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IMMUNOMORPHOLOGICAL, NEUROENDOCRINE AND METABOLIC SHIFTS IN TARGET ORGANS AT CRUSH SYNDROME

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Abstract

The character and peculiarities of pathological processes resulting from experimentally induced crush syndrome (CS) have been investigated from complex (morphological, histo-enzymatic, immunopathologic, endocrine, biochemical and bacteriologic) standpoints. For the first time the important role of the immune system mediators and hypophysial growth hormone in pathogenesis of CS manifestations in organs and systems was established.

The immune enzyme analysis has revealed that on the background of acute inhibition of mediatory function in immunocompetent cells of central and peripheral organs of immunogenesis a structural and functional reorganization of lungs, liver, and pancreas takes place. The process of remodeling in "target organs" in many ways depends on the quantitative and qualitative composition of locally secreted immunocytokines (IL-I, IL-II, IL-VI and γ-IFN), and, probably, of prolactin.

The cells of lymphomacrophagial line perform the role of a source for local synthesis of pro-inflammatory cytokines in lungs and liver, whereas in the pancreas it s supported by the acinar cells. The phenomenon of intestinal bacterial translocation of Gram-negative resident microorganisms was revealed under CS, with their further persistence in the "target organs".

The state of endotoxicosis arising at the early stages of CS, in development of which, as established by our study, a significant role belongs to the process of bacterial translocation and NO activation, should be considered from qualitatively new positions.

Due to the complex studies, it has been revealed for the first time that at the early stages of CS on the background of disturbed bacterial homeostasis earlier unknown immunoendocrine loops participate, functioning in accordance with the paracrine and autocrine principle.

Keywords: crush syndrome, immunopathological disorders, cytokines, target organs.

Introduction

In many countries of the world due to World Health Organization (WHO) approval and financial support a complex of scientific-organization measures is thoroughly worked out within the frames of "Medicine of Catastrophes" basic direction.

Medical aspects connected with the consequences of earthquakes are rather topical for the Republic of Armenia as well. The wide program of scientific-preventive and medical measurements worked out in our country already in 1988 after the natural disaster serves as an evidence of their high relevance. This program was carried out under the aegis of the Ministry of Health of the Republic of Armenia. A special place among the complications resulting from the earthquake is given to the long-term crush syndrome (LCS). From this point of view the investigations, which are directed to study different aspects of its pathogenesis in the aspect of defining the character and peculiarities of systemic and organic injuries in case of LCS, are quite promising.

The socioeconomic significance of LCS is obvious: because of earthquakes young and middle-aged people who are the most able-bodied citizens creating material values are affected.

For the last 50 years clinical approaches for solving these problems have had two directions: radical surgical approach and research of effective means of general and local conservative therapy.

At the same time, we should mention that

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methodological approaches implemented in treatment of LCS are far from being perfect. Postoperative complications are frequent and, as a rule, are related to development and/or prolonged course of local aerobic-anaerobic infectious process. The antitoxic therapy is frequently ineffective due to a number of reasons. Firstly, in our opinion, the notion of LCS is, to a known extent, a generalized one involving symptom complex of general and local manifestations, the emergence of which greatly depends on the character and duration of the affecting factor, exact sites of body affection, degree of its spread and depth of affection in soft and hard tissues in the compressed/squeezed areas. Secondly, many pathogenetic aspects of local and general impairments in LCS have remained not investigated. Particularly, immunopathological, metabolic, microcirculatory and bacteriological aspects of the studied problem have not been entirely revealed. This latter hinders the treatment, choice of exact means of symptomatic and pathogenetic therapy of LCS.

As more serious complications having not infrequently a lethal termination, painful emotional shock and toxemia should be mentioned. The intoxication process has an increasing character already at the early stages of LCS. At present, the point of view that the intoxication process is related to rather rapid entry of toxic products into blood (devitalized tissue products, tissue and microbial toxins) after decompression of affected areas claims to be self-dependent.

Not in the least underessimating the fact that decomposition products partially participate in induction of endotoxicosis we consider that genesis of toxemia is also conditioned by involvement into the general pathological process and other provoking factors, the realization of which might occur at the organ and system levels. However, no information on partial participation of exact target organs in development of shock reaction and toxemia under LCS is available in appropriate literature so far.

With the help of our own investigations, we have determined that at primary stages of LCS the structural reconstruction of lungs occurs with significant immune components [*Sahakyan K., 2000*]. We mean the presence of peripheric areas of bronchopulmonary tissues of large macrophageal and lympholeukocytic infiltrates.

It is not excluded that "immunological re-arrangement" of lymphoid apparatus in lungs under conditions of LCS is accompanied by local production of anti-inflammatory cytokines and NO high concentrations, which might have cytotoxic effect on the structural components of bronchopulmonary tissues, to a large extent aggravated the process of endotoxicosis. Partial participation of liver in development of systemic immunological disorders and intoxications in LCS is not sufficiently studied; however, it is known that mainly this organ can serve as a source for increased synthesis of a number of anti-inflammatory immunocytokines and NO under conditions of dysadaptation of the organism.

The investigation on morphofunctional shifts in the pancreas is of utmost importance as the pancreatic gland is quite early involved in the general pathological process under stress conditions of different genesis resulting from the increased entry into blood of toxic products originating due to dystrophy and death of excretory cells of the gland. Meanwhile, the problem of partial participation of "pancreatogenic enzymes" remains open, as well as immunocytokines and NO produced *in situ* in intoxication processes, developing at specific stages of LCS.

The necessity for thorough investigation of the adrenal glands is dictated by the circumstance that the stress-inducing and stress-limitating role of glucocorticoids at the specified stages of LCS has not been established yet, whereas many organ and system level manifestations in this suffering can be caused by hypercorticism state of the adrenal glands. It should be mentioned that the study on immunopathological impairments in LCS, as a rule, was carried out from the point of partial participation of reactions characterizing only the state of humoral immunity. Issues relevant to activation of T-mediated immunity reactions are insufficiently studied. Thus, in spite of the fact that an important role is given to the mediators of immunogenesis (interleukins) in the development of systemic and organ impairments in stresses of different genesis, this important link, as well as neuroendocrine shifts, fell out of the researchers' field of view.

The expediency of studying growth hormones of pituitary gland (hypophysis): prolactin and insulin-like growth factor 1 (IGF-1) is dictated by the fact

that both neurohormones have dose-dependent influence on the processes of anti-inflammatory cytokines modulation in the animal organism [*Yarilin A., Beliakov E., 1996; Clapp C. et al., 1998; Richards S. et al., 1998; Zellweger R. et al., 1998; Holstad M., Sandler S., 1999*].

Simultaneously, as a result of such hormonal stimulation the enhanced synthesis of IL-I, IL-II; IL-VI is accompanied by further activation of prolactin and IGF-1 synthesis in hypophysis. One cannot exclude that in this exact case, i.e. under conditions of such an acute combined stress like the LCS a similar mechanism is engaged underlying general and local immunopathological and endocrine disorders.

As known, in a number of diseases of infectious and non-infectious genesis and in emergency conditions the process of bacterial translocation occurs due to migration of microorganisms from the econiches of the intestine by hematogenic and lymphogenic ways into internal organs [*Berg R., 1985; Wells C. et al., 1988; Deitch E., 1994; Fukushima R. et al., 1994; De Souza L. et al., 1996; Lemaire L. et al., 1997; Demetriades D. et al., 1999; Nikitenko V. et al., 2001*]. It is not excluded that such translocation takes place in conditions of LCS. The investigation of this process from the bacteriological standpoint was also the subject matter of the present investigation, as endotoxins of Gram-negative residents of microorganisms can serve as both an independent source of intoxication and a modulator of synthesis of exact pro-inflammatory immunocytokines.

Material and Methods

The experiments were done in 280 puberal male mice with body weight of 40-50 *g*. The animals of trial series were divided into 3 groups. Intact animals formed the control group. On a special device the experimental group animals underwent squeezing for one hour. The area of affection was the internal surface of the thigh. The pressure on the mentioned area was 280 *kPa.* The animals of experimental groups I-III were taken away from the experiment in 1 hour, 24 hours and 7 days after decompression, appropriately.

The thymus, spleen, lymphatic nodes, lungs liver, and pancreas underwent morphological analysis. Paraffin sections were stained by hematoxylin eosin. The freshly frozen cryostat-derived sections from these organs underwent histoenzymatic analysis for determination of acid phosphotase (APh), succinate dehydrogenase (SDG) and lactate dehydrogenase (LDG) activity. The activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was determined photometrically with the help of an appropriate kit ("Delta", Armenia). The activity of α-amilase was determined with the help of the kit ("Lachema", Czech Republic) and was expressed in *u/l.*

By the method of immune enzyme analysis (ELISA) in blood serum and supernatants prepared from lungs, liver and pancreas the content of cytokines was determined: interleukins (IL)-I, II and VI, γ-interferon (γ-IFN), prolactin, insulin-like growth factor –1 (IGF-1), and nitric oxide (NO). To perform the reaction tissues of examined organs after drying on filter paper and weighing were homogenized in physiological solution with the use of homogenizer of glass-teflon type at 1000 *rpm* and freezing at -4º C. Then the suspension was centrifuged at 15000 *g* for 15 *min* at freezing. The content of cytokines was defined with the help of a corresponding anti-mouse kit (DRG-International Inc., USA-Germany) and was expressed in *pg/ml.*

The content of prolactin was determined using reagents of Syntron Bioresearch Inc. (USA), for cortisol the kit of DRG-International Inc. (USA-Germany) was used, while for NO the kit from Assay Design Inc. (USA) was applied. The content of prolactin and cortisol was expressed in *pg/ml*, NO - in *mmol/l*. The level of cytokines, prolactin, and NO was measured by automatic spectrophotometer Stat-Fax 303 Plus (USA) in the range of 420-450 *nm* of absorption wave length. IGF-1 was determined using the radiation counter "Gamma 1" with the help of kits of Amersham Biotech Co. (USA). The content of IGF-1 was expressed in *imp/min*.

The technique is based on concurrency principle of endogenous IGF-1 in blood serum with administered IGF-I^{125}. Therefore, high indices of impulses signify to IGF-1 low level in studied biomedia and vice versa.

Bacteriological investigations were carried out according to the generally accepted scheme, with inoculation of the content of the large and small intestines, blood, regional (mesenterial) lymphatic nodes, lungs, pancreas, liver on Endo's and Ploskirev's media.

The bacteriological analysis was simultaneously carried out to define *E. coli* growth in colonies.

The statistical analysis was done with the use of Student's criteria according to SPSS-12.

Results

Primary stages of the present work were devoted to investigation of mediator link of immunity defining pro-inflammatory cytokines level both in blood serum and in the central and peripheral immunity organs. Due to immune enzyme analysis, we succeeded to reveal that at LCS relatively early stages the expressed inhibition of cytokines IL-I, IL-II, IL-VI and γ-IFN synthesis took place in thymus, spleen, and lymphatic nodes. At the same time direct correlation dependence between the levels of IL-I, IL-VI and γ-IFN was established in the studied tissues and blood serum (Table 1). It should be also mentioned that data of immune

Table 1.

Shifts in immunocytokines contents in organs of immunogenesis and blood serum at early stages of LCS

Object of Investigation	Control	In 24 hours after decompression	In 7 days after decompression
Interleukin Iα (IL-Iα)			
Serum	2.4±0.7	3.1±0.2 0.25>p>0.1	1.3±0.3 p=0.1
Thymus	131.2±14.1	30.6±2.9 p<0.0005	14.8±3.1 p<0.0005
Spleen	83.2±7.7	56.7±4.9 0.025>p>0.01	45.4±5.6 0.005>p>0.0005
Lymph node	94.6±5.6	35.9±1.8 p<0.0005	48.0±2.1 p<0.0005
Interleukin II (IL-II)			
Serum	232.0±17.6	486.4±27.6 p<0.0005	949.4±124.1 0.005>p>0.0005
Thymus	6543.8±182.3	1985.2±193.6 p<0.0005	1175.9±196.6 p<0.0005
Spleen	674.7±14.8	245.3±11.9 p<0.0005	376.3±18.2 0.01< p<0.0005
Lymph node	768.6±54.1	365.4±21.9 p<0.0005	591.6±25.2 0.025> p>0.01
Interleukin VI (IL-VI)			
Serum	1661.2±109.9	290.7±25.3 p<0.0005	295.7±23.7 p<0.0005
Thymus	1013.3±51.0	85.45±9.1 p<0.0005	433.1±12.9 p<0.0005
Spleen	117.4±15.4	48.8±3.1 0.005>p>0.0005	166.6±9.5 0.025>p>0.01
Lymph node	103.9±9.7	54.4±2.6 0.005>p>0.0005	186.7±15.6 0.005>p>0.0005
γ-Interferon (γ -IFN)			
Serum	6.4±0.5	0.3±0.09 p<0.0005	3.3±0.7 0.005>p>0.0005
Thymus	16.2±2.6	5.5±0.5 0.005>p>0.0005	11.4±1.3 0.1>p>0.05
Spleen	1.9±0.2	0.33±0.04 p<0.0005	0.25±0.05 p<0.0005
Lymph node	2.9±0.8	0.56±0.06 0.01>p>0.005	0.43±0.07 0.01>p>0.05

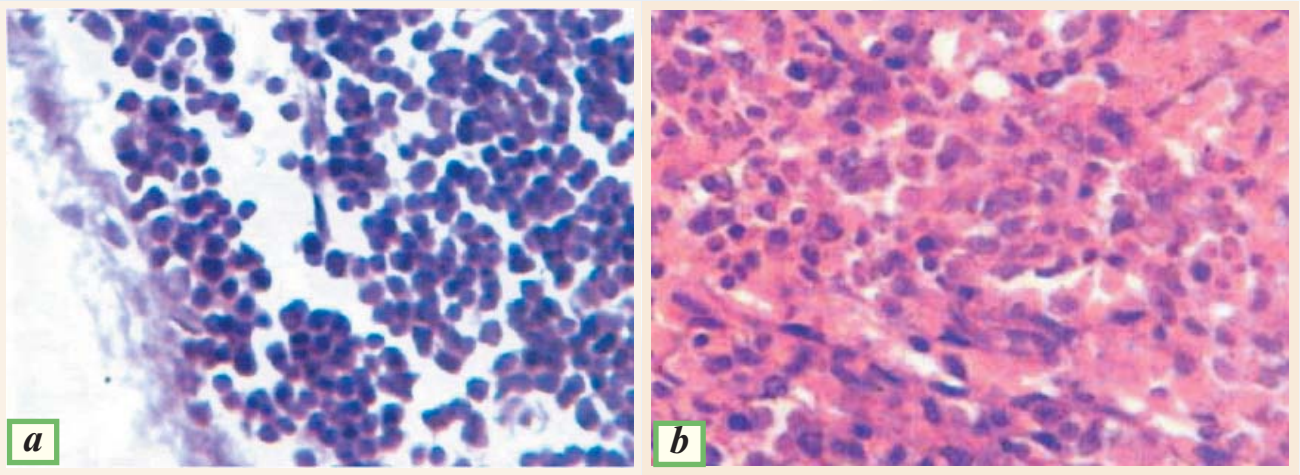


Figure 1. *Structural shifts in thymus and spleen 24 hours after decompression. Ob. 40; oc. 10.*
a) *Process of thymus "accidental" involution. Lymphocytes lose the compact, dense orientation and are characterized with loose localization in deep parts of the cortical layer. Optically light sites are identified overall.*
b) *Expressed hypoplastic processes in lymphoid tissue of the red pulp of spleen with "bearing" (uncovering) of the reticular stroma.*

enzyme analysis correlated with the results of morphological investigations, at which the process of "accidental" involution of the cortical layer was revealed in the thymus. Hypoplastic processes were also revealed in peripheral organs of immunogenesis: in the spleen and lymphatic nodes (Figure 1a;b).

Thus, for the first time we managed to establish that at early stages of LCS hypoplastic processes occur in the central and peripheral organs of immunogenesis, being accompanied by inhibition of secretory activity of lymphoid and macrophageal cells in the aspect of selective synthesis of cytokines IL-I, IL-II, IL-VI, and γ-IFN.

We should note that the high level of IL-II observed in blood serum is not resulting from its increased secretion in immunity organs. The investigation on morphofunctional state of certain target organs was realized by comparing the results of immune enzyme and morphological analyses of organs of immunogenesis, as only due to this methodological approach it became possible to define partial participation of specific target organs in development of symptom complex of systemic manifestations in case of LCS.

As obvious from results of immune enzyme analysis, at early stages of LCS rather high indices of IL-I, IL-II, IL-VI and γ-IFN content were determined in supernatants prepared from lung tissues; high levels of IL-I and IL-II were observed during the entire period of the experiment (Table 2).

A slightly different picture was observed in defining γ-IFN: in 24 hours the content of γ-IFN decreased 3.4-fold, whereas on the 7th day the level of the mentioned cytokine did not differ from that in control mice. It should be mentioned that we have established direct correlation dependence between the high level of pro-inflammatory immunocytokines and the growth hormones. During the immune enzyme analysis high indices of prolactin and IGF-1 were registered in lungs at the early stages of LCS (Table 3).

As it is known, in a mature organism alongside with somatotrophic hormones prolactin and

Table 2.

Shifts in immunocytokines contents in lungs of mice at early stages of LCS

Study Groups	Studied Indices			
	IL-Iα	IL-II	IL-VI	γ-IFN
Control	46.7±3.4	631.4±34.4	11.7±1.6	19.4±2.3
Experimental Group II (in 24 hours)	179.9±19.6 p<0.0005	1660.7±164.5 p<0.0005	22.8±1.75 0.005>p>0.0005	5.7±0.9 0.005>p>0.0005
Experimental Group III (in 7 days)	96.3±12.4 0.005>p>0.0005	1661.8±89.45 0.005>p>0.0005	68.8±3.7 p<0.0005	22.5±2.1 0.25>p>0.1

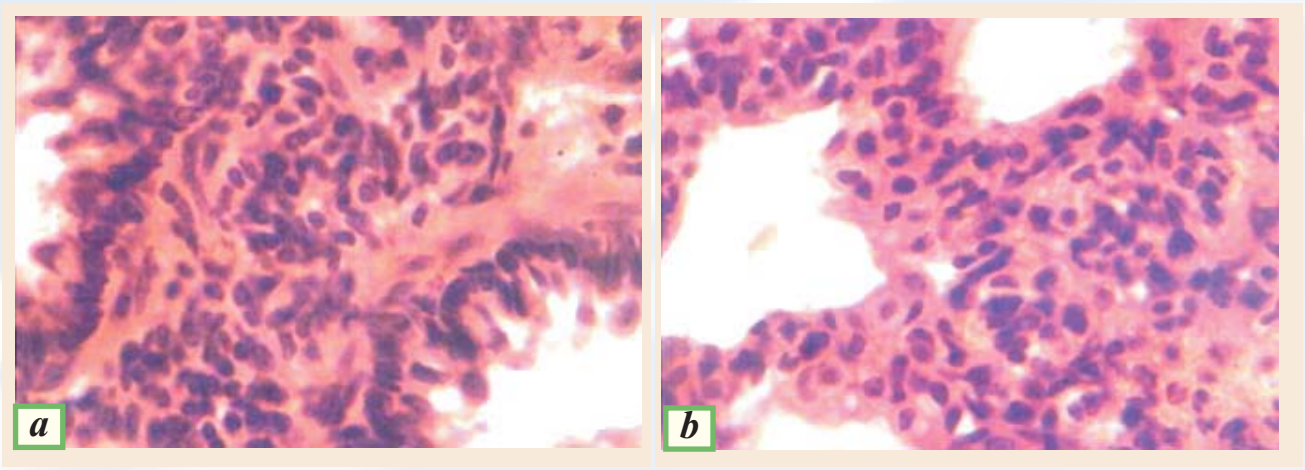


Figure 2. *Structural shifts in bronchopulmonary apparatus on day 7 of observation after decompression. Staining with hematoxylin eosin. Ob. 40; ok. 10.*
a) *and* ***b)*** *Peribronchiolar and perialveolar lymphomacrophageal infiltration.*

IGF-1 serve as stimulating factors that selectively activate metabolic processes in parenchimatous organs [*Rozen B., 1994*].

At the same time both growth hormones (prolactin and IGF-1) are modulating factors in the mammalian organism and selectively activate IL-I, IL-II, IL-VI and γ-IFN synthesis. This is why it is not excluded that the high level of studied cytokines in lungs is to a certain extent conditioned by local modulating influence of prolactin and IGF-1, as at early stages of LCS in lungs we have revealed direct correlation dependence between the studied immunocytokines and the growth hormones.

Our further investigations were aimed to study the level of NO in bronchopulmonary tissues of experimental animals. The necessity of carrying out such investigations was explained by the fact that high concentrations of NO in mammals exerted an expressed toxic influence on parenchymatous and stromal cells. Therefore, it is not excluded that in the mechanism of endotoxicosis development in case of LCS a cascade of pathochemical reactions eventually bringing forth a sharp increase of NO level is involved.

According to the results of immune enzyme analysis (Table 3) at early stages of LCS high indices of NO were registered in lungs being in direct correlation dependence on the level of studied cytokines and growth hormones.

Which are the possible mechanisms underlying the significant increase of pro-inflammatory cytokines level in lungs?

In the mentioned aspect, results of our morphological and histoenzymatic investigations were a great help. Thus, on the background of the expressed activation of catabolic processes in the broncho-

Table 3.

Shifts in content of prolactin, insulin-like growth factor (IGF-1) and nitric oxide (NO) in lungs and blood serum of mice at early stages of LCS

Study Groups	Object of Investigation					
	Serum			Lungs		
	Studied Indices			Studied Indices		
	Prolactin	IGF-1	NO	Prolactin	IGF-1	NO
Control	5.3±1.2	318.2±24.5	1.3±0.3	3.4±1.0	519.0±24.3	1.0±0.3
Experimental Group II (in 24 hours)	3.9±0.5 0.25>p>0.1	440.4±26.1 0.01>p>0.005	6.5±0.6 p<0.0005	2.2±0.25 0.25>p>0.1	332.2±17.6 p<0.0005	5.7±0.6 p<0.0005
Experimental Group III (in 7 days)	3.1±0.8 0.1>p>0.05	307.6±26.9 0.4>p>0.25	4.8±0.7 0.005>p>0.0005	10.7±2.1 0.005>p>0.0005	406.3±15.8 0.005>p>0.0005	2.3±0.8 0.1>p>0.05

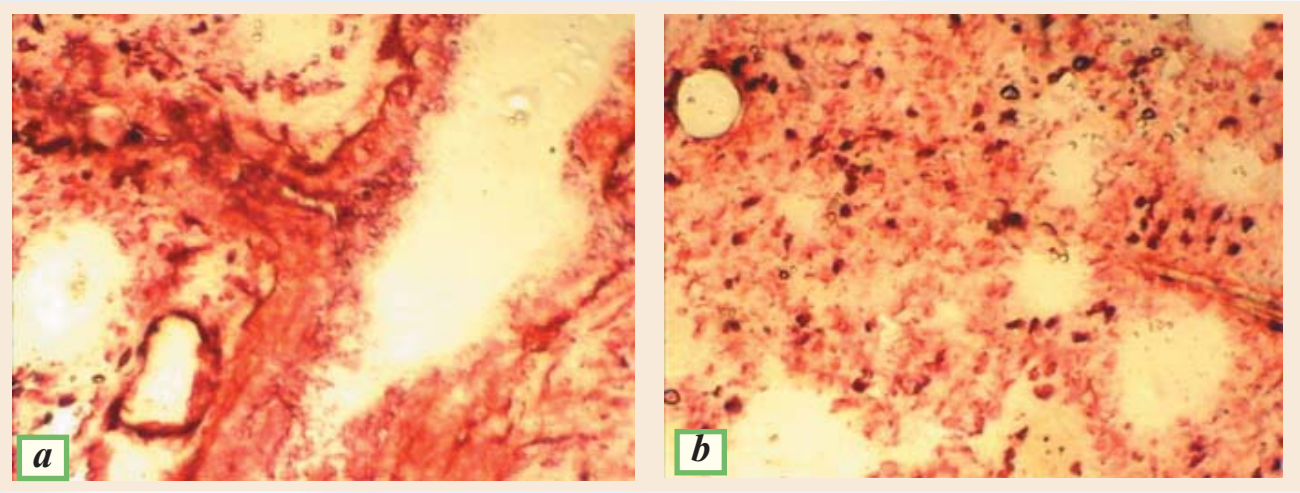


Figure 3. *Acid phosphatase activity in bronchopulmonary tissue 24 hours after decompression. Histoenzymatic analysis.*
a) *Acid phosphatase high activity in peribronchiolar and perivascular sites. Ob. 10; oc. 10.*
b) *High activity of the enzyme in perivascular localized lymphocytes and macrophages. Ob. 40; oc. 10.*

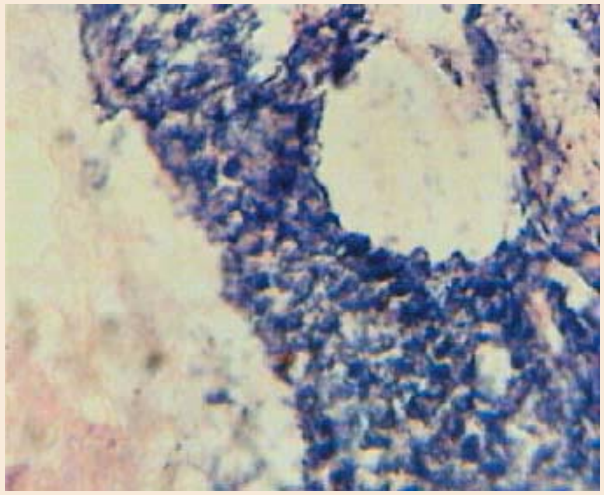


Figure 4. *Succinate dehydrogenase (SDG) activity in bronchopulmonary tissue on day 7 of observation. Histoenzymatic analysis. Ob. 40; oc. 10.*
Voluminous lymphomacrophageal infiltrates in stroma of lungs are characterized with rather high activity of SDG.

pulmonary tissue, organ stroma, mainly in peribronchial sites, the massive lympho-macrophageal infiltrates of lymphoid follicles formation type were revealed (Figure 2a;b). It is also important to mention the fact that in histoenzymatic analysis high activity of succinate dehydrogenase (SDG) was revealed in the lymphoid line cells, whereas in macrophages high activity of SDG and acid phosphatase (APh) were simultaneously registered (Figures 3a; b; 4). The results of morphological and histoenzymatic analyses, to a certain extent, testify in favour of their high metabolic activity. Therefore, on the base of the performed complex investigations we can conclude that immunocompetent cells of peribroncheal and perialveolar infiltrates serve as possible sources of local synthesis of cytokines.

An important argument in favour of our suggested assumption became the results of immune enzyme analysis of the organs of immunogenesis, as in its course unlike the analysis of lungs there were registered rather low indices of studied pro-inflammatory cytokines. In favour of local synthesis of certain pro-inflammatory cytokines, in particular, IL-I, IL-VI and γ-IFN, testifies also their low level in blood serum of experimental mice.

Thus, pro-inflammatory immunocytokines (IL-I, IL-II, IL-VI), prolactin, IGF-1 and NO perform an important role in the processes of structural-and-functional re-arrangement of bronchopulmonary tissue at the early stages of LCS formation. The comparative analysis of immunocytokines produced in the thymus alongside with the structural-and-functional changes observed in the lungs allowed to draw a conclusion that the process of re-modeling bronchopulmonary tissue at specific stages of LCS greatly depends on quantitative and qualitative composition of locally produced immunocytokines and, probably, prolactin and IGF-1 as well.

Such conclusion finds its confirmation also in a number of works, which show that certain structural elements of the bronchial tissue under conditions of the organism normal functioning serve as source of cytokines synthesis [*Norman J. et al., 1996*].

Thanks to our investigations we succeeded to determine that in pathogenesis of intoxication in

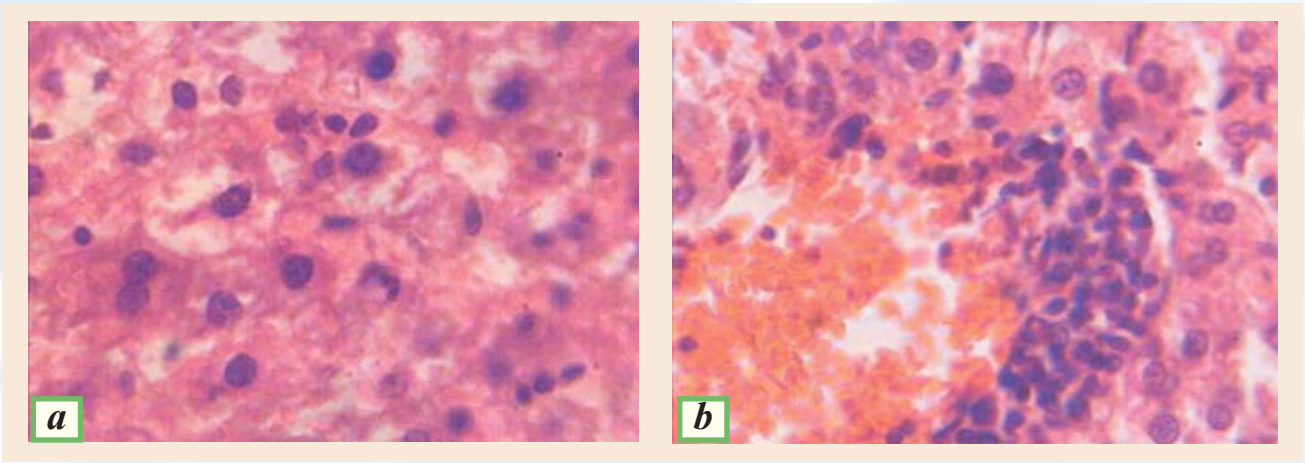


Figure 5. *Structural shifts in liver at the early stages of crush syndrome. Hematoxylin eosin. Ob. 40; oc. 10.*
a) *In 1 hour after decompression. Processes of hepatocytes discomplexation, dystrophy, and degradation on the background of the moderate edema.*
b) *In 24 hours after decompression. Processes of hepatocytes discomplexation, dystrophy on the background of the expressed hemorrhagic component. Presence of single lymphocytes in the immediate vicinity and the "site" of distrophically changed hepatocytes.*

case of LCS the processes of free radical activity have an important role in the system of reactions cascade providing an increased synthesis of NO. However, based on our investigations it is not possible to define certain mechanisms bringing to a marked increase of NO level in the lungs. Cytokines IL-I, IL-II, and IL-VI produced by the infiltrate cells, as well as γ-IFN can serve as a potential source of NO synthesis. Such mechanism directed to NO stimulation by the mentioned cytokines is described in scientific publications in a number of extremal states.

It is not excluded that the high level of NO in bronchopulmonary tissue is the result of its hematogenic entrance from other target organs as at the early stages of LCS high indices of nitrogen oxide were registered in blood serum of the experimental group animals.

We have also studied morphofunctional shifts in liver at the early stages of LCS from the complex standpoints. As manifested by results of morphological investigations, during the entire course of the experiment catabolic processes dominated in liver, whereas the degree of their expressiveness was more explicitly observed in 1 hour and 24 hours after decompression (Figure 5 *a, b*). On the 7th day of observation certain activation of reparative-proliferative reactions took place on the background of alterative changes expressed in focal protein-lipoid dystrophy processes. Direct correlation dependence was also revealed when comparing the results of morphological and histoenzymatic analysis with findings of biochemical examination for defining blood serum ALT and AST activity considered as informative markers of hepatocytes affection. Thus, on the background of expressed dystrophic changes, high activity of APh and LDG there took place a simultaneous significant increase of ALT and AST in blood serum of both experimental groups (Table 4; Figure 6 *a; b*).

On the background of expressed dystrophic changes 24 hours after decompression in different parts of liver the massive lymphocytar infiltrates were revealed; this latter, likewise those in lungs, does not exclude the possibility of directed local synthesis of cytokines by immunocompetent cells (Figure 7 *a; b*).

The results of immune enzyme analysis for determination of cytokines are presented as Table 5.

As obvious from the Table, on the 5th day of LCS rather high indices of IL-I, IL-II and γ-IFN were revealed in liver. In 24 hours after decompression, the IL-VI level in experimental animals decreased 1.7-fold, while on day 7 it practically did not differ from the control one.

Similar dynamics was observed upon determination of NO in liver. Thus, 24 hours after decompression NO level in liver decreased 2.7 times (2.1±0.3 *mcat/l* versus 5.7±0.6 *mcat/l* in control). On day 7 of observation NO practically did not differ from that in control group mice (4.1 ± 0.35 *mcat/l* versus 5.7 ± 0.6 *mcat/*l in control).

Thus, we can suppose that at LCS early stages

Table 4.

ALT and AST activity in blood serum of mice at the early stages of LCS

Studied Indices	Control groups	Experimental groups (after decompression)	
		24 hours	Day 7
ALT	26.4±4.4	68.4±6.3 0.005 >p>0.0005	52.5±4.0 0.005 >p>0.0005
AST	22.0±3.9	72.3±7.6 0.005 >p>0.0005	48.1±5.2 0.005 >p>0.0005

NO cannot be considered as a local factor inducing both catabolic processes and processes of regional synthesis of a number of pro-inflammatory cytokines studied by us.

A high level of IL-I, IL-II and γ-IFN correlated with the high level of prolactin (6.9±1.2 *ng/ml* against 2.5±0.6 *ng/ml* in the control) on day 7 of observation.

IGF-1 indices at early stages of LCS practically did not differ from the control ones (in 24 hours: 441.4±31.3 *imp/min*, on day 7: 403.9±2.9 *imp/min* compared to 319.1±20.4 *imp/min* in the control).

Analyzing data obtained, we can draw a conclusion that at early stages of LCS the shifts in immunocytokines and prolactin content take place in liver. These changes in total with the results of morphohistochemical and histoenzymatic analysis allow defining the character and peculiarities of the regional pathological process. The results obtained due to complex investigations allow us to consider the role of certain representatives of cytokines class in induction of the pathological process and formation of adaptive reaction in precise target organs from qualitatively new positions. For this reason, one should firstly consider the role of IL-VI in liver not as a provoking factor but as an adaptogen. Particularly, its synthesis by Kupffer cells at "physiologic concentrations" is conditioned by stimulating effect of prolactin of liver providing the structural-and-functional reconstruction of the organ in the aspect of reparative-and-proliferative processes activation *in situ.*

Which is the biological significance of increased entry of prolactin into liver at early stages of LCS?

An assumption might be drawn that high concentrations of prolactin in liver provide for reparative-proliferative processes under conditions of developed protein-lipoid dystrophy. In this aspect, the influence of prolactin on reparative-proliferative processes in liver was studied by a group of Japanese researchers [*Tokada T. et al, 1997*]. It was found that in partial resection of liver under conditions of multiple introduction of prolactin on the background of caused prolactinemia no activation or inhibition of processes of hepatocytes proliferation and stromal elements was

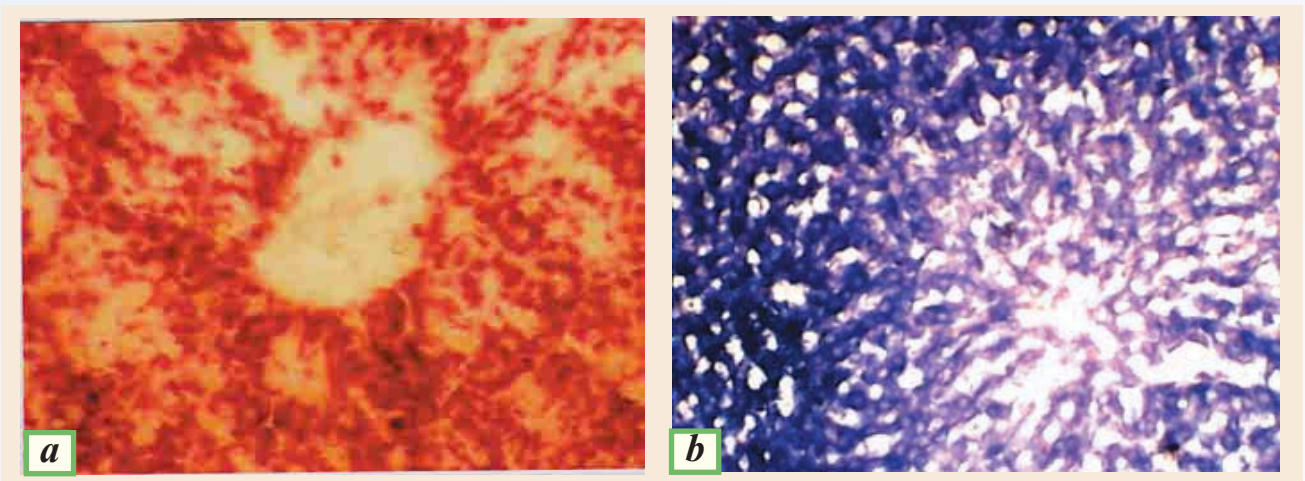


Figure 6. *Acid phosphatase and lactate dehydrogenase activity in liver of experimental mice under crush syndrome. Histoenzymatic analysis. Ob. 10; oc. 10.*

a) *High activity of acid phosphatase in all parts of the lobe in hepatocytes cytoplasm alongside the single linearly oriented intercellular capillaries. In 2 hours after decompression.*

b) *High activity of lactate dehydrogenase in peripheral parts of the hepatic lobe. On day 7 after decompression.*

Table 5.

Shifts in immunocytokines content in liver of mice at early stages of LCS

Study Groups	Studied Indices			
	IL-Iα	IL-II	IL-VI	γ-IFN
Control	146.0±12.3	399.0±20.5	415.9±24.3	0.8±0.07
Experimental Group II (in 24 hours)	42.3±6.6 p<0.0005	600.6±37.9 0.005>p>0.0005	248.8±13.7 p<0.0005	not determined
Experimental Group III (in 7 days)	234.6±17.6 0.005>p>0.0005	3763.0±118.7 p<0.0005	404.5±15.8 0.4>p>0.25	2.8±0.51 0.005>p>0.0005

observed in the preserved parts of liver.

It is necessary to mention that in another series of experiments introduction of prolactin was done under conditions of ovariectomy. Thus, the authors excluded a possible pathway of prolactin entry from the ovary into the liver. Therefore, according to our data, under conditions of LCS the "accumulation" of prolactin in liver should not be considered as a factor ensuring the course of formation of local reparative-proliferative processes. This latter is confirmed by the morphological changes, which we revealed in liver at the early stages of LCS, i.e. the process of protein-lipoid dystrophy without any visible signs of activation in either hepatocytes or fibroblastic cells. At the same time, it is not excluded that the high level of prolactin in liver is provided first of all by the entry from the main source of its synthesis, i.e. the hypophysis, as well as due to migration of certain lymphocytar populations, which also produce prolactin, into liver [*Reber P., 1993; Matera L., 1996*].

The fact that in healthy liver prolactin is revealed only in the perivascular parts of "arteriolovenular triad", central veins and epithelium of the biliary tract testifies in favour of this circumstance [*Kloehn S. et al., 2001*].

Moreover, the same authors establised that no receptors to prolactin were revelaed in the parenchymatous or stromal cells of normally functioning liver. In this concern, the authors draw a conclusion that, to our mind, is rather important: at the site of liver prolactin does not act through autocrine and even paracrine mechanisms.

Taking into consideration this quite valuable information obtained by the mentioned authors, we can suppose that at the early stages of LCS "realization" of prolactin entry into the liver is apparently done by the effect of the immune system on target cells *in situ*. Local macrophages, Kupffer cells, as well as alien cells of monocytar-lymphocytar line cells, which are revealed in liver as perivascular infiltrate served as such cells under

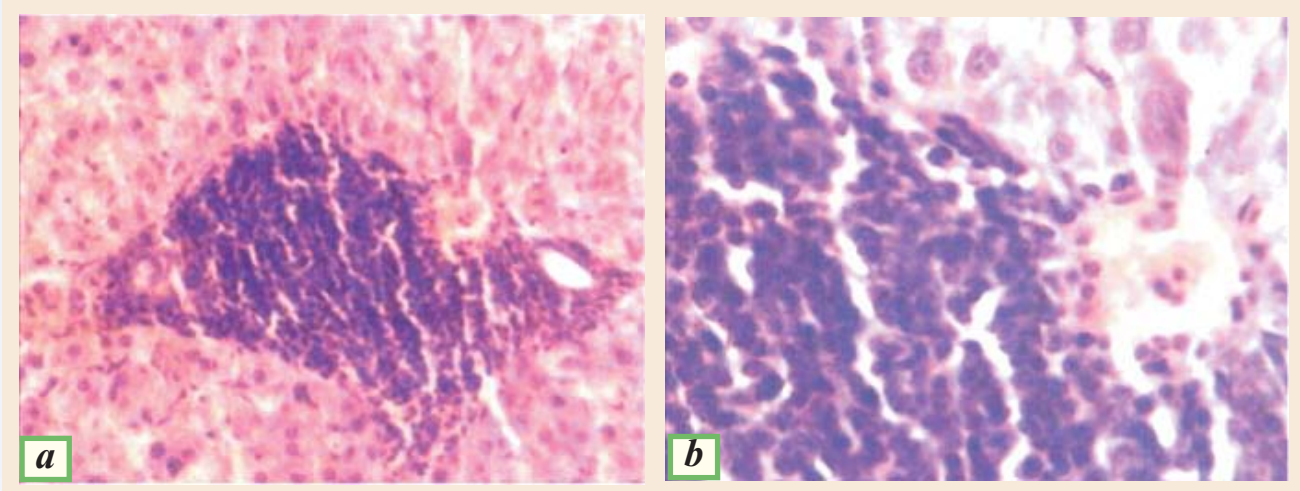


Figure 7. Structural shifts in liver in 24 hours after decompression.
a) Voluminous infiltrates in immediate vicinity of the central vein resembling in their structural organization the follicular apparatus of spleen. Ob. 10; oc. 10.
b) Image detail. Ob. 40; oc. 10.

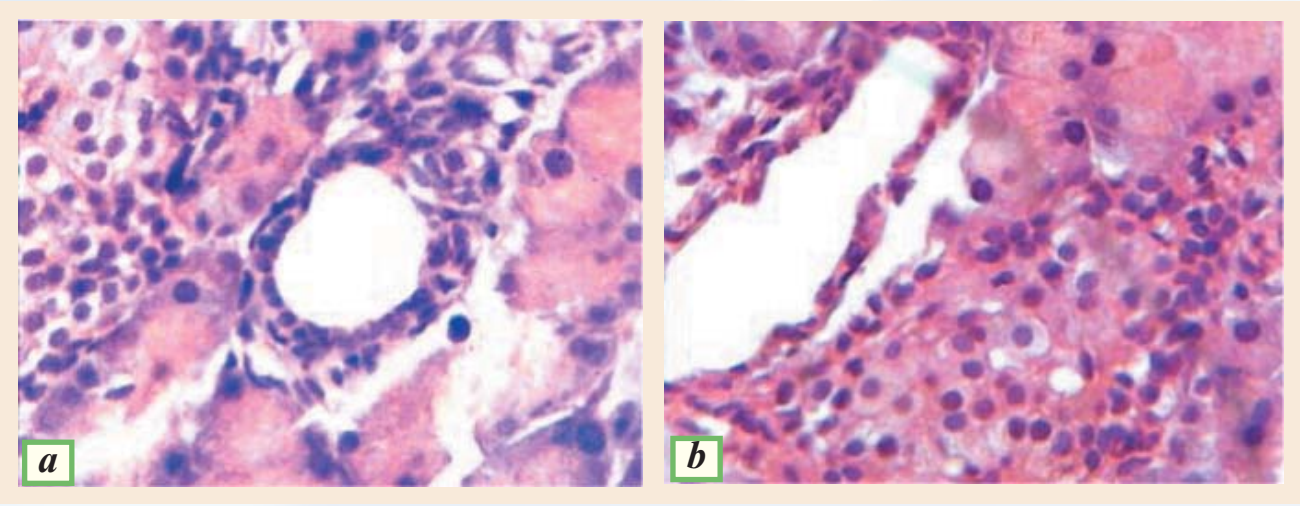


Figure 8. *Structural shifts in pancreatic gland in 1 hour after compression.*
a) *The process of acinar apparatus discomplexation on the background of periductal edema and moderate lymphocitar infiltration.*
b) *Moderate perivascular infiltration, presence of isolated lymphocytes in sites of acinar epithelium dystrophy.*

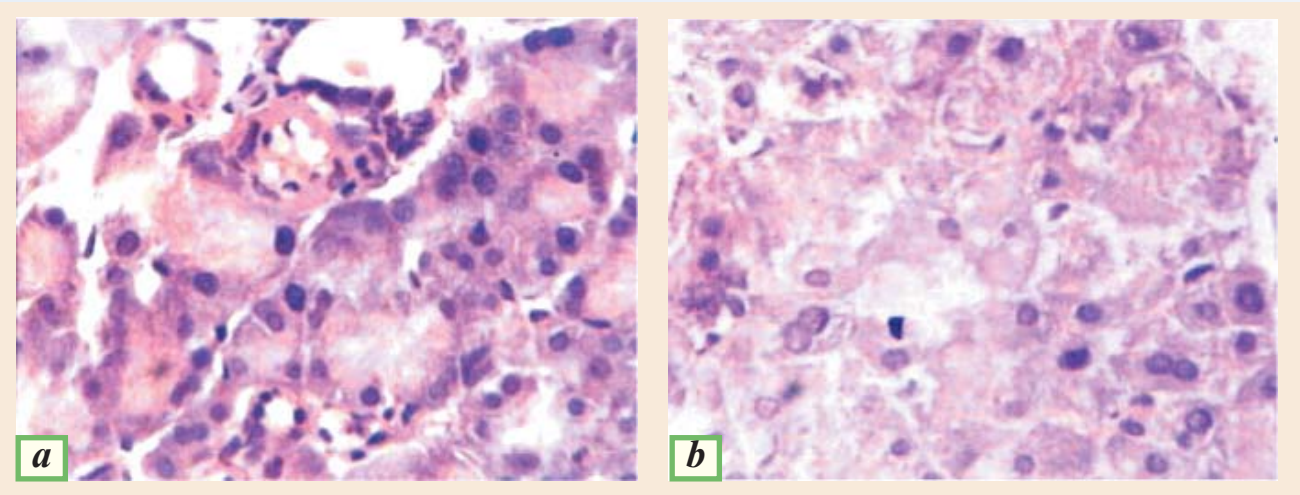


Figure 9. *Structural shifts in pancreatic gland 24 hours after compression. Hematoxylin eosin. Ob.40; oc. 10.*
a) *and* **b)** *expressed pericellular edema on the background of dystrophy and degradation of exocrine pancreacytes.*

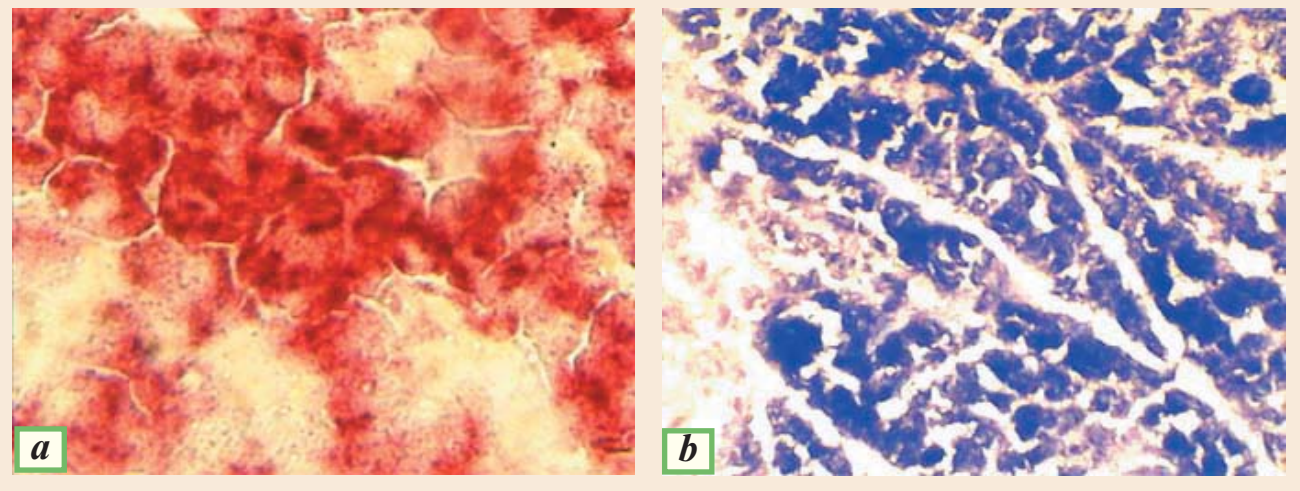


Figure 10. *Acid phosphatase and lactate dehydrogenase activity in the pancreatic gland 24 hours after decompression.*
a) *Extreme activation of the enzyme in acinar cells evidenced as the homogeneous red mass and small grains in cytoplasm of distrophically changed epitheliocytes. Ob. 40; oc. 10.*
b) *High lactate dehydrogenase activity in acinar cells. Ob. 20; oc. 10.*

the conditions of our experiment. Therefore, it is not excluded that in case of LCS in the background of liver affection such immunopathological processes, which manifest in enhanced local synthesis of a number of pro-inflammatory immunocytokines exerting the cytotoxic effect on the hepatocytes, also underlie.

As evident from results of morphological and immune enzyme analyses, in animals exposed to LCS there occurred structural-functional changes in the cortical layer of adrenal glands. Being the response to such an acute stress as LCS, these changes were nonspecific in character. Moreover, at the early stages of LCS the state of hypercorticism of the adrenal glands developed and was accompanied by the increased entry of cortizol into blood flow.

Thus, on the background of significant dilatation of fascicular zone, where "hypertrophied" optically light adrenocorticocytes dominated, 24 hours after decompression the level of cortizol in adrenal glands and blood serum increased more than twice: in adrenal glands: 275.0±19.9 *ng/ml* versus 134.8±12.8 *ng/ml* in control; in blood serum: 34.8±3.7 *ng/ml* versus 16.8±0.5 *ng/ml* in control.

It should be noted that the process of hypercorticism in adrenal glands had a transitory character: it was a response to acute combined stress, as on day 7 after decompression there was observed a tendency to normalization of architectonics and secretory function of the organ and on day 15 morphofunctional indices of the organ practically never differed from those in control group mice.

According to results of morphological investigation, in the earliest period of LCS (in 1 hour and 24 hours) an expressed activation of catabolic processes took place in the pancreas with their primary localization in the excretory part of the gland. Dystrophic changes were manifested by the signs of discomplexation of acinar cells, vacuolization of cytoplasm, pycnosis and rexis of their nuclei. Foci of micronecrosis were revealed throughout. It should be noted that mentioned changes in pancreatic acini were revealed on the background of microhemocirculatory disorders manifested by the signs of endotheliocytes dystrophy, erythrodiapedesis, and moderated perivascular lympholeukocytar infiltration (Figures 8a;b; 9a;b).

On day 7 after decompression, the intensity of catabolic processes significantly weakened. On this background an expressed activation of reparative-proliferative reactions took place being characterized by the proliferation processes of epithelial cells of interlobular and intralobular ducts and accompanied by formation of small pancreatic acini, which were at different stages of differentiation.

The results of morphological analysis correlated with data of performed histoenzymatic investigations. At a certain stage of LCS, i.e. in the period when catabolic processes dominated in the pancreas (1 hour and 24 hours after decompression), high activity of APh, LDG was observed on the background of a significant decrease of SDG (Figure 10a;b). On day 7 of LCS, i.e. in the period, when the expressed activation of anabolic processes directed to recovery of cytoarchitechtonics of excretory apparatus took place in the pancreas, APh activity significantly decreased overall.

The intensity of catabolic processes in excretory apparatus correlated with α-amylase level in blood serum and precisely in pancreas during the entire course of the experiment. Almost similar high indices of amylase activity were determined in both experimental groups. Thus, 24 hours later, the blood serum amylase level made 300.05 ± 33.4 *mcat/l*, on day 7 it achieved 360.0 ± 30.1 *mcat/l* versus 216.0 ± 18.4 *mcat/l* in the control. In 24 hours the amylase content in pancreas of mice was 443.8 ± 32.3 *mcat/l*, and on day 7 of the experiment it mounted 428.4 ± 34.5 *mcat/l* versus 140.0 ± 11.8 *mcat/l* in the control.

As manifested by the results of immune enzyme analysis at the early stages of LCS an expressed increase of IL-I level took place in the pancreas (Table 6).

We did not succeed in defining other studied cytokines in the pancreas of mice of experimental and control groups by immune enzyme analysis method, i.e. only their trace quantities were revealed. At all stages of LCS rather low indices of prolactin were registered in pancreas (Table 8).

IGF-1 indices in the pancreas practically did not differ from those in the control. In 24 hours IGF-1 content made 398.6 ± 32.1 *imp/min*, on day 7 it was 363.5 ±2 9.3 *imp/min* versus 348.0 ± 28.6 *imp/min* in the control.

As shown by the results of immune enzyme analysis for nitrogen oxide, 24 hours after decom-

Table 6.

Shifts in content of interleukin I and prolactin in pancreas of mice at early stages of LCS

Study Groups	Studied Indices	
	IL-Iα	Prolactin
Control	14.6±2.8	8.2±1.6
Experimental Group II (in 24 hours)	89.5±7.2 p<0.0005	1.8±0.5 0.005>p>0.0005
Experimental Group III (in 7 days)	62.7±7.2 p<0.0005	2.6±0.9 0.025>p>0.01

pression NO level in the pancreas increased 3-fold as compared with the control (95.3±8.4 *pg/ml* versus 31.4±6.5 *pg/ml* in the control), but on day 7 of observation only the tendency directed to its decrease (54.8±6.3 *pg/ml*) was registered.

For many years it was considered that the progression of pancreatitis was related to the primary activation of digestive enzymes in acinar cells that brings to autolysis of the pancreas, and, consequently, to development of endotoxicosis.

However, the mechanisms of this "activation" are insufficiently studied. Apparently, they should not be related to a category of initial affecting factors, as on the background of extensive clinical and experimental material the inefficacy of symptomatic therapy with the wide use of anti-proteases and preparations inhibiting synthesis of lipase and amylase in the pancreas was convincingly proved. As fairly grounded by J.G. Norman, the direction on studying the role of inflammation mediators and first of all of pro-inflammatory cytokines in the mechanism of pancreas affection turned to be the most promising [*Norman J. et al., 1996*]. In this respect, it is necessary to mention that in a number of emergency conditions, including experimentally induced pancreatitis, rather high levels of IL-I and NO are observed in the pancreas and blood serum. As noted by numerous authors, acinar cells are the source of their synthesis [*Fink G., Norman J., 1997; Gukovskaya A. et al., 1997; Norman J. et al. 1997; Klar E., Werner J., 2000; Zaninovic V. et al., 2000*].

We should particularly emphasize highly informative results obtained by J.G. Norman and co-authors [*Norman J. et al., 1996; 1997; Norman J., 1998*] in several models of pancreatitis. Thus, the authors clearly state that IL-I and TNF-*α* induced by the most different provoking factors in case of experimental pancreatitis actually are the first to release in the pancreas parenchyma. In favour of the local synthesis of mentioned sources signified the authors' investigations at genetic, immune enzyme and immunohistochemical levels. Particularly, gene induction of pancreatic IL-I and TNF-α and the formation of albumin took place already in 30 minutes after initiation of pancreatitis under conditions of the experiment.

The following circumstance should be specially mentioned: after a certain period of time following the expressed increase of cytokines secretion in the pancreas, IL-I and TNF start to be produced in great amounts in lungs, liver, spleen, but not in kidneys, myocardium and skeletal muscles. Many authors consider certain concentrations of IL-I and TNF in the pancreas tissue to be toxic for parenchymatous and stromal cells.

Therefore, due to performed complex investigations we can suppose that a possible reason for severe dystrophic processes in the pancreas is the high level of IL-I, which is secreted especially *in situ,* i.e. in acinar pancreacytes.

The high level of nitrogen oxide in the pancreas at early stages of LCS is conditioned by its inflow from blood, as in 24 hours and on day 7 after decompression in blood serum, lungs and organs of immunogenesis we registered their rather high indices. On the other hand, its possible synthesis *in situ* due to directed stimulating action of locally produced IL-I is not excluded. Unlike lungs and liver, in the pancreas prolactin does not act as a factor providing directed synthesis of IL-I, as the indices of its concentration are rather low in the gland: manyfold lower than those in the control.

Our bacteriological and bacterioscopic investigations allowed to determine that one-hour exposure of the posterior extremity of mice provoked translocation process of residential Gram-negative microflora from econiches of distal parts of the gastrointestinal tract (jejunum and ileum) into the internal medium of the macroorganism. The positive results obtained upon sowing Gram-negative microorganisms, including *E. coli* as well, from the regional mesenterial lymphatic node tissues, blood and a number of parenchymatous organs testify in favour of this circumstance.

Already at early stages of LCS (1 hour after decompression), "retrograde" translocation of Gram-negative microorganisms is quite precisely

observed within the gastrointestinal tract. The degree of bacterial dissemination increased 10^8 colony forming units (CFU), while on the background of a decrease in number of colonies in the ileum it made 10^7 CFU.

It is worth mentioning that Gram-negative microflora began to get sowed from the pancreas in the mentioned period of observation (10^4 CFU), whereas inoculation in control mice was sterile. Gram-negative microflora with the presence of *E. coli* began to be sowed from blood as well (10^3 CFU). Data of bacteriological investigations obtained at relatively early stages of LCS, though indirectly, testify that the econiches of residential Gram-negative microorganisms localized in the corresponding parts of the large intestine (10^7 CFU against 10^9 in the control) served as a possible source of increased dissemination of the jejunum and duodenum and colonization in the pancreas.

The following picture was observed in the studied organs of the gastrointestinal tract 24 hours after decompression. The number of colonies in large intestines tended to decrease (10^5 CFU) even compared with the control indices (5 x 10^7 CFU).

We must note that such nature of the process of intestinal Gram-positive microflora translocation was observed in mice in the conditions of their 4-hour stiff immobilization [*Gritsenko V. et al., 2000*].

According to results of bacterioscopic investigation, *E. coli* made 8-10% of the general number of iliac Gram-negative microorganisms.

It should be mentioned that in this period of LCS the pancreas persisted to serve as a source of colonization of microflora translocated from small and large intestines. Moreover, the general number of colonies practically did not differ from that of the pancreas in mice of the previous experimental group. However, upon bacterioscopic investigation amongst the Gram-negative microorganisms *E. coli* was revealed much more frequent: in 20% cases. On day 7 of observation further "colonization" of the pancreas took place by Gram-negative microorganisms (10^5 CFU), among which *E. coli* was determined in 35-40% of cases.

In ileum a tendency to normalization of the bacterial landscape (10^8 CFU) was observed. A similar tendency was observed in the jejunum (10^6 CFU).

Rather low dissemination (10^3 CFU) was registered in blood inoculation in nutrient media of Endo and Ploskirev. On day 14 of observation bacterial landscape (we mean only the resident Gram-negative microorganisms, which are revealed in econiches of exact parts of the gastrointestinal tract) got normalized: 5 x 10^7 CFU in the jejunum and 10^9 CFU in the ileum.

The degree of bacterial dissemination in the pancreas significantly decreased (10^2 CFU), when the sown Gram-negative microflora in its subsequent bacterioscopic analysis was exceptionally presented as a monoculture of *E.Coli.*

As demonstrated by results of bacteriological analysis, the process of bacterial translocation at early stages of LCS was also accompanied by colonization of liver and lungs by Gram-negative microflora with *E.Coli* identified in 15-20% of cases. Only single pinpoint colonies were defined in lungs and liver in 1 hour after decompression. In 24 hours Gram-negative microflora (10^5 CFU) was intensively sown from the lungs. In the subsequent period of observation a tendency directed to the decrease of dissemination of the bronchopulmonary tissue (10^3 CFU) was quite distinctly observed, whereas on day 7 as well as 1 hour after decompression only single pinpoint colonies were determined in the nutrient media.

Unlike the lungs, colonization of liver by Gram-negative microorganisms took place only 24 hours after decompression (10^3 CFU). In further period of observation (on days 7 and 14 after decompression) only single pinpoint colonies were sown.

It should be emphasized that in regional (mesenterial) lymphatic nodes at all the stages of LCS a picture, similar to that in bacterioscopic analysis of bronchopulmonary tissue, was observed.

Discussion

On the base of own investigations and comparison of findings with the recent literature data a conclusion might be drawn, according to which LCS can be considered as a syndrome of polyorganic insufficiency (SPOI). In this aspect, we should note that partial participation of target organs in LCS development and state of endotoxicosis is not equal. As mentioned above, until present the kidney and muscular tissue were considered as the main target organs in case of LCS. It is due to our investigations that we made a conclusion: in LCS there are no specific "priority"

target organs, which might predetermine the character and degree of severity of the pathological process overall. Our complex investigations enabled us to establish that lungs and liver as well as the pancreas are very early involved in the general pathological process of systemic inflammatory reaction thus predetermining the character and the course of LCS and the degree of severity of increasing intoxication.

Due to comparison of immune and endocrine research findings, we managed to determine that in every studied target organ the pathological process differs by a certain originality manifested in each exact case in a characteristic symptom complex of immunological and endocrine disorders. Particularly, in some parts of the target organs there was determined the precise correlation dependence between the high level of certain pro-inflammatory cytokines and NO, which leaves its mark on the course of endotoxicosis, as their high concentrations are known to be toxic. In other target organs a distinct correlation dependence is observed between the levels of studied cytokines and growth hormones and, hence, for the first time there is determined the important role of impairment in a certain link of neuroendocrine system, which assumes the formation of new functional immune-endocrine loops in case of probably acting by both the paracrine and autocrine principle.

The role of pancreas in LCS induction should be considered from qualitatively new positions. On the base of data obtained by us this organ should be considered as one of the main target organs in case of the given suffering as the character of affection and the determined functional loop of NO *in situ* can be defining at the most initial stages of endotoxicosis development under LCS. On the other hand, not at all underestimating the significance of existing conception of endotoxicosis, in development of which the defining role belongs to the devitalized tissues and microorganisms entering the blood channels and lymphatic system after decompression, the mechanism of organ disturbances and especially development of intoxication is more difficult and many-sided. Thus, to our mind, the state of endotoxicosis greatly aggravates, if not induces, immunopathological impairments in specific target organs being accompanied by increased entry of cytotoxic concentrations of pro-inflammatory cytokines and NO into blood.

The next focal moment that we succeeded to establish is the translocation process of residential conditionally pathogenic microorganisms from the intestine econiches with their subsequent persistence in a number of internal organs. This is why the state of endotoxicosis is conditioned by microorganism translocation not only from the crushed area, but also from the intestine econiches.

Apart from this, we have found that the process of intestinal translocation is accompanied by relatively long-term persistence of *E. coli* in the pancreas. Taking into account the fact that in the animal organism lipopolysaccharide of *E. coli* serves as a powerful immunomodulator, which selectively stimulates synthesis of certain pro-inflammatory cytokines, we can assume that the increased synthesis of IL-I occurs mainly due to *E. coli* in the pancreas.

It is beyond any doubt, that the translocation process observed at early stages of LCS can be considered as a factor aggravating the course of intoxication. At the same time, peculiarities and the character of *E. coli* persistence in certain organs can be considered local factors stimulating selective synthesis of cytokines by immunocompetent cells. When comparing the results of bacteriological analysis in every precise organ, one might draw a conclusion that the observed process of translocation of intestinal microflora under LCS was characterized by relatively long-term colonization only in lungs and pancreas. Therefore, the symptom complex of structural, immune, and endocrine shifts that we succeeded to reveal earlier should be considered taking into account this latter. It is not excluded that local "immunological" re-arrangement of the above mentioned organs accompanied by activation of their mediatory function is greatly conditioned by *E. coli* persisting in them.

The practical value of this research is that on the base of the obtained experimental data the significant correction will be done in the general scheme of pathogenetic and symptomatic therapy of LCS.

Data and findings of complex investigations allow defining to what extent and at which stage of LCS development the pancreatic toxic enzymes, which enter the blood flow when the excretory

apparatus of the pancreas is affected, also participate in occurrence and development of intoxication. It is precisely this reason why at a certain stage of LCS special symptomatic therapy becomes necessary with the use of preparations inhibiting activity of chemotripsin, α-amylase, and lipase.

Since under LCS the pancreas is a source for synthesis of IL-I and NO, which at high concentrations are considered toxic factors, it is necessary to carry out local therapy with preparations inhibiting IL-I and NO synthesis *in situ.*

We used the similar methodological approach to study the immunopathological reactions in the lungs as well. Thus, as we have already revealed, lymphocytar and macrophageal cells of infiltrate were the source of secretion of immunocytokines of pro-inflammatory spectrum of action; in case of pancreas already at early stages of LCS local symptomatic therapy is needed being directed to inhibition of synthesis in bronchopulmonary tissue of pro-inflammatory immunocytokines, because these latter promote development of local dystrophic processes, particularly by NO activation.

Revealing the character of structural-and-functional shifts in the terminal link of hypothalamic-hypophysial-adrenal system (HHAS), in adrenal glands, allows to answer the question to what extent hypoplastic processes in the organs of immunogeneses are connected with hyperfunction of adrenocorticocytes of cortical layer and increased entry of glucocorticoids (cortisol) into blood. It is not excluded that it is at early stages of LCS that the scheme of complex treatment with preparations inhibiting the directed synthesis of glucocorticoids by the adrenal glands becomes necessary. On the other hand, according to literature data at relatively later stages of LCS, when autoimmune processes occur, on the contrary, introduction of glucocorticoids is necessary as well, alongside with other preparations. Thus, based on data obtained, for the first time it becomes possible to carry out directed hormonal therapy by supporting optimal level of glucocorticoids in blood serum, on the one hand, and inhibition of autoimmune processes by introduction of glucocorticoids in the period of autoagression mechanisms formation, on the other hand.

The revealed shifts in content of hypophysis growth hormones (prolactin, IGF-1) allow planning application of symptomatic therapy directed also to support their optimal level in blood and internal organs, as their high content, in spite of metabolic processes activation in all organs and tissues, can simultaneously bring to activation of synthesis of pro-inflammatory immunocytokines IL-I, IL-II, IL-VI and, indeed, this happened in case of LCS.

The earlier revealed phenomenon of bacterial translocation and processes of NO synthesis, which alongside with pancreatogenic enzymes, greatly pre-condition the state of increasing intoxication should be considered from qualitatively new positions. Therefore, at the early stages of LCS (immediately after decompression) symptomatic and pathogenetic therapy appears necessary and should be directed to normalization of blood serum NO level and bacterial homeostasis of the intestine.

On the base of our own investigations we consider it expedient to present the summarizing scheme of the possible pathogenetic links of immunopathological, endocrine and metabolic disorders arising in the target-organs in LCS.

For the first time we have defined the important role of the immune system and the growth hormone mediators in the pathogenesis of organ and system manifestations in LCS.

Due to the carried out immune enzyme analysis we succeeded in finding out that on the background of acute inhibition of the mediatory function of immunocompetent cells of the central and peripheric organs of immunogenesis the structural-functional reconstruction of lungs, liver, pancreas took place.

Remodeling process in the "target organs" greatly depends on the quantitative and qualitative composition of the locally secreted immunocytokins (IL-I, IL-II, IL-VI and γ-IRN) and might be of prolactin as well.

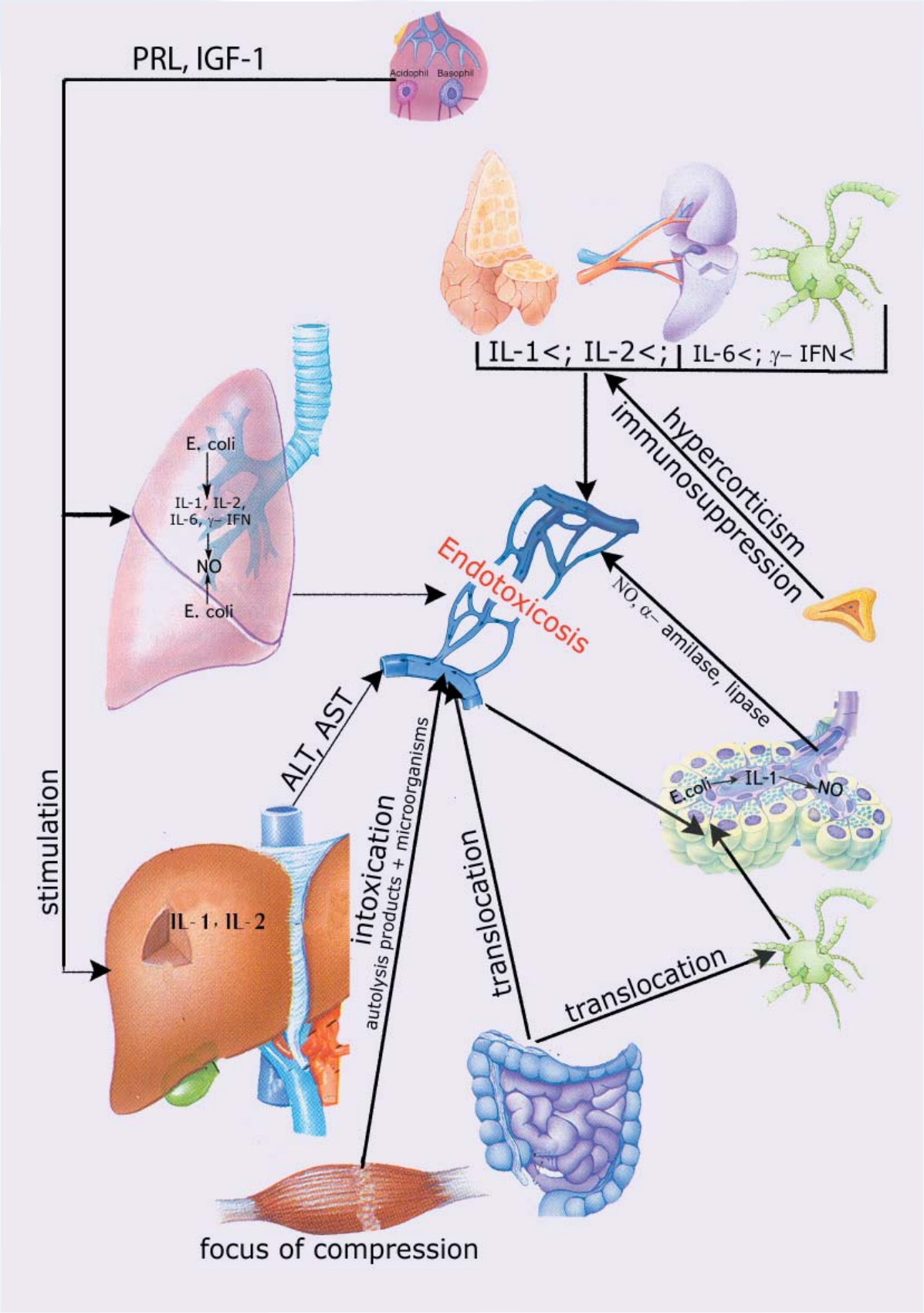
In lungs and liver the cells of lympho-macrophageal line served as a source of local synthesis of pro-inflammatory cytokines, while in the pancreas acinar cells had the same function.

In long-term crush syndrome the phenomenon of intestinal bacterial translocation of Gram-negative resident microorganisms was defined with their further persistence in the "target organs".

As a result of a relatively prolonged persistence of microorganisms in lungs and pancreas their endotoxins (lipopolysaccharides) might act

Scheme.

Pathogenetic links in development of immunopathologic, endocrine and metabolic disorders in target organs at initial stages of crush syndrome.



alongside with prolactin as the stimulators of pro-inflammatory cytokines synthesis *in situ.*

The state of endotoxicosis arising at the early stages of LCS, in development of which the role of processes of bacterial translocation and NO activation is great, should be considered from qualitatively new positions.

Due to the complex investigations we were the first to define that at the early stages of LCS on the background of the impaired bacterial homeostasis there are engaged earlier unknown immune-endocrine loops functioning according to paracrine and autocrine principle.

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