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# Biomechanical Aspects of the Auto-digestion Theory

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**Abstract:** Increasing evidence suggests that most cardiovascular diseases, tumors and other ailments are associated with an *inflammatory cascade*. The inflammation is accompanied by activation of cells in the circulation and fundamental changes in the mechanics of the microcirculation, expression of pro-inflammatory genes and down-regulation of anti-inflammatory genes, attachment of leukocytes to the endothelium, elevated permeability of the endothelium, and many other events. The evidence has opened great opportunities for medicine to develop new anti-inflammatory interventions. But it also raises a fundamental question: What is the *origin of inflammation*? I will discuss a basic series of studies that was designed to explore trigger mechanisms for inflammation in shock and multi-organ failure, an important clinical problem associated with high mortality. We traced the source of the inflammatory mediators to the powerful digestive enzymes in the intestine. Synthesized in the pancreas as part of normal digestion, they have the ability to degrade almost all biological tissues and molecules. In the lumen of the intestine, digestive enzymes are fully activated and self-digestion of the intestine is prevented by compartmentalization in the lumen of the intestine facilitated by the mucosal epithelial barrier. Under conditions of intestinal ischemia, however, the mucosal barrier becomes permeable to pancreatic enzymes allowing their entry into the wall of the intestine. The process leads to auto-digestion of the intestinal wall and production of inflammatory mediators. The hypothesis that multi-organ failure in shock may be due an auto-digestion process by pancreatic enzymes is ready to be tested in a variety of shock conditions.

**Keyword:** shock, intestinal ischemia, digestive enzymes, transport, epithelial barrier

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## 1 Introduction

One of the great opportunities for modern biomechanics is its application to the *analysis* of *human disease*. Biomechanical analysis of a living tissue that is subject to a disease process can fill an important gap between traditional epidemiological analysis and molecular and genetic analysis. This opportunity for bioengineering will be the focus of the following discussion.

I will outline some of the unique contributions that a biomechanical analysis can make while focusing on one of the most difficult medical conditions: *Physiological Shock and Multi-organ Failure*. Multi-organ failure is associated with high mortality and therefore is of extraordinary clinical importance, almost second to none.

The following discussion will be focused on the initial step in any biomechanical analysis, i.e. identification of the key players and main transport events that may be involved in this medical problem. Identification of these issues is merely the first step, before a more advanced quantitative analysis can be carried out in the future. But the first step is the most important, if one makes a mistake in the important first assumptions of the analysis, the validity of any further step in the analysis is in question.

## 2 The Inflammatory Process

While for centuries recognized as an important sequence of events in living tissues, the inflammatory cascade has in the past decade moved center stage in medical research and practice. Following decades of *experimental* studies in which in-

dividual steps in the inflammatory cascade have been identified and characterized in ever increasing detail, in the past decade *clinical* studies have come forward that show that human disease is accompanied by an inflammatory cascade. It is possible to demonstrate telltale markers for inflammation in diverse diseases (diabetes, chronic diseases, hypertension, cancer, aging and many others) even though there is no conclusive documentation for an association or causal effect of classical infections by bacteria, viruses or fungi. Today the question may be asked, which disease does *not* have markers for inflammation? Even patients with medical risk factors (e.g. smokers), but not yet full manifestations for cardiovascular diseases, have markers for inflammation. The recognition that human disease may be accompanied by inflammation has opened an unprecedented opportunity for medical research to create new interventions and test existing interventions against inflammation in diverse and apparently unrelated diseases.

One has to keep in mind that over a lifetime the inflammatory cascade serves as a *tissue repair mechanism* after injury. Most tissues can mount an inflammatory response. The inflammatory process constitutes a cascade of events whose outcome is the *repair* of injured tissue and generation of new tissue, such as during healing of a scratched or burned skin and its replacement with a scar. The inflammatory cascade includes steps that involve initial removal of injured tissue and de-novo synthesis of extracellular matrix with new tissue cells by local mitosis and also derived from stem cells of different origins (e.g. bone marrow precursor cells).

The individual steps in the inflammatory cascade serve to eliminate injured tissue (and therefore the inflammatory cascade has steps that by themselves cause tissue injury) and provide replacement by new connective tissue matrix and functional cells. The tissue that is regenerated after an inflammatory reaction may or may only in part (e.g. in the case of a scar tissue) have the mechanical properties and the activities of the tissue that it replaces.

## 3 Biomechanics of Inflammation

During inflammation major changes occur and an entire gene expression profile is brought into action, damaged cells are removed by apoptosis and necrosis and new cells are generated. The changes can be observed in the tissue parenchyma, the extracellular matrix, and in the microcirculation (1). A profile of repair genes (cytokines and lymphokines, membrane adhesion molecules, growth factors, intracellular signaling genes, and many other families of proteins) are expressed and their proteins synthesized to facilitate a spectrum of cellular reactions that are part of the repair mechanism. The genomics of the inflammatory process is today an extraordinary active field of investigation (2-10) including the development of mathematical models (11).

From a biomechanics point of view, key steps in the inflammatory cascade include an elevation of the permeability in the endothelium, attachment of platelets and leukocytes to themselves and to the endothelium, migration of different cell types into the tissue across the endothelial barrier, obstruction of microvessels, blood clotting, actual apoptotic loss of blood vessels and parenchymal cells, and eventual cell mitosis and infiltration of stem cells into newly forming tissue. It is a rich field of opportunities for biomechanics and I will highlight here only a few selected aspects.

One of the ways to study the transition from normal tissue homeostasis into an inflammatory state is by study of individual cell types. Among the circulating cells, one of the few cell types readily available for analysis from patients with inflammation, the red cells exhibit a change in membrane deformability, a shift in membrane resting shape (e.g. membrane crenation), red cell aggregation into rouleauxs, in some diseases (e.g. sickle cell, diabetes, thalassemia) adhesion to the endothelium (12-15), and eventually even hemolysis.

Sensitive and early markers for inflammation can be readily detected in leukocytes or platelets, cells that respond within seconds to inflammatory mediators. Upon stimulation, leukocytes undergo a transition from a passive spherical state into an

activated state. They change their typical passive spherical shape in the circulation into a shape with clearly visible pseudopod extensions due to local actin polymerization (16, 17). Many different biochemical receptor agonists control the process (e.g. peptides, lipid mediators) but also fluid mechanical stresses (18-20). Upon stimulation, leukocytes also release cytoplasmic granules, a process that causes on one hand release of proteolytic granules and cleavage of selectin adhesion molecules while on the other hand it causes integrin adhesion molecule release from the membrane of these granules into the plasmalemmal membrane. The end result of this action is that the leukocytes transition into an adhesive cell type that is ready to adhere to postcapillary venules. Details of these reactions depend on specific leukocyte types (21). In the microcirculation, adhesive leukocytes adhere to the endothelium of postcapillary venules on which they roll and adhere in a sequential fashion depending on the specific membrane adhesion molecules that are present (22). Interstitial cells, such as mast cells, may also degranulate early during inflammation, a process that in turn triggers a cascade of secondary events since their granules contain a number of mediators (23).

Another event, readily detectable in inflammation, is a defect in the normal circulation of blood cells through the microcirculation. Reduced perfusion at normal central blood pressure is in part the result of the well-recognized vascular smooth muscle contraction in arterioles, in part pseudopod formation by endothelial cells, especially in capillaries (24, 25), and in part due to blood rheological effects controlled by cell activation (26-30) with leukocyte adhesion to the venous endothelium (31). When leukocytes form pseudopods and produce elevated hemodynamic resistance in the capillary network (32, 33), they interfere with the free motion of red cells in narrow capillaries raising the hemodynamic resistance (34). In the limit, activated leukocytes will obstruct capillaries and cause capillary endothelial and interstitial apoptosis and necrosis (32, 35-37). Leukocyte and platelet aggregates interfere with normal microvascular perfusion. Enzymatic shedding of the endothelial glycocalyx invivo plays an important role in adhesion of blood cells to the endothelium (38).

## 4 Chronic Markers for Inflammation as a Sign of Incomplete Tissue Repair

How is it possible then that markers for inflammation accompany so many diverse diseases? Why doesn't the inflammatory cascade in these diseases come to a resolution with healing of the tissue? There may be a variety of reasons, including the loss of an ability to synthesize all the molecular species (cytokines, adhesion molecules, growth factors, glycocalyx, etc.) and cells (stem cells, mitotic cells) that are required for the inflammatory repair. There could be defects in gene expression and protein synthesis.

I will discuss here evidence for another mechanism. The basic idea is that the lack of a resolution of inflammation may be an indication that the *original stimulus* responsible for tissue injury in the first place may continue to be present and may require identification for development of an effective intervention. What could be such an injury mechanisms? Besides the well-known mechanisms for inflammation, such as infections, heat or cold exposure, allergic reactions or mechanical trauma, there exists another mechanism that we recently described and refer to as *auto-digestion* (39, 40).

## 5 Shock and Multi-organ Failure

Shock is an acute, life-threatening condition in which rapid progression of organ failures occur, a condition that is referred to as multi-organ failure. In relative short time intervals (days and hours), normal functioning organs become under perfused, individual cell types exhibit loss of normal function, and many physiological organ functions begin to fail. Shock is also a condition with extra-ordinary high levels of inflammatory markers. It can be produced in young and old and by a variety of situations, such as a burn, tissue trauma, an allergic reaction with antigen – antibody formation, toxins, and many other tissue insults.

I will address in the following *hemorrhagic shock*,

a condition in which no extraneous biochemical mediators are involved. The only requirement is withdrawal of the blood volume (typically of the order of 2 to 3 ml/kg blood volume) to reduce the femoral blood pressure from a normal level of 100 mmHg to about 40 mmHg for a period of 1 to 2 hours. This is then followed by return of all blood volume during which time period the blood pressure is observed. In a typical experiment the blood pressure returns initially to near control values in order to fall thereafter to lower and lower values. This model of shock (known as Wigger's hemorrhagic shock, (41)) leads to multi-organ failure and death within hours. Even before return of the blood volume there are increasing levels of markers for inflammation in plasma.

These markers for inflammation can be effectively detected by means of a cell mechanical approach. For example, naïve donor leukocytes are exposed to fresh plasma of animals in shock, and within minutes the characteristic transformation from passive spherical cells to activated cells with pseudopods and enhanced membrane adhesion are generated. An interesting consequence of the cell activation is that leukocytes start to disappear from the free circulation and become trapped in the microcirculation (e.g. in organs like the lung, liver and many others (42)). The central blood becomes leukopenic and as a consequence a form of immune suppression sets in that is due to the lack of leukocyte transport and immune surveillance, i.e. deficient transport in the circulation of leukocytes to lymph nodes and lymphoid organs, inflammatory cells to sites of infections, lack of bone marrow stem cell transport. Control animals with low levels of inflammation exhibit no such immune deficiency.

This evidence then shows that circulating cells become activated when exposed to the plasma derived from an individual in hemorrhagic shock. No significant transformation can be detected when control plasma is used (43). Plasma contains a mediator that is responsible for the activation. There have been numerous proposals what these mediators may be (e.g. fragments from the clotting cascade, complement fragments, lipid products, bacterial derived products, cytokines), but all proposals are missing conclusive proof (e.g. biochemical identification, elimination of the activity upon removal and restoration of the activity upon return of the mediator to a plasma). We have to identify the biochemical nature of the mediator and locate its source in the circulation.

Both biomechanics - in terms of transport processes - and biochemistry – in terms of the identification of the particular molecular species - have to make a contribution to understand this important medical problem.

## 6 The Endotoxin/Bacteria Translocation Hypothesis

There is long-standing and compelling evidence that the intestine may play a central role in shock. The idea has its origin in ancient times and has lead to extraordinary experiments (44) in which the entire intestine was removed during periods of shock. But there is no universal agreement why the intestine plays this role.

The lumen of the intestine is home to a natural bacterial culture (Gram-positive colonies) in considerable amounts. The barrier that protects against this bacterial culture is provided by the mucosal epithelium that lines the lumen of the intestine. This epithelium is generated by stem cells at the base between individual villi in the cryst (45-47). The transport barrier is generated by tight junction attachment between the epithelial cells by E-cadherin adhesion molecules (48). There is also a group of goblet cells positioned in regular intervals between the epithelial cells. Goblet cells secrete a mucus composed in large part of a highly hydrated mucin molecule (49, 50) forming a continuous thin viscoelastic layer on top of the epithelial cells. This mucous layer facilitates peristaltic transport of food along the intestine. It may also form a transport barrier to larger molecules and colloid material but the transport properties of different molecular species through mucin remain to be studied in greater detail.

There is evidence to support the idea that the permeability properties of the intestinal barrier are compromised in hemorrhagic shock (51, 52). The tight junctions between epithelial cells, keep-

ing large molecular weight material from passing across the epithelial layer, start to separate (53), a mechanisms that may involve depletion of ATP in epithelial cells due to reduction of oxygen transport to the intestine during the hypotensive period. This observation gave rise to the idea that bacteria from the lumen of the intestine may be transported ("translocate") (54-56) from the lumen into the wall of the intestine where they are taken up by microvessels or by a system of lymphatics located inside the villi and also in deeper cell layers of the intestinal wall. Since bacteria may shed their membrane coat in form of endotoxins (large aggregates of membrane carbohydrates, with a lipid core and cytoplasmic proteins, (57)), the proposal was advanced that after compromise of the intestinal barrier not only whole bacteria but also endotoxin enters into the wall of the intestine and from there into the circulation and into the lymphatics.

The hypothesis has been tested in a variety of ways in experimental models of shock and in patients, but no consensus has been developed to either confirm or completely deny its validity (58-61). Interventions with antibiotics or antibodies against different epitopes of the bacteria have yielded mixed results in terms of clinical outcome in shock patients (62-64). Further analysis of the situation in individual patients may shed new light on this hypothesis.

## 7 A New Hypothesis: Auto-Digestion

In the meantime we explored the possibility that another mechanisms may be at work in shock and multi-organ failure (40). The entry point for this analysis was a set of basic experiments designed to identify potential sources of inflammatory mediators. If inflammatory mediators can be detected within less than one hour after initiation of hemorrhagic shock, then there must be a preexisting source(s) of these mediators. It is less likely that they are newly synthesized by gene expression and gene product synthesis in such a short period. The idea is to identify particular organs (lung, liver, intestine, muscle, etc.) or even specific cell types that may serve as the source of inflammatory mediators.

Thus testing the ability of different tissue homogenates to generate pseudopod projection on fresh naïve leukocytes showed that *pancreatic digestive enzymes* were involved (65, 66). These are the same enzymes involved in degradation of food items into a form that can be transported by specific membrane transporters across intestinal epithelium (e.g. amino acids, nucleotides, monosaccharides, fatty acids). Digestive enzymes (proteases, lipases, amylases, nucleases) are synthesized in the pancreas by exocrine cells in a proform, and then released via an endocytotic process and the pancreatic ducts into the upper most part of the small intestine, the duodenum. Once in the duodenum and upper ileum, the digestive enzymes are converted from their inactive proform into their active form. These are powerful enzymes, present in higher concentrations than in other organs, and capable to digest most food items (proteins, lipids, carbohydrates, nucleotides). On the epithelial mucosal membranes there are also additional enzymes (e.g. carbohydrolases like maltase, lactase and sucrase) to process sugars. It is the very digestive process we rely on over a lifetime.

Experiments with different tissue homogenates indicate that most organs have the ability to generate low levels of inflammatory mediators, while in contrast the pancreas with its digestive enzyme or the intestine in the presence, but not absence, of active digestive enzymes generate high levels of inflammatory mediators (66). Even individual purified digestive enzymes in the intestine can generate inflammatory mediators (67).

## 8 The Epithelial Barrier in the Intestine

Anybody who has eaten a tasty traditional sausage may think about how it is possible to eat an intestine, digest this intestinal tissue, but not digest one's own intestine. What prevents auto-digestion of the intestine by pancreatic enzymes? At this stage we can identify two mechanisms to prevent auto-digestion: Biochemical blockade outside the lumen of the intestine or compartmentalization of the highly active digestive enzymes by a barrier.

So far we have found no conclusive evidence to suggest that the wall of the intestine contains sufficient concentrations of endogenous enzyme in-

hibitors to prevent auto-digestion. Instead our current evidence suggests that auto-digestion is prevented by compartmentalization of active pancreatic digestive enzymes in the lumen of the intestine. The barrier for this compartmentalization is provided by the mucosal epithelium lining the intestinal wall. Neighboring epithelial cells have tight junctions forming a cell layer with low permeability. The mucus layer may provide additional barrier properties, preventing entry of digestive enzymes into the interstitial tissue of the intestine.

Under ischemic conditions, however, the usually tight epithelial barrier is compromised and high molecular weight molecules may penetrate the tight junction regions in the inter-epithelial gaps and enter into the interstitial space of the intestinal wall (51, 53, 68-71). There may also be a reduction of mucin secretion from goblet cells thereby reducing a potentially significant diffusion barrier for active digestive enzymes. Instead, fully activated digestive enzymes enter into the submucosal space and initiate self-digestion of unprotected villi structure, a process that leads to complete destruction of their tissue matrix (72). Digestive enzymes may be carried into the smooth muscle tissue layers of the intestinal wall (73); they can enter the portal venous circulation, the intestinal lymphatics, and may escape across the serous collagen coat into the peritoneal fluid (Figure 1). Thus, in shock the entire intestinal wall is under the threat of autodigestion, and digestive enzymes that have even entered into the peritoneal space (74) put other organs in the peritoneal space under jeopardy of autodigestion.

The microvessels in the wall of the small intestine cause development of leukocyte/endothelial interaction in the venules of the intestinal circulation with accumulation of neutrophils, which in turn carry proteases (e.g. gelatinase) in their cytoplasmic granules. Measurement of the enzyme activity with zymography (73) and immunofluorescence reveals co-localization is derived from MMP-9 released by neutrophils accumulating in the venules. The MMP activation is greatly enhanced when trypsin is present in the wall of the intestine, as compared to an intestine with low protease activity. The transport of active trypsin into the wall of the small intestine serves to convert proMMPs into active MMPs (73) further amplifying the proteolytic degradation of the intestinal wall. Basic biomechanical properties of the intestinal wall and its structure are now subject to proteolytic degradation. The normal peristaltic smooth muscle function of the intestine as well as molecular uptake of nutrients by the epithelial cells and blood flow in the microcirculation of the intestinal wall is compromised.

## 9 Blockage of Pancreatic Enzymes in the Lumen of the Intestine

The analysis brings to light the question whether blockade of the digestive enzymes, readily accessible in many clinical situations, may help prevent auto-digestion of the intestinal wall and consequently development of inflammatory mediators with multi-organ failure. We have tested this potentially important idea in a variety of shock models.

Our first experiments designed to reduce pancreatic digestive enzyme activity in the lumen of the intestine were carried out using a serine protease and lipase inhibitor with broad specificity (6-amidino-2-naphthyl-p-guanidinobenzoate dimethanesulfonate, ANGD). This inhibitor produces a reduction in the inflammatory mediator levels in shock, reduced morphological damage to the intestinal villi and prevents blood pressure reduction (Figure 2) as well as failure of several organs in a model of shock due to occlusion of the splanchnic (celiac and superior mesentery) artery supplying blood to the small intestine (75). The fluid requirements to maintain the blood pressure at physiological levels after hemorrhagic shock, is also reduced after blockade of the digestive enzymes (76).

A similar reduction of inflammatory mediator formation is also seen with other inhibitors of pancreatic proteases (gabexate mesilate, aprotinin) if administered via lavage into the lumen of the intestine (72, 77). Blockade of superoxide production by xanthine oxidase in the ischemic intestine (78) does not provide additional protection beyond that provided by protease inhibition itself

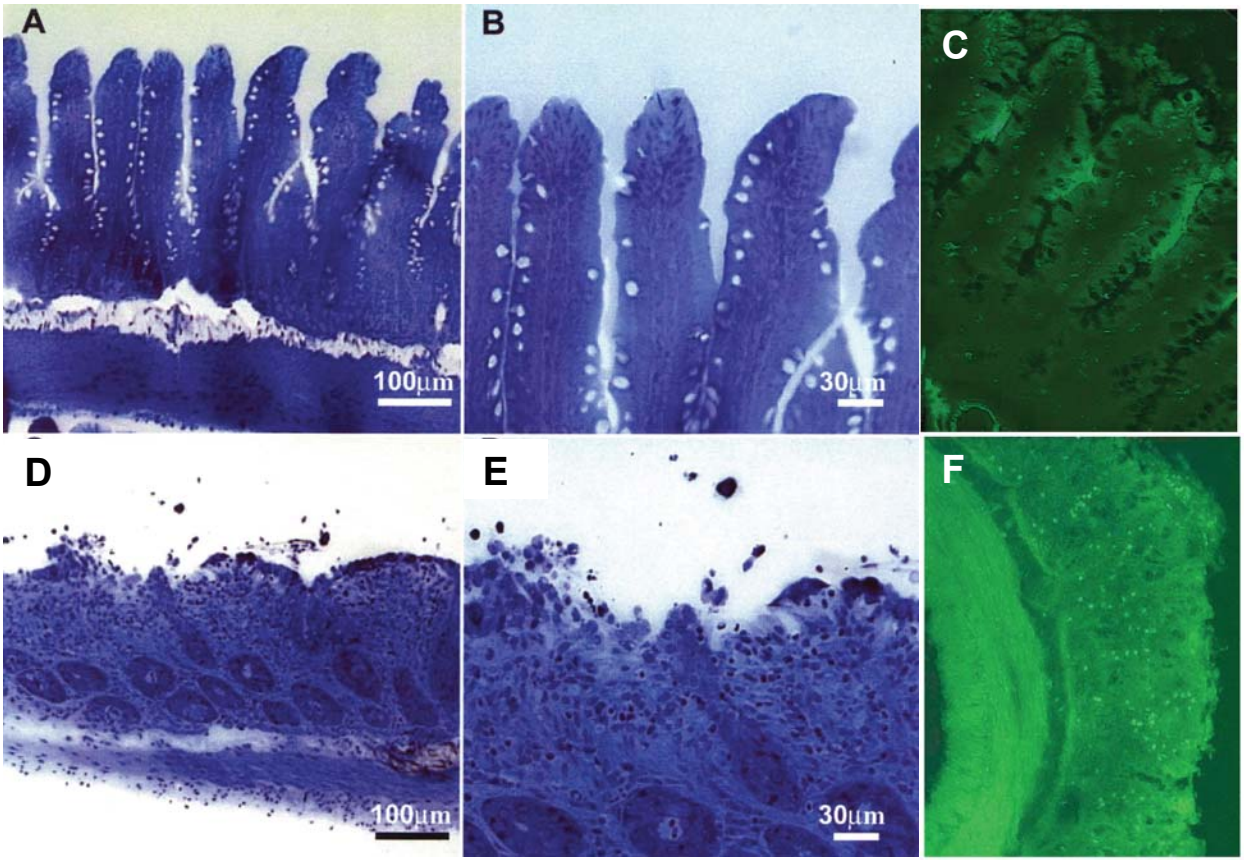


Figure 1: Micrographs of rat intestinal wall morphology (semi-thin section stained with toluidine blue) and zymographic image with trypsin substrate (green fluorescence) before (Panels A,B,C, respectively) and after 45 min intestinal ischemia (D, E, F). Note the extensive damage to the microvilli and mucosal epithelium (D,E,F) and penetration of activated trypsin across the full thickness of the intestinal wall (F). Adapted from (73, 87).

(79). As a clinically relevant situation in which treatment has to *follow* an initial shock insult, we also showed that a delayed intestinal protease inhibition serves to improve experimental superior mesenteric artery occlusion (SMAO)-induced shock by reducing intestinal injury, the level of cell activation in plasma and in the microcirculation, and by restoring the blood pressure (80).

The protection rendered by inhibition of pancreatic digestive enzymes is only provided if the enzymes are blocked *inside the lumen* of the intestine (75). If the enzyme inhibitors are administered directly into the circulation (i.v., i.a., i.m.), less protection, and in some cases no protection, is achieved (72, 81) irrespective of the protease inhibitors used. This observation supports the hypothesis that digestive enzymes in the lumen of the intestine - where they are fully activated and in high concentrations as part of normal digestion - are the major source of degradive enzymes in acute intestinal ischemia.

Digestive enzymes that enter into the intestinal wall appear to produce inflammatory mediators that are carried towards the central circulation via the portal venous system (75), but also via the intestinal lymphatics (82-86). Besides the portal venous circulation and the intestinal lymphatics, inflammatory mediators can also be carried directly across the intestinal wall into the peritoneal cavity (74). The details of this process are,

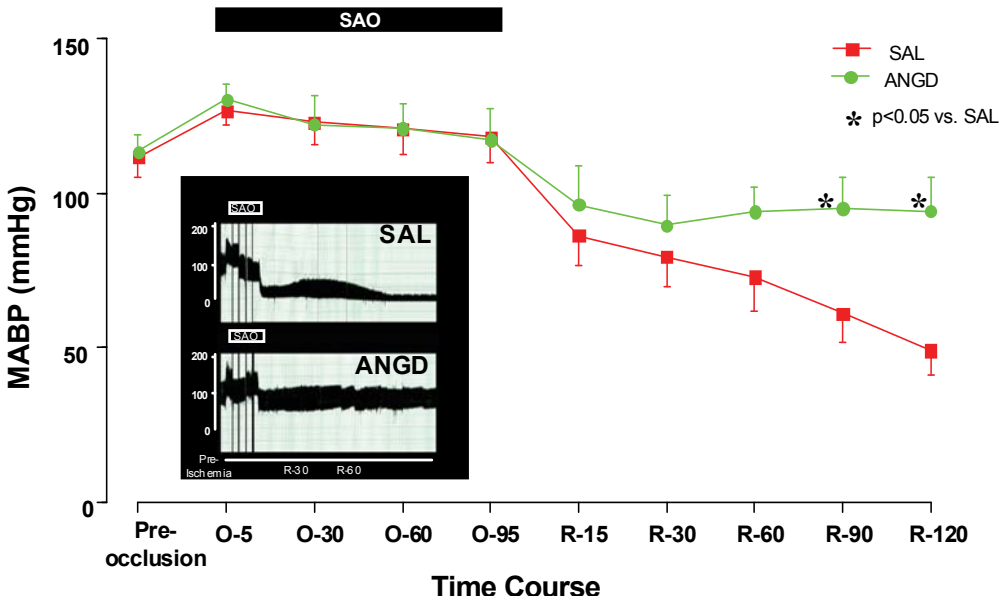


Figure 2: Mean femoral arterial pressure before (pre-occlusion), during occlusion of the splanchnic artery (SAO) (times O-5 toO-60), and upon reperfusion (R-15 to R-120). Time instances are indicated in minutes. The mean blood pressure in a control group is show whose lumen of the intestine was rinsed with saline (SAL group) and a group whose intestinal digestive enzymes were blocked with a digestive enzyme inhibitor (ANGD-group). Note the preservation of the mean blood pressure upon reperfusion in the group with the digestive enzyme blockade. The insert shows a typical tracing of the pulse pressure, indicating that the digestive enzyme blockade also serves to maintain the pulse pressure. Adopted from (75).

however, unexplored. Once inflammatory mediators generated in the intestine by digestive enzymes are released into the circulation they contribute to the severe *systemic inflammatory response* during shock, with increase in leukocyte-endothelium interactions in post-capillary venules and parenchymal cell death in remote peripheral organs. Here again, intra-intestinal pancreatic protease inhibition reduces cell activation and leukocyte-endothelial interactions and cell death in the microcirculation of a peripheral skeletal muscle that in this shock model is not exposed to the low flow state (87).

As an alternative approach to attenuate the effect of digestive enzymes in shock several studies have used ligation of the pancreatic duct to prevent discharge of digestive enzymes into the duodenum. This procedure, which does not immediately eliminate the digestive enzymes already present in the intestine, is of limited value as long as the digestive enzymes already present in the intestine are still active (88, 89).

## 10 Digestive Enzymes Mediate Microvascular Inflammation in *Septic Shock*

Septic shock is one of the most important forms of shock encountered in clinical medicine. Sepsis is accompanied by severe inflammation whose origin and mechanisms of generation remains unknown. We examined the possibility that pancreatic digestive enzymes may also be involved in inflammation in an experimental form of septic shock with a lethal dose of gram-negative endotoxin in the rat. In this shock model a lethal dose of endotoxin is administered intravenously. After endotoxin administration, control rats develop hypotension, tachycardia, hyperventilation and leukopenia and within hours multi-organ failure and death. The intestine and plasma contains mediators that activate leukocytes. The leukocyte-endothelial interaction within the cremaster muscle microcirculation is enhanced. In contrast, blockade of pancreatic proteases in the intestinal lumen improves hemodynamic parameters and reduces all indices of inflammation in

plasma as well as cell injury in peripheral skeletal muscle microcirculation. This experiment indicates the first time that inflammatory mediators derived from the intestine by digestive proteases may be involved in the prolonged inflammatory response and may sustain symptoms of sepsis after exposure to a short endotoxin bolus (90). The administration of endotoxin causes a *transient* inflammation response and elevated intestinal permeability. But the *sustained* inflammation that leads to multi-organ failure in this situation may again be caused by auto-digestion due to escape of pancreatic digestive enzymes from the lumen of the intestine due to the elevated mucosal permeability.

## 11 Generation of Inflammatory Mediators by Pancreatic Enzymes in the Wall of an Ischemic Intestine

In ischemic tissues, such as in shock, inflammatory and even cytotoxic factors are generated by a variety of biological activities. A famous example is the "myocardial depressing factor", an undefined biochemical mediator that appears in plasma and can be shown to depress contraction of the heart (91-93). There are several other examples of cytotoxic factors derived from plasma with a variety of pathophysiological activities (26). The technology was not readily available until recently to isolate and identify peptide factors from complex mixtures in sub-microgram quantities, and so these factors remain characterized mostly by means of cell experiments after exposure.

The current analysis points towards a central role played by the digestive enzymes. This can be seen in homogenates of pancreas and of intestine that have been incubated with active digestive enzymes. Homogenates of pancreatic tissue contain relatively small protein and lipid fragments (94). The lipid mediators derived from the pancreas exhibit a high level of toxicity in-vivo, similar to the hydrophobic peptide fragments. But in-vitro the peptidic fragments generate lower levels of cell activation (measured by degranulation) compared to the lipid fragments. The great majority of peptide fragments are below $\sim$15 kDa in molecular weight, supporting the hypothesis that they are generated by proteolytic degradation of tissue proteins in the pancreas (94). The lipid and protein fragments may have a different pathophysiological mechanism of action.

In contrast to the cytotoxic mediators derived from the pancreas, mediators from the intestine exhibit a different pattern. Generation of cytotoxic mediators in the intestine requires the presence of digestive enzymes. But it is interesting that the particular source of the digestive proteases (pancreatic trypsin, chymotrypsin, elastase) does not seem to play a major role (67). In contrast, few mediators are generated by amylases or nucleases (66). The intestinal homogenate when incubated with trypsin, chymotrypsin, or elastase form powerful mediators that cause rapid death of control cells while the enzymes without intestinal homogenate had no such effect (67, 95). Separation into lipid and protein fractions shows that the major cytotoxic factors from the intestine are lipid in nature and are unbound free fatty acid (i.e. soap, a major detergent and at the same time a major building block of bilipid membranes). The presence of a lipase inhibitor (1-(3-hexyl-4-oxo-oxetan-2-yl)tridecan-2-yl 2-formylamino-4-methyl-pentanoate, Orlistat), prevents cell death after protease digestion, confirming that free fatty acids are the major source of cytotoxicity in intestinal tissue under attack from pancreatic digestive enzymes. There is almost no cytotoxic activity in the protein fraction. If the protein fraction is remixed with the lipid fraction its cytotoxicity is attenuated, unless the protein fraction has previously been digested by proteases. This surprising evidence indicates that cytotoxicity of a major lipid mediator, free fatty acids, is attenuated by binding to a fatty acid-binding protein. One of the proteins that binds and carries free fatty acids in the circulation is albumin. It binds 11 free fatty acids per molecule. The addition of albumin to the lipid fraction of the ischemic intestine attenuates its cytotoxicity (96).

In summary, a major cytotoxic mediator derived from an intestine under in-vitro conditions is due to *lipase generated free fatty acids*, which are neutralized by binding to free fatty acid binding-proteins. Breakdown of the free fatty acid-binding

proteins by proteases causes release of free fatty acids as powerful cytotoxic mediators.

## 12 Summary

Digestive enzymes in the intestine are fully activated and in higher concentrations than in most other organs. They have the ability to break down almost all biological molecules as a requirement until the last day of a life. Auto-digestion of the intestine is usually prevented by restriction of the digestive enzymes to the lumen of the intestine by the mucosal epithelial barrier. Any disruption of the mucosal barrier by hypoxia, inflammatory mediators and other mechanisms, permits entry of the powerful digestive enzymes into the wall of the intestine, precipitating auto-digestion. Escape of digestive enzymes or the cytotoxic mediators generated by digestive enzymes in the wall of the intestine into the central circulation serves to initiate tissue injury and multi-organ failure. The preclinical evidence in favor of this auto-digestion hypothesis remains to be tested in a clinical setting.

From a biomechanics point of view it is apparent the specific details of the transport of digestive enzymes is at the center of the problem that transport will have to be an integral part of a quantitative theory in the future.

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