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AN ELECTRON MICROSCOPE STUDY OF THE SMOOTH MUSCLE CELL IN ACQUIRED AND CONGENITAL MEGACOLON

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SUMMARY

Biopsies taken from the muscle layer of the colon of 5 patients without colonic disease (control group), 10 patients with acquired megacolon and 2 patients with congenital megacolon, were studied with the electron microscope. Specimens were submitted to double fixation in glutaraldehyde 2 per cent and in 1 per cent phosphate-buffered osmium tetroxide; dehydration was carried out in ascending grades of ethyl alcohol; embedding was made in Araldite. A Siemens Elmiskop I electron microscope was used. Conspicuous ultrastructural changes were observed in the smooth muscle cells of the hypertrophied rectum and sigmoid in acquired megacolon in relation to the control group: increase in the number of points of close contact between neighboring cells; cytoplasmic vacuolization; dilatation of the endoplasmic reticulum and disorganization of myofilaments. The transverse colon, although normal macroscopically, showed the latter 3 alterations, but in a lesser grade of intensity and affecting a smaller number of cells than in the rectum and sigmoid. All the alterations described in the acquired megacolon involved isolated cells or groups of cells, so that there was always a large number of cells similar to those in the control group. The ultrastructure of the smooth muscle cells in the congenital megacolon was significantly different from the acquired megacolon and control groups; large areas of cytoplasmic destruction around the nucleus were seen in the majority of cells of the hypertrophied rectum as well in the normal appearing intestine.

INTRODUCTION

Acquired megacolon is a common disease among rural workers in certain geographic areas of Brazil, and has been related to Chagas Disease, on account of epidemiological and serological data.

The lesion of the nervous myenteric plexuses in acquired megacolon and megaesophagus was first described by Amorim & Corrêa Neto [1], and by Vasconcelos & Botelho [24], and has been confirmed by Authors like Köberle [12] and Okumura [17] in a vast clinical and experimental material.

The degeneration and destruction of the intramural autonomous plexuses have been admitted to cause muscular incoordination, resulting in hypertrophy of the muscular layer in the "megas".

Vasconcelos [21], however, observed some contradictions and uncertainties of this "plexular theory", and has insisted on the fact that in the "megas" there must be pathological changes affecting all the structures of the organ's wall, involving not only the myenteric nervous plexuses, but the smooth

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muscle cells too. Favouring this point of view, arise VASCONCELOS [22, 23] studies on megacolon and megaesophagus with the light microscope, and on megaesophagus with the electron microscope.

Conspicuous alterations of the muscle layer like focal intersticial myositis and degeneration of muscle fibers, were related by BRITO & VASCONCELOS [4] in acquired megacolon and megaesophagus and by OKUMURA et al. [16] in mice experimentally infected by *Trypanosoma cruzi*, which later developed megacolon.

The smooth muscle cell in the "megas" have not been sufficiently investigated by the majority of the Authors, who focus their studies on the muscle hypertrophy and the focal intersticial myositis.

We decided then to study the smooth muscle cell in acquired and congenital megacolon with the electron microscope, considering that the light microscope coul hardly give any new information.

The scanty literature dealing with the subject was a further stimulus for the accomplishment of this work. As a matter of fact, we could not collect any article concerning the ultra structure of the smooth muscle cell in acquired or congenital megacolon.

The literature is extremely poor even in relation to the smooth muscle cell of the normal human intestine. YAMAMOTO [25] studied the smooth muscle cell in the cecal appendix; GANSLER [9] investigated the small bowell, and TOSI et al. [20] worked with the smooth muscle cell of the ascending colon of one human case.

The great majority of the electron microscopic studies on smooth muscle cells has been conducted on a large series of different species of animals, and in several other organs than the intestine.

Finally, this is a merely morphological study, so that physiopathological considerations can not here be advanced.

MATERIAL AND METHODS

1. MATERIAL

We studied three groups of patients.

The first group, entitled "Control", was composed of 4 patients with chronic duodenal ulcer and 1 patient with gastric lymphosarcoma, in whom there was not any clinical or macroscopical sign of colonic disease. These patients, hereon designated by the letter C followed by numbers from 1 to 5, serve to characterize the smooth muscle cell of the normal human colon. There were 4 men and 1 woman in this group; the age ranged from 19 to 62 years, with a medium of 40.4.

The second group, named "Acquired Megacolon", comprises 10 patients with acquired megacolon, from now on designated by the letter M followed by numbers from 1 to 10. The rectum and sigmoid colon were always hypertrophied in this group, whilst the cecum, ascending, transverse and descending colon were macroscopically normal. There were 6 men and 4 women in this group, and the age ranged from 21 to 54 years, with a medium of 40.0.

The third group, entitled "Congenital Megacolon", consists of 2 patients, a 5-years old boy and a 6-years old girl, affected by congenital megacolon, designated by H 1 and H 2, respectively. The operation disclosed in H 1 a normal rectum and a very dilated sigmoid, while in H 2 only a small segment of the perineal rectum had a normal caliber, the sigmoid and the major part of the rectum appearing very dilated.

2. METHODS

Fragments of the muscle layer of the colon were obtained at operation, and submitted to electron microscopic examination.

In the control group, specimens were taken from the anti mesenteric taenia of the recto-sigmoid junction and of the transverse colon.

In the patients with acquired megacolon the biopsies were obtained from the anti mesenteric taenia of the transverse and sigmoid colons, and from the anterior wall of the intra peritoneal rectum.

In H 1 specimens were taken from the anti mesenteric taenia of the sigmoid and transverse colon and from the anterior wall of the intra peritoneal rectum. In H 2 the fragments were obtained from the muscle layer of the perineal rectum, and from the anti mesenteric taenia of the sigmoid and transverse colon.

Double fixation of the tissues was carried out, first in 2 per cent glutaraldehyde at pH 7,3 during 2 hours, and then in 1 per cent phosphate-buffered osmium tetroxide (MILLONIG [14]) at pH 7,3 during 60 to 90 minutes. The fixed specimens were then transferred into a 0.5 per cent uranyl acetate solution, resting there overnight.

Specimens were then dehydrated in ascending grades of ethyl alcohol, then embedded in Araldite. Blocks were sectioned by a Sorvall MT-2, Porter-Blum ultratome, and the sections were mounted on copper grids.

Sections were triple stained with a hydroxide lead solution during 10 minutes (KARNOVSKY [11]), then with a saturated solution of uranyl acetate during 15 minutes, and finally with a lead cytrate solution during 5 to 10 minutes (REYNOLDS [18]).

Grids were examined with a Siemens Elmiskop 1 electron microscope at the "Instituto Butantan de São Paulo".

The level of significance for the rejection of the nullity hypothesis was fixed at 5 per cent. Considering the little number of cases, we used the Fisher's "exact" test for the association tests (*).

RESULTS

1. The ultrastructure of the smooth muscle cell in the Control Group.

The ultra structural pattern was the same in all the cases of this group; there was not any significant difference between the specimens obtained from the transverse colon and those taken from the recto-sigmoid junction.

1.1 *Plasma membrane*

It was seen as a single osmiophilic line, without any evidence of a double membrane, even at high magnification studies.

The "dense plaques" are a very characteristic feature of the smooth muscle cell; they are areas of electron-dense material attached to the inner surface of the plasma membrane. Myofilament insertion occur at the dense plaques (Fig. 1).

These dense plaques alternate with clear areas containing small "pinocytotic vesicles", arranged in a single row, parallel to the cell membrane. The diameter of these vesicles ranges from 200 to 700 A (Fig. 1).

The smooth muscle cells run parallel to each other, so that the neighboring cell membranes are separated by a fairly constant extracellular substance. Very infrequently points of close contact between adjacent membranes can be seen, but there is always a space of at least 100 A separating them. Direct protoplasmic continuity could never be detected in the control group (Fig. 1).

Desmosomes were rather frequently observed, as a result of the apposition of dense plaques of adjacent cell membranes.

1.2 *Cytoplasm*

In a homogenous, clear matrix, several structures are immersed.

The endoplasmic reticulum, with its smooth and rough components, is very inconspicuous in the control group, and is located mainly at the nuclear poles (Fig. 1).

The Golgi Complex was infrequently seen in the control group; when present, it was located close to the nuclear contour.

The mitochondria can be easily detected throughout the cytoplasm, but they concentrate mainly at the nuclear poles (Fig. 1).

Ribosomes occur sparsely in the cytoplasm, showing as clusters or insulated particles of ribonucleoprotein, with some trend to concentrate at the nuclear poles, where they can be seen attached to the membranes of the granular endoplasmic reticulum (Fig. 1).

Occasionally, lamellar structures resembling "myelin figures" can be observed in the cytoplasm, appearing as a strongly osmiophilic spiral.

Fine myofilaments run across the cytoplasm along its longitudinal axis, in straight or slightly ondulated parallel bundles (Fig. 1).

(*) SIEGEL, S. — Non Parametric Statistics for the Behavioral Sciences. New York, Mac Graw-Hill Book Comp. Inc., 1956, pp. 96-104.

These myofilaments do not show any striation at and their diameter is approximately 50 A.

The "dense bodies" are a very characteristic aspect of the smooth muscle cells; they are numerous electron-dense, spindle shaped structures, scattered in the cytoplasm, closely resembling the dense plaques of the cell membrane. Their long axis coincide with the longitudinal direction of the cell, and may measure up to 0.5 micron (Fig. 1).

The dense bodies of the cytoplasm are traversed by myofilaments, so that they may possibly serve as insertion points, like the dense plaques of the cell membrane.

1.3 *Nucleus*

It is located at the centre of the cell, its long axis coinciding with the longitudinal axis of the cell.

The nucleus is bound by a double mem-

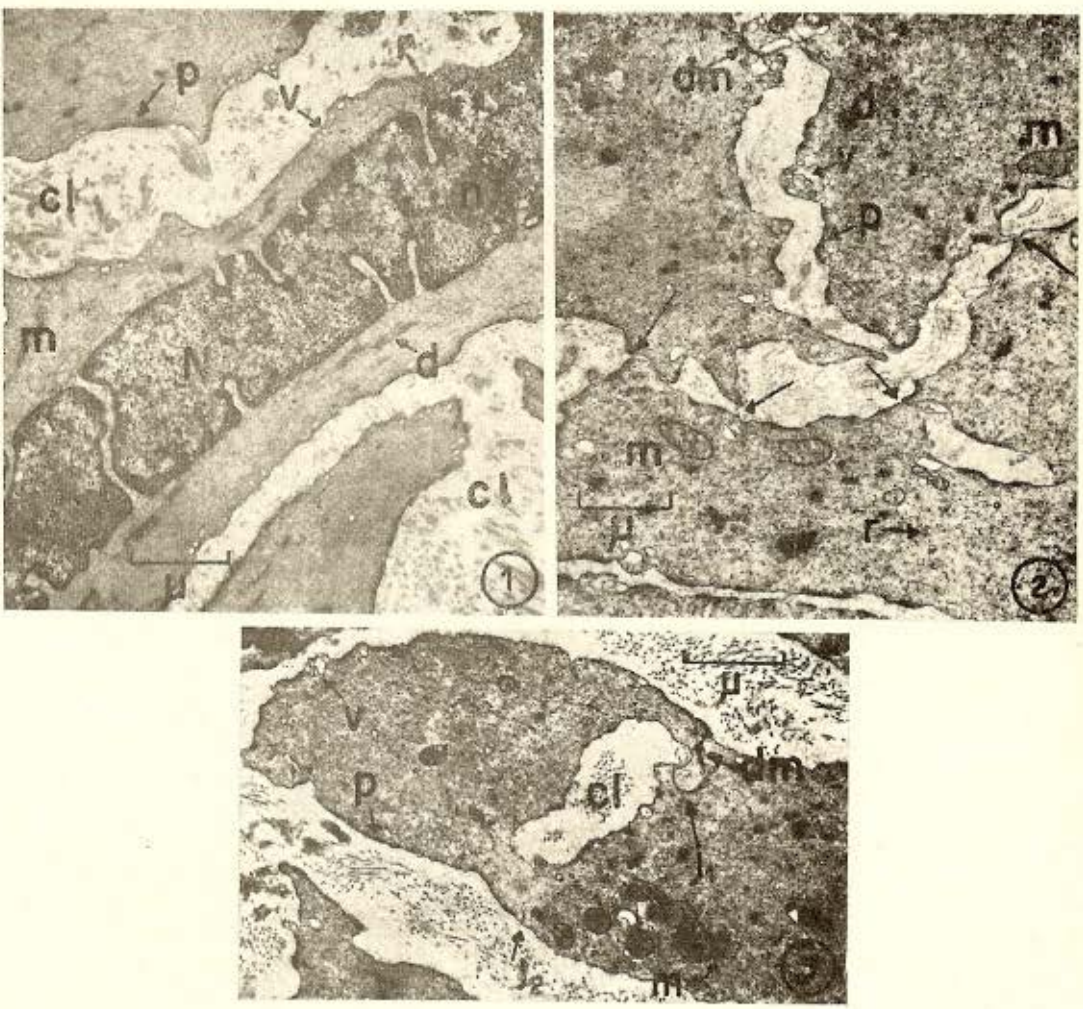


Fig. 1 — Smooth muscle cell in the control group (C1 — transverse colon)
Fig. 2 — Several points of close contact between neighboring muscle cells in acquired megacolon (arrows). (M — 5 — sigmoid)
Fig. 3 — Intimate relationship between two smooth muscle cells in acquired megacolon. In j 1, there is close apposition between adjacent cell membranes. In j 2, there is fusion of adjoining cell membranes and direct protoplasmic continuity (M 1 — sigmoid)

brane, and the inner aspect of the internal membrane is coated by a rather thick, almost continuous band of a highly electron dense chromatin. The chromatin still appears as irregular filaments or granules scattered through the nucleoplasm (Fig. 1).

Pores in the external layer of the nuclear membrane communicate the nucleoplasm and the cytoplasm.

An excentrically located nucleole was frequently seen in the nucleus; we could never observe more than one nucleole in a single nucleus (Fig. 1).

1.4 *Interstitial Space*

A very large number of collagen fibers can be seen running in different directions through the interstitial space, so that in a single section, some fibers are cut transversally, others longitudinally and still others, obliquely. In the first case they appear as dense granules; when cut longitudinally they show the characteristic collagen periodicity. (Fig. 1).

2. The ultrastructure of the smooth muscle cell in acquired megacolon

Some preliminary remarks must be taken into consideration:

a) Ultrastructural alterations were seen in all the patients of this group; b) There was not any difference in ultrastructure between the specimens obtained from the rectum and those from the sigmoid colon. The alterations observed in the transverse colon, however, were much less intense than those in the rectum and sigmoid; c) The ultrastructure alterations occur in groups of cells or in single cells, so that many of them or even the majority, do not show any morphological change, but an increase in volume, consequence of the hypertrophy, already well known from the light microscope studies; d) The ultrastructure changes that will be described did not always occur altogether in a particular cell.

2.1 *The plasma membrane. Interrelationship alterations*

There was not any significant alteration of the plasma membrane in relation to the control, but an increase in size and number of the pinocytotic vesicles, which reached up to 0.3 micron in diameter. The rectum and sigmoid showed a much more intense pinocytotic activity than the transverse colon.

A very striking feature of acquired megacolon is alteration of the interrelationship pattern between neighboring smooth muscle cells. In contrast to the control group, there is formation of a large number of points of close contact between adjacent cells in acquired megacolon (Fig. 2).

Smooth muscle cells send out lateral cytoplasmic processes that make intimate contact with similar cytoplasmic processes or with flat areas of the adjacent cell membrane. Sometimes there is intrusion of a cytoplasmic process of one cell into a depression of the adjacent cell membrane (Fig. 3).

It is not infrequent to observe one muscle cell sending out multiple cytoplasmic processes, which make contact with several neighboring cells (Fig. 2).

The intercellular space is notably reduced at these contact points, but there is always a distance of at least 100 A separating the opposing cells.

Occasionally the intercellular space disappears as a consequence of the fusion of adjacent cell membranes (Figs. 3, 4).

In 4 cases (M 1, M 5, M 9 and M 10), we could detect direct protoplasmic continuity between smooth muscle cells, in specimens obtained from the rectum and sigmoid (Figs. 3, 4).

Increase in the number of points of close contact between smooth muscle cells, was observed in the rectum and sigmoid of 7 patients with acquired megacolon (M 1, M 2, M 3, M 4, M 5, M 6 and M 8), which was statistically significant in relation to the control group ($p = 0.018$) (Table I).

As for the transverse colon, increase in the number of close contacts was observed in only two cases (M 3 and M 6), which was not significant in relation to the control group ($p = 0.085$) (Table II).

2.2 *Cytoplasm*

The cytoplasm was the site of the most conspicuous morphological alterations in the acquired megacolon group.

TABLE I

Comparison between the Control and Acquired Megacolon Groups concerning the number of patients with a large quantity of points of intimate contact between smooth muscle cells of the rectum and sigmoid colon

Group	no. of cases with many contacts	no. of cases with little contacts	Total
C	0	5	5
A.M.	7	3	10
Total	7	8	15

Fisher's "exact" test = significant (p = 0.018)
C = Control
A.M. = Acquired Megacolon

TABLE II

Comparison between the Control and Acquired Megacolon Groups concerning the number of patients with a large quantity of points of intimate contact between smooth muscle cells of the transverse colon

Group	no. of cases with many contacts	no. of cases with little contacts	Total
C	0	5	5
A.M.	2	8	10
Total	2	13	15

Fisher's "exact" test = non significant (p = 0.085)
C = Control
A.M. = Acquired Megacolon

The cytoplasmic matrix showed oedema dissociating the myofilaments, particularly in those cells with evident ultramicroscopic changes.

2.2.1 *Endoplasmic reticulum*

It is much more developed and conspicuous in acquired megacolon than in the control group, so that the granular and agranular components can be easily recognized in the rectum and sigmoid or in the transverse colon.

In single sparse cells or in groups of cells, the canaliculi of the endoplasmic reticulum suffer different grades of dilatation, ranging from a minimum increase in their size up to the formation of large vacuoles, containing a substance either homogenous and amorphous, or finely granular and with low density (Fig. 5).

In all the 10 patients of this group, the specimens from the rectum and sigmoid revealed the described alterations of the endoplasmic reticulum, which was statistically significant (p = 0.00033) in relation to the control group, where the endoplasmic reticulum was always normal (Table III).

Ultrastructural changes of the endoplasmic reticulum were more conspicuous and were seen in a larger number of cells in the rectum and sigmoid than in the transverse colon. Only in two patients (M 3 and M 10) did the endoplasmic reticulum show intense dilatation and formation of vacuoles.

Seven patients (M 1, M 3, M 4, M 5, M 6, M 7 and M 10) with acquired megacolon showed dilatation of the endoplasmic reticulum in the transverse colon which was significant (p = 0.018) in relation to the control group (Table IV).

2.2.2 *Cytoplasmic vacuoles and myofilament disorganization*

Vacuoles could be observed in the cytoplasm of the smooth muscle cells of the rectum and sigmoid in all the 10 patients of the Acquired Megacolon group, which was significant (p = 0.00033) in relation to the Control group, where vacuoles were never seen (Table III).

Cytoplasmic vacuoles were also seen in the smooth muscle cells of the transverse colon in 7 patients of this group (M 1, M 3, M 4, M 5, M 6, M 7 and M 10), which was significant (p = 0.018) in relation to the control (Table IV).

The vacuoles occur isolated or in groups, diffusely scattered throughout the cytoplasm. They are rounded or irregular, and may

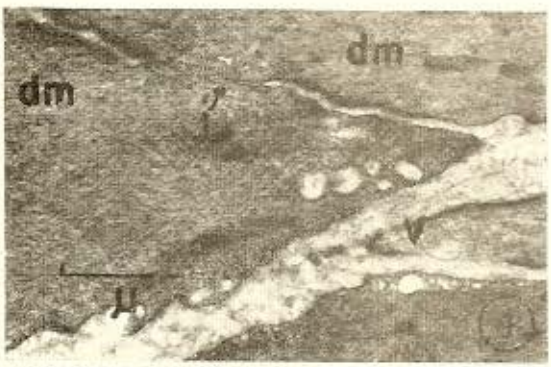
reach 1 micron of diameter. They are smooth-surfaced and contain a substance, either homogenous and amorphous, or finely granular and with low electron-density. Occasionally the vacuoles are transparent (Figs. 6, 7).

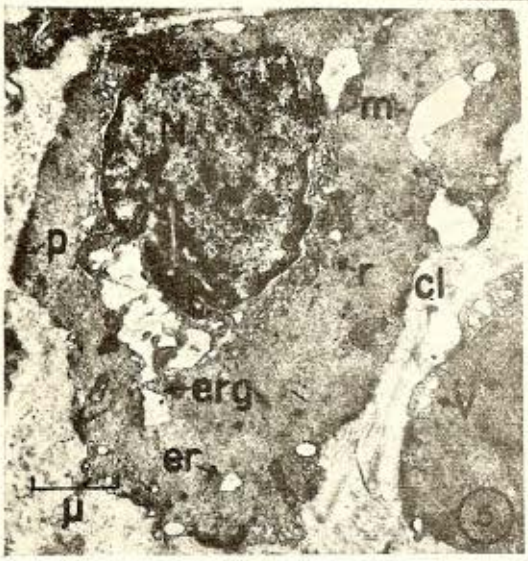
The rectum and sigmoid showed a much higher grade of cytoplasmic vacuoles formation than the transverse colon, with the exception of M 3 and M 10 where all these intestinal segments were equally affected.

Cytoplasmic vacuoles were seen in single cells or in groups of cells.

In those cells with conspicuous cytoplasmic alterations, the myofilaments suffered a process of disorganization and interruption at those zones harboring vacuoles and dilated endoplasmic reticulum.

In the rectum and sigmoid of some patients (M 5, M 6, M 7, M 8 and M 9), myofilament disorganization was particularly intense, with formation of cytoplasmic lacunar areas, with low electron density, where fragments of myofilaments and "dense bodies" could hardly be recognized (Fig. 7).





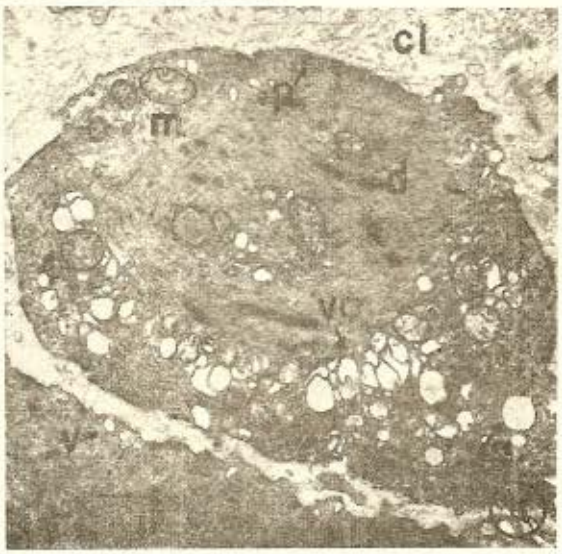


Fig. 4 — Close apposition and fusion of adjoining cell membranes (j), and direct protoplasmic continuity between two muscle cells in acquired megacolon (M 5 — sigmoid)

Fig. 5 — Intense dilatation and vacuolization of endoplasmic reticulum in acquired megacolon. Myofilament insertion at dense plaques of the cell membrane (arrow). (M 3 transverse colon)

Fig. 6 — Cytoplasmic vacuoles in acquired megacolon (M 8 sigmoid)

Disorganization of myofilaments was observed in the sigmoid and rectum of all the patients of this group, which was significant ($p = 0.00033$) in relation to the control (Table III).

As for the transverse colon, myofilament disorganization was observed in 7 patients (M 1, M 3, M 4, M 5, M 6, M 7 and M 10), which was significant ($p = 0.018$) in relation the control (Table IV).

2.2.3 *Golgi complex, mitochondria, ribosomes and "myelin figures"*

The Golgi complex was not constantly detected in this group; in some cases its ve-

TABLE III

Comparison between the "Control" and "Acquired Megacolon" groups concerning the number of cases with cytoplasmic vacuoles, dilatation of the endoplasmic reticulum and myofilament disorganization in the smooth muscle cells of the rectum and sigmoid colon.

Group	no. of cases with cyt. vac., dilatation of the e.r. and myof. disorg.	no. of cases without cyt. vac., myof. disorg. or dilatation of the e.r.	Total
C	0	5	5
A.M.	10	0	10
Total	10	5	15

Fisher's "exact" test = significant ($p = 0.00033$)
C = Control
A.M. = Acquired Megacolon
cyt.vac. = cytoplasmic vacuoles
e.r. = endoplasmic reticulum
Myof. disorg. = myofilament disorganization

TABLE IV

Comparison between the "Control" and "Acquired Megacolon" groups concerning the number of cases with cytoplasmic vacuoles, dilatation of the endoplasmic reticulum and myofilament disorganization in the smooth muscle cells of the transverse colon.

Group	no. of cases with cyt. vac., dilatation of the e.r. and myof. disorg.	no. of cases without cyt. vac., dilatation of e.r. or myof. disorg.	Total
C	0	5	5
A.M.	7	3	10
Total	7	8	15

Fisher's "exact" test = significant ($p = 0.018$)
C = Control
A.M. = Acquired Megacolon
cyt.vac. = cytoplasmic vacuoles
e.r. = endoplasmic reticulum
Myof. disorg. = myofilament disorganization

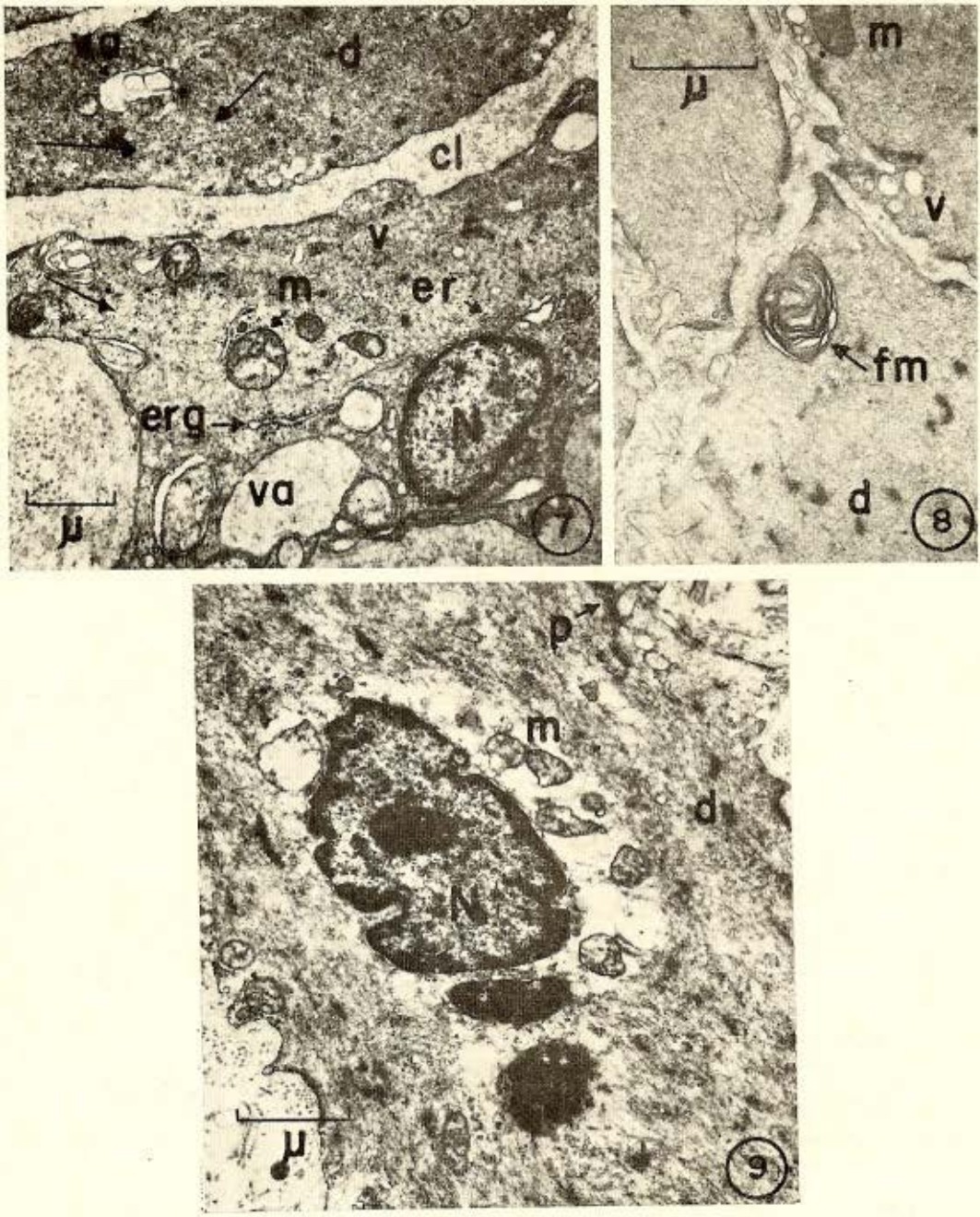


Fig. 7 — Cytoplasmic oedema and vacuoles; myofilament disorganization (arrows). (M 8 — sigmoid)

Fig. 8 — Myelin figure in a smooth muscle cell in acquired megacolon (M 9 — rectum)

Fig. 9 — Intense cytoplasmic destruction around the nucleus in congenital megacolon (H 1 — rectum)

p = dense plaque; v = pinocytotic vesicle;
d = dense body; r = ribosomes; m = mitochondria;
N = nucleus; n = nucleole; cl = collagem;
dm = desmosome; er = agranular endoplasmic reticulum;
e r g = granular endoplasmic reticulum;
v a = vacuole; m f = myelin figure.

sicles showed evident dilatation, making it difficult, even impossible, to distinguish them from dilated canaliculi of the smooth-surfaced endoplasmic reticulum.

The mitochondria and ribosomes did not differ from the control.

"Myelin figures" were observed, apparently, rather more frequently in acquired megacolon than in the control, but this impression could not be proved (Fig. 8).

2.3 *Nucleus*

The nucleus did not differ significantly from the control. There were only more indentations of the nuclear membrane, so that the nucleus frequently had a scalloped appearance.

3. "Congenital Megacolon" Group

In congenital megacolon the alterations in ultrastructure were always intense and affected the majority of cells, involving equally the narrowed rectum and the dilated and hypertrophied sigmoid.

3.1 *Plasma membrane*

As in the control and in acquired megacolon, the plasma membrane in congenital megacolon appears as a single osmiophilic line, with electron dense plaque alternating with clear areas harboring pynocitotic vesicles (Fig. 9).

As in acquired megacolon, we observed increase in the number of points of close contact between neighboring cells, sometimes with fusion of adjoining membranes.

Protoplasmic continuity between adjacent cells was not seen.

3.2 *Cytoplasm*

Here are the most conspicuous changes. They occur in large areas around the nucleus, with a wide destruction of cytoplasm and myofilaments, resulting in the formation of great, irregular, clear spaces (Fig. 9).

In these electron-transparent areas, we can see normal appearing mitochondria and Golgi complex, as well as fragments of myofilaments and dense bodies (Fig. 9).

Outside these areas of cytoplasmic destruction the cytoplasm looked exactly like in the control group (Fig. 9).

No cytoplasmic vacuoles or dilatation of endoplasmic reticulum were seen in congenital megacolon.

3.3 *Nucleus*

No particular difference regarding the nucleus could be observed in relation to the previous groups of patients.

DISCUSSION

There was a great increase of the number of points of close contact between smooth muscle cells in the rectum and sigmoid of patients with acquired megacolon in relation to the control group; furthermore, direct protoplasmic continuity was observed in acquired megacolon (M 1, M 5, M 9 and M 10), in contrast to the control, where no evidence of such communication could ever be detected.

The exact role played by this alteration of interrelationship between smooth muscle cells in acquired megacolon must still be determined. Our morphological data do not allow firm statements regarding this subject, but hypothesis to be investigated in future researches must here be considered.

As a matter of fact, it is a well established fact that in acquired megacolon there is an extensive lesion of the intra mural autonomous nervous system along the whole digestive tract.

The formation of a large number of points of contact between smooth muscle cells, could be a compensatory mechanism for the nervous lesion, trying perhaps to facilitate the intercellular transmission of the stimulus.

It is interesting that HARMAN et al. [10] observed an increase in the number of direct communications between neighbor cells in megaesophagus, in relation to normal human esophagus; this was not confirmed by the studies of CASSELLA et al. [6], who rarely saw protoplasmic continuity in normal human esophagus or in megaesophagus.

The apparent increase in the number and dimension of the pynocitotic vesicles in acquired megacolon, may possibly reflect a

greater metabolic activity of the smooth muscle cells in the rectum and sigmoid of patients affected by this disease.

The most conspicuous changes in acquired megacolon occur in the cytoplasm.

The cytoplasmic alteration affected the rectum and sigmoid of all the 10 patients of the A. M. group, and the lesions were much more intense in these segments than in the transverse colon.

The most evident ultrastructure changes consist of dilatation of the endoplasmic reticulum, cytoplasmic vacuoles and myofilament disorganization which were present in the rectum and sigmoid of all the patients of the A.M. group, a highly significant statistic event ($p = 0.00033$).

Such frequent and significant lesions in acquired megacolon, specially when associated with extensive areas of myofilament destruction and fragmentation, as in M 5, M 6, M 7, M 8 and M 9, may possibly cause important impairment of cellular contraction, but future researches must be accomplished to confirm this hypothesis.

Although Tosi et al. [20], working with normal human colon, described near the nucleus, many cavities with various grades of dilatation, vacuoles were never seen in the cytoplasm of our control group.

The "myelin figures" observed in both the Control and Acquired Megacolon groups, resemble the "membranous bodies" described by Neil et al. [15] in the smooth muscle cells of the guinea pig's deferens, and the "lamellar structures" seen by Brunner & Vallejo-Freire [5] in reticulocytes of guinea-pigs with lead poisoning. All these Authors emphasize that these structures are of unknown origin and function.

A very interesting and important fact is that all the alterations observed in acquired megacolon occurred in single cells or in groups of cells, so that there was always a great number of cells, sometimes the majority, that looked exactly like the control. If these changes in ultrastructure were a consequence of cellular hypertrophy, all the smooth muscle cells in acquired megacolon should exhibit them, for they are all enlarged in this disease, as already known from light microscopy.

Furthermore, Mark [13], Gansler [8], Dessouky [7], Bergman [2], Bo et al. [3], working with smooth muscle cells of pregnant and non pregnant uterus of different animals, including the human being, could never observe alterations similar to those seen by us in acquired megacolon. If we consider the tremendous hypertrophy of the smooth muscle cells in pregnant uterus, we must assume that the alterations observed in acquired megacolon are not really related to cellular hypertrophy.

Some of Tafuri's [19] observations in smooth muscle cells of the colon of mice with acute experimental Chagas Disease, resemble some of our results in acquired megacolon, for this Author related in his material, dilatation of the endoplasmic reticulum and myofilament disorganization in single cells or in groups of cells.

The presence of ultramicroscopic changes in the transverse colon, which was never hypertrophyed in our Acquired Megacolon group, shows that there is no relation between the alterations described and cellular hypertrophy.

In congenital megacolon there is hypertrophy of the muscular layer of the rectum and sigmoid, above a narrowed normal looking rectal segment. This narrowed rectal segment does not contain any myenteric plexus so that it is supposed act as a functional obstacle.

The study of the patients with congenital megacolon showed a striking difference in relation to acquired megacolon; the lesions observed in the first disease were always very intense and diffuse, affecting all the smooth muscle cells either in the hypertrophyed sigmoid and rectum or even in the narrowed rectal segment. The cytoplasmic lesions were much more conspicuous and constant in congenital megacolon, where large areas of cytoplasmic destruction around the nucleus were always seen.

RESUMO

Estudo ao microscópio eletrônico da célula muscular lisa no megacólon congênito e adquirido

Obtiveram-se biopsias da camada muscular do cólon de 5 pacientes sem doença cólica (grupo contrôle), 10 doentes com megacólon adquirido e 2 com megacólon congênito, para estudo com o microscópio eletrônico. Os espécimens foram submetidos à

dupla fixação em glutaraldeído a 2% e em tetróxido de ósmio a 1% em tampão fosfato; a desidratação foi feita em álcool etílico de concentrações crescentes; inclusão em Araldite. Usou-se para o exame das peças um microscópio eletrônico Elmiskop I.

Observaram-se evidentes alterações de ultra-estrutura das células musculares lisas do reto e sigmóide hipertrofiados no megacólon adquirido, em relação ao grupo contrôle: aumento do número de pontos de íntimo contato entre células vizinhas; vacuolização citoplasmática; dilatação do retículo endoplasmático e desorganização de miofilamentos. O cólon transverso, se bem que macroscòpicamente normal, foi sede das três últimas alterações, mas em menor grau de intensidade, e comprometendo menor número de células do que no reto e sigmóide.

Tôdas as alterações descritas no megacólon adquirido ocorreram em células isoladas ou em grupos de células de modo que sempre havia um grande número de células com aspecto idêntico ao contrôle.

A ultra-estrutura das células musculares lisas no megacólon congênito foi significantemente diferente dos grupos megacólon adquirido e contrôle; observaram-se grandes áreas de destruição citoplasmática em tôrno do núcleo na maioria das células do reto hipertrofiado assim como também no intestino macroscòpicamente normal.

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