres and their transverse orientation along the axis of the muscle fibres.

Figure 11b; Photomicrograph of the transverse section of the sub mucosa and circular muscle layer of the mid segment of the sigmoid colon. Weigert's resorcin and fuchsin stain. Magnification×200. Note the distinct elastic fibre lamina at the musculo-sub mucosal junction (arrow).

Figure 11c, d, e; Photomicrographs of the transverse section of the junction between the taenia coli and the circular muscle layer of the proximal, middle and distal segments of the colon wall. Weigert's resorcin and fuchsin stain. Magnification×400. Note the distinct elastic fibre clusters at the junction. Note also the elastic fibre bundle around the nerve fibre bundle at the junction (arrow). There is a relative increase of density of elastic fibre cluster at the junction proximodistally.

igure 11a

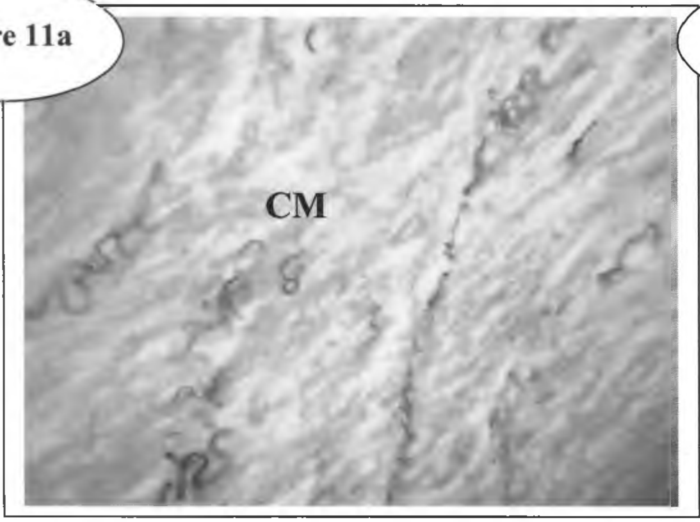
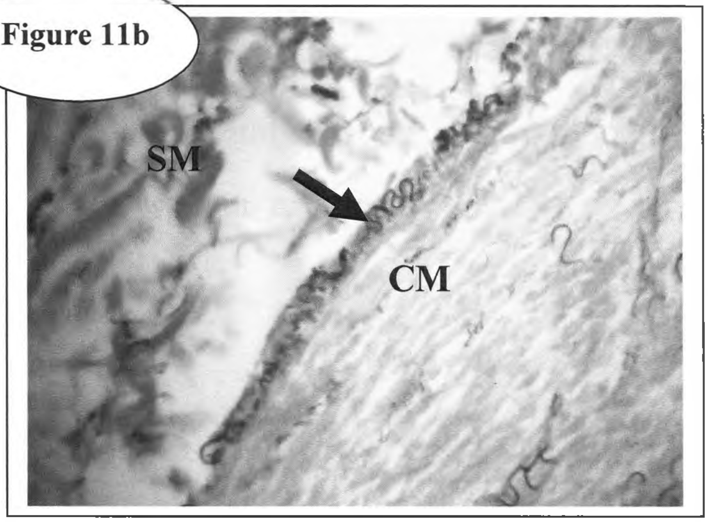


Figure 11b



igure 11c

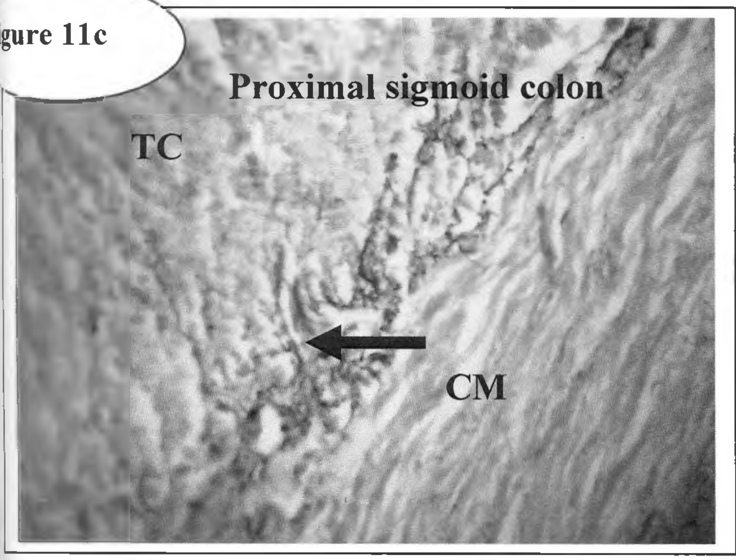
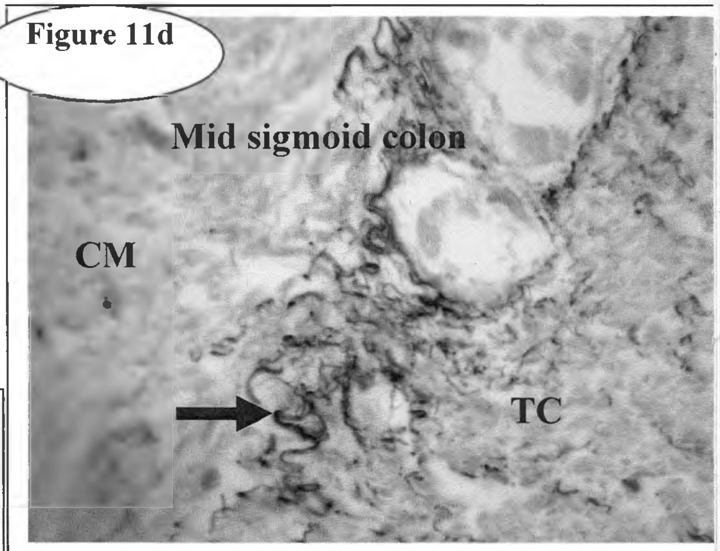
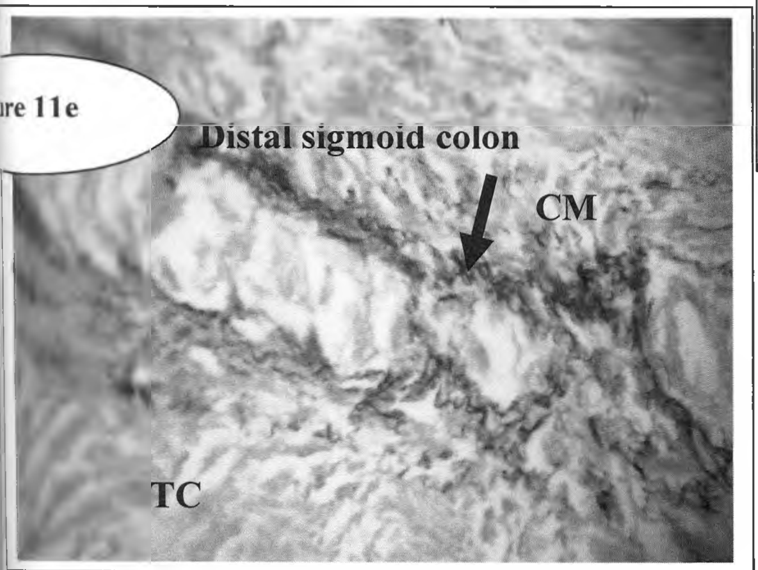


Figure 11d



ure 11e



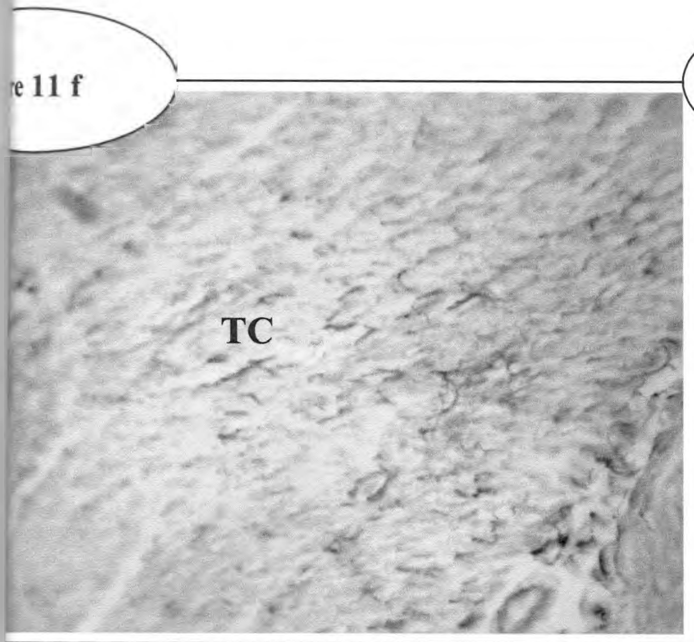
KEY
SM= Submucosa
CM= Circular muscle layer
TC= Taenia coli

SEROSA

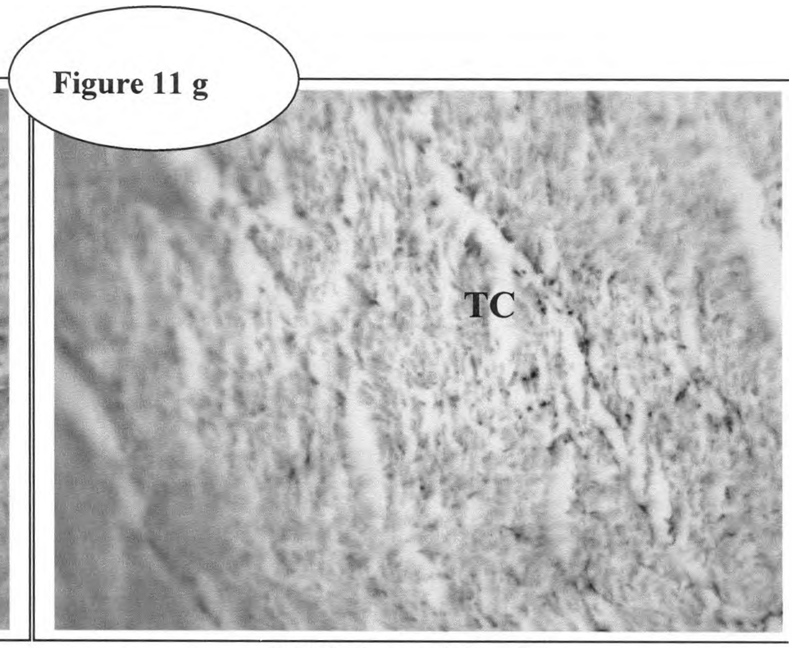
The serosa is predominantly elastic. The elastic fibres run mainly transversely (figure12). Bundles of collagen fibres are clustered around the elastic fibres. Similar arrangement was observed in all segments without any observable regional differences.

INFANT SIGMOID COLON WALL HISTOLOGY

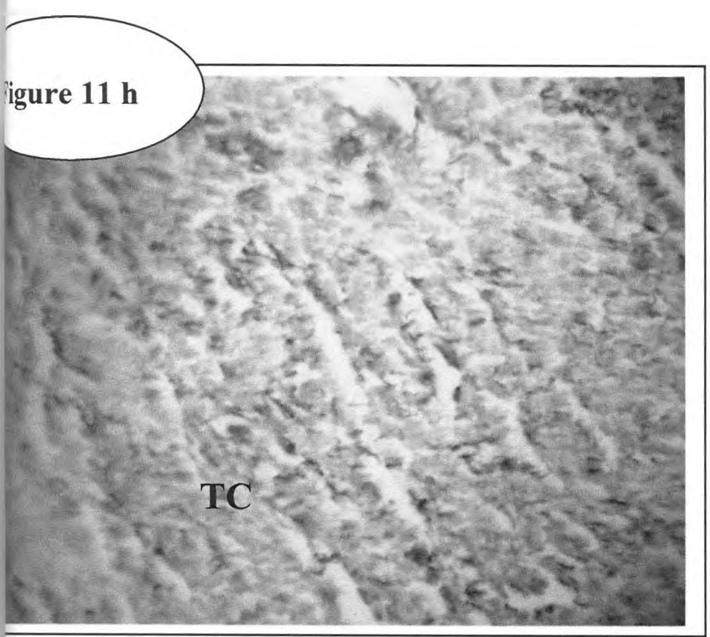
The connective fibre composition of the sigmoid colon wall of a one year old male infant showed scarcely visible elastic fibres between the wall layers as compared to the sigmoid colon wall of the adult (Figure 13).

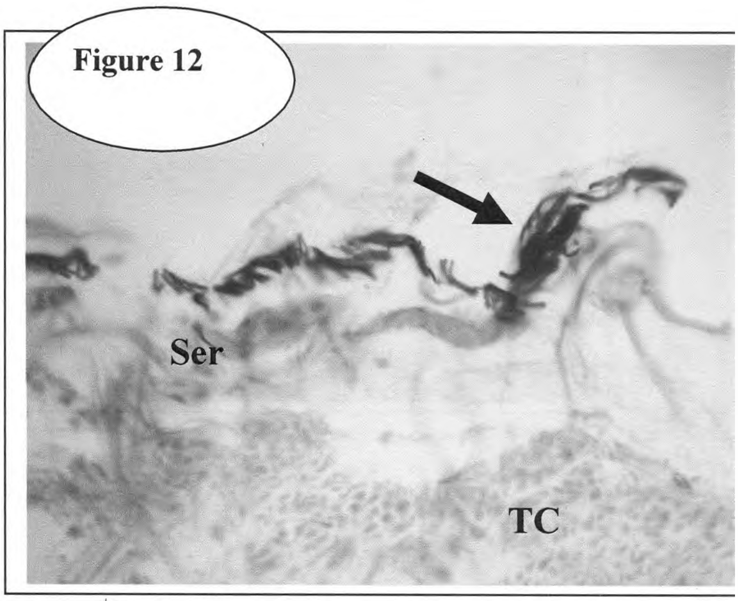


Proximal sigmoid colon



Mid sigmoid colon





Distal sigmoid colon

KEY
TC= Taenia coli
Ser=Serosa

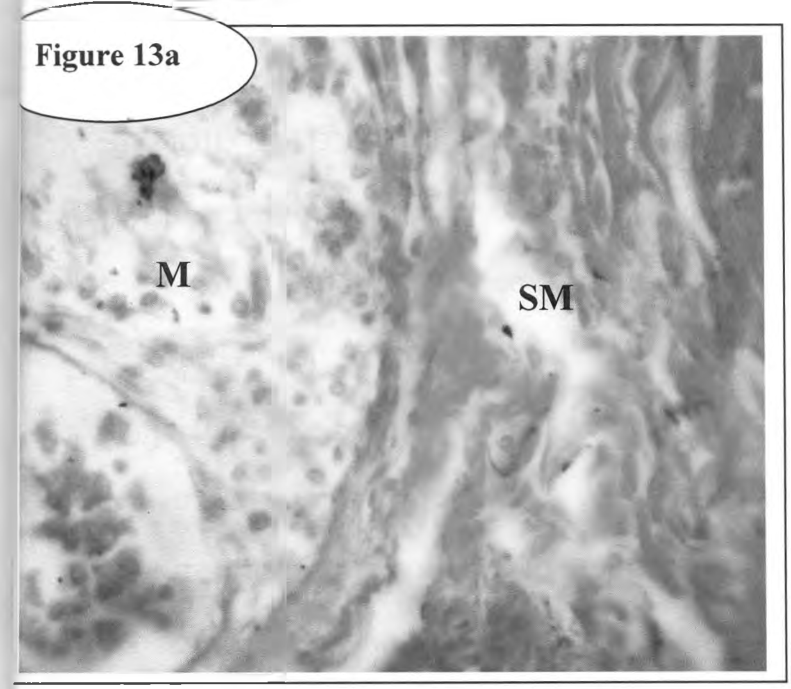
Fig 11f, g, h; Photomicrographs of the transverse section of the taenia coli in the proximal, middle and distal sigmoid colon. Weigert's resorcin and fuchsin stain. Magnification×400. Note the relative increase in density of the elastic fibres and their vertical orientation along the axis of the muscle fibres.

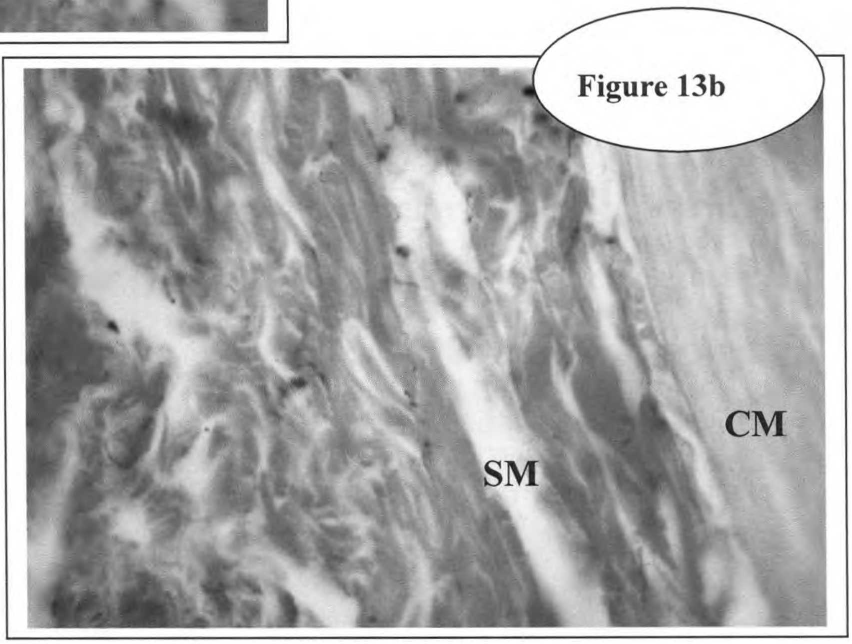
Figure 12; Photomicrograph of the transverse section of the serosa in the proximal sigmoid colon wall. Weigert's resorcin and fuchsin stain Magnification×400. Note the predominance of the elastic fibres (arrow).

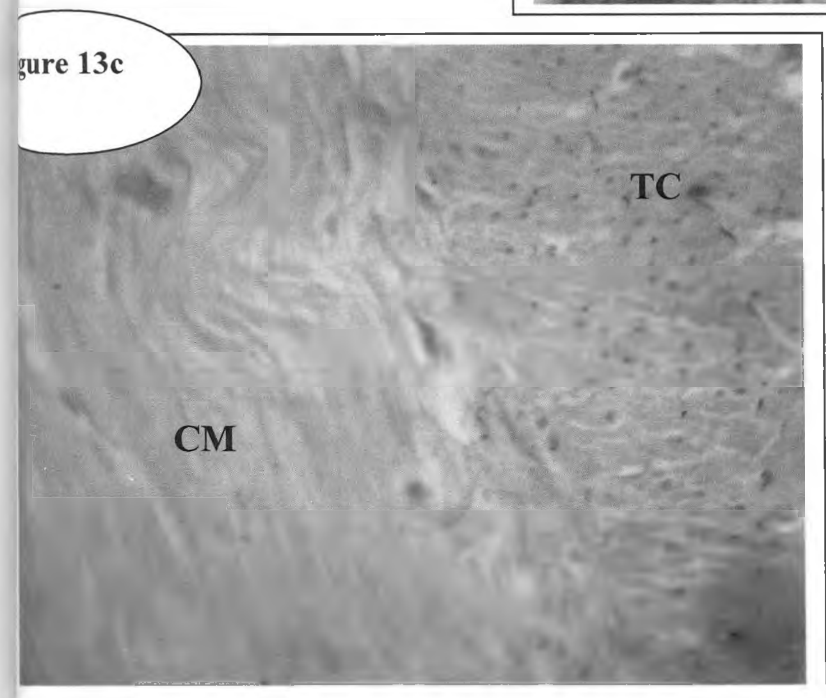
Figure 13a;Photomicrograph of a transverse section of the mucosal-submucosal junction of the mid portion of the sigmoid colon wall of a one year old male infant. Weigert's resorcin and fuchsin stain. Magnification×400. Note the scarcity of the elastic fibres.

Figure 13b; Photomicrograph of a transverse section of the submucosal-muscular junction of the mid portion of the sigmoid colon wall of a one year old male infant. Weigert's resorcin and fuchsin stain. Magnification×400. Note the scarcity of the elastic fibres.

Figure 13c; Photomicrograph of a transverse section of the junction between the circular muscle layer and the taenia coli of the mid portion of the sigmoid colon wall of a one year old male infant. Weigert's resorcin and fuchsin stain. Magnification×400. Note the presence of collagen fibres and the relative scarcity of the elastic fibres.







KEY
M= Mucosa
SM= Submucosa
CM= Circular muscle layer
TC= Taenia coli

DISCUSSION

SIGMOID COLON MORPHOMETRY

Observations of the present study have revealed that the mean length of the sigmoid colon is 36.3cm for adult males and 32.3cm for adult females (average mean length, 34.3cm) with the gender difference being statistically significant (p=0.007). These findings that there is gender difference support and extend those of previous workers on other populations. Ertem (1995) reported a mean sigmoid colon length of 38.9cm for adult males and 34.2cm for adult females in Turkey[3]. Bhatnagar et al (2004) also describes a gender difference in mean length of the human sigmoid colon in adult Indians but he calculates the mean length of the population as 46.6 cm[1]. Both workers used similar morphometric methods. Fifty-one live subjects were used in Bhatnagar's study. The male mean length was 46.5cm and the female mean length was 36.8cm (Table 2). The same group calculated a mean length of 26.8cm for males and 36.5cm for females for 16 fixed cadaver subjects. The marked difference in mean lengths between the cadaver and the live subjects indicate shrinkage of the sigmoid colon after fixation.

Table2; Mean lengths of the sigmoid colon in different populations.

Author	Country	Sigmoid length (cm)	
		Males	Females
Anangwe, 2006	Kenya	36.3	32.3
Bhatnagar, 2004	India	46.5	36.8
Ertem, 1995	Turkey	38.9	34.2

There are few population studies on the length of the sigmoid colon for statistical comparison[1, 3]. Even so, the few that have been quoted seem to reveal an interpopulation difference but with a generally longer sigmoid colon for males than for females. Within these populations, it has been shown that sigmoid volvulus is more common in males than in females[1, 3] implicating a morphometric basis in the susceptibility to this pathology. Population specific anatomic dimensions of the sigmoid colon may be of value in designing colonoscopic tools[19].

Morphometric dimensions of the sigmoid mesocolon were also studied and these showed a statistically significant gender difference. These are the sigmoid mesocolon root length which was 14.3cm for males and 18.4cm for females (average, 16.4cm) ($p=0.012$) and the sigmoid mesocolon vertical height which was 16.8cm for males and 13.2cm for females (average, 15.0cm). The measurements taken showed no significant age differences although all the studied subjects were considered adults (ranging from twenty to fifty five years of age). Bhatnagar recorded the mean vertical height of the sigmoid mesocolon as 13cm in the previously stated study using in situ metric measurements. Saunders et al (1995) radiographically compared the vertical height of the sigmoid mesocolon in Caucasians and Orientals and recorded a small difference where the mean mesocolic height in Caucasians was 11cm and that in Orientals was 12 cm[19] (Table3). In adults, gender difference in morphometry of the sigmoid colon and mesocolon are more obvious than age differences[20]. These studies show little difference in the mesocolic height regardless of the methodology used.

Table3; Mean sigmoid mesocolon height in different populations.

Author	Country	Sigmoid mesocolon height (cm)
Anangwe, 2006	Kenya	15.0
Bhatnagar, 2004	India	13
Saunders, 1995	Undefined Caucasians	11
Saunders, 1995	Undefined Orientals	12

Previous studies suggest that individuals with a long sigmoid colon length and short mesocolon root length are more susceptible[1]. Therefore indices were calculated from ratios of the sigmoid colon length to the mesocolic root length and the mesocolic vertical height as an indicator of the tendency to volvulus formation. Index1 was calculated and found to be 2.68 for males and 1.76 for females while index2 was 1.22 for males and 0.74 for females. Both ratios indicate that males in the studied population are more prone to volvulus formation than females.

Observations of the present study support and extend those of Bhatnagar that the sigmoid mesocolon in the male is dolichomesocolic (longer than wide), whereas the female mesocolon is brachymesocolic (wider than long). According to Ertem et al (1995), it is the narrower mesocolic root with a greater vertical length of the mesocolon (dolichomesocolic pattern) that makes the male sigmoid colon more prone to volvulus[3]. It is plausible therefore that like in other populations[1, 3], the gender difference in the morphometry of the sigmoid colon and mesocolon may provide part of the of the explanation for the gender disparity in the prevalence of sigmoid volvulus among Africans[5, 6, 7]. The variations in the configuration of the sigmoid loop could also have implications for colonoscopy of this segment of the bowel[1].

HISTOMORPHOLOGY

CIRCULAR MUSCLE LAYER

The current observations have revealed that the distal third of the sigmoid colon was characterized by a relatively thicker circular layer of muscle in comparison to more proximal sigmoid segments. This result is consistent with published literature[12]. Physiological studies have demonstrated a high pressure segment, 3.5 –4.5cm long in the rectosigmoid area suggesting the presence of a physiological sphincter - the rectosigmoid sphincter[13]. Recent anatomical studies have provided evidence that a thickened circular muscle in this segment is the anatomical basis for the high pressure zone[13].

The sigmoid colon is considered a site of fecal storage[21]; upon contraction, it delivers the stools to the rectum. As the rectum receives the feces, the recto-anal inhibitory reflex is evoked with a resulting rectal contraction, relaxation of the internal anal sphincter and stool evacuation[21]. It is assumed that the stools arriving from the colon are halted at the rectosigmoid junction by the existing high-pressure-zone. Shafik, 1999, suggests that this could contribute to involuntary fecal continence[12]. Our results on the thickened circular muscle towards the rectum provides further evidence of the existence of an anatomical sphincter at the recto sigmoid junction which may regulate the passage of stools from the sigmoid colon to the rectum[12].

The regional organization of the sigmoid smooth muscle layer may relate in some way to the genesis of diseases in the rectosigmoid area. The precise etiology of diverticula disease of the colon is not known[18], but four factors have been recognized to play important roles in its pathogenesis, namely; structure of the colon wall, intracolic pressure, fibre content of the diet and genetic influences[22]. Intracolic pressure depends on the load within the colon and the thickness of the colonic wall. The semisolid nature of feacal matter in the distal colon exerts higher intraluminal pressures than the proximal parts[22]. Further, the proximo-distal increase in the thickness of the colonic wall would narrow the lumen and contribute to raised intraluminal pressure, based on Laplace' law[17]. This pressure may cause out pouching of colonic wall especially at weak points in the circular muscle layer, where the blood vessels enter to supply the mucosa[23]. It is proposed that dysfunctions in the rectosigmoid sphincter may further increase intracolic pressure and play a role in the formation of diverticular disease.

The similarity of the distal sigmoid circular muscle for the subjects studied in the current study with prevailing data limits its potential in the genesis of the disease. Other population differences may be key. The African diet has long been suggested as an important factor. High fibre diets in Africa are associated with fewer incidences of diverticulas in Africans[8].

The pathway through which diet affects the integrity and tensile strength of the colon wall is thought to be collagen fibres. Wess et al, (1996) fed a high fibre diet to rats and observed that this protected against cross linking of collagen fibres and this was related to decreased incidence of diverticulas[24]. The authors postulated a similar pattern in man.

Collagen composition

Observations of the present study have revealed a lamina propria of the colonic mucosa with a loose matrix of collagen fibres except at the base of the crypts near the mucosal – sub mucosal junction where these fibres form bundles that are circumferentially arranged around the muscularis mucosae. Similar collagen formation has been described for the rat esophagus where collagen constituted 40% of connective tissue fibers at the mucosa-submucosal junction[25]. This organization is thought to be the mechanism by which the mucosa is attached to the submucosa and the transmission of stress from the mucosa to the submucosa[25].

The current study reports a random array of collagen in the submucosa that exhibits no recognizable change in orientation in all relevant segments of the sigmoid colon wall. The main framework of the submucosa in intestines is classically composed of two arrays of collagen fibre bundles running diagonally around the intestinal wall, one set in a clockwise direction, the other counterclockwise[26]. Scanning electron microscopic investigations of the framework have however revealed an interwoven lattice sheet with fibers running in different directions rather than two separate layers[25]. This arrangement is thought to play a significant supportive role in the wall of the intestine and in other parts of the gastrointestinal tract [25].

The morphology and the mechanical properties of the submucosa have been described in the esophagus[27]. The esophageal submucosa is collagen-rich. In-vivo inflation experiments have demonstrated increases of radii of the intact esophageal wall as well as the individual layers of the esophagus with increase of intra luminal pressure[28, 29, 30, 31]. The sub mucosal layer is demonstrably stiffest[29], an observation in concordance with the fact that submucosa contains large amounts of collagen.

Collagen provides structural support for most tissues within the extra cellular matrix and pays a vital role in cellular proliferation, migration and differentiation[32]. There are at least 19 different vertebrate collagens with tissue-specific distributions and unique functional properties. These unique types of collagen can be subdivided into the following classes based on function or size: fibrillar; fibril-associated; network forming; filamentous; short chain; and long chain[33]. Crosslink formation between collagen micro fibrils is largely responsible for the tensile strength attributed to collagen[34]. As an animal ages, collagen cross linking is progressive, and fiber size increases[35].The collagen type found in the submucosa of the gastrointestinal tract is mostly collagen type III with small amounts of collagen type IV[16]. As observed earlier, the collagen fibres are arranged in a honeycomb fashion and are extensively branched. This arrangement contributes to the strength, natural tension and extensibility of the colon wall[36].

The present study has revealed a relative proximo-distal reduction of the width of the submucosa relative to muscular layer. This result supports the observation by Watters (1985) that the tensile strength of the colon wall falls distally[8]. It is plausible that distally, the collagen fibres become smaller and more tightly packed than in proximal segments of the sigmoid colon. This may be associated with relative weakness of the sigmoid colonic wall.

We contend that the similarity of collagen data in the present study to other published works forces consideration of non-anatomical factors in the genesis of diverticular disease. The experiments by Wess et al, (1996) indicated the protective role of high fiber diet[24]. Collagen seems to be more compact as the rectum is approached. Presumably then, the protection against cross linking of collagen afforded by high fiber in the African diet could not be proved by the current descriptive study. Other factors implicated in the causation of diverticular disease include genetic collagen fibre disorders like Marfan's and Ehlers-Danlos syndrome[37, 38]. Seventy seven percent of colonic lesions caused by these diseases occur in the rectum and the sigmoid colon[39].

Using in vitro tensiometric techniques, Lord et al illustrated that African sigmoid colons have greater tensile strength than European sigmoid colons but no inference to the inherent structural basis of this difference was made[16]. Collagen is known to exist in viable structural and functional relationship with elastic fibers. These specific interactions may determine the ease with which tissues accommodate tensile stresses.

Elastic fibre distribution

Within the submucosa, glands were enclosed in a distinct elastic capsule almost similar to the internal elastic lamina of adjacent blood vessels. The current study also describes a laminar arrangement of elastic fibres at the submucosal surface of the circular muscle layer. Elastic fibres also formed bundles at the junction of the inner circular muscle layer and the outer longitudinal muscle layer. Density of elastic fibres was higher in the outer longitudinal muscle layer and also seemed to increase proximodistally in the taenia coli.

Elastic fibres have the ability to deform reversibly without loss of energy; an index for high resilience. Elastin works well as a strain-energy storage fibre and plays a big role in the transmission of stress[40]. The predominant elaboration of elastic fibers in the taenia coli with a graded increase proximodistally parallels the elastic fiber morphology in the outer muscular part of stomach[41]. The elastic fibres ran parallel to the gastric muscle fibres and increased in density distally towards the pylorus. The length and density of the elastic fibers in areas of high intraluminal pressure were found to be statistically greater than those in areas of low pressure. It was speculated that these elastic fibers played a role in the sphincteric function by increasing the tonus and elastic recoil of the muscle tissue[41]. Since the distal sigmoid colon wall is thought to be part of the rectosigmoid sphincter[12] the similarity in regional change of density of elastic fibres proximodistally could imply a similar functional role as in the pyloric sphincter.

The observation of distribution of elastic fibers in all layers of the colon is consistent with the observations by[18]. The latter observation described an intersecting arrangement of elastic fibers in the submucosa and a fascicular arrangement in muscularis externa. These earlier accounts did not mention the laminar arrangement of elastic fibres in between the wall components[17, 18] as described in the present study. This laminar disposition between wall components of the sigmoid colon wall may play an important role in the transmission of stress from one wall component to the next and protect against the formation of diverticuli.

This suggestion is however at variance with contentions by Smith et al (1976) and Whiteway (1995)[14, 18]. Smith et al have demonstrated an increase in quantity and fibre diameter in with age[14]. Colonic diverticular disease showed a striking correlation with advancing age[42]. The colon wall properties also change with a decrease of the tensile strength[8]. Could the reason for this change in colonic strength relate to alteration of elastic and collagen fiber configuration? Increased elastic fiber content has been postulated to result in contracture or shortening of the taenia coli with secondary corrugation of the circular muscle layer predisposing to diverticula formation [14, 18]. The preponderance of elastic fibers in the African colon as reported here would however seem to make this contention untenable.

The observation that elastic fibers were sparsely distributed in the infant is a general theme in elastogenesis. This suggests that the development and appearance with demand for a tissue with high resilience, large strains and low stiffness. This temporal sequence of appearance of visible fibers may be related to elastogenesis and elastic tissue types. Structural studies on the elastic fibers reveal a complex structure consisting of 2 distinct components[43]. The major component, which has an amorphous appearance, represents the elastin protein. The amorphous elastin is surrounded by distinct fibrillar structures (microfibrils), organized into 10 to 12 nm fibrils of beaded appearance[44]. During embryonic development; the newly developed fibers are composed exclusively of the microfibrillar component, but during development the proportion of elastin increases progressively and in a fully developed fiber more than 90% is elastin[43].

CONCLUSIONS

The adult male sigmoid colon is longer in males than in females with a shorter root length. The height of the sigmoid mesocolon is also longer in males showing a clear gender specific morphometric pattern. These morphometric differences may be the basis of the higher incidence of sigmoid volvulus in males than in females in Africans.

Although the distribution and arrangement of elastic fibres in the muscular layers in adult Kenyans is similar to that described in Caucasians, the former has elaborate elastic bundles in between the colon wall layers not described in Caucasian colons. This distinction may improve the capacity of the wall to withstand intracolic pressure - a possible postulation as to why diverticulosis is rare in Africans as compared to Caucasians.

The distal sigmoid colon wall is characterized by thickening of the circular muscle. This feature is consistent with recent reports describing an anatomical rectosigmoid sphincter.

LIMITATIONS OF THE STUDY

Post mortem changes in morphometric parameters of the sigmoid colon and mesocolon may have affected the accuracy of the measurements and therefore the outcome of the results. To mitigate this, the measurements were taken from subjects that had died within forty eight hours. In some cases the where records were absent; the post demise period was difficult to ascertain. Microscopic pathologic changes in the sigmoid colonic wall, like inflammatory collagenous colitis, could have affected the outcome of the histology results.

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APPENDIX I

CONSENT BY RELATIVES OF THE DECEASED (English)

Study Number: ____

Aim and benefit of the study:

To study the form and structure of the end part of the large intestines in an attempt to explain why certain diseases in Kenyan Africans are rare.

Method of the study:

The end part of the large intestines will be obtained during postmortem opening of the abdomen. The item of study will be carefully removed, cleaned and taken to the laboratory for measurement and microscopic observation. The study items will then be collected and taken to Lang'ata cemmetry for mass burial or incineration as a means of safe disposal.

Humble request:

I, hereby, humbly ask for consent from the next of kin to allow the study to be carried out. The consent is formalized by a confidentiality signature below. Denial of consent by next of keen will be duely respected.

Confidentiality:

The identity of the deceased will be concealed and no information concerning him will be published.

I, ____, the relative/next of kin, have been explained to and understood the above and willingly accept to let the deceased participate in the study.

Signature / Thumbprint: ______________________________________

Date: ____

I, as the investigator, hereby accept that the data be collected and recorded during the post mortem and understand that the data will be used in a study whose findings can be published. I understand that privacy of the data record shall be maintained and the researcher in this or any other study will reveal no other details, apart from those related to the study.

Signature: ____

Date: ______________________

APPENDIX II
RUHUSA KUTOKA KWA WALIOFIWA (Swahili)

Nambari ya uchunguzi ____

Lengo na umuhimu wa uchunguzi huu
Kuchunguza maumbile ya matumbo kubwa ili kueleza uadimifu wa magonjwa fulani ya matumbo kubwa katika waKenya wa asili ya kiAfrika.

Namna ya uchunguzi
Matumbo kubwa yatafikiwa wakati wa upasuaji wa mfu. Chombo cha uchunguzi kita safishwa kabla ya kupimwa. Baada ya uchunguzi, maumbile haya yatachomwa.

Usiri
Mambo yote kuhusu marehemu yatawekwa kwa siri na hayatachapishwa wala kufichuliwa.

Mimi ____, jamaa ya marehemu, nimeelezwa na kuelewa haya, na kwa hiari yangu namruhusu mchunguzi autumie mwili wa marehemu katika uchunguzi wake.

Sahihi/ kidole ____

Tarehe ____

Mimi kama mchunguzi naelewa ya kwamba siri za marehemu ni muhimu na hazitafichuliwa. Mambo ambayo yana uhusiano na uchunguzi huu ndiyo yatatazamwa na kuandikwa.

Sahihi ____

Tarehe ____

APPENDIX III
DATA SHEET 1

Morphometric features

Study no……………………………………………………………………….……..

Subject age ………………………………………………………………………………

Sex: male…………………………… female …………………………….............

Day after demise……………………………………………………………………

Sigmoid length; S1…………………………..…………………………………..……

Mesocolon vertical length; S2……………….……………………………………………

Mesocolon root width; S3………………………………………………………………..

KENYATTA NATIONAL HOSPITAL
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Ref: KNH-ERC/ 01/ 3559

Date: 7th June 2006

Anangwe D
3rd year medical Student
Dept. of Human anatomy
Univeristy of Nairobi

Dear Duncan

RESEARCH PROPOSAL: MORPHOLOGY AND CONNECTIVE TISSUE COMPOSITION OF THE SIGMOID COLON IN BLACK KENYANS (UP64/3/2006

Refer your above proposal.

This is to inform you that permission has been granted by the KNH-Ethics & Research Committee to conduct research on study titled "Morphology and connective tissue composition of the sigmoid colon in black Kenyans"

By a copy of this letter the relevant persons are requested to avail the relevant information and materials that you will require.

Yours sincerely

Guantai

PROF A N GUANTAI
SECRETARY, KNH-ERC

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The Deputy Director CS, KNH
The Chairman, Department of Human anatomy, UON.
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