



# PATHOLOGICAL PROCESSES IN LIVER AND INTUSSUSCEPTIVE GROWTH OF CAPILLARY ASSOCIATED WITH IT

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The growth of new blood vessels contributes to numerous malignant, ischemic, inflammatory, infectious and immune disorders. There are two forms of vascular remodeling associated with physiological and pathological processes; angiogenesis and arteriogenesis. It is shown in this study that, practically in all investigated models, the process of intussusceptive angiogenesis, etc. is the first step. Chemical agents lead to the development of intussusceptive angiogenesis in liver that could be the main reason for hemodynamic disturbance.

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## INTRODUCTION

Blood vessels constitute the first organ in the embryo and form the largest network in our body. When deregulated, the formation of new blood vessels contributes to numerous malignant, ischemic, inflammatory, infectious and immune disorders. Angiogenic stimulation promotes intense structural and functional changes in liver architecture and physiology.

Angiogenesis and arteriogenesis are two forms of vascular remodeling associated with physiological and pathological processes. Angiogenesis is a process of growth of new capillaries from the existing capillaries through capillary sprouting or intussusceptions.<sup>16,33</sup> Non-sprouting angiogenesis by means of intussusception – "growth within itself", is an important mode of capillary formation and is termed intussusceptive microvascular growth. Intussusceptive angiogenesis plays a role in tumor vascularisation.<sup>4,13,14,11</sup> The presence of numerous tiny holes in vascular corrosion casts are shown, upon serial sectioning of tissue and subsequent transmission electron microscopy, to correspond to slender transcapillary tissue pillars or posts.<sup>9</sup>

Among features of the vasculature of the liver, not found in other tissues, are the hepatic sinusoids, characteristics of which include the presence of hepatic sinusoidal endothelial cells that possess distinctive fenestrations and pericytes or hepatic stellate cells.<sup>52,34</sup> Additionally to the induction of proliferation of EC, effective angiogenesis also requires stabilization of nascent blood vessels, and formation of interendothelial cell junctions and lumens.<sup>30</sup>

Pathological angiogenesis is different from that associated with physiological processes.<sup>28</sup> While physiological hepatic angiogenesis during liver regeneration leads to the formation of new functional sinusoids, pathological angiogenesis,

present in many chronic liver diseases, is characterized by the appearance of capillarized vascular structures.<sup>12,26</sup> Angiogenesis is a recurring factor in the disease progression.<sup>8</sup>

Vascular imaging makes possible to quantify the number and spacing of blood vessels, measure blood flow and vascular permeability, as well as analyze cellular and molecular abnormalities in blood vessel walls. Microscopic methods ranging from fluorescence, confocal and multiphoton microscopy to electron microscopic imaging are particularly useful in elucidating structural and functional abnormalities of angiogenic blood vessels.<sup>25</sup>

## Goal

The aim of this study is to compare the mechanism of angiogenesis at different types of pathological influence on the liver.

## MATERIALS AND METHODS

### Reagents

Powdered paraformaldehyde; OsO<sub>4</sub>; sodium cacodylate; 90% ethyl alcohol, acetone, epon 812, epon hardener MNA, epon hardener DDSA, epon accelerator DNP-30, uranyl acetate, sodium citrate, and lead nitrate, were of analytical grade from Sigma.

### Animals

All procedures involving animals were approved by the Institutional Review Board/ Institutional Animal Care and Use Committee (H. Buniatian Institute of Biochemistry, Yerevan, NAS RA) and conformed to the European Communities Council directives (86/609/EC).

For experiments (Crush Syndrome, Intoxication by CCL<sub>4</sub>) the liver of two-month-old male rats weighing 150-200 g and for additional study the liver of frog from Yerevan water area was used.

### Treatment of material

The bioplates were put at 4°C in a mixture of paraformaldehyde in cacodilate buffer and glutaraldehyde for 12 hours, followed by post fixation in 1% OsO<sub>4</sub> solution for 2 hours; dehydration in ascending series of spirits; saturation in a mixture of acetone and epon resins of different proportions and pouring in gelatinous capsules into epon.

### Obtaining of ultrathin slices and its treatment:

The ultrathin slices (up to 500 Å) were made using ultracut LKB (Swedish) and Reichert (Austria). Ultrathin slices were double contrasted with uranyl acetate and lead citrate.

### Observation under transmission electron microscopy (TEM) and optical microscope

The ultrathin slices were observed under the transmission electron microscope (Phillips CM 10) with resolution X 10-20.000. Semithin slices 0,5-1 μm were stained for further light optical investigation.

## RESULTS

The results of our study, from two types of angiogenesis intussusceptive, have shown that angiogenesis was dominant. Practically in all investigated groups the process of intussusceptive angiogenesis was observed.

However there were still some differences of the development of this process depend on the type of influence.

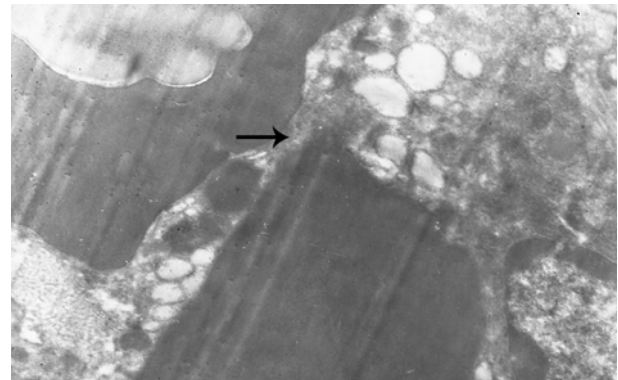
So, in the liver of frog lived in Yerevan water area polluted with different elements the process of angiogenesis was presented. It was shown that as a response on ecological influence etc water pollution alterations among the organelles of hepatocytes were observed this could be a physiological reaction of the tissue to the pathological influence.<sup>19</sup>

The sinusoid was divided by the way of transluminal bridge formation, and it's important to say that such new capillaries are quite different in size and asymmetric. Practically very close to the transluminal bridge Kupfer cells were observed. This could be explained as a protective response of liver on the influence of pollutant of water (Fig. 1).

Several cells appear to play a role in the process of intussusception, such as the endothelial cells, pericytes, macrophages and blood cells.<sup>5</sup> The part of the cell body of the endothelial cells that formed the pillar frequently contained a high density of microfilaments, excluding all other cellular organelles.<sup>27</sup>

Endothelial cells are quite large and the Disse space is observed. We opine that endothelial cells take part in the formation of transluminal bridge cover from two opposite sides. In the zone of close contact of endotheliocytes of two

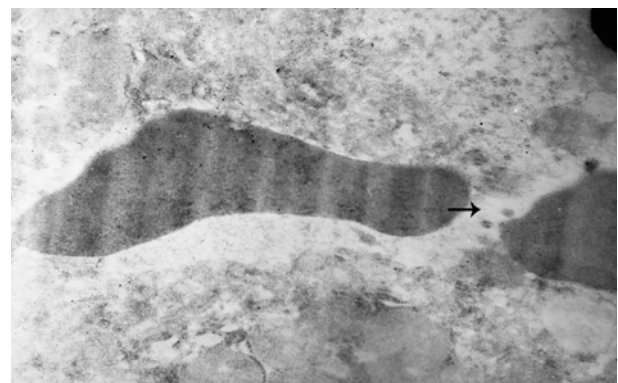
opposite walls of lumenar cover of sinusoid the subplazmalemmas structure composed of thin filaments, was observed. Thin microfilaments organized in structure in the place of close contact of transluminal bridge made by endotheliocyte were observed in liver and in heart.<sup>19</sup>



**Figure 1.** Formed transluminal bridge in frog liver. X 20.000

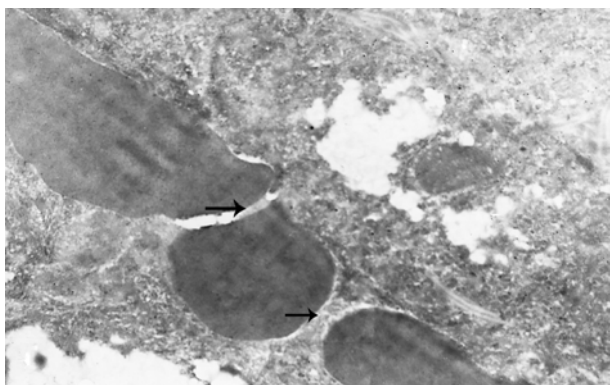
Organ's toxic injuries are widespread cause of the diseases and death in populations.<sup>2,15,31,29,22,3</sup> We, therefore, studied two different types of influence on the liver particularly on the angiogenesis process that takes place in it. As a first model we studied the experimental model of Crush Syndrome etc 2h compression.

It is well known that at the compression period the catecholamine level increases which leads to cramp of arterioles and precapillaries lead to the retardation in the speed of blood flow, endovascular aggregation of erythrocytes and development of thrombus formation. Progression of microcirculation dysfunction lead to development of acute hypoxia that results in ischemia of different organs and tissues.<sup>21,23,17</sup> At the experimentally induced Crush syndrome (2h of compression) the process of the transluminal bridge formation - the key of intussusceptive angiogenesis - takes place following the aggregation of erythrocytes in sinusoids, which become more critical at 4 hr. of decompression period (Fig. 2). The angiogenesis process takes place by the same mechanism mentioned above.

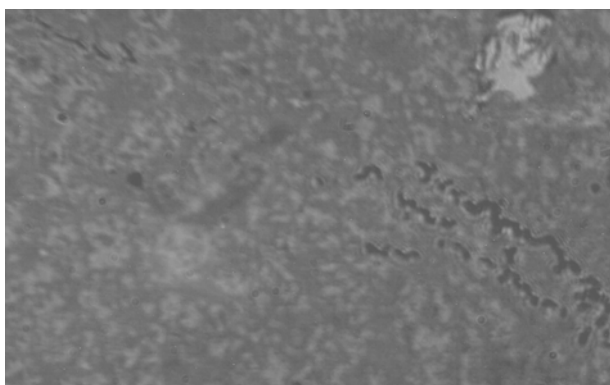


**Figure 2.** Formed transluminal bridge at Crush Syndrome. X 10.000

The experimentally induced intoxication by  $\text{CCl}_4$  was chosen as the second model. It is well known that  $\text{CCl}_4$  is an industrial toxin which leads to different pathological alteration in organism and in liver particularly.<sup>18</sup> In this case we aimed to study if there are any changes observed in sinusoids structure. The results of our study have shown that the influence of  $\text{CCl}_4$  is a leading factor in the development of angiogenesis process. The process of transluminal bridge formation occurs. At the same time numerous bridges could be formed, leading to the formation of further capillaries with a different lumen diameter (Fig. 3 and 4).



**Figure 3.** Formed transluminal bridge at  $\text{CCl}_4$  toxicity. X 10.000



**Figure 4.** New vessel formation by the way of intussusception at  $\text{CCl}_4$  toxicity. Semithin slices stained by Azur II, X 1.250

The results of current study have shown that the process of intussusceptive angiogenesis is observed in all investigated groups. This could be a response to acute influence as it does not need much energy to process it. Therefore it must be more economical and preferable for tissues.

## DISCUSSION

Liver has different important functions in the organism and one of the most important is its detoxication function. Therefore it's also a target organ for many toxic agents which lead to its injury.

The influence of different pathological agents on the organism, especially those on the liver lead to microcirculation dysfunction. Such dysfunctional blood vessels are unable to provide expanding tissues and organs

with oxygen and nutrients, as well as remove the metabolic waste. This could be a reason for further progression of microcirculation dysfunction that leads to the development of acute hypoxia and as result ischemia of different organs and tissues.<sup>21,23,17</sup>

Ischemic damage of different organs and tissues is still one of the most acute problems in medicine and biology. In ischemia the organism reacts by activation of angiogenesis process to provide normal metabolism processes to tissues. However, if in some case this process is normal, known as physiological angiogenesis, the influence of pathological agents is quite different. It must be mentioned that the role of angiogenesis etc., its different types are very important in the progression and development of different diseases such as diabetes, cardiovascular ischemic complications, cancer etc.<sup>24,5,7</sup>

During angiogenesis, endothelial cells from intact blood vessels quickly infiltrate avascular regions via vascular sprouting. This process is fundamental to many normal and pathological processes such as wound healing and tumor growth, but its initiation and control are poorly understood. Sprouting angiogenesis is believed to be a major type of vasculature development in both liver regeneration and cancer development.<sup>1</sup>

Another type of angiogenesis, different from sprouting, is intussusceptive angiogenesis. In contrast to sprouting angiogenesis, which is a well established mode of new blood vessel formation, intussusceptive angiogenesis is a relatively new concept in vascular biology. Intussusceptive angiogenesis is wide spread process and could be recognized as a ubiquitous phenomenon in vertebrates.<sup>4</sup> This process was first observed in postnatal remodeling of capillaries in the lung.<sup>6</sup> In this developmental process, a new concept of vessel formation was found where preexisting vessels split in two new vessels by the formation of transvascular tissue pillar into the lumen of the vessel. Intussusception is believed to take place after vasculogenesis or angiogenesis to expand the capillary plexus. Intussusceptive microvascular growth is a fast process that can take place within hours or even minutes with a little amount of energy, because it doesn't need proliferation of endothelial cells.

Our data indicate the fact that intussusceptive angiogenesis may play an important role in the malignant angiogenesis and chronically liver diseases.

The process of intussusceptive angiogenesis is found to occur practically in all investigated models (groups). However, some differences were observed in some studied processes. It must be noted that the transluminal bridge formation points to the presence of intussusceptive form of angiogenesis. It is well known that  $\text{CCl}_4$  is a strong industrial toxin and has its negative toxic influence on liver as well as ischemia. Toxins released during Crush Syndrome also have a damaging influence on liver.

Transmission electron microscopy revealed four consecutive steps.<sup>5</sup> Pillar formation and remodeling is not only observed in capillary plexuses but also within smaller arteries and veins.<sup>10</sup> It was mentioned in literature that alteration in blood flow dynamics in arterial branches could

stimulate the process of angiogenesis etc that could promote pillar formation.<sup>1</sup> Several cells appear to play a role in the process of intussusception, such as the endothelial cells, pericytes, macrophages and blood cells.<sup>5</sup>

The part of the cell body of the endothelial cells that formed the pillar frequently contained a high density of microfilaments, excluding all other cellular organelles.<sup>27</sup>

## CONCLUSION

The influence of different pathological processes lead to intussusceptive angiogenesis development in liver, that could be the main reason for hemodynamic disturbance.

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