

**Analysis of the effects of the interruption of the flow  
in the normal arterial endothelium morphology and the correlation with  
the ischemia duration in rabbits<sup>1</sup>**

Análise dos efeitos da interrupção do fluxo na morfologia  
do endotélio arterial normal e a correlação com  
o tempo de isquemia em coelhos

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**ABSTRACT**

The study of acute arterial insufficiency of the extremities is an area of continuing interest and investigation, in light of the possible effects stemming from the evolution of the disease and the necessity for urgent treatment. **Purpose:** To analyze the effects of the interruption of the flow in the normal arterial endothelium morphology and correlate them with the ischemia duration. **Methods:** We submitted 30 rabbits to the ligation of the right external iliac artery for 6 hours or 72 hours and observed specific morphological variables in the endothelial layer under optical and electronic microscopy. **Results:** In the optical microscopic study, no statistically significant results were observed in the comparison of the groups (control, 6- and 72-hour occlusions). With electronic microscopy, we observed alterations in the endothelial cell characterized by hyperpigmentation with detachment of the same from its bed; and rupture of the internal elastic membrane, with the exposure of the subendothelial material to the vascular lumen. **Conclusions:** The optical microscopy was not an effective method for the determination of endothelial morphological alterations; the electronic microscopy allowed us to observe initial signals of the endothelial cell and layer injury 72 hours after the interruption of the normal arterial flow.

**Key words:** Endothelium. Microscopy, electron. Ischemia. Rabbits.

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**RESUMO**

O estudo da insuficiência arterial aguda das extremidades é área de contínuo interesse e investigação, devido possibilidade de eventos catastróficos na evolução da doença e necessidade de tratamento cirúrgico de urgência. **Objetivo:** Analisar os efeitos da ausência de fluxo na morfologia do endotélio arterial normal segmentar, como os que ocorrem na porção imediatamente abaixo da área que sofreu oclusão arterial aguda por embolia, e correlacioná-los com o tempo de isquemia. **Métodos:** Submetemos 30 coelhos à ligadura da artéria ilíaca externa direita por 6 horas ou 72 horas e observamos variáveis morfológicas específicas da camada endotelial e subendotelial à microscopia óptica e eletrônica. **Resultados:** No estudo da microscopia óptica não foram observados resultados estatisticamente significativos quando comparados os grupos entre si. À microscopia eletrônica observamos alterações da célula endotelial caracterizadas por hiperpigmentação e descolamento da mesma de seu leito, e ruptura da membrana elástica interna com exposição do material subendotelial para luz vascular. **Conclusões:** A microscopia óptica não foi procedimento eficaz na determinação das alterações morfológicas endoteliais estudadas; a microscopia eletrônica mostrou sinais iniciais de sofrimento da célula endotelial e lesão da camada endotelial após 72 horas da ausência de fluxo na artéria normal.

**Descritores:** Endotélio. Microscopia eletrônica. Isquemia. Coelhos

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## Introduction

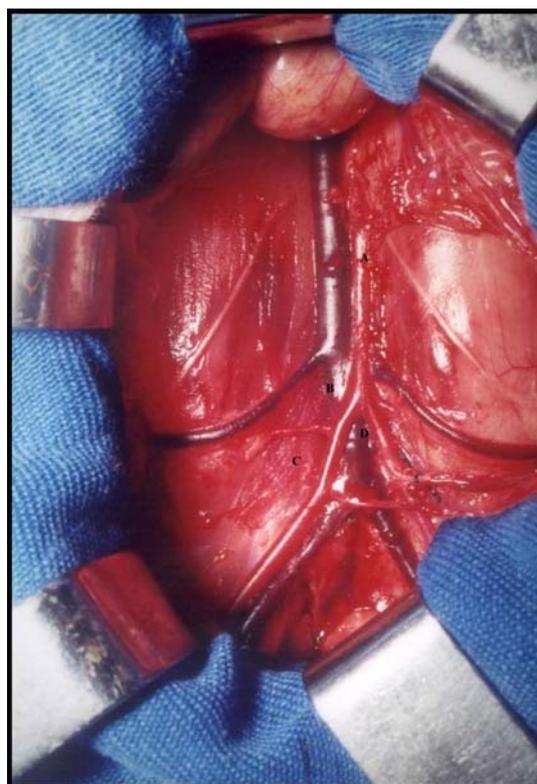
The acute interruption of the arterial flow due to the presence of thrombi, emboli or spasms, causes the sudden loss of tissue perfusion and the functional harming the affected organ.<sup>1</sup> After the event, a series of enzymatic metabolic processes are triggered, determining a variable clinical condition, possibly culminating in the loss of a member, when it is an ischemia in the extremities, or even in the loss of life.<sup>2</sup> Due to the deleterious effects of the occlusion, the reperfusion must be reestablished as soon as possible, once the reversibility of the process is directly proportional to the ischemia duration.<sup>3,4</sup> Upon analyzing specifically the segmentary arterial alterations caused by the absence of arterial flow we observed exuberant regional physiological and morphological events.<sup>5-8</sup> The literature is vast in its documentation of physiological alterations due to the interruption of the arterial flow. However, little is known about its effects on the normal arterial endothelium morphology, such as those which occur in the normal arterial port<sup>9-12</sup>

## Purpose

Analyze the effects of the interruption of the flow in the normal segmental arterial endothelium morphology to the area suffering acute arterial occlusion due to emboly as well as correlate them to ischemia duration.

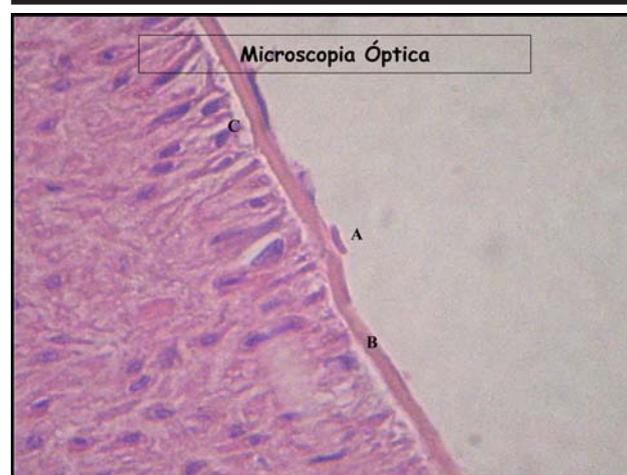
## Methods

Thirty young adult rabbits of the New Zealand lineage were used. After anaesthetizing the animal with 1% Xilazine 5mg./kg and Chetamine Hydrochlorate 8 mg./kg., a median infra-umbilical abdominal incision was made. With the opening of the abdominal cavity, the splanchnic viscera was shifted to the right) and the visceral peritoneum was moved to permit the visualization of the retroperitoneal structures. The dissection and isolation of the abdominal aorta, common iliac arteries, internal and external, was performed. With the objective of interrupting the flow in the right external iliac artery, the ligature was performed with a 4.0 cotton thread on the following vessels: common iliac artery, immediately before its bifurcation; right internal iliac artery, right after its origin; right external iliac artery, immediately before its trajectory through the inguinal ligament; and small collateral branches of the right external iliac artery (Figure 1). According to the group to which they belonged, the animals were submitted to a determined procedure: control group underwent the resection of the right external iliac artery; occlusion group, 6- and 72- hour closure of the abdominal cavity and after 6 or 72 hours, arterial resection Following preparation and fixation, the material was sent to optical and electronic microscopes analysis.

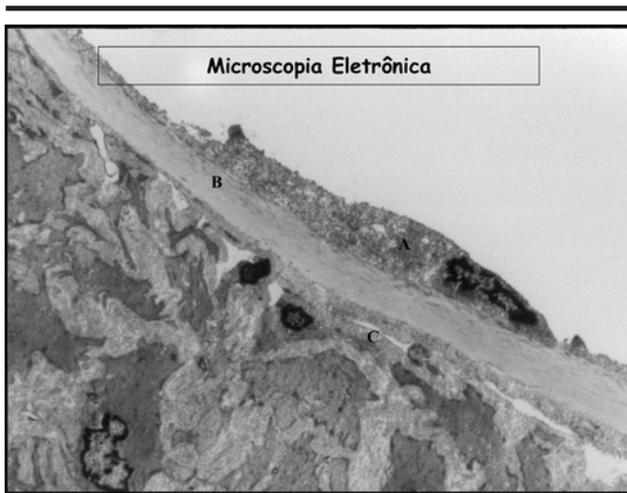


**FIGURE 1** - Retroperitonium anatomical aspect post-dissection. A - aorta, B - right common iliac artery, C - right external iliac artery, D - right internal iliac artery

The optical microscope was used to evaluate the presence of thrombus and discontinuity of the internal elastic membrane in the endothelial layer. The electronic microscope was used to research alterations in the nucleus, presence of adhered thrombus and the presence of intracytoplasmatic pinocytotic vacuoles in the endothelial cell; discontinuity, the presence of subendothelial material, alterations in the width and hyperpigmentation in the internal elastic membrane were also evaluated (Figures 2 and 3).



**FIGURE 2** - Arterial paries aspect normal to optical microscopy. A - endothelial cell, B - internal elastic membrane, C - subendothelial region



**FIGURE 3** - Arterial paries aspect normal to electronical microscopy A - endothelial cell, B - internal elastic membrane, C - subendothelial region

*Statistical analysis*

We used descriptive statistical analysis to describe the qualitative variables, calculating absolute and relative frequencies. We used comparative analysis to test the homogeneity among the groups, utilizing the exact Fisher test, allowing for a significance level of 5%.

**Results**

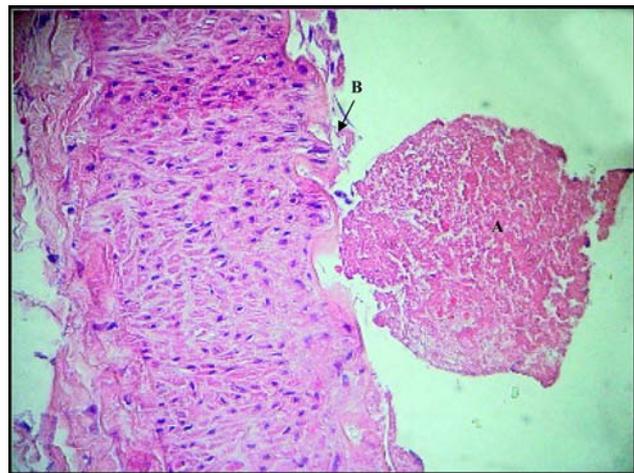
*Optical microscopy*

The statistical analysis of the variables presence of thrombus and discontinuity of the internal elastic membrane did not reveal a statistically significant difference among the groups (Figures 4 and 5).

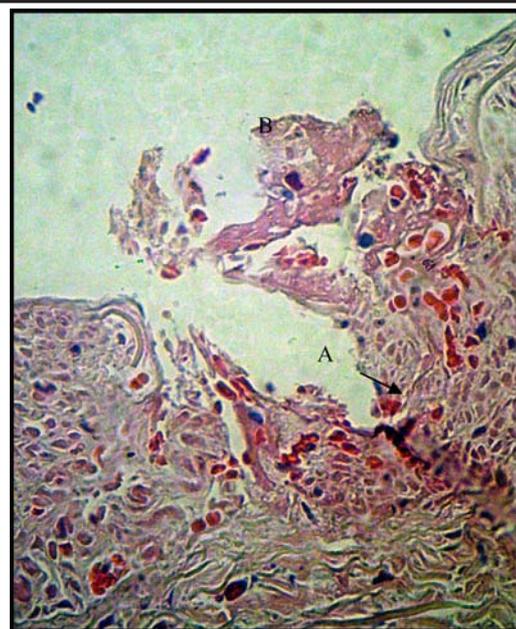
*Electronic microscopy*

The analyzed variables in the endothelial cell alterations in the nucleus, presence of adhered thrombus and presence of intracytoplasmatic pinocytotic vacuoles; and in the internal elastic membrane discontinuity, presence of subendothelial material, alteration in width and hyperpigmentation presented statistically significant differences when performed a comparative analysis among the groups. As for the characteristics of the nucleus of the endothelial cell, it was observed that in the control group all of the samples presented a normal aspect. In that of 72-hour occlusion, alterations were characterized by detachment or hyperpigmentation ( $p = 0.001$ ). As for the presence of thrombus, a greater incidence was observed in the 6- and 72-hour occlusion groups ( $p = 0.017$ ). Regarding the presence of intracytoplasmatic pinocytotic vacuoles, a greater incidence was observed in the control and 72-hour occlusion groups, with predominance in the later (66.7%)

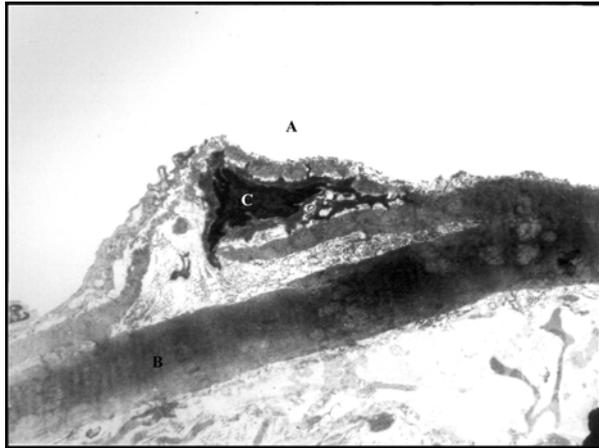
( $p = 0.010$ ). As for the discontinuity in the internal elastic membrane, integrity was observed in all of the control group samples, only 20 % in the 6-hour occlusion group and 0 in the 72-hour occlusion group ( $p < 0.001$ ). As for the presence of subendothelial material in the internal elastic membrane, it was observed that the 6 and 72-hour occlusion groups presented 70 and 100 % presence of subendothelial content in their layers, respectively; while the control group did not present anything similar ( $p < 0.001$ ). The analysis of the variables width and coloration revealed an alteration in the 6 and 72-hour occlusions, with no alteration in the control ( $p < 0.001$ ) (Figures 6 to 10).



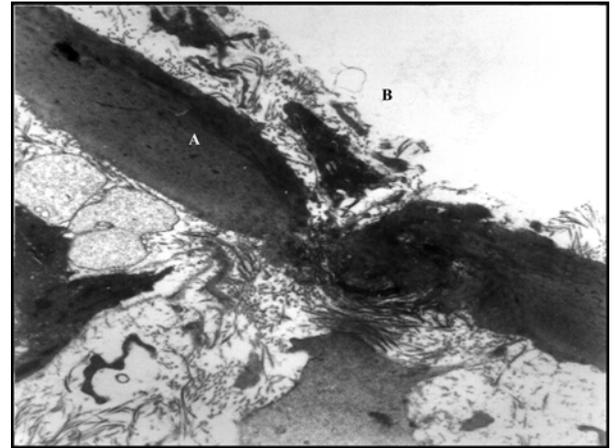
**FIGURE 4** - Arterial paries optical microscopy aspect with adhered thrombus to endothelial layer. Group Thromboses 72 hours. A - thrombus, B - endothelial layer



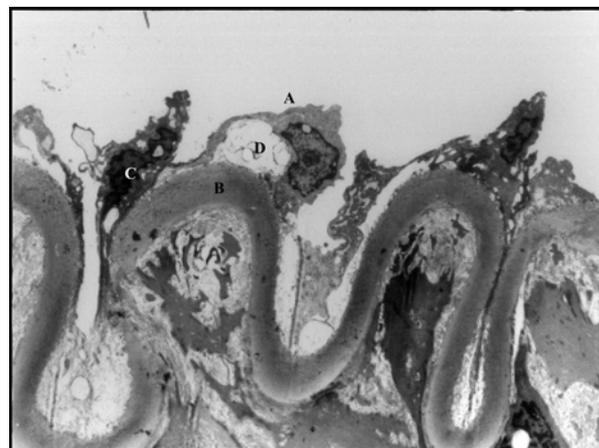
**FIGURE 5** - Arterial paries optical microscopy aspect with subendothelial material exposure to vascular light. A - ruptures internal elastic membrane, B - subendothelial region components exposed to vascular light



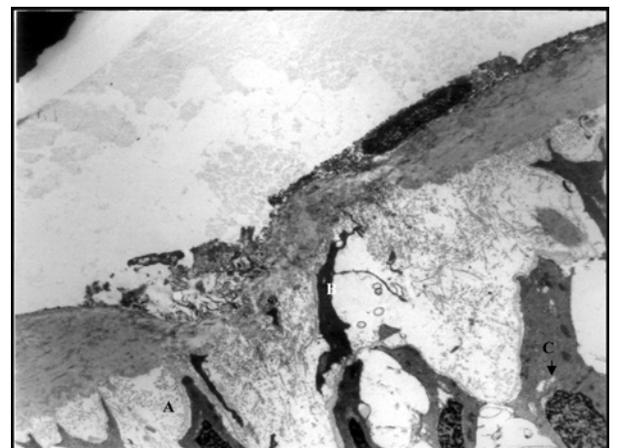
**FIGURE 6** - Endothelial layer aspect to electron microscopy. Group Thromboses 72 hours. A - altered endothelial cell, B - internal elastic membrane endothelial cell displacement, C - cell nucleus hyperpigmentation



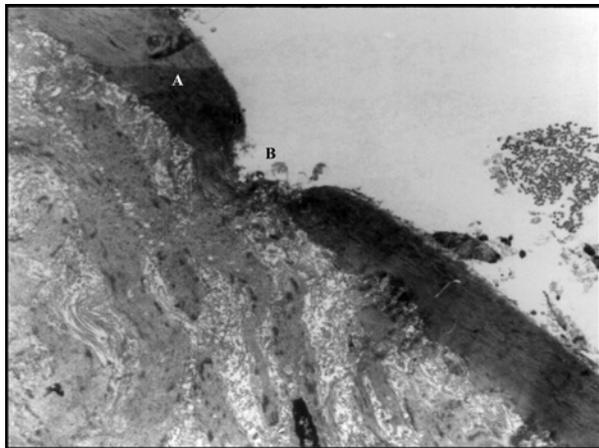
**FIGURE 9** - Endothelial layer aspect to electron microscopy. Group Thromboses 72 hours. A - hyperpigmented internal elastic membrane, B - Rupture with extravasation of subendothelial layer content to vascular light



**FIGURE 7** - Endothelial layer aspect to electron microscopy. Group Thromboses 72 hours. A - altered endothelial cell, B - internal elastic membrane endothelial cell displacement, C - cell nucleus hyperpigmentation, D - intracytoplasmic vacuoles



**FIGURE 10** - Subendothelial region aspect to electron microscopy. Group Thromboses 72 hours. A - intercellular discontinuity, B - hyperpigmentation, C - vacuoles



**FIGURE 8** - Endothelial layer aspect to electron microscopy. Group Thromboses 6 hours. A - internal elastic membrane, B - Rupture

### Discussion

The choice of the right external iliac artery was based on the fact that it is accessible and that it constitutes a muscular conduction artery, as those of the inferior members where the majority of the thromboembolic phenomena are located. The arterial ligation with a cotton thread is one of the simplest models of thrombosis. Its applicability is based on innumerable papers in the literature, in addition to permitting easy manipulation and representing low cost.<sup>13</sup>

In our research, we performed optical microscopic study, with the aim to analyze the real contribution that this method brings to the diagnosis of ischemic alterations, by comparing the results obtained with this method to those obtained with an electronic microscope. As for the use of the electronic microscope, we know that it constitutes a consecrated method and an immeasurable contribution to the evolution of knowledge in pathologies of the circulatory system. Analyzing

our results, we found that the optical microscopy was not an effective method for determining the alterations in the endothelial artery observed in this study, once there are no statistically significant results from the comparison of the groups. Therefore, the discussions which follow are related to the results obtained by using the electronic microscope. We observed that even after the right external iliac artery had undergone ligation, there was no formation of thrombus in the vascular lumen in any of the samples. This interruption can be explained by the reflex contraction of the arterial wall following ligation without the formation of thrombus or reabsorption of the same. Analyzing the endothelial cell, we observed that with the evolution of the ischemia there was an increase in the number of intracytoplasmic vesicles, appearance of subendothelial vacuoles and the presence of signs of cellular death, characterized by hyperpigmentation and detachment. The presence of the vacuoles and vesicles is shown by the increase in the endothelial cell permeability for ions and fluids, suggesting the intensification of exchanges; and the beginning of apoptosis is due to the duration of local anoxia, this being an intensifying factor in the local thrombogenesis. Analyzing the results related to the membrane, we observed that all of the samples from the control group had intact membranes, and all of the samples from the 72-hour occlusion group showed a loss in the integrity of the membrane, suggesting that the ischemia 72-hour duration factor is related to the rupture of the internal elastic membrane. This result is identical when the variable presence of subendothelial material in the internal elastic membrane is analyzed. Alterations in the internal elastic membrane coloration and width were present in some of the 6- and 72-hour occlusion samples, suggesting initial tissue duress.

## Conclusions

The optical microscopy was not an effective method in determining the studied endothelial morphological alterations, while the electronic microscopy allowed us to observe the initial signals of endothelial cell duress as well as the injury in the endothelial layer 72 hours after the interruption of the normal arterial flow.

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