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Adaptive Protection of the Heart: Protecting Against Stress and Ischemic Damage

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Adaptive Protection of The Heart: Protecting Against Stress and Ischemic Damage

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CRC Press

Taylor & Francis Group

Boca Raton London New York

CRC Press is an imprint of the
Taylor & Francis Group, an informa business

CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

Reissued 2019 by CRC Press

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ISBN 13: 978-0-367-24625-9 (hbk)
ISBN 13: 978-0-429-28356-7 (ebk)

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CRC Press Web site at <http://www.crcpress.com>

FOREWORD

In our age of expeditious information, any book of some volume which would take a while to read is in itself somewhat antiquated; it is especially so for a treatise devoted to such a wonted problem as protecting the heart from stress and ischemia. Therefore the author feels a need to explain himself going back to the good old days when a manuscript submitted for judgment to a respectable university used to be prefaced with a brief listing of the basic propositions, "theses" that had not theretofore been set forth in other works. I believe that just as in the past, this enables the reader to decide whether to spend time on further reading or to make better use of it. Hence I state that this book has been written to substantiate the following ideas concerning heart damage and heart protection.

1. Stress and ischemia, and most often combinations thereof, are of paramount importance in the development of major heart diseases, and nevertheless these two factors are deeply unequal both in their place in cardiac pathology and in the extent of previous study. Indeed, genetic defects in the hepatic cholesterol-eliminating systems and excess cholesterol in food with ensuing atherogenic dyslipidemia and stenosing coronary atherosclerosis, are generally recognized as the key link in the pathogenesis of ischemic heart disease (IHD).^{*} The position of stress in heart pathology proves to be less definite. On the one hand, an excessively long and strong stress reaction is known to provoke atherosclerosis, spasm, thrombosis, overloading of the heart, and other conditions engendering or aggravating ischemia. On the other hand, are there indeed self-contained, noncoronarogenic, not ischemia-linked, direct stress damages to the heart? What are the concrete regulatory, biochemical, morphological, and physiological substrata of such damage? Can such damage to the heart evolve because of stress disorders in other organs? Finally, what is the clinical significance of such nonischemic stress heart damages, in particular, for the pathogenesis of noncoronarogenic cardiosclerosis, arrhythmias, and sudden cardiac death? Diagnoses such as "stress heart" and "stress arrhythmic heart disease" are not found in the accepted nosology. Yet the thought that cardiac stress damage is a reality in many a patient and not at all need be associated with ischemic disease is most obviously present in the minds of more than a few cardiologists. The idea haunts the clinics and conferences. Therefore, Chapter 1 of this monograph is devoted to analyzing the noncoronarogenic stress damage to the heart, and considering their probable role in the pathogenesis of chronic stress cardiopathies, nonischemic arrhythmias, and sudden cardiac death. In other words, in this Chapter we sought to unfold the concept of "primary stress damage of the heart" and to bring it into use in modern cardiology.

2. The decisive role of coronary atherosclerosis in the pathogenesis of ischemic heart disease does not at present raise any doubt, but still leaves open some important questions. Thus, it has been shown that at the same degree of coronary stenosis, the clinical manifestations of IHD can be vivid — or altogether lacking. The fatal sequelae of IHD — myocardial infarction or sudden cardiac death — may occur in moderate coronary atherosclerosis that is of no such consequence for other persons. Moreover, these dramatic events happen even when the coronary arteries are intact. Facts of this kind make inevitable the question of the weight of the stress factor, first, in the pathogenesis of dangerous arrhythmias, fibrillation, and sudden cardiac death, and second, in the formation of atherosclerosis, thrombosis, and spasm, i.e., in the pathogenetic progression of the ischemic disease proper. Therefore, Chapter 2 deals consecutively with these matters, using experimental, epidemiological, and some clinical data to discriminate, on the one hand, the stress arrhythmic disease of the heart from the ischemic disease as such, and to demonstrate, on the other hand, that the two conditions are interconnected.

* References to all works used in this Foreword are given at the end of the relevant Chapters.

The notions substantiated in the first two Chapters have laid the foundation for devoting all further discourse to the problem of heart protection. In so doing, to protect the heart from stress, ischemic, and reperfusion damage, to correct the disturbances of the cardiac contractile function and electric stability in infarction, and postinfarction cardiosclerosis, we made use of a somewhat unorthodox approach, namely gradual adaptation of the organism to environmental factors, as well as biologically active substances accumulating in the organism during adaptation and taking part in heart protection.

To identify the basic issues outlined in these studies it is necessary to dwell briefly on the evolution of our views in such a general physiological problem as stress and adaptation.*

The stress reaction which we have hastily represented on the very first page as a detrimental factor, in point of fact becomes such only if exceedingly strong and protracted, but in the general biological context it is one of the greatest evolutionary achievements, an obligate link of all without exception adaptive reactions of the healthy organism. The stress, naturally and necessarily, contributes both to such primitive reactions as standing still to avoid danger and to such supreme adaptive reactions as seeking scientific truth. Thus, H. Selye, who created the concept of stress, was undoubtedly right in designating his brainchild as the general adaptive syndrome. Only in the most harsh conditions of the biological or social reality is this neurohormonal reaction inordinately amplified, changes its meaning, and turns into the common pathogenetic link of "endogenous" diseases. Indeed, now we have unambiguous proof that the excessive stress reaction is involved in the pathogenesis of duodenal and gastric ulcerous diseases, diabetes, skin, and allergic diseases, in the evolution of immunodeficient states and escalation of blastomatous growth and, just as certainly, in the pathogenesis of arrhythmias and ischemic heart disease which we are dealing with in this book. This well-founded idea of the role of excessive stress in pathology in essence means that designing the methods for preventing stress damage is a necessary step in the prophylaxis of endogenous diseases which are a standing problem of contemporary medicine. At the same time, this idea had gathered such a psychological momentum as to prevent many researchers from appreciating the extremely important fact that animals and people become afflicted with "classical" stress diseases by far more rarely than they encounter most severe and protracted stress situations in the environment. For animals, such stress situations occur widely in the form of long periods of starvation and cold, natural calamities, and interspecific and intraspecific conflicts. For man, the qualitatively more complex, socially determined stress situations occur no less widely. Thus, in a relatively short stretch of its history, mankind went through slavery, serfdom, and World Wars, but did not (hopefully) degrade in the least. So *a living organism possesses a highly developed ability to adapt to stress situations*.

3. Mankind has survived owing to successful adaptation to stress, but only recently has it begun to study it objectively under laboratory conditions.

The basic technique used in our and other laboratories consisted of exposing animals to relatively mild and brief emotional pain or immobilization stress, to which they successfully adapted, acquiring therewith a remarkably high resistance to severe, previously lethal stress.

Such adaptation to stress further turned out to have a notably wide and potent cross-protective effect, i.e., to enhance the resistance not only to severe stress and total stress damage of internal organs usual in such situations, but also to the lethal action of electric current, direct chemical, ischemic, and reperfusion injuries to tissues, to cold, and even to ionizing radiation. In conformity with the nature of this book, Chapter 3 deals first with the neuroendocrine mechanism of the adaptation to brief stress episodes and with its cross-protective effects, and then the main attention is focused on protecting the heart through such adaptation. Among the results, it has for the first time been found that prior adaptation to repeated short stress exposures, besides completely preventing primary stress damage to

* These matters are considered in detail in a previous monograph, Meerson, F. *Adaptation, Stress and Prophylaxis*, Springer-Verlag, Berlin, 1984.

the heart, also efficiently protects the heart from ischemic and reperfusion arrhythmias and averts animal death due to cardiac fibrillation. Further, adaptation to stress has been shown to wholly lack any anti-ischemic effect, but at the same time to significantly attenuate the necrosis upon coronary occlusion owing to its cytoprotective effect. Simultaneously, it prevents or abolishes the disorders in the electric stability of the heart in myocardial infarction and postinfarction cardiosclerosis.

It has been obvious from the very beginning that the mechanism of this generalized protective (and specifically, powerful cardioprotective) effect of adaptation deserves the most detailed study at the systemic, the organic, and the cellular levels, since stress situations are actually the most common ones in our individual lives.

4 to 5. Studies on the mechanism of the protective effect of adaptation to stress, the results of which are presented in Chapters 4 and 5, have shown that the stress reaction never occurs in isolation. In the evolution of animals and man, it came to be coupled with activation of several central and cell regulatory systems that modulate the stress reaction, restrict its duration and intensity, and that we have designated as *stress-limiting systems*. The central stress-limiting systems at the brain level are represented by an association of GABA-ergic, opioidergic, serotonergic, dopaminergic, and parasympathetic neurons, which by their mediators control the excitation of the adrenergic neurons and the hypothalamic output of releasing factors triggering the stress reaction, and thereby limit the duration of the reaction itself. At the level of target organs operate the local stress-limiting systems, namely the antioxidant, the adenosinergic, the prostaglandin systems tempering the effect of stress hormones. Coordinated action of central and local stress-limiting systems ensures optimal modulation of the stress reaction and, in most cases, prevents stress damages to the organism.

Repeated stress exposures augment the power or the efficiency of the stress-limiting systems as manifest by enhanced biosynthesis and direct accumulation of their mediators, from opioid peptides and dopamine to prostaglandins and antioxidant factors. This and this exactly constitutes the material basis of increased direct and cross-resistance in adaptation to stress. Chapters 4 and 5 demonstrate that this novel issue has quite weighty clinical consequences.

The first one is that a genetically determined or acquired deficiency of the stress-limiting systems is a major element in evolvement of stress diseases, and in particular stress cardiac injuries, arrhythmias, and fibrillation mentioned above.

The second consequence is more optimistic, consisting of the protective benefits of adaptation which can be achieved without such drastic measures as immobilization or emotional pain stress, but using procedures like acupuncture or adaptation to other factors such as physical load or hypoxia that initially also elicit a stress reaction. Furthermore, it turned out that a protective effect in all respects similar to the adaptive one can be obtained by administration of activators, mediators of the stress-limiting systems, and their synthetic analogs. Such examples of success in the pharmacology of the stress-limiting systems are analyzed in Chapters 4 and 5.

6. Further experiments produced an unexpected result, i.e., that the protective effects of adaptation to stress are not only exhibited in the whole organism, but also in great measure retained in the isolated hearts of adapted animals, which display markedly enhanced resistance to ischemia, reperfusion injury, and high catecholamine and calcium concentrations. Analysis of this impressive cytoprotective effect of adaptation revealed that myocardial organelles and enzymic systems of adapted animals — mitochondria, sarcoplasmic reticular calcium-pumping vesicles, and creatine kinase system — are significantly more resistant to autolysis than those of controls.

There is quite a body of evidence that this phenomenon, described and examined for the first time here in Chapter 6, i.e., *adaptive stabilization of cell structures*, is associated with increased intracellular production of what is known as stress proteins, and the genetic mechanism involved requires further study.

7. The concluding Chapter concerns the use of adaptation to intermittent hypoxia in the altitude chamber, not only for heart protection from stress and ischemic damage in animals, but also for therapy and prophylaxis of some serious human diseases. The very practicability of such a clinicophysiological study stems from the general concept of environmental adaptation that has been substantiated in recent years. The gist of this concept is that stable, long-term adaptation to, let's say, physical loads, hypoxia, heat, cold, toxic agents, or novel complex situations that demand learning, cannot be provided by simple activation of the functional system mainly affected by the given factor. The principal element of stable adaptation is the enhanced expression of certain genes (and accordingly enhanced synthesis of certain nucleic acids and proteins) in the cells of the system predominating in the organismic adaptive reaction. The resulting selective activation of protein synthesis for and proliferation of just those structures that are limiting the cell function, i.e., growth of the populations of quite definite enzymes, membranes, and organelles, ultimately gives rise to the *systemic structural trace* which is the material basis of adaptation. Sometimes this trace is quite miniature and clearly demarcated. For instance, in adaptation to barbiturates and other diverse chemicals, it manifests itself as increased population and activity of the hepatic cytochrome P₄₅₀-linked enzymes. As a result, the organism acquires high resistance to toxic compounds and, therewith, greater capacity for oxidizing and eliminating cholesterol. In other cases, the systemic structural trace is, on the contrary, marked by a wide scope and ramified architectonics.

Adaptation to intermittent hypoxia in the altitude chamber is consistently attended with respiratory muscle hypertrophy, increased number of lung alveoli, a 1.5 to 2-fold greater coronary bed capacity, enhanced erythropoiesis and oxygen capacity, and other shifts directly testifying to a step-up in the physiological power of the oxygen-uptake systems. In recent years, it has been found that the long-term response to hypoxia is not limited to this increasing capacity of the systems responsible for oxygen uptake and transport, but is also revealed in physiologically significant changes at the regulatory level. These include growth of pyramidal neurons and altered neuroglial relations in the brain cortex; increased brain and adrenal amounts of opioid peptides and serotonin; partial atrophy of the adrenal glomerular zone and of the hypothalamic supraoptic nucleus, i.e., of the structures responsible for secretion of aldosterone and vasopressin; as well as moderate hypofunction of the thyroid gland and attenuated basal metabolism. It is easy to see that in the architectonics of this ramified, systemic, structural trace there are elements that certainly can enhance the organismic resistance not only to hypoxia, but also to other factors as well, i.e., there is a basis for the cross-protective effect of adaptation.

The contents of Chapter 7 furnishes evidence that adaptation to intermittent hypoxia not only diminishes the necrotic zone in ischemic and adrenergic cardiac injury, but also protects the heart, the liver, and other organs from stress damage, prevents stress atherogenic dyslipidemia, averts ischemic and reperfusion arrhythmias, abolishes the disturbances of cardiac electric stability in acute infarction and postinfarction cardiosclerosis, and hinders the development of hereditary hypertension in rats of the SHR line. Furthermore, such adaptation proved to be beneficial for animal behavior in extremal situations and brain resistance to hallucinogens, epileptogens, etc. So evidently, adaptation to hypoxia is highly promising in regard to therapy and prophylaxis.

Along this line, the last Chapter presents, besides the experimental data, a body of clinical testimony that this potential has been successfully deployed to lessen the risk factors of IHD, to treat the so-called idiopathic and in essence neurogenous arrhythmias, grave forms of neurasthenia, and some allergic diseases of man.

* * *

Once I. P. Pavlov wrote, "The seeking of truth by mathematical analysis and the movement of plants to light, are these not extreme links of the infinite chain of adaptations

that constitutes Life on Earth". Indeed, adaptation is an indispensable property of living matter, hence its study is one of the most far-flung — and maybe final — goals of biology. On the other hand, heart protection from stress and ischemic damage is a most actual and often dramatic everyday problem, with the clinical cardiologist devoting the better part of his abilities and time to treating advanced cases, struggling for minutes, days, months, at best, years of the patient's life. Writing a book in an attempt to bring together two such different problems, the author inevitably takes a risk of looking amateurish, and I don't think I have avoided this altogether. Nevertheless, intrusion of biological patterns of adaptation into cardiology had always seemed fruitful to me. The benefits are that such natural merging of sciences, on the one hand, allows us to use the power of controlled and rationally dosed adaptation to make the medicine really preventive, and on the other hand, may catalyze the development of novel, physiologically sound approaches in pharmacology.

PREFACE

Adaptation is a fundamental process in evolution. Dawkins has called Natural Selection the “blind watch maker” because it has no foresight, works at random, and does not plan for the future. The genetic information, in Crick’s words, “needs to do something useful, or to produce other things that will be useful for jobs, to help it to survive and to produce fertile offsprings with a good chance of survival.” Adaptation is a key factor in survival of the individual, therefore of the species. But adaptation does not and cannot change the genetic material. It only acts on the individual, the link in the chain of generations. But by doing so, it preserves the entire chain of generations, making possible a continuation and transmission of genetic material.

Professor Meerson has undertaken to study the physiological effects of adaptation. For many years, Professor Meerson has been interested in the physiopathology of the heart and the circulation. His work on the stages of heart failure, how the heart adapts itself to changes in load, has lead him to his various studies on the effects of adaptation. The present monumental book contains much more than the title suggests. There are detailed descriptions of the effect of stress on the heart, beginning with cardiac function, and continuing with the effect of stress on biochemical and biophysical processes, with changes occurring on the molecular level and with clinically related events. In its wide scope, it encompasses the cardioprotective effects of adaptation to stress with particular reference to adaptation of the heart to hypoxia. Here we will find a significant message: adaptation to one particular stress factor, such as intermittent hypoxia, results in profound changes of many different and seemingly unrelated functions. For example, stepwise adaptation to hypoxia alters RNA synthesis and produces changes in lung, heart, muscle, bone marrow, coronary vessels, and sympathetic neurons innervating the heart. Further, stepwise adaptation to hypoxia results in adaptational changes in the central nervous system and the spleen. It causes cessation of cardiac arrhythmias, and more generally, leads to profound changes in immune responses mediated by T-lymphocytes. The clinical ramifications of these adaptive changes are surprising: stepwise adaptation to hypoxia results in cessation of arrhythmias, diminished body weight, and increased working capacity.

What are the mechanisms which cause adaptation? Naturally, we must first consider the stimulus for adaptation, namely stress. Stress has become a much used and sometimes abused term. We speak of psychological stress, environmental stress, and physical stress. But stress can be quantitated only by its effect on the target organ. Our genetic heritage, whether expressed as personality or as physical characteristics, can become both source and target of stress. In the former, it may have very divergent effects. Stress originating from within can spark creative impulses or it can lead to destructive conflicts. Therefore, stress originating from our genetic heritage, may be both curse and privilege. Can one imagine creative activity without stress which originates from the genetic instruction inherited by our minds? Yet, predisposition of a vulnerable mind may, as stress increases, lead to physical and mental deterioration.

Meerson’s thoughts are on firmer and better definable grounds: physical stress such as hypoxia, exercise, etc., force the organism to adapt itself physiologically. This certainly is of more immediate concern to the physiopathologist who is only too happy to leave behaviorism or cognitive science to others. Professor Meerson has presented us with challenging concepts which, when based on firm quantitative studies, should keep physiopathologists occupied for many years to come.

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Among other awards he has received the State Award of the U.S.S.R. for research in molecular cardiology in 1978 and The Gold Medal of the Exhibition of Economic Achievements of the U.S.S.R. for development and grounding of the method of adaptation to hypoxia in altitude chamber for therapy and prophylaxis of human diseases. He became "One Distinguished in Sciences of Russia" in 1988.

Dr. F. Z. Meerson is the author of 15 books, published in the U.S.S.R., U.S., Japan, Germany and more than 500 research papers.

His current major research interests include the basic mechanisms of adaptation of the organism to environmental factors and application of the adaptation to the environmental factors for therapy and prophylaxis of human diseases.

ACKNOWLEDGMENTS

I am deeply grateful to my friends E. E. Ustinova, M. G. Pshennikova, and E. B. Manukhina for their invaluable help in manuscript preparation. The book is in large measure based on the experimental and clinicophysiological work carried out by N. A. Abdykaliev, Yu. V. Arkhipenko, L. M. Belkina, V. M. Boev, T. N. Bukina, V. I. Vovk, E. Ya. Vorontsova, L. Yu. Golubeva, V. V. Didenko, S. S. Dyusenov, Yu. N. Kopylov, V. I. Kuznetsov, A. V. Lapshin, N. P. Lyamina, E. V. Malysheva, I. Yu. Malyshev, V. V. Malyshev, E. B. Manukhina, A. A. Nikonorov, M. G. Pshennikova, T. G. Sazontova, V. A. Saltykova, V. P. Tverdokhlib, E. E. Ustinova, V. A. Frolov, A. B. Shneider, and other colleagues of mine, whose contribution is gratefully acknowledged. The book has gained much from having been translated into English by Dr. A. V. Galkin, who did not restrict himself to mere translation, but quite often acted as a scrupulous editor of matter and style.

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ABBREVIATIONS

ACTH	adrenocorticotrophic hormone
ALT	alanine transaminase
AP	action potential
AP_s	systolic arterial pressure
AST	aspartate transaminase
ATPase	adenosine triphosphatase
cAMP	3',5'-cyclic adenosine monophosphate
cAMP-PK	cAMP-dependent protein kinase
cGMP	3',5'-cyclic guanosine monophosphate
CIC	circulating immune complexes
CM	calmodulin
CNS	central nervous system
CP	creatine phosphate
CPK	creatine phosphokinase
DAG	diacylglycerol
dopa	dihydroxyphenylalanine
E	epinephrine
EA	electroacupuncture
ECG	electrocardiogram, electrocardiography
EDRF	endothelium-derived relaxing factor
ES	extrasystole
EPS	emotional pain stress
FDA	fructose 1,6-diphosphate aldolase
GABA	gamma-aminobutyric acid
GABA-TA	GABA transaminase
GDC	glutamate decarboxylase
GHBA	gamma-hydroxybutyric acid
GPO	glutathione peroxidase
HDLP	high-density lipoproteins
HSP	heat-shock proteins
HR	heart rate
IFS	intensity of the functioning of structures
Ig	immunoglobulin(s)
IHD	ischemic heart disease
IP₃	inositol triphosphate
LCAT	lecithin: cholesterol acyltransferase
LDH	lactate dehydrogenase
LDLP	low-density lipoproteins
LPO	lipid peroxidation
MDA	malonic dialdehyde
MDH	malate dehydrogenase
NCE	negative chronotropic effect
NE	norepinephrine
OP	opioid peptide(s)
PAF	platelet activating factor
PG	prostaglandin(s)
PIP₍₂₎	phosphatidylinositol (di)phosphate
PK C	protein kinase C
RP	rest potential

SL	stress load
SOD	superoxide dismutase
SR	sarcoplasmic reticulum
TXA₂	thromboxane A ₂
VEL	veloergometric load
VFT	ventricular fibrillation threshold
VLDLP	very-low-density lipoproteins

*To the memory of my father,
who resolutely passed through those ordeals,
I dedicate this book.*

Chapter 1

PRIMARY STRESS DAMAGE OF THE HEART

I. INTRODUCTION

Stress that damages the previously normal heart will be the focus of our attention in this Chapter. To assess correctly the facts bearing directly on this issue, it should be borne in mind that by no means did the stress response evolve as a detrimental reaction, but, on the contrary, it constitutes one of the evolutionary achievements, a necessary link in adaptation of the organism to the environment.

The scheme in Figure 1 illustrates the concept of the mechanism of individual adaptation established in our laboratory¹ and shows that a disturbance of homeostasis brought about by an environmental factor activates, through higher levels of regulation, the systems responsible for adaptation. This results in two chains of events: firstly (the upper part of the scheme) mobilization of the functional system specifically responsible for adaptation to the given particular factor, for example physical load, cold, or hypoxia; secondly (the lower part) a wholly nonspecific, "ordinary" activation of the stress-effecting system elicited by any strong or novel stimulus.

Further on, in the cells of the functional system specifically responsible for adaptation, the enhanced physiological function turns out to be coupled with activation of the genetic apparatus: nucleic acid and protein synthesis is increased to augment the key subcellular structures limiting the cell function. Selective growth of these key structures leads to the formation of the "systemic structural trace" which increases the functional power of the systems responsible for adaptation and allows transformation of the initial, immediate, but unreliable adaptation into a stable and long-term one.

As this takes place, depending on the architecture of the functional system, the structural trace may be quite ramified as in adaptation to hypoxia or, conversely, relatively confined as in adaptation to chemicals when the adaptive goal is achieved mainly at the expense of elevated enzymes of the cytochrome P₄₅₀ system in the liver and hypertrophy of this organ.^{2,3}

The scheme shows that formation of the systemic structural trace and stable adaptation is potentiated by the stress reaction which plays an especially important part just at the transition from immediate to long-term adaptation. After the systemic structural trace has completely formed and become the basis of, for instance, training to physical loads, temporary connection, or enhanced resistance to hypoxia, the stable adaptation rectifies the homeostatic disturbance, with ensuing disappearance of the now needless stress reaction.

It is essential that the stress reaction not only precedes stable adaptation, but also plays a prominent role in its formation. In this context it should be remembered that the originator of the teaching on stress, Selye, with fascinating sagacity defined this link of the adaptive mechanism as the general adaptive syndrome.

At present many circumstances concerning the mechanism of stress and its adaptive and damaging effects have become clear. As regards higher animals, it is now evident that emotions are decisive in the origin of stress. According to the information theory of emotions developed by Simonov⁴ they are brought about by the "conflict" between the actual needs of the organism (alimentary, sexual, self-preservation, self-assertion, domination) and the lack of sufficient information on whether or not these needs can be satisfied in the particular environment. This discrepancy between the need and the information necessary to fulfill it is perceived at the level of the brain neocortex and gives rise to descending cortical influences which activate the special neural apparatus of emotions: a system of nervous centers located in the area of hypothalamus, amygdaloid complex, and paleocortex. This system, according to numerous data, acts as a connecting link between the neocortex, which primarily senses

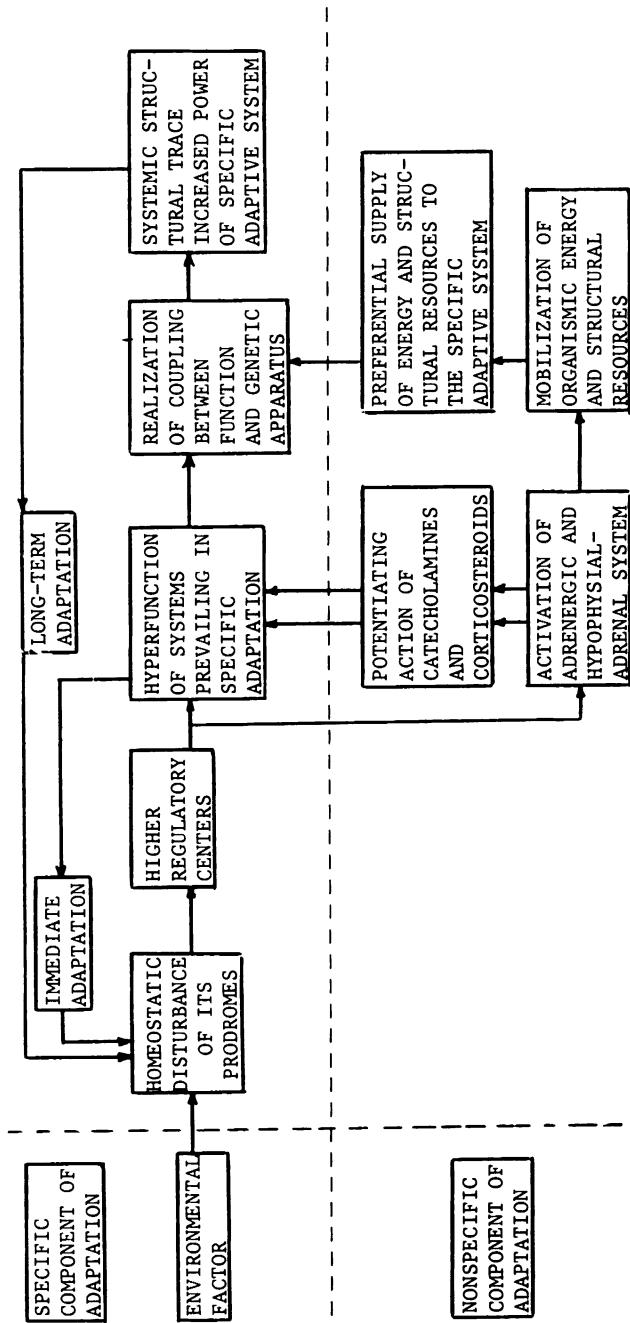


FIGURE 1. Mechanism of long-term adaptation. For explanations see text.

the information deficiency, and the centers in the hypothalamus. Excitation of the latter centers is subjectively realized as emotions of fear, anxiety, rage, pleasure, etc. In our opinion, this actuates two interconnected regulatory mechanisms which together constitute the main content of the stress reaction.

The first mechanism manifests itself as ascending activatory, mostly adrenergic influences from the emotion centers to the brain cortex, and development of diverse behavioral responses as if the organism is trying to fill in the "information gap" between the need to realize an instinct and the possibility of its realization. A relatively simple example of an emotional attempt to compensate for, to fill in the information gap is the defensive dominant formed in frightened children or in adults encountering a new and complex situation. The dominant of this kind is reflected in diverse, often exaggerated defense reactions to various and in many cases biologically insignificant environmental factors. By chance one of such reactions may turn out to be exact; in this case it is reinforced and a new environmentally adequate habit is formed: adaptation takes place.

In the more complex processes constituting the basis of human mentality, emotions, through the mobilizing adrenergic and other effects of stress, can ensure rapid and varied recombination of memorial traces preserved in the brain and thereby create new associations, some of which are reinforced by the reality and become the basis of new notions of the surrounding world. Thus Archimedes or Newton, inspired by mighty emotion, in painstaking thought, perceived fundamental physical laws behind simple things like a body immersed in water, or a falling apple. Initially, each such event comes to that in the brain of a single man and a new temporary connection is established, a new structural trace. This trace is first expressed, but in the experience of this one man, and only later, through relatively simple practices of imitation and education, becomes the property of mankind, i.e., part of the progress which is the supreme form of human adaptation. It is obvious, however, that such creative stress-based thinking, like any other stress event, is attended by great expenditures for the population, since the overwhelming majority of new associations prove to be of very modest value, or may be altogether erroneous, but nevertheless take complete possession over the person and find their imitators and followers. Ultimately, the sequelae of this sort of "creative errors" find their place in the case records of psychiatric clinics or constitute the dark pages of the history of mankind.

The second chain of events contributing to the matter of stress is directed inside the organism and is the basis of timely energy and structural provision for thinking and behavior. With all this it may become decisive for the pathology of internal organs, in particular stress damage to the heart. In this connection one should as a first approximation answer the questions, under what circumstances does the adaptive effect of stress become detrimental? what particular damages have been demonstrated in the heart? what clinical significance might these damages have?

In its general form the answer stems from the concept developed above that stress is a link in adaptation and ends as soon as adaptation is achieved (see Figure 1). Accordingly, if a functional system adequate to the environment is not formed, then the homeostatic disturbances caused by the environment in the organism persist. The stress also persists and becomes unusually long and intense. It is in this situation that stress may evoke stress diseases from duodenal ulcers and immunodeficient activation of blastomatous growth to psychic disorders and cardiac damage.

In other words, excessively intense and prolonged activation of the descending branch of the stress reaction also involves heavy costs for individuals and for the population as a whole, since the major noninfectious diseases constituting a standing medical problem are mostly elicited or aggravated by stress.

The diverse situations causing heavy and prolonged stress fraught with internal organ damage ultimately boil down to the conflict between an imperative need to immediately carry out a defense, alimentary, sexual reaction, or reflexes of "freedom" or domination,

and an insurmountable ban on carrying them out. In its most elementary form this conflict is reproduced by subjecting animals to a pain or immobilization stimulant and at the same time depriving them of a possibility to evade these factors. In a qualitatively more complex form this conflict is realized when a person is under some socially determined influence jeopardizing his existence or dignity while the natural response is prohibited by other also socially determined conditions requiring self-restraint to avoid still greater dangers. The self-restraint is indeed ensured through critical exertion of the cortical inhibitory mechanisms, but as this takes place it is only the external behavioral component of the response that is suppressed or modified. Its internal vegetative component, i.e., the stress reaction with mobilization of the circulatory and respiratory functions, etc., is retained and may even turn out to be more intense and protracted than upon execution of the behavioral response. This situation, considered from various angles in the works of Pavlov, Lang, and Anokhin, is characterized in particular by the intense stress reaction manifesting itself as a manifold increase in blood catecholamine and glucocorticoid levels. It is just under such conditions that the adaptive effect of stress on internal organs becomes a damaging one and a basis of disease. We shall further consider this fatal transformation as applied to the heart.

II. CONVERSION OF THE CARDIOTROPIC ADAPTIVE EFFECT OF STRESS INTO A DETRIMENTAL ONE

In response to the emotiogenic stress situations in the environment, the brain centers determining the stress reaction evoke a significant increase in the secretion of releasing factors and hormones and in the liberation of neuromediators. The hypothalamus produces corticoliberin, somatoliberin, and other releasing factors activating the hypophysial secretion of ACTH and other tropic hormones.⁵⁻⁷ There is a rise in the “output” of corticosteroids from the adrenal glands^{5,6,8} and of catecholamines from the adrenergic terminals and adrenals.⁹⁻¹¹

The release of tropic hormones, catecholamines, and corticosteroids stimulates or suppresses the secretion of hormones of the next line of regulation. Secretion is stimulated for glucagon,¹² thyroid and parathyroid hormones,^{5,13,14} somatotropic hormone,^{5,15} aldosterone, angiotensin, renin,^{5,16,19} vasopressin,^{14,16} and thyrocalcitonin,¹⁴ and suppressed for insulin¹⁷ and sex hormones,¹⁸ and so on.

A detailed description of the secretion and action of hormones and mediators during stress is not the subject of this exposition, but it should be emphasized that it is just these regulatory factors that carry out the adaptive or — at excessively high concentrations — the damaging effects of stress on the heart. The general adaptive influence of stress on the heart is naturally realized during any behavioral reaction from physical work to solving mathematical problems. Analysis of the components of this phenomenon yields at least five cardiotropic adaptive effects of stress.

The first adaptive effect of stress is the potentiation of organ and tissue function through activation of the most ancient signal pathway, namely the rise in the cytoplasmic concentration of the universal mobilizer of cell function, Ca^{2+} . Penetration of Ca^{2+} into the cell, besides other factors, depends on its extracellular concentration. In the initial phase of the stress reaction a rapid rise in the blood level of the parathyroid hormone draws Ca^{2+} from the bones so that its content in the blood may increase by 40 to 50%.¹⁴ Since there is a certain interrelation between the Ca^{2+} concentrations in the extracellular space and in the cardiomyocytes,¹⁹ this effect may entail enhanced entry of Ca^{2+} through the potential-dependent Ca^{2+} channels and the $\text{Na}^+/\text{Ca}^{2+}$ exchange mechanism.

On entering the cell, Ca^{2+} combining with its main intracellular receptor calmodulin (CM) passes into the active state (Ca-CM), activates the CM-dependent protein kinases (Ca-CM-PK), and thereby stimulates most intracellular processes from myofibrillar contraction and relaxation to glycolysis and lipolysis.²⁰

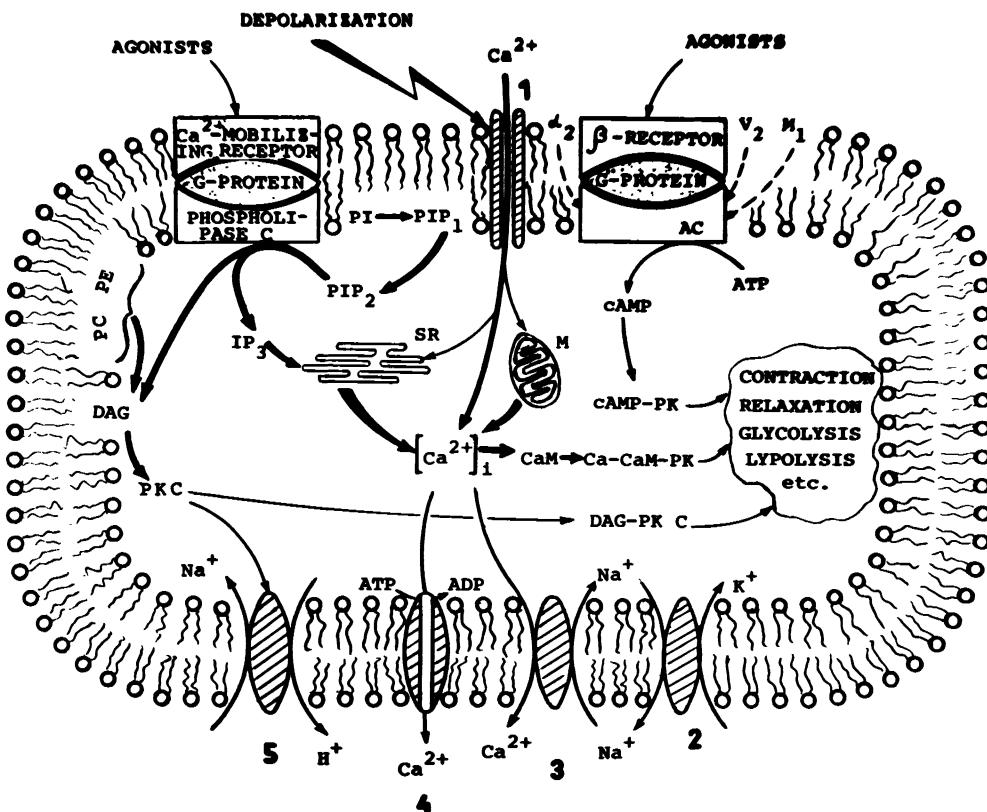


FIGURE 2. Activation of the cell regulatory mechanisms in stress. For explanations see text.

Figure 2 displays the basic regulatory mechanisms that, at the cardiomyocyte membrane level, control the Ca²⁺ entry into the cell, the changes in its sarcoplasmic content, and thus the myocardial function. Firstly, propagation of the pulse generated by the sinus node, i.e., cell membrane depolarization, entails the opening of the potential-dependent Ca²⁺ channels and Ca²⁺ entry which, until recently, has been believed to cause Ca²⁺ release from the sarcoplasmic reticulum (SR) through some as yet obscure mechanism. Secondly, as shown in Figure 2, the action of catecholamines via activation of adenylate cyclase produces cAMP, which, through the cAMP-dependent protein kinase (cAMP-PK), effects phosphorylation of the proteins of the potential-dependent Ca²⁺ channels. This prolongs their "opened state" during depolarization and thereby increases Ca²⁺ entry into the cell. Similarly, i.e., through cAMP-PK, cAMP potentiates the major cellular processes activated by CM. Thirdly, Figure 2 shows the comparatively recent discovery of a receptor-dependent way of Ca²⁺ mobilization from the SR.²⁰⁻²² The gist of this important mechanism is that catecholamines, vasopressin, angiotensin, and other agonists of the Ca²⁺-mobilizing receptors activate phospholipase C coupled with these receptors in the cell membrane.²³ The enzyme hydrolyzes phosphatidyl inositol 4,5-diphosphate (PIP₂), a derivative of the minor phospholipid phosphatidyl inositol (PI) found in the interior of the membrane lipid bilayer. PIP₂ hydrolysis yields two compounds: inositol triphosphate (IP₃) and diacyl glycerol (DAG).^{23,24} At a certain stage of the process, another isoenzyme of phospholipase C is activated which hydrolyzes other phospholipids — phosphatidyl choline and phosphatidyl ethanolamine — which also produce DAG.²³ IP₃ quickly reaches the SR and, acting as a ligand for a specific receptor localized therein, causes Ca²⁺ release from the SR to the sarcoplasm. DAG is a specific activator of

protein kinase C (PK C) which in great measure “duplicates” the functions of Ca-CM-PK and cAMP-PK in the activation of cell processes.^{20,24} Besides, PK C is important for the initiation of cell growth; this function of DAG will be considered later.

In the context of our presentation it is essential that during stress, besides the parathormone-elevated Ca^{2+} concentration, quite a number of other factors become operative which finally activate at three ways of Ca^{2+} entry into the cytoplasm depicted in the scheme (Figure 2) as well as the mechanism of cAMP formation. This augments the Ca^{2+} and cAMP contents in the cytoplasm and enhances their PK-mediated stimulatory effect on the functions of the cardiomyocytes and of the heart as a whole.

Indeed, excitation of the stress-determining brain centers and increased release of catecholamines from adrenal glands and adrenergic terminals actualize the positive chronotropic adaptive effect of stress on the heart: the number of “depolarization — repolarization” cycles and therefore the Ca^{2+} entry into cardiomyocytes are increased per unit time. As the duration of the “opened state” of the Ca^{2+} channels is longer at elevated cAMP concentrations, with each cycle this potential-dependent Ca^{2+} entry increases still more. Besides, as already mentioned, the stress raises the blood levels of hormones that are specific agonists of the Ca^{2+} -mobilizing receptors, namely vasopressin, angiotensin, histamine, et seq. Essentially similar agonists are catecholamines acting on α -adrenoreceptors not linked with adenylate cyclase. They activate phospholipase C coupled to these receptors and other phospholipases, and consequently the regulatory “ IP_3 -DAG” cycle. The elevated acting concentration of IP_3 augments the power of the Ca^{2+} “discharge” from the SR.^{20,24}

Increasing cAMP concentration in the sarcoplasm results in a greater degree of phosphorylation by cAMP-PK of the proteins of the myofibrillar contractile apparatus, which enhances the velocity and force of myofibrillar contraction: *the positive inotropic adaptive effect of stress on the heart is realized*. The scheme in Figure 2 also shows that the effect of Ca-CM potentiated by cAMP is not restricted to mobilizing the contraction, but is involved in the *prompt energy provision* for it through activation of glycolysis. The more *stable energy supply* for enhanced function is known to be ensured as the increased ATP hydrolysis in myofibrils via the creatine kinase system stimulates the oxidative ATP resynthesis in mitochondria. It is quite important that the rising concentrations of Ca-CM and cAMP can increase the rate of transition from contraction to relaxation by activating the Ca^{2+} pumps in the SR and sarcolemma. Here, cAMP activates relaxation not only by potentiating the Ca-CM action at the level of the membrane Ca^{2+} pumps, but also at the level of myofibrils where phosphorylation of troponin I by the cAMP-PK decreases the affinity between troponin C and Ca^{2+} and thereby increases the relaxation velocity.²⁰ The result is *the other component of this adaptive effect of stress on the heart: activation of the relaxation process*.

Obviously the cardiotropic effect of stress, mediated by the Ca-CM signal pathway and the modulating regulatory circuits (cAMP and IP_3 -DAG), may play a prominent part in the mobilization of cardiac function and therefore in the adaptation of the organism to various environmental factors.

At the same time it should be emphasized that the now well-proven stress-induced adrenergic increase of Ca^{2+} levels in cardiomyocytes²⁵⁻²⁷ can be an adaptive factor, only provided sufficient power of the membrane calcium and sodium pumps and of the sodium-calcium exchange mechanism which, as shown in Figure 2, ensure timely removal of Ca^{2+} from the cells to the extracellular space and maintain the normal sarcoplasmic Ca^{2+} concentration that is 10,000 times less than that in plasma.²³ This evolutionarily established concentration gradient is necessary not only to preserve the role of Ca^{2+} as the prime signal controlling the cell function, but also to prevent excessive activation of Ca-dependent phospholipases and proteases, free-radical lipid oxidation, myofibrillar contracture, etc., i.e., to prevent the well-known cardiotoxic effect of calcium.^{26,28}

Under congenital deficiency of the cation-pump enzymes or excessively long stress, when the amount of calcium and sodium increases in the cytoplasm, the excess of these cations first of all causes a more or less pronounced depolarization of the sarcolemma which is later manifested in a decreased rest potential, lower threshold of ventricular fibrillation, and higher probability of arrhythmias. Further buildup of Ca^{2+} leads to incomplete relaxation of myofibrils in diastole and development of the myocardial "poststress rigidity" which in principle can interfere with diastolic relaxation and hence with the Starling mechanism, thereby attenuating the cardiac contraction force. Simultaneously, the excess of calcium combined with the catecholamine action on the β -receptors may cause overmobilization and sometimes exhaustion of the glycogen reserves; excessive calcium entry into mitochondria may impair oxidative phosphorylation and thus ATP resynthesis. The result is a certain complex of disturbances to the energy supply for cardiac function.

In limited groups of cardiomyocytes the buildup of Ca^{2+} can be responsible for the "calcium triad" of damage to the cell structures comprising irreversible contractural damage of myofibrils, activation of phospholipases and proteases, and free-radical lipid oxidation. The experimental material characterizing these phenomena will be considered below. Here it is important to say that the transformation of the Ca-CM-induced adaptive mobilization of the cardiac function into the cardiomyocyte damage by excess Ca^{2+} is usually not an isolated event, but is intrinsically linked with an immoderate augmentation of the adaptive lipotropic effect of stress in biomembranes (see below). In other words, *the excess of calcium and its detrimental effect usually result from impaired functioning of the membrane-bound lipid-dependent cation pumps*.

The second adaptive effect of stress is that the "stress hormones" — catecholamines, vasopressin, and others — either through corresponding receptors or directly, affect the activity of lipases,^{29,30} phospholipases,^{23,29,31} and the intensity of free-radical lipid oxidation,^{32,33} i.e., the main processes responsible for the renewal of the membrane lipid bilayer. The *lipotropic effect of stress* alters the structural organization, the phospholipid and fatty acid composition of the membrane lipid bilayer, and thereby the lipid milieu of the membrane-bound functional proteins. This allows comparatively rapid changes of the activity of the main membrane proteins, i.e., vital enzymes, receptors, ionic-transport channels localized in the cell membrane (see Figure 2).

Thus, for example, catecholamines and other hormones and mediators on contact with appropriate receptors have been shown to activate the phospholipid methyl transferase methylating the membrane phospholipids surrounding the receptors. This results in phospholipid migration and decreasing viscosity (or increasing "fluidity") of the membrane.³⁴ Membrane "fluidity" is also increased upon phospholipid hydrolysis by phospholipase A₂ owing to the detergent action of lysophospholipids.^{35,36}

Such moderate alterations of the membrane lipid bilayer promote the "mobility" of the peptide chains of the functional membrane-bound proteins and enhance their activity.

The adaptive significance of the lipotropic effect of stress appears to be great: this effect can rapidly optimize the activity of all key membrane-bound proteins and thereby the cell and whole organ function, thus facilitating the immediate adaptation of the organism to environmental factors. However, in excessively long and intense stress it is the amplification of just that very effect — overactivation of phospholipases, lipases, lipid oxidation — that causes membrane damage and acquires the key role in the transformation of the adaptive effect of stress into the detrimental one. The damaging factors here are free fatty acids accumulating from excessive triglyceride hydrolysis and from phospholipid hydrolysis by phospholipases C, A₁, and A₂, as well as lysophospholipids formed upon phospholipid cleavage by phospholipase A₂. The high fatty acid level increases the content of their long-chain derivatives acyl coenzyme A and acyl carnitine.^{36,37} These compounds and lysophospholipids are amphiphiles, possessing hydrophilic (polar) and hydrophobic (nonpolar) moie-

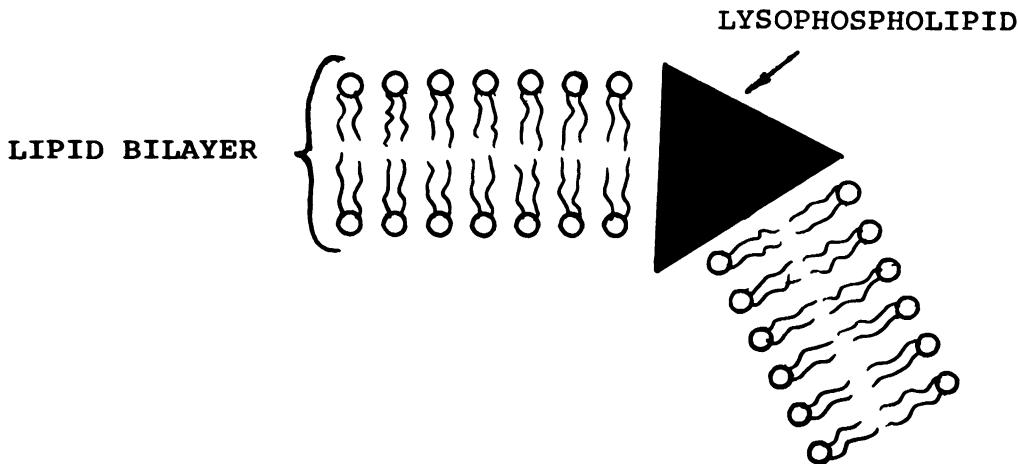


FIGURE 3. Sarcolemmal damage by lysophospholipids. For explanations see text.

ties. This feature allows them to interact with the phospholipids of the lipid bilayer and to alter its structure by penetrating into the bilayer and substituting for phospholipids. At high concentrations the amphiphiles form micelles which "break up" the membrane and ruin its integrity. The most "toxic" in this respect are lysophospholipids because of their wedge-like structure (Figure 3), and especially lysophosphatidyl choline.^{31,36,38} Such action results in enhanced permeability of the cell membranes to ions and particularly to Ca^{2+} . Specifically, it has been shown that when catecholamines act on the heart, activation of phospholipase A₂ and lysophospholipid formation induce increased permeability of the cardiomyocytal SR membranes and a release of Ca^{2+} therefrom,³⁹ with ensuing overloading of the sarcoplasm with this cation and cell damage.³¹ This mechanism appears to be also involved in the phenomenon of desensitization: a decrease in the number of operative β -adrenoreceptors in the heart under stress or excess catecholamines. Indeed, this phenomenon is prevented by phospholipase A₂ inhibitors suppressing phospholipid hydrolysis⁴⁰ and, conversely, induced by phospholipase A₂ activation.³⁵

Another detrimental factor of the lipotropic effect in intense or protracted stress are the products of lipid peroxidation (see Chapter 5).

In general, the overenhancement of the stress lipotropic effect in biomembranes may lead to their damage and be involved in the inactivation of ionic channels, receptors, and especially calcium and sodium cation pumps. As applied to the heart, the latter alteration increases Ca^{2+} and Na^+ concentrations in the sarcoplasm, decreases the membrane potential, and thereby becomes the cause of impaired electric stability of the heart, arrhythmias, and the above-considered cardiomyocyte damage by excess Ca^{2+} . In the final analysis the ionic disturbances in cardiomyocytes and their hypercalcium damage induced by the immoderate lipotropic effect apparently constitute the key link of cardiac damage in stress and the major cause of the damage to the contractile function of the heart and development of arrhythmias.

The third adaptive effect of stress is the mobilization of the energetic and structural resources of the organism as manifested by elevated blood levels of glucose, fatty acids, nucleotides, amino acids, and enhanced respiratory and circulatory functions. This effect comes to increasing the availability of oxidizable substrates, biosynthetic precursors, and oxygen for the organs performing extra work.

In assessing this adaptive effect of stress, it should be borne in mind that the chief role in mobilizing the carbohydrate reserve in the organism is played by catecholamines and glucagon which activate glycogenolysis and glycolysis via the adenylate cyclase system in

the liver, the skeletal muscle, and the heart.^{5,41} As this takes place, catecholamines are released at the earliest stage of the stress response, whereas glucagon is produced somewhat later^{42,43} and as if it "Duplicates" and "backs up" the effect of catecholamines. This last point can be of importance when the catecholamine action is not fully realized because of desensitization of β -adrenoreceptors by excess catecholamines, and the adenylate cyclase is activated through the glucagon receptors.⁴⁴ These hormones cause glucose to be supplied into the circulation directly from the carbohydrate depots of the liver and skeletal muscle. Another source of glucose comes from the effect of glucocorticoids and to some extent of the parathormone,⁴⁵ activation of proteolysis, increasing free amino acid pool, and activation of gluconeogenesis in the liver and skeletal muscle.^{7,8,45} Glucocorticoids act on their receptors to stimulate at the nuclear level the synthesis of gluconeogenetic enzymes glucose 6-phosphatase, phosphoethanol pyruvate carboxykinase, etc.⁷ The outcome of this activation is transamination of amino acids and production of glucose. It is in principle important that both hormonal mechanisms of glucose mobilization in stress ensure the timely supply of glucose to such vital organs as the brain and the heart. Under stress associated with acute physical load, especial significance is again acquired by the glucocorticoid-induced activation of the glucose — alanine cycle providing for glucose formation from amino acids directly in muscle tissue.⁴⁶

Mobilization of the lipid depots in stress is chiefly ensured by catecholamines and glucagon which, through the adenylate cyclase system, activate lipases and lipoprotein lipases in the adipose tissue, the skeletal muscle, and the heart.^{29,47,48} Hydrolysis of plasma triglycerides seems to involve the parathormone and vasopressin, secretion of which is substantially increased in stress.^{14,49,50} The fatty acid pool thus liberated is utilized in the heart and skeletal muscle.

A peculiar role in the mobilization and utilization of the energy resources in stress is played by the well-known phenomenon that catecholamines suppress insulin secretion and stimulate glucagon secretion.^{12,17} On the one hand, the increasing glucagon/insulin ratio promotes activation of gluconeogenesis in the liver; on the other hand, the decreased insulin level limits glucose utilization in the tissues. Under such conditions a certain advantage in the glucose supply is gained by the organs and tissues with adaptively enhanced blood flow, i.e., by the systems bearing the main load.

On the whole, mobilization of the energy and structural resources in stress is quite pronounced. Assessing this phenomenon, however, one should keep in mind that its adaptive role can be realized only on condition of the timely formation of a functional system responsible for adaptation which would utilize these resources to sustain the organ and tissue hyperfunction and then to form the systemic structural trace. Under conditions when the organism "cannot find a way out" of the situation and an environmentally adequate functional system (i.e., an adequate behavioral stereotype) is not formed, the protracted stress and attending intensive mobilization of resources cease to be an adaptive factor and entail progressive exhaustion of the organism.

Besides simple exhaustion, mobilization of the energy depots may lead to direct cardiac damage. This happens because in protracted stress the catecholamine activation of lipases and phospholipase A in the adipose tissue produces high blood levels of fatty acids and lysophospholipids, which by the mechanism considered above can alter the membrane lipid bilayer of cardiomyocytes and impair their function. The significance of this factor is directly confirmed by the experiments in which the heart was damaged by the known method of Rona et al.⁵¹ with large doses of the β -adrenomimetic isoproterenol. The size of cardiac necroses and lethality proved to be substantially greater in animals with excess adipose tissue. Recent studies⁵² using the same means of cardiac damage have shown that to a large extent the damage is effected through accumulation of the fatty acid derivative acyl carnitine, and correspondingly can be averted with an agent inhibiting carnitine synthesis, thereby

precluding acyl carnitine accumulation. These facts are very important for understanding the mechanism of cardiac damage in behavioral stress, since in such cases the β -adrenergic effect on the heart, i.e., essentially the isoproterenol-like effect, has been proved to be predominant.⁵³

Another possible cause of impaired cardiac function in prolonged stress is the significant decrease in the number of specific glucocorticoid receptors in the liver, which leads to attenuated hepatic synthesis of gluconeogenetic enzymes and attenuated glucose production.⁷ This increases the proportion of fatty acids among the cardiac substrates and may deepen their damaging effect.

The fourth of the main adaptive effects of stress can be defined as the “addressed transfer of the energy and structural resources from inactive systems to the functional system carrying out the adaptive reaction.”

An important factor of this selective redistribution of resources is the well-known locally originating “working hyperemia” in the organs of the system responsible for adaptation, with simultaneous narrowing of the vessels of “inactive” organs. It is indeed known that under stress induced by acute physical load the portion of the minute volume of blood flowing through the skeletal muscles increases fourfold to fivefold, whereas in the digestive organs, kidneys, and other tissues this index, conversely, decreases fivefold to sevenfold as compared to the resting state.⁵⁴ Nonanesthetized animals under heavy behavioral stress are known to consistently develop an enhanced coronary flow.⁵⁵ This also appears to happen at the expense of other organs.

The given mechanism ensures preferential supplies of oxygen and substrates to the organs that take the main load. The leading role in carrying out this stress effect belongs to catecholamines as well as to vasopressin and angiotensin II, whose secretion is activated in stress.^{14,16} These hormones narrow the vessels in those organs and tissues where this is not counteracted by the “working” hyperemia and mobilization of the closed (reserve) capillaries.

Some observations give grounds for thinking that the enhanced blood supply to the working organs at the expense of the “idle” ones does not exhaust the problem of the radical redistribution of resources in stress. Thus it has been noted that after surgical aortic ostial stenosis in rabbits, when the stress elevation of blood glucocorticoids activates hepatic gluconeogenesis and causes an almost complete involution of the thymus, this “catabolic effect” of stress in no way hinders the immense activation of nucleic acid and protein synthesis in the myocardium, and the cardiac mass in operated animals may increase by 80%.⁵⁶ Such facts prompt an idea that, besides the flow redistribution, the organs with enhanced function become less sensitive to glucocorticoids and are therefore capable of activating their nucleic acid and protein synthesis in proportion to the enhanced function.⁵⁷

Obviously, the redistribution of resources of the organism in stress, aimed at priority supplies to the system responsible for adaptation, regardless of its mechanism is an adaptive phenomenon.

Yet in an excessively pronounced stress reaction this may be attended by functional disturbances in and even damage to other systems not involved in the given adaptive reaction; for instance, ischemic ulceration of the gastrointestinal tract under heavy physical loads,⁵⁸ etc. A conversion of the redistributive effect from an adaptive to a detrimental one at the cardiac level seems hardly probable at first glance; indeed, behavioral stress studies with intact animals have shown that the β -adrenergic effect of catecholamines prevails, and owing to the vasodilatory action of cAMP ensures dilation of the coronary vessels and myocardial hyperemia even in pronounced stress.^{55,59} However, prolonged stress and high blood concentrations of epinephrine, vasopressin, and histamine may cause partial desensitization of β -adrenoreceptors and a coronary spasm developing under the action of these agonists on the Ca^{2+} -mobilizing receptors (see Figure 2).

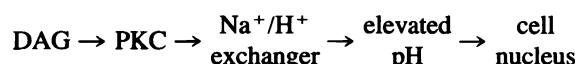
Such a coronary spasm has been experimentally demonstrated with vasopressin⁵⁰ and has also been observed in apparently healthy persons under stress.⁶⁰

Another way in which the effect of resource redistribution may turn into the effect of cardiac damage is that a drastic striction of the vessels of internal organs, in particular liver and pancreas, may cause a deep functional disturbance in these organs. The resulting seepage of trypsin into the blood from the pancreas⁶¹ and impaired cholesterol oxidation in the liver⁶² can in their turn directly damage the myocardium or be conducive to atherosclerosis as will be shown further.

The fifth adaptive effect of stress worth attention is that upon a single sufficiently heavy exposure to stress the above-mentioned "catabolic phase" of the stress response is followed by a much longer "anabolic phase". It manifests itself, in particular, as a generalized activation of nucleic acid and protein synthesis in different organs.⁶³ Such activation can promote the formation of systemic structural traces and development of stable adaptation to most various environmental factors. Thus, for example, it speeds up elaboration and fixation of temporary connections in the brain or provides for a more efficient response of immunocompetent organs to a foreign antigen.⁶³ To explain this adaptive effect of stress one should bear in mind the dynamics of hormonal shifts in stress and the function of the above-mentioned IP₃-DAG link in the regulation of cell processes. It has been shown that as the stress response develops, the initially elevated catecholamine and glucocorticoid concentrations decline with a concurrent rise in the levels of other hormones and specifically thyrocalcitonin, which restricts catecholamine and glucocorticoid secretion, causes deposition of Ca²⁺, and normalizes its concentration in the blood, thus acting as an "antipode" of the parathormone^{14,64} and as one of the stress-limiting mechanisms which will later be considered in more detail. Simultaneously, secretion is enhanced for hormones of direct anabolic action: somatotropic hormone, insulin, as well as thyroxin, which is known to promote the biosynthesis of mitochondrial structures.

Clearly, the action of these hormones on the cell can be of importance in the development of the anabolic phase of the stress response and in the growth of particular cellular structures that had taken the main load during the mobilization of the cell function.

Recent data give grounds for believing that a substantial part in the "delayed" anabolic effect of stress is played by DAG, shown above to be produced on phospholipid hydrolysis when agonists of the Ca²⁺-mobilizing receptors act on the cell (see Figure 2). DAG has been found to be a messenger which in the cells of different animals, by agency of PK C, activates the Na⁺/H⁺ exchange mechanism in the plasma membrane, thereby raising the cytosolic pH.^{24,65} According to present-day notions, the elevation of cytosolic pH activates nucleic acid and protein synthesis to the extent of cell proliferation.^{20,24,65} Thus, DAG is one of the main intracellular signals relaying information from the cell surface to the nucleus and playing a key role in cell growth initiation. It is conceivable that the stress-activated regulatory circuit



is involved in the delayed adaptive anabolic phase of the stress response. Moreover, it cannot be excluded that this regulatory mechanism ensures the coupling between the extent of function and the growth of cell structures at any load on the organ, e.g., in the development of cardiac hypertrophy caused by valvular diseases or hypertension as well as by repeated stress situations. In the general case, excessive stress activation of this regulatory circuit, combined with the stress immunodeficiency, may contribute to the oncogenic effect of stress.

As applied to the heart, of great interest is the possible role that the excessive activation of the cardiomyocyte genetic apparatus through this regulatory circuit may play in the

evolution of myocardial hypertrophy induced by catecholamines, and especially in the pathogenesis of incurable idiopathic cardiomegalias whose nature is obscure.

In assessing the transformation of the adaptive effects of stress on the heart into the detrimental effects, it should not be forgotten that we are dealing with a vitally important organ. Considering that all animal or human beings many times in their lifespan encounter grave, almost hopeless stress situations, one must admit that if all such basically possible transformations were actualized, then the mammals and specifically Homo sapiens should have long vanished off the face of the earth. In reality, a primary stress death from damage to the heart or other organs is a far rarer occurrence than the most severe and protracted stress situations. Most humans and animals experiencing such situations do not die and either do not fall ill at all or recover, i.e., the stress reaction and the effects of stress factors in the cells happen to be opportunely limited. Accordingly limited are the stress damages themselves. With the heart, for instance, even in the most severe stress it goes no further than a total but transitory impairment of the whole organ, and death of only separate cardiomyocyte groups with ensuing microfocal cardiosclerosis.⁶⁶ This means that the organism possesses potent mechanisms ensuring adaptation to stress situations. We shall further show that this is provided for by important features inherent in the very design of the stress reaction as well as by the modulatory stress-limiting systems linked with this reaction and restricting its intensity and duration. In this chapter, basing on the above concept of the transformation of the adaptive cardiotropic effects of stress into the detrimental ones, we shall concentrate on the cardiac stress damage as such.

Before proceeding to a systematic consideration of the functional, metabolic, and structural disorders of the heart under the damaging effect of stress, we should first deal with two essential points. The first one is to draw a clear discriminating line between the cardinal component of the stress response of a healthy organism and the genuine stress damage to the heart; the second is to demonstrate experimentally the very phenomenon of the transition of the stress effect from adaptive to detrimental, i.e., to prove the real existence of what we have been so liberally postulating.

Differentiating the cardinal component of the stress response from cardiac stress damage, we shall not delve into the rather complicated question of to what extent the stress situations causing human disease can be reproduced in animals. There seem to be sufficient grounds for the generally established opinion of researchers that such situations can be approximately simulated mainly in the mentioned conflict between a "need" to carry out a defense reaction and a "ban" on this reaction. In the course of such simulation studies it very soon became clear that the extent of stress and ensuing damage substantially increase if the basic elementary conflict is overlaid with additional inputs potentiating the activation of the emotional apparatus. Such additional inputs are introduction of signals notifying of an inevitable pain shock, and delivery of shocks at random and not too short intervals, engendering the expectation of pain associated with anxiety and fear. Of similar significance is the creation of additional conflicts: delivery of pain stimulation in response to a conditioned alimentary reaction or to a reaction by which the animal previously managed to avoid danger. The importance of these additional emotiogenic factors in the stress damage to internal organs has been first proved with ulcerous lesions of the gastric mucosa, which are the most convincing, visible, and reliably measurable stress damages.

Most clearly, the role of additional emotiogenic factors has been assessed in the studies where the stress intensity was judged from the decline in brain catecholamines and the stress damage from the area of the ulcerous lesions of the gastric mucosa developing in stress because of arteriolar spasm.⁶⁷ In the experiments of all researchers the introduction of emotiogenic factors further reduced the catecholamine concentration in the brain and appreciably expanded the ulceration area.^{68,69}

The comparatively easy induction of stress ulcers in the gastric mucosa make it in most cases clear enough whether the gastric stress damage has occurred or not. The heart does not usually develop macroscopically observable necroses even in quite serious stress situations. Therefore the question of the presence or absence of a stress damage proves much more complicated. Indeed, the mobilization of cardiac function is an indispensable link in the defensive, aggressive, sexual reactions in animals and in the major socially determined reactions in humans. It can be envisaged that in the basic conflict between these vital reactions and bans on their realization, i.e., in intense and protracted stress, the cardiovascular component of the reaction is not simply sustained despite the lack of reactions as such, but can be attended by myocardial damage. However, there is a far way to go from this assumption to the actual proof of stress damage to the cardiac muscle. This is so because, unlike the stomach where the ulcers are well seen, the heart provides much less definite indications of the damage to its structures. The changes in its performance even under heavy stress exposures often manifest themselves as quite normal phenomena like tachycardia or bradycardia. The numerous studies revealing such changes mean much for evaluating the cardial component of the behavioral reactions,⁷⁰⁻⁷² but do not per se solve the question of whether or not the heart is damaged by stress. Electrocardiographic shifts, even when there are stress disturbances to cardiac metabolism and structure, may be relatively small owing to a diffuse pattern of damage. The alterations of the contractile function are not infrequently limited to its mobilization. Thus, Randall et al.⁷³ used catheterization to study the contractile function of the left ventricle in nonanesthetized primates placed in a classical stress situation: a great "urge" for a defense reaction was created (an audio stimulus followed by electric pain stimulation) while the reaction itself was precluded by fixation. The response to the conditional audio stimulus was manifest in the rise of systolic pressure, higher contraction velocity, and tachycardia. Denervation experiments showed that this cardial component of the organismic reaction was determined mainly by excitation of the sympathetic cardiac innervation and to a much lesser extent by catecholamines in the circulating blood. Obviously these and numerous similar data sufficiently characterize the cardiac contractile function in stress conditions, but do not answer the question of whether or not the stress causes damage to the myocardium.

Later, the role of emotional stress was quite definitely proved in the studies by Miller and Malov.⁷⁴ There the stress situation was created for rats by electric pain stimulation through the chamber floor. Myocardial damage revealed itself in the pronounced leakage of enzymes from isolated hearts of stressed animals and in enhanced myocardial incorporation of technetium-labeled pyrophosphate routinely used to diagnose myocardial infarction. Upon administration of nembutal the same "dosage" of electric stimulation produced no cardiac damage. Thus, in a sufficiently long and intense stress we observe not merely an inclusion of the cardial component into the reaction, but genuine cardiac damage, caused not at all by such physical factors as electricity, mechanical pressure, etc., but by pain and impossibility to escape it, i.e., by an emotional factor.

The other point of interest — the transformation of the adaptive effect of stress into the detrimental one — was dealt with in our studies of the contractile function in isolated hearts taken from animals subjected to emotional pain stress (EPS) in the anxiety neurosis model according to Desiderato et al.⁷⁵ The duration of exposure to stress was varied to discern the phenomenon of transformation.

In the EPS model, male rats were starved for 1 d and then put into a special chamber in which electric current (4 mA) could be passed through the floor. The rats could avoid electric pain stimulation only by going to a platform in the center of the chamber. Consequently, the conditioned defense reflex of avoidance was developed quickly: the animals were constantly on the platform. Afterwards, short strong currents (6 mA for 2 s) were passed through the floor of the platform at random intervals for 6 h. The main features of

this EPS model consist in the conflict between the conditioned reflex of avoiding the current by going to the platform and the unconditioned pain stimulation on the platform, attended by tense and fearful expectation of pain because electric shocks on the platform occurred at random intervals.

This EPS strongly activated the hypophysial-adrenal and adrenergic systems, as shown by a threefold to fourfold increase in the amount of corticosteroids in the adrenal cortex, plasma, and cardiac muscle. Simultaneously, the amount of catecholamines decreased in the hypothalamus and the striate body as well as in the executive organs: cardiac muscle and small intestine (Table 1). This well-known phenomenon is due to the fact that in stress the release of catecholamines into the blood and their utilization in the target organs considerably outstrips their resynthesis. Subsequent restoration of the catecholamine content, as follows from Table 1, is much sooner achieved in the sympathoadrenal system itself (i.e., in the hypothalamus and the adrenal glands) than in the target organs where the norepinephrine concentration is still depressed 5 d after the stress exposure. In whole, the restoration process is conditioned by the poststress activation of catecholamine biosynthesis.⁷⁶ Accordingly, Table 2 shows that after EPS the auricles increase ^3H -norepinephrine synthesis from ^3H -tyrosine by 40% over the control. Conversely, the neuronal uptake of ^3H -norepinephrine declines by 42% immediately after EPS and remains at that level for 1 d. This apparently provides for increased mediator concentration in the synaptic spaces, i.e., for enhanced adrenergic effect.

It should be noted that the activation of the adrenergic and hypophysial-adrenal system in EPS consistently leads to stress damages. In our experiments, as in the experiments of other researchers, the gastric mucosa rapidly developed ulcerous lesions typical of stress. Thus, these experiments reproduced a serious detrimental stress situation.

In these experiments the hearts were obtained under urethane anesthesia 2 h after the end of the stress exposure and studied for their contractile function in isovolumic conditions according to Fallen et al.⁷⁷ After a 60-min perfusion of the heart under normal oxygenation, the oxygenated and glucose-containing Krebs-Henseleit solution was replaced with the non-oxygenated and glucose-lacking one. This hypoxic exposure lasted 20 min and was followed by reoxygenation. The coronary flow was assessed as the amount of liquid passing through the coronary bed per unit time.

As follows from the data of Table 3, under aerobic conditions the hearts of animals subjected to short-term stress do not differ from the controls in the developed and diastolic pressure, but the pressure drop rate and the coronary flow increase by about 40%. On the contrary, the cardiac muscle of animals exposed to prolonged stress exhibits a depressed contractile function, with the developed pressure and the pressure buildup and drop rates decreased roughly by one third. The diastolic pressure, which in the given experimental conditions reflects the dynamics of the diastolic myocardial tension, proved to be 2.5 times higher.

It is evident in Table 3 that the major difference between the effects of short and long stress reveals itself in myocardial hypoxia. Thus, under hypoxic conditions the contractile function and coronary flow parameters in the hearts of animals subjected to a short stress exceeded the controls, while the hypoxic contracture was abated by a factor of 2.6.

During reoxygenation all the myocardial contraction and relaxation parameters in animals exposed to a short stress were completely restored within the first minute, and the hypoxic contracture disappeared. In the control animals at the same stage of reoxygenation the developed pressure and the pressure buildup and drop rates remained diminished by more than half.

The reaction to hypoxia in animals that had undergone a long stress was marked by a decline in the basic contraction and relaxation parameters as in controls, but the hypoxic contracture at 20 min of hypoxia was more pronounced. During reoxygenation the hypoxic

TABLE 1
Norepinephrine Content (ng/g) in Rat Tissues Upon Emotional Pain Stress (EPS)

Conditions	Myocardium	Small intestine	Hypothalamus	Striate body	Adrenal gland		
Control	1117 ± 8	732 ± 42	900 ± 43	244 ± 38	157	285 ± 14	213
During EPS	864 ± 45 ^a	477 ± 32 ^a	688 ± 61 ^a	167 ± 32	133	692 ± 12	082
After EPS				—	109	622 ± 10	372 ^a
2 h	841 ± 31 ^a	434 ± 31 ^a	633 ± 72 ^a				
24 h	616 ± 81 ^a	415 ± 61 ^a	757 ± 49	166 ± 41	242	747 ± 26	754 ^a
2 d	658 ± 41 ^a	574 ± 42 ^a	786 ± 138	218 ± 23	199	695 ± 22	758
5 d	924 ± 49	616 ± 49	823 ± 108	204 ± 39	157	319 ± 15	029
10 d	—	694 ± 68	834 ± 52	251 ± 18	143	547 ± 14	384

^a $p < 0.01$.

TABLE 2
Uptake and Synthesis of ³H-Norepinephrine (cpm/g) in Auricles Upon EPS

	Uptake	Synthesis
Control	90 031 ± 6470 (n = 15)	7155 ± 677 (n = 12)
During stress	52 227 ± 13 541 ^a (n = 10)	9772 ± 1182 ^b (n = 6)
After stress		
2 h	—	9590 ± 579 (n = 6)
24 h	54 064 ± 6011 ^c (n = 9)	9555 ± 1119 ^c (n = 6)

^a $p < 0.02$.

^b $p < 0.05$.

^c $p < 0.01$.

contracture, as indicated by the diastolic pressure, disappeared much more slowly in long-stressed than in control animals. Thus, a brief exposure to stress enhances the resistance of the cardiac contractile function to hypoxia, whereas prolonged stress produces an opposite effect.

The data in Table 4 show the release of creatine phosphokinase from the isolated heart into the perfusate under aerobic, hypoxic, and reoxygenation conditions. As follows from Table 4, the loss of the enzyme by the heart is minimal under normal oxygenation and increases in hypoxia. During reoxygenation still more enzyme is released into the perfusate. Notably, in both hypoxia and reoxygenation the enzyme leakage from the hearts of animals subjected to a short stress is 36 and 26% lower as compared to the controls, whereas with the hearts of animals subjected to a long stress it is, conversely, almost twice as high.

The loss of enzymes by the cardiac muscle results from energy-deficient damage to the cardiomyocyte sarcolemmal membrane and correlates quantitatively with the decline in myocardial ATP.⁷⁸ Therefore, the data obtained testify that a preliminary short exposure to stress attenuates the hypoxic damage to the membrane apparatus of cardiomyocytes, while a long one aggravates it.

By and large, the results of the experiment show that an intense but brief EPS exposure elicits quite stable alterations in the cardiac muscle, which are retained when the organ is separated from the neurohumoral influences of the whole organism. If the stress is protracted

TABLE 3
Effects of Short and Long EPS on Myocardial Resistance to Hypoxia and Reoxygenation

Indices	Series	Aerobic	Hypoxia 20 min	Reoxygenation 1 min
Developed pressure mmHg	Control	100.3 ± 4.1	22.9 ± 1.4	53.5 ± 5.1
	1 h EPS	102.2 ± 3.9	38.6 ± 2.1 ^a	100.9 ± 6.9 ^a
	6 h EPS	68.4 ± 4.9 ^a	14.9 ± 1.2 ^a	44.6 ± 3.8
Diastolic pressure mmHg	Control	4.3 ± 1.3	40.3 ± 3.2	20.3 ± 2.5
	1 h EPS	4.4 ± 2.1	15.0 ± 2.4 ^a	6.1 ± 1.4 ^a
	6 h EPS	8.1 ± 0.8 ^a	48.5 ± 3.5 ^a	32.6 ± 6.7 ^a
Max pressure buildup rate mmHg/s	Control	1900 ± 89	400 ± 27	875 ± 69
	1 h EPS	2150 ± 120	875 ± 92 ^a	2016 ± 190 ^a
	6 h EPS	1380 ± 110 ^a	286 ± 92 ^a	586 ± 80 ^a
Max pressure drop rate mmHg/s	Control	1000 ± 84	188 ± 22	538 ± 55
	1 h EPS	1451 ± 120 ^a	550 ± 20 ^a	1125 ± 108 ^a
	6 h EPS	840 ± 45 ^a	126 ± 12 ^a	375 ± 108 ^a
Coronary flow ml/min	Control	8.0 ± 1.4	6.0 ± 0.8	6.5 ± 0.5
	1 h EPS	11.0 ± 1.2	11.3 ± 1.1 ^a	11.2 ± 0.7 ^a
	6 h EPS	7.5 ± 0.6	4.9 ± 0.3	6.5 ± 0.8

Note: Control (n = 12), 1 h EPS (n = 9), 6 h EPS (n = 12).

* Difference from control significant at $p < 0.05$.

TABLE 4
Effects of Short and Long EPS on The Leakage of Myocardial Creatine Phosphokinase into the Perfusate in Hypoxia and Reoxygenation

Series	CPK activity in the perfusate (IU/l)		
	Perfusion 90 min	Hypoxia 20 min	Reoxygenation 2 min
Control (n = 10)	9.8 ± 0.9	34.0 ± 2.4	49.5 ± 2.8
1 h EPS (n = 18)	10.0 ± 0.9	21.5 ± 3.5 ^a	29.6 ± 3.9 ^a
6 h EPS (n = 17)	12.4 ± 1.2	72.9 ± 5.0 ^a	87.3 ± 4.1 ^a

* $p < 0.05$.

(duration increased sixfold) these positive changes are completely abolished, displaced by the decreasing resistance of the heart to hypoxia.

Thus, these experiments made it possible to observe directly how the adaptive effect of stress turns into the detrimental one. The significance of this phenomenon for pathology in general, and cardiac pathology specifically, cannot be overemphasized; we therefore thought it worthwhile to verify it using a basically different technique which allows the cardiac contractile function under isotonic conditions (and primarily the cardiac resistance to the contractural and arrhythmogenic action of excess Ca^{2+}) to be compared upon brief and prolonged exposures to stress created with the same method of Desiderato et al.⁷⁵

The curves in Figure 4 depict a typical experiment with heart perfusion according to Langendorf. Here it should be borne in mind that the upper outline of the mechanogram corresponds to maximal relaxation and the lower to maximal contraction of the isotonic heart. It can be seen that at normal Ca^{2+} concentration the 1-h stress, unlike the 6-h one, slightly increases the amplitude and velocities of contraction and relaxation. An immense difference between the 1- and 6-h stresses is observed under Ca^{2+} loading. After the 1-h stress, contracture and extrasystoles are virtually absent in contrast to the control; there is

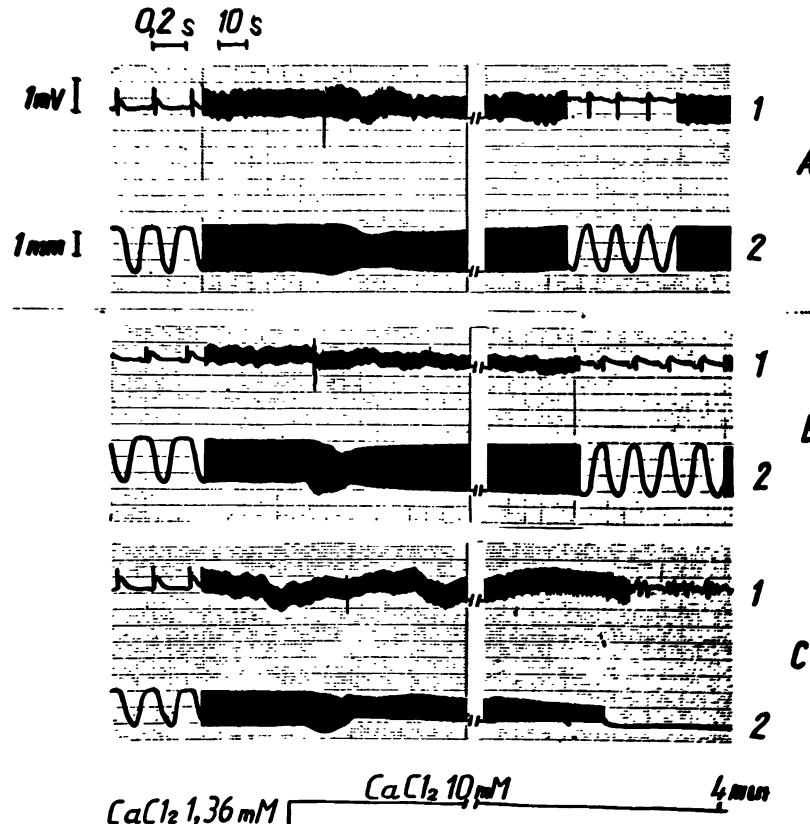


FIGURE 4. Effect of an increase of calcium concentration from 1.36 to 10 mM on the function of isolated hearts from control rats (A) and rats subjected to a 1-h (B) and a 6-h (C) EPS. 1, ECG; 2, mechanogram of the isotonic heart. For explanations see text.

also no cardiac fibrillation. Contrariwise, after the 6-h stress the scope (percent of initial contraction amplitude) and area (the product of scope by duration, percent of the contraction area) of contracture are about twice those in the control; there are numerous extrasystoles, and at the third minute of exposure to Ca^{2+} irreversible cardiac fibrillation develops.

This result provides unequivocal evidence that a short stress, through its adaptive effects described earlier, enhances the cardiac muscle resistance to Ca^{2+} overloads. A long stress, because of the detrimental shifts also described earlier, entails an opposite result, reducing the efficiency of the membrane calcium-transferring mechanisms and ultimately decreasing the resistance of the heart to the contractual and arrhythmogenic action of excess Ca^{2+} .

Here we must underline the significance of this phenomenon at the level of the whole organism, where the damaging effect of excess Ca^{2+} is the common final link of the pathogenetic chain in most various cardiac conditions from ischemic disease to hereditary cardiopathies and, as we shall see, a key link in the pathogenesis of primary stress damage to the heart.

The adaptive effect of stress on the heart and its transformation into the detrimental effect constitute an important reality for the cardiologist.

However, notwithstanding the elegance and instructiveness of this concept, the most recent experiments in our laboratory show that it pertains to the "stress of struggle", the one most widely represented in nature and experiment, when animals or people finding themselves in a difficult, hazardous, or even injurious situation can carry out some active

reactions in search for a way out. Other stress conditions that absolutely exclude even seeking an escape, for example, when the animal is fixed by all four extremities, bring about the "stress of capitulation" where the dynamics of events in the heart is different, and in a sense opposite to that just described for the emotional pain stress. The data in Table 5 present the experiments where one group of rats was subjected to 2 h and the other to 6 h of immobilization stress by four-limb fixation. As can be seen, the hearts obtained from animals after 2 h of the immobilization stress were distinguished by a definite decrease in the contraction amplitude and in the contraction and relaxation velocities at physiological Ca^{2+} concentrations. When the Ca^{2+} concentration was raised by a factor of 7.3, the maximal contracture scope and area were increased threefold as compared to the controls. Finally, at the fourth minute of hypercalcium perfusion more than half of these hearts developed ventricular fibrillation. There is thus no doubt that after 2 h of the capitulation stress the membrane mechanisms of calcium transport in cardiomyocytes had been quite deeply impaired and the heart resistance to the arrhythmogenic and contractual action of excess Ca^{2+} had been reduced.

The hearts obtained after the 6-h immobilization stress did not differ from the controls (Table 5). This of course does not mean that all damages there had disappeared, but demonstrates that the decline in the resistance to the adverse effects of excess Ca^{2+} had worn off in animals that had capitulated long before their hearts were taken for examination.

That such dynamics of the heart resistance to excess Ca^{2+} is directly linked with the performance of the SR calcium pump will be shown by the results of biochemical studies presented later. Yet the whole question of the mechanism of such immediate adaptation to forced capitulation, as well as the more general issue of the neurohumoral, cellular, and molecular differences between the stress of struggle and the stress of capitulation are, in our opinion, an important subject for further studies.

Hereinafter we shall consider the experimental data from this laboratory on the stress damages to the contractile function, electric stability, ionic transport, and energy metabolism constituting the basis of the primary cardiac stress damage. Then, using clinical data we shall assess the significance of such damage from the clinical point of view.

III. STRESS DAMAGES TO THE CARDIAC FUNCTION

The studies conducted for the last 15 years in our laboratory show that even upon prolonged and heavy stress or administration of a large dose of the synthetic β -adrenoreceptor agonist isoproterenol, in conditions of the whole organism the disturbances of cardiac contractile function prove to be moderate and may often be only demonstrable with such special techniques as imposing a maximal load on the heart by aortic clamping, imposing a high contraction frequency, etc. At the same time with the isolated heart these disturbances, which in the whole organism would have been masked owing to the regulatory mobilization of compensating factors, can be revealed definitely enough.

In accordance with this, we shall now consider the disturbances of the contractile function of the isolated right auricle and ventricular papillary muscle caused by prolonged (6 h) EPS.

The atrial contractility and elasticity were studied in isometric spontaneous contractions with the use of a DMP-4F Physiograph, Narco Biosystems, U.S. The auricle was placed in a constant-temperature (34°C) bath with oxygenated Krebs-Henseleit solution (95% O_2 /5% CO_2) where the auricle itself was fixed immovably while the top of the auricula was attached to an F-50 myograph coupled with a spring micrometer. The auricle was left to contract spontaneously for 40 to 50 min and then gradually stretched with the micrometer to a length at which it developed the maximal systolic contraction force (T_{\max}), i.e., to the maximal physiological length (L_{\max}). The changes in length were recorded for each 100-mg increment of the stretching load. The load (mg) corresponding to L_{\max} was taken as the maximal tension at rest (T_r).

TABLE 5
Effect of the Duration of Immobilization Stress on the Indices of Contractility, Arrhythmias, and Contracture of Isolated Hearts at a 7.3-Fold Increase of the Ca^{2+} Concentration in the Perfusing Solution

Series	Heart rate	Contraction amplitude (mm)	Contraction velocity (mm/s)	Relaxation velocity (mm/s)	Contracture %		Ventricular fibrillation (cases)
					Scope	Area	
Control (n = 15)	274 ± 8	2.60 ± 0.08	51.0 ± 2.8	57.6 ± 3.0	21 ± 4	10.0 ± 1.8	5 ± 2
2-h stress (n = 11)	269 ± 8	2.07 ± 0.16 ^{p < 0.01*}	42.8 ± 3.4	46.8 ± 4.2 ^{p < 0.05*}	59 ± 17 ^{p < 0.05*}	35.8 ± 10.1 ^{P < 0.02*}	7 ± 3
6-h stress (n = 8)	272 ± 6	2.66 ± 0.14	54.6 ± 3.6	64.6 ± 3.8	18 ± 6	9.4 ± 3.9	3 ± 2

* Student's criterion.

† χ^2 Criterion with the Yate's correction.

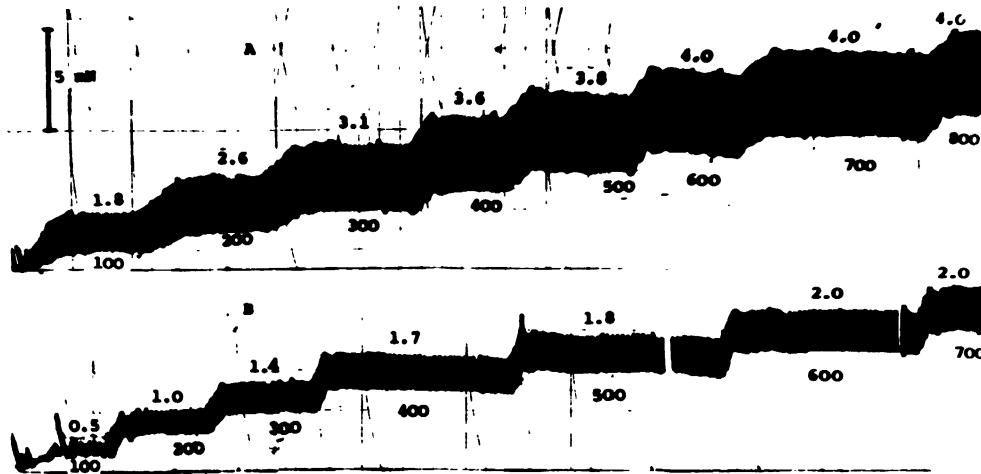


FIGURE 5. Mechanograms of the isometrically contracting right auricles from (A) control and (B) EPS animals. The numbers above the tracing denote the developed force in mN; the numbers below the tracing denote the muscle-stretching load in mg.

The mechanograms in Figure 5 present a typical experiment and provide an estimate of the depression of the myocardial contraction force in animals subjected to stress.

Atrial extensibility was judged from the "load — length" dependence in the course of extension to L_{max} . Atrial contractility was judged from the maximal contraction force (mN), frequency of spontaneous atrial contractions (min^{-1}), and the integrative index "maximal intensity of functioning of structures" calculated as the ratio of the product of T_{max} and contraction frequency to the atrial mass and expressed in mN/mg·min. The state of the self-regulatory mechanism of myocardial contractility was assessed by the dependences "load — force" and "length — force" (the Frank-Starling dependence) and by the index $\Delta T/\Delta L$ (mN/mm). The $\Delta T/\Delta L$ index denotes the increment of atrial contraction force in response to each 1-mm increment of atrial length during stretching, and reflects the "functional value" of the extension of the auricle by 1 mm. This index, together with the Starling curve, characterizes the realization of the Frank-Starling mechanism.

The curves in Figure 6 show that auricles of the animals that have undergone EPS exhibit lower extensibility and contraction force. The decreased myocardial extensibility would impede the elongation of cardiomyocyte sarcomeres in diastole and therefore restrict the realization of the Frank-Starling mechanism. Indeed, it is evident from Figure 6 that the auricles of rats exposed to EPS when stretched develop about half the contraction force than do the controls. Thus, obviously, one of the causes of the poststress depression of the atrial contractile function is the lesser extensibility that handicaps the Frank-Starling mechanism (Figure 6D).

The two basic facts established in these studies, namely the decreased atrial extensibility and the depressed Starling curve, give grounds for believing that one of the main causes of impaired cardiac contractile function in stress is the upset of the myocardial self-regulatory mechanisms. Reduced myocardial extensibility may hinder optimal sarcomere elongation in diastole, decrease the diastolic volume, increase the diastolic pressure, and interfere with ventricular filling; combines with the lesser specific increase in force per unit muscle length, all this can apparently impair the pump function of the heart in whole.

The data presented pertain to the myocardium of the auricles, so to prove the validity of the above assertion it was necessary to check whether or not the poststress reduction of extensibility and the attending depression of contractility also occur in the myocardium of

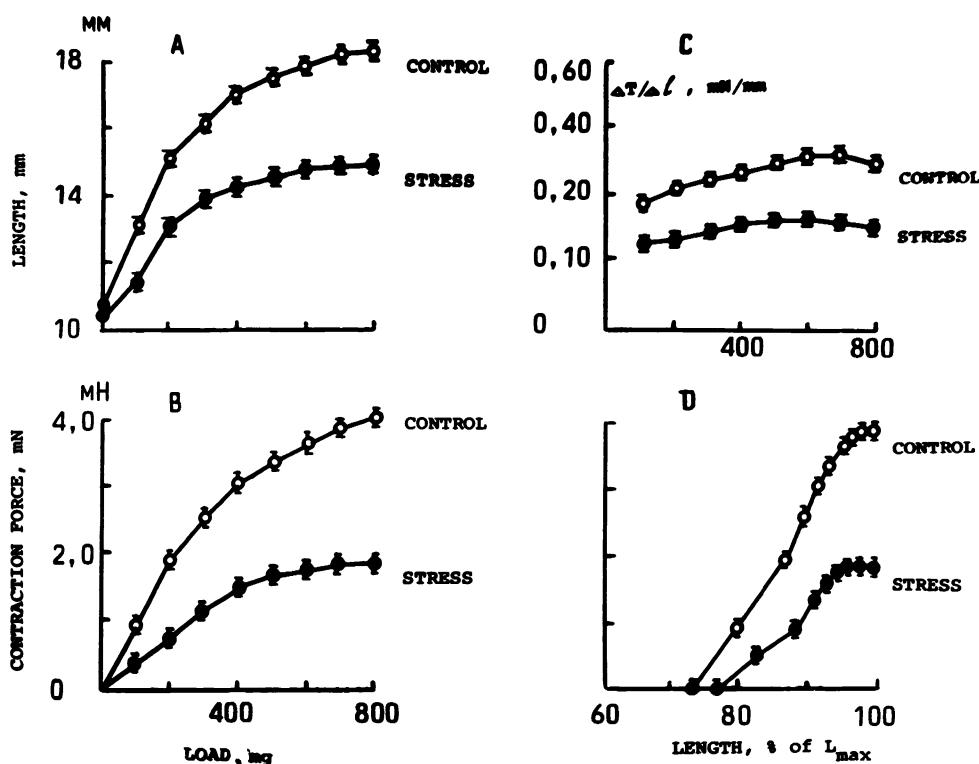


FIGURE 6. Effect of EPS on the rat right atrial extensibility and contraction force. The stress can be seen to depress (A) atrial extensibility, (B) contraction force at equal stretching load, (C) increment of force per unit increment of atrial length ($\Delta T/\Delta L$), and (D) the Starling curve.

the ventricles which are central in the pump function of the heart. To this end, in the next series of experiments we studied the effect of EPS on the extensibility and contractile function of the left ventricular posterior papillary muscle.

The relevant results are represented by curves in Figure 7 and testify that the alterations caused by EPS in the left ventricular myocardium are similar to those in the auricles. It is shown that in rats subjected to EPS the elongation of the papillary muscle under a similar load is 1.5 to 2 times less than in controls. Reduced extensibility of the ventricular myocardium combines with depressed contraction amplitude. Thus, in EPS rats the amplitude of isotonic contractions of the papillary muscle is on average 18 to 24% lower compared to the control ($p < 0.01$).

The data obtained provide evidence that reduced extensibility and hampered operation of the Frank-Starling mechanism constitute a typical manifestation of the poststress damage to both atrial and ventricular myocardium, and allow one to think that these alterations may be involved in the impairment of the pump function of the heart.

In further studies it was deemed important to elucidate the extent to which the stress damage to the elasticity and contractility of the atrial myocardium was reversible. The experiments showed that both the poststress myocardial rigidity and the reduction in its contractile force were transitory: they were most pronounced 2 h after the end of stress, still definite but much abated in 1 d, and not observable in 3 d.

Prior administration of the α -adrenoblocker phentolamine to animals proved to have no effect on the development of the complex of myocardial functional disorders typical of stress. By contrast, administration of the β -adrenoblocker propranolol completely abolished the upset of extensibility and contractile function. The curves in Figure 8 reflect this protective

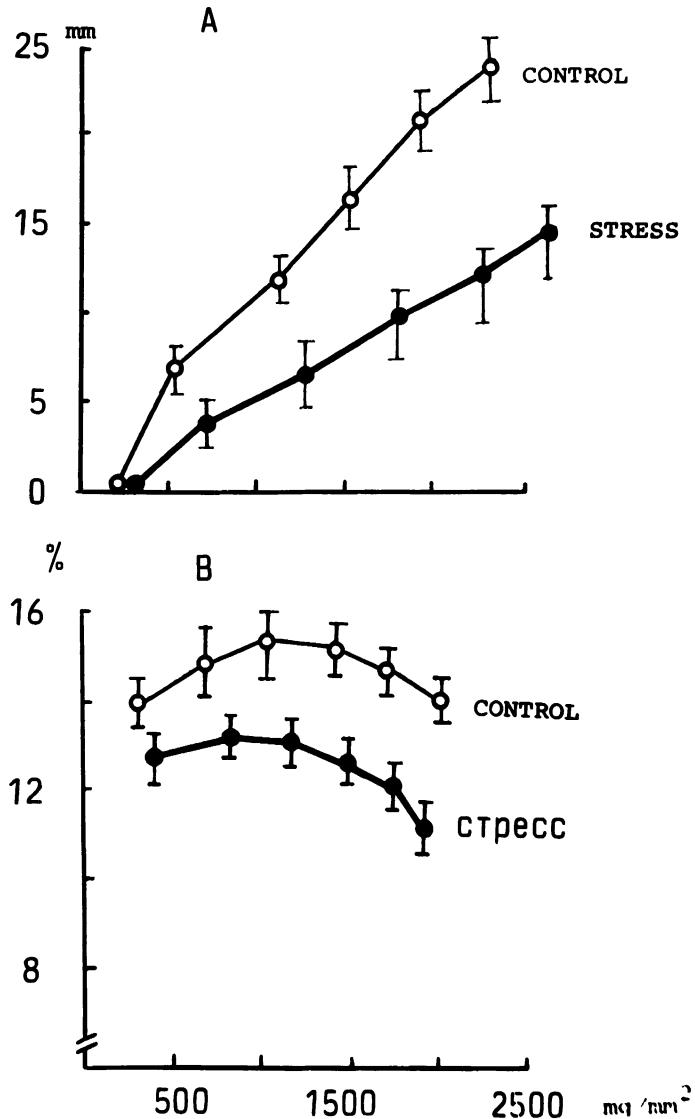


FIGURE 7. Effect of stress on the (A) extensibility and (B) contraction amplitude of the left ventricular papillary muscle. The abscissa is the load in mg/mm²; the ordinates are (A) increase in muscle length (length at the 250 mg/mm² load taken as 100%); (B) contraction amplitude in mm.

effect of the β -blocker and indicate that the discovered phenomenon of stress rigidity is due precisely to the excessive β -adrenergic action of surplus catecholamines.

The question of how this action leads to the observed impairment of extensibility and contractility is rather complicated. Discussing it we should bear in mind that in accordance with the present day notions of cardiac contractility⁷⁹ the physiological extensibility of the myocardium and the realization of the Frank-Starling mechanism are in great measure determined by the state of the relaxation mechanism. The "residual" actomyosin bridges retained in the sarcolemma during diastole can affect the tension of rest. Obviously the increasing number of residual bridges, whatever their cause, may promote the "internal shortening" of myofibrils and reduce myocardial extensibility. The process of relaxation

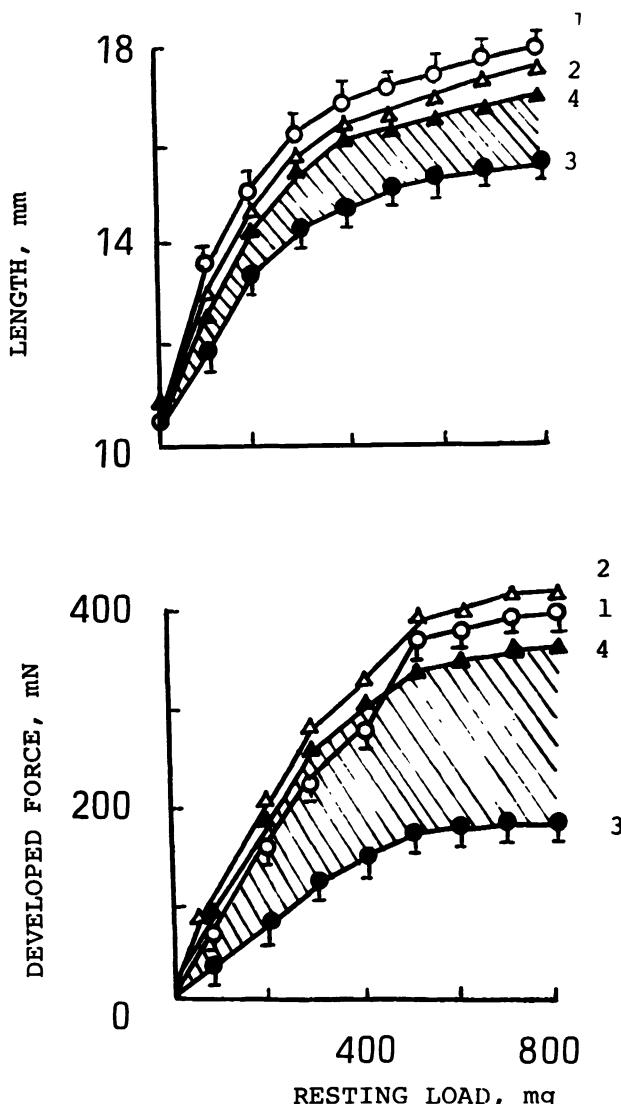


FIGURE 8. Effect of stress and propranolol of the rat right atrial (A) extensibility and (B) contraction force. The abscissa is the load at rest in mg; the ordinates are (A) atrial length in mm, (B) developed force in mN. 1, Control; 2, propranolol; 3, stress; 4, propranolol plus stress.

and dissociation of the actomyosin bridges formed in systole is known to be provided for by two major factors: (1) energy supply to the myofibrils, comprising ATP synthesis by the mitochondrial and glycolytic systems, energy transfer through the creatine kinase system, and ATP resynthesis in the contractile apparatus,⁸⁰ and (2) prompt removal of Ca^{2+} from this apparatus, performed by the calcium pumps of the sarcolemma and SR. The two factors are inseparable since the calcium pumps need ATP.

It will be further shown that both the calcium pump operation and the myocardial energetics are impaired in stress. While studying the myocardial stress rigidity we tried to make a rough assessment of these factors, and to this end compared the influence of EPS on the development of calcium and hypoxic contracture.

Myocardial resistance was estimated from the dynamics of contracture development after the end of exposure to hypoxia and excess Ca^{2+} , at the plateau of the Starling curve.

Figure 9 demonstrates that upon EPS the hypoxic and calcium contractures are reliably more pronounced than in control. These shifts agree with the observations (to be considered below) of the impairment of the calcium pumping and energy supplying systems in primary cardiac stress damage and, as well as stress rigidity, are virtually completely prevented by blocking the β -receptors.

A more comprehensive study of the damaging effect of stress was then carried out by the method of Neely et al.⁸¹ with hearts isolated 2 h after the end of EPS. The stroke volume and the systolic pressure in the hearts of stressed animals proved to be decreased by 10 to 20%. As a result, the external work of such hearts in steady-state conditions was one third lower. Oxygen consumption by the cardiac muscle was the same as in control, hence the cardiac efficiency was also one third lower.

However, the major result of the experiment was the dramatically enhanced response of the hearts of animals that had undergone stress to the changes of Ca^{2+} concentration in the perfusing solution (Figure 10). When the Ca^{2+} concentration was lowered from 2.5 to 1.25 mM the decrease in the stroke volume of the isolated heart was less than 40% in the controls, but more than twice was great after EPS. In response to a subsequent increase of the Ca^{2+} concentration from 1.25 to 7.5 mM the stroke volume was restored by 20 to 30% of the initial value in the controls and by 70% in the hearts of animals subjected to stress. These alterations were vivid in the first 3 to 5 min after Ca^{2+} shifts, whereas further on, the cardiac functions in both groups gradually returned to the initial levels owing to the adaptation of the ion transporting mechanisms.

Thus, stress damages to the membrane calcium pump drastically enhance the dependence of the cardiac contractile function on the Ca^{2+} level in the liquid flowing through the heart. This is in accord with the comparative physiological data that the reaction of muscle to a changing external concentration of Ca^{2+} depends on the capacity of the mechanisms for its transport. So in the skeletal muscle the well-developed SR system ensures that almost all Ca^{2+} released during the action potential into the sarcoplasm to initiate myofibrillar contraction is then returned to the SR. Thanks to such nearly 100% recycling, the skeletal muscle can contract in a calcium-free solution for several hours. In the myocardial cells of mammals the Ca^{2+} transporting mechanisms of the SR and sarcolemma are not so powerful and the recycling is not complete, hence in a calcium-free solution the heart ceases functioning after a few dozen contractions. In the frog heart the SR is negligible, and it stops instantly upon removal of Ca^{2+} from solution.⁸²

Taken together, the available facts evidence that the greater dependence of the cardiac function on the external Ca^{2+} concentration in animals that have undergone stress results from impaired membrane transport of Ca^{2+} .

The results of experiments on the papillary muscle of animals subjected to EPS are in accord with this conclusion and, besides, furnish some additional information on the primary stress damage to myocardial contractility.⁸³

The contractile function of the isolated left ventricular papillary muscle was studied under electric pacing of its isotonic contractions at an optimal load providing maximal physiological length, in the Krebs-Henseleit solution with 95% O_2 /5% CO_2 at $29 \pm 1^\circ\text{C}$. At imposed contraction frequency the amplitude and the velocity of contraction and relaxation of the papillary muscle from animals that had undergone stress proved to be decreased 2 to 3-fold.

The experiments were mostly aimed at elucidating the effect of stress on the response of papillary muscle to the shifts of Ca^{2+} , Na^+ , and H^+ concentrations in the perfusing solution.

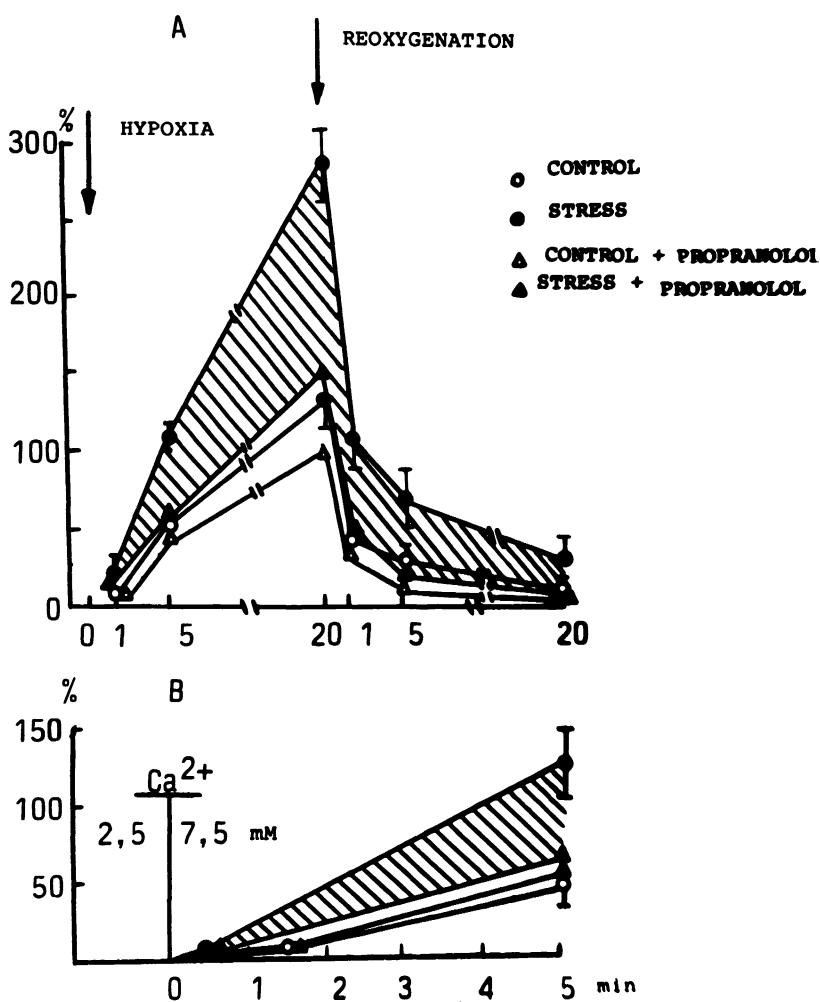


FIGURE 9. Effect of stress and propranolol on the contracture caused by (A) hypoxia and (B) increased Ca^{2+} concentration. The abscissa is time in min; the ordinate is the contracture index (ratio of the increment in tension of rest to the initial developed tension, percent).

It has been shown that upon stress the depressed myocardial contractility is accompanied with its higher sensitivity to the changes in extracellular concentrations of Ca^{2+} as well as of the natural antagonists of this cation, Na^+ and H^+ , which are known to compete with Ca^{2+} for the binding sites in the sarcolemmal structures and to be, at increasing concentrations, capable of displacing Ca^{2+} from these binding sites and thereby reducing the contractile function of the cardiac muscle.^{84,85}

Figure 11 presents the results of studying the negative inotropic effect of Ca^{2+} antagonists — Na^+ and H^+ — after stress. Increasing the H^+ concentration (lowering the pH from 7.4 to 6.85) or the Na^+ concentration (adding sodium chloride to 165 mM) in the Krebs-Henseleit solution flowing around the papillary muscle elicits a typical decrease in the myocardial contractile indices (the negative chronotropic effect due to competitive displacement of Ca^{2+} by these cations). The stress potentiates this effect considerably, as seen in Figure 11: excess Na^+ or H^+ depresses the myocardial contractile function approximately twice more in animals that have undergone stress than in the controls.

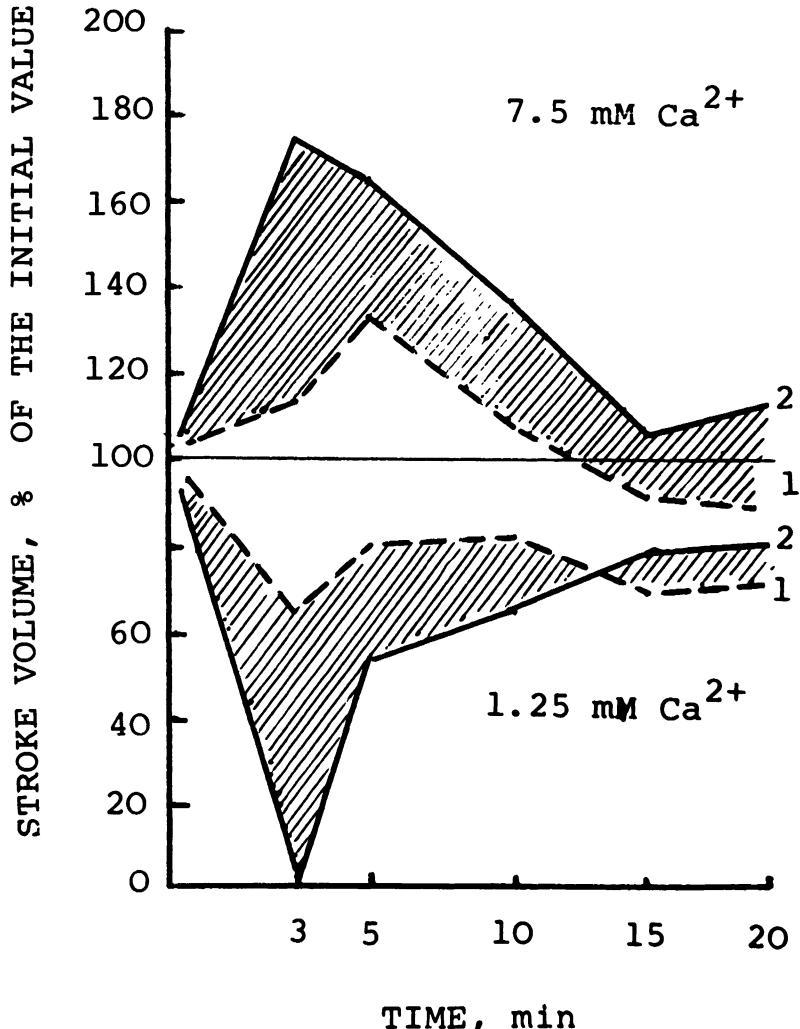


FIGURE 10. Dynamics of the response of isolated working hearts from (1) control and (2) stress animals to the changes of calcium concentration in the perfusing solution. The abscissa is time in min; the ordinate is the stroke volume, percent of initial. The shaded zone reflects the effect of stress. (A), Response to an increase from 1.25 to 7.5 mM Ca^{2+} ; (B), response to a decrease from 2.5 to 1.25 mM Ca^{2+} .

In assessing the results of these experiments, it should be emphasized that the cationic tests for the Ca^{2+} binding and transporting ability of the membrane apparatus to a certain extent mimic the cationic disturbances in the myocardium typical both of maximal physical loads (where they limit the enhancement of cardiac function)⁸⁶ and of cardiovascular diseases.^{87,88} In particular, the experimentally created excess of H^+ (lowered pH) in the extracellular space mimics the condition attending many diseases including ischemic heart damage, namely metabolic acidosis in which excessive amounts of HCO_3^- and H^+ ions appear in the blood and pass through the membranes into the cells.⁸⁵ The lower myocardial resistance to excess Ca^{2+} and Na^+ in stress, as will be shown in Section VII, is caused by the impaired performance of the calcium and sodium membrane pumps in the cardiomyocytes. By analogy it can be thought that the lower resistance of myocardial contractility to excess protons is caused by impaired performance of the sarcolemmal Na^+-H^+ pump responsible for timely removal of H^+ from the sarcoplasm.

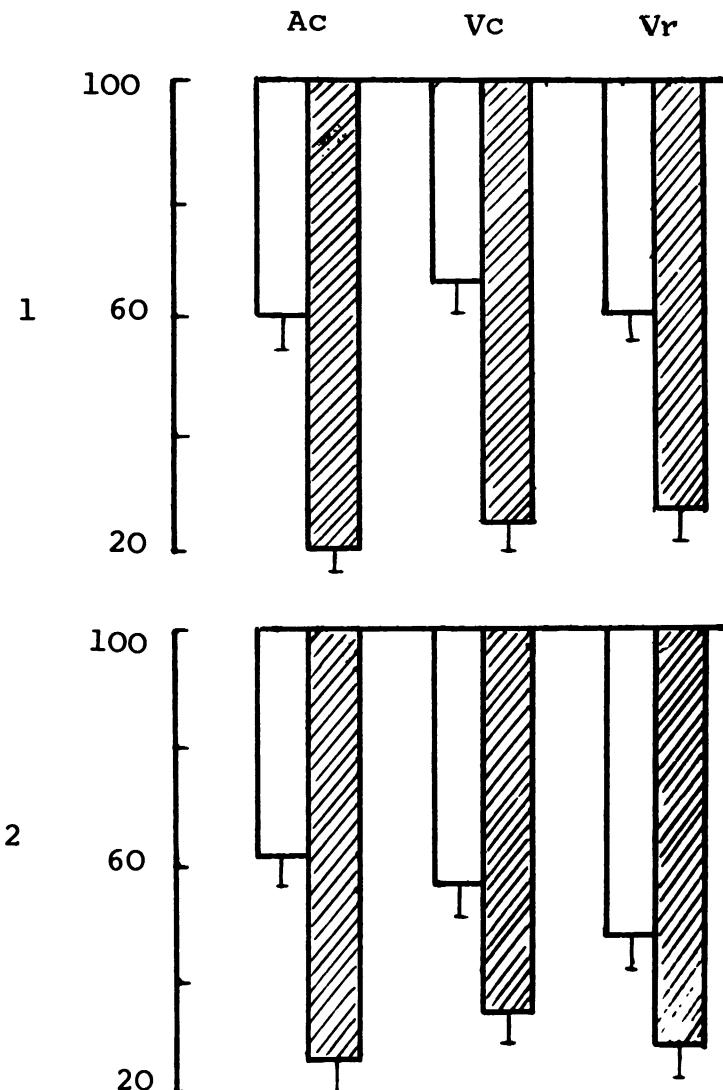


FIGURE 11. Effect of stress on the inotropic response of the papillary muscle to shifts in Na^+ and H^+ concentrations. The ordinate is the relative contractile indices, with 100% assigned to those at physiological ion levels. Ac, Contraction amplitude; Vc, contraction velocity; Vr, relaxation velocity. Empty columns, control; shaded columns, stress. 1, Increase of Na^+ concentration to 165 mM. 2, Decrease of pH to 6.85.

In the aggregate, there is unequivocal evidence that a single, long enough stress impairs the contractile function of the cardiac muscle, gives rise to a peculiar syndrome of myocardial stress rigidity, decreases the force, amplitude, and velocity of myocardial contractions, depresses the Starling curve, and drastically upsets the resistance of cardiac function to deviations from ionic homeostasis in the milieu. Special studies concerned with the effect of stress on the cardiac papillary muscle hypertrophied because of aortic stenosis have shown that the myocardial contractile function, already initially reduced under such conditions, is further depressed by stress to a critical level that may predetermine cardiac insufficiency.⁸³ Thus, *in certain circumstances the stress-induced depression of the contractile function may be involved in the development of heart failure.*

Such functional disorders are obviously underlined by alterations in energy metabolism, cation exchange, etc. Accordingly, in the next section we shall consider the disturbances caused by stress in the myocardial energetics.

IV. STRESS DAMAGES TO ENERGY PROVISION IN CARDIAC MUSCLE

The state of the energy-providing system in cardiac stress damage is incomparably less studied than in ischemia. In a sense this gap is somewhat filled in by the already mentioned data on the β -adrenergic injury to the heart caused with isoproterenol according to Rona, where, besides membrane disruption, excessive accumulation of cytoplasmic calcium, and its pronounced cardiotoxic action, there are marked deterioration of mitochondria and exhaustion of creatine phosphate, ATP, and glycogen. However, in elucidating the question of to what extent such a complex of β -adrenergic damage is actualized during true stress in animals, and in this context what should be expected to happen in the human cardiac muscle, we had to start from scratch.

A work carried out by Malyshev et al.⁸⁹ with mitochondria isolated from the hearts of animals subjected to stress revealed defects in oxidation and oxidative phosphorylation. The data presented in Table 6 show the changes in the basic parameters of oxidation and phosphorylation in mitochondria isolated from cardiac muscle at different moments after exposure to stress. It can be seen that already in 2 h after stress both oxidation and phosphorylation are depressed. The most affected parameter is the phosphorylation rate (ADP/t), which, in 2 d after stress, is decreased by 50% in glutamate plus malate oxidation and by 47% in succinate oxidation.

According to the present day notions, such defects in oxidative phosphorylation can be brought about by two interconnected factors: direct harm to the mitochondrial membranes by the "lipid triad" and calcium accumulation in mitochondria. It was important to find out whether or not these defects result in decreasing ATP concentration in the working heart at relative rest and under maximum load. To solve this question L. Yu. Golubeva determined the concentrations of ATP, ADP, AMP, and inorganic phosphate in the left ventricular myocardium of the hearts obtained under aerobic conditions from anesthetized animals and instantly frozen in liquid nitrogen with the Wollenberger forceps. At physiological rest the concentrations of ATP, other adenine nucleotides, and inorganic phosphate in the left ventricular myocardium of animals that had undergone stress did not differ from those in the control, and at maximal load imposed by aortic clamping for 90 s the ATP content declined also to the control level.

Hence it follows that the above-described defects in oxidation and phosphorylation in the cardiac mitochondria from stressed animals do not, however, affect the overall ATP content in the myocardium. This apparent discrepancy between the *in vitro* and *in vivo* data may have several explanations. Thus it is possible that the damage to the mitochondrial membrane lipid bilayer by lipid peroxidation and related processes is limited *in vivo* by natural antioxidants, but highly pronounced in isolation.

The works of Jacobus⁹⁰ and Saks et al.⁹¹ allow one to think that the defects in oxidative ATP resynthesis revealed upon isolation of myocardial mitochondria from stressed animals are, under conditions of the whole organism, not sufficient to decrease the overall ATP level, but they attenuate the creatine phosphate (CP) content and hinder the transport of energy-rich phosphate groups from mitochondria to myofibrils and sarcolemmal and SR membranes, which in principle may impair myofibrillar contraction and relaxation.

In this connection, together with L. Yu. Golubeva, we have specially determined the CP content and creatine phosphokinase (CPK) activity in the hearts of animals subjected to EPS.

TABLE 6
Oxidation and Phosphorylation in Animal Cardiac Mitochondria Upon EPS

Time after EPS (h)	V ₃	V ₄	V ₃ /V ₄	ADP/O	ADP/t
Glutamate + Malate					
Control	173 ± 12	48 ± 5.9	3.95 ± 0.3	2.9 ± 0.2	469 ± 20
2	123 ± 5.2 ^a	38 ± 1.1	3.20 ± 0.1 ^b	2.4 ± 0.2	301 ± 30 ^c
24	133 ± 9.2 ^b	55 ± 3.0	2.45 ± 0.1 ^c	2.0 ± 0.1 ^a	275 ± 29 ^c
45	104 ± 4.5 ^c	42 ± 4.2	2.40 ± 0.2 ^c	2.0 ± 0.1 ^a	234 ± 11 ^c
72	154 ± 6.1	58 ± 4.6	2.70 ± 0.2 ^a	2.6 ± 0.2	434 ± 44
96	171 ± 7.8	39 ± 2.6	4.00 ± 0.3	2.5 ± 0.2	422 ± 48
Succinate					
Control	251 ± 17	88 ± 5.7	2.85 ± 0.2	2.0 ± 0.2	524 ± 38
2	226 ± 8	75 ± 4.8	2.90 ± 0.2	1.7 ± 0.1	413 ± 27 ^b
24	215 ± 20	118 ± 9.6 ^b	1.85 ± 0.2 ^a	1.4 ± 0.2 ^b	323 ± 27
45	189 ± 13 ^a	102 ± 9.8	1.80 ± 0.1 ^c	1.3 ± 0.2 ^b	280 ± 24 ^c
72	241 ± 15	107 ± 11	2.25 ± 0.2	1.9 ± 0.2	469 ± 32
96	218 ± 20	98 ± 5.1	2.20 ± 0.2	2.0 ± 0.2	439 ± 32

Note: V₃, Respiration rate (natoms 0/min per mg protein) upon ADP addition; V₄, same after ADP exhaustion; V₃/V₄, respiratory control ratio; ADP/O, phosphorylation ratio; ADP/t, phosphorylation rate (nmol ADP/min/mg protein).

The data in Table 7 demonstrate that prolonged EPS did not affect the myocardial content of adenine nucleotides, whereas the CP content fell by one third while the total CPK proved to be almost 40% lower. It also follows from Table 7 that the decline in CPK activity in myocardial tissue was only in small measure due to its decrease in the postmitochondrial supernatant, which was just 12%. In essence this fact means that we observed the loss of CPK associated with mitochondria. A rough calculation shows that in the control the activity of structure-bound CPK (difference between total and postmitochondrial CPK) amounted to some 45% of the total, which agrees with the data of other works,⁹³ whereas after stress its portion decreases by a factor of 2.5. Stress is known to activate lipid peroxidation, lipases and phospholipases, impair mitochondrial function, damaging thus the cardiomyocyte membranes and promoting the leakage of CPK and other membrane-bound enzymes.¹ It can be thought that mitochondrial and other membranes damaged by stress (as compared to the control) more readily lose CPK during standard treatment, and the enzyme goes into the soluble fraction. Of course this suggestion must be verified by assaying CPK in mitochondria and determining the isoenzyme spectrum in the cytosolic fraction. Thus, in stress the disturbances in the CPK system occur earlier than direct damage to ATP resynthesis. Such impaired transport of high-energy phosphates may contribute to the depression of cardiac contraction and relaxation in stress.

This suggestion is in accord with the results of a recent study of Kupriyanov et al.,⁹⁴ who have shown that in the course of recuperation from transitory intoxication with deoxylucose the heart can function at quite low ATP concentrations, but its functional level correlates with a high enough concentration of CP in cardiomyocytes. In another work the same group⁹⁵ made a detailed study of the function of isolated hearts from animals who had been fed guanidine propionate, an inhibitor of creatine transport into cardiomyocytes, to depress the myocardial CP level. It turned out that at normal ATP, but lowered CP content, the cardiac contractile function was impaired, and mainly affected were the relaxation velocity

TABLE 7
Effect of EPS on Myocardial Energy Metabolism

	Metabolite content ($\mu\text{mol/g}$ tissue)					Creatine phosphokinase activity (nmol/min/g tissue)	
	ATP	ADP	AMP	CP	Total	Postmitochondrial supernatant	
Control (n = 15)	5.4 \pm 0.15	1.2 \pm 0.09	0.43 \pm 0.04	8.8 \pm 0.20	959 \pm 39	537 \pm 14	
Stress (n = 10)	5.1 \pm 0.11	1.0 \pm 0.20	0.50 \pm 0.03	6.0 \pm 0.20	566 \pm 18	473 \pm 12	
Reliability					p < 0.05	p < 0.01	p < 0.05

and myocardial extensibility, i.e., the situation was familiar to the one that several years before we had described in hearts exposed to stress.¹ In other words, the decreasing CP content at normal ATP content that we have found upon exposure to stress may be essential to the described impairment of myocardial extensibility in stress — the phenomenon of stress rigidity and corresponding disturbances of the contractile function.

The disorders in glycogen metabolism in EPS that we have found further are likely to play a no less important part in these phenomena. Table 8 presents the data that we have obtained with Golubeva and Pavlova on the effects of stress on phosphorylase activity and glycogen content and synthesis in the cardiac muscle.

It follows that stress caused activation of phosphorylase and an almost twofold decrease in the glycogen concentration. This consumption of the cardiac glycogen reserve was not accompanied by activation of glycogen synthesis: its specific radioactivity did not change, and the rate of glycogen synthesis (judged from total radioactivity) per unit cardiac mass was almost twice lower. This shift was apparently due to the marked decrease in the glycogen content and accordingly in the number of glycogen granules or glycosomes which harbor both hydrolysis and biosynthesis of this polysaccharide; a similar phenomenon had been earlier shown in liver upon pain stress.⁹⁶ Activation of phosphorylase to mobilize glycogen is well known as one of the first results of the β -adrenergic effect in stress, with the phosphorylase A/A + B ratio⁹⁷ and the decline in myocardial glycogen⁹⁸ being proportionate to the probability of arrhythmias and cardiac fibrillation. This fact was quite consistently observed in our experiments. Table 9 presents analogous data, with the difference that glycogen and lactate were determined in the left ventricular myocardium both at physiological rest and maximum load imposed by aortic clamping for 90 s.

It can be seen that stress decreases the glycogen content almost by half. With the maximum cardiac load further reducing it by 120 to 130 mg in both poststress and control animals, after 90 s of aortic clamping the myocardial glycogen content in poststress animals proves to be 2.2 times lower than in controls. This is direct evidence that a longer loading or hypoxia would sooner exhaust the myocardial glycogen reserve after stress. Such a depletion of the endogenous glycolytic substrate should not and does not tell on the dynamics of the overall myocardial ATP level, since even with glucose as the sole substrate the portion of glycolytic ATP in its overall resynthesis is small, about 1/20 of the total amount of ATP produced by oxidative phosphorylation in mitochondria. In point of fact, besides carbohydrates mitochondria utilize fatty acids as well as pyruvate and lactate from the blood; accordingly, the contribution of glycolytic phosphorylation to the energy provision for the heart working in aerobic conditions would be still smaller. Nevertheless, it has been definitely shown that blocking the glycolysis with low doses of monooiodoacetate or 2-deoxyglucose impairs the cardiac contractile function despite unlimited supplies of oxygen and pyruvate, the end product of glycolysis.⁹⁹ There is thus no doubt that glycolysis, on the one hand, provides an insignificant portion of ATP used by the cell, but on the other constitutes an indispensable link in the mechanism of energy supplies for the cardiac contractile function.

TABLE 8
Effect of EPS on Phosphorylase Activation and Glycogen Content and Biosynthesis in the Rat Myocardium

	Phosphorylase A/A + B ratio	Glycogen content (mg/g tissue)	¹⁴ C incorporation into glycogen, cpm	
			Specific per mg glycogen	Total per g tissue
Control (n = 10)	0.43 ± 0.03	6.40 ± 0.31	205 ± 22	1310 ± 201
EPS (n = 10)	0.61 ± 0.04	3.60 ± 0.23	215 ± 17	776 ± 95
	p <0.01	p <0.01		p <0.01

TABLE 9
Effect of EPS on Myocardial Glycogen and Lactate at Physiological Rest and Maximal Cardiac Load

Indices	Control		EPS	
	Rest	Load	Rest	Load
Glycogen (mg/100 g)	640 ± 31 (n = 9)	519 ± 18 (n = 7)	360 ± 23 ^a (n = 17)	288 ± 27 ^a (n = 10)
Lactate (μmol/100 g)	88 ± 19 (n = 7)	548 ± 34 (n = 13)	105 ± 12 (n = 8)	638 ± 50 (n = 8)

^a Difference from control significant at p <0.01.

This has prompted a suggestion that the small amount of ATP formed in glycolysis is a unique, irreplaceable source of energy for a certain vital function of the muscle cell. The suggestion is favored by several facts.

1. Blocking the glycolysis with moniodoacetate consistently impairs myocardial relaxation, eliciting cardiac contracture.¹⁰⁰ In this connection an idea has been put forth that glycolysis generates the ATP fraction used for the operation of the SR calcium pump, i.e., for removal of Ca²⁺ from myofibrils to allow diastolic relaxation.
2. The multienzyme glycolytic system is for the most part localized on the SR membranes¹⁰¹ and, accordingly, muscles with more developed SR always possess a more powerful glycolytic system as, in particular, do skeletal muscle compared to the cardiac muscle. Substantial amounts of glycogen — the main cell glycolytic substrate — are found along the longitudinal SR, i.e., in immediate proximity to the calcium pump.¹⁰²
3. The processes of glycogenolysis-glycolysis and Ca²⁺ removal from the sarcoplasm are regulatively dependent on the sarcoplasmic Ca²⁺ content. Indeed, a rise in Ca²⁺ and active calmodulin during excitation simultaneously causes phosphorylation of phosphorylase B to phosphorylase A (activation of glycolysis) and phosphorylation of the sarcolemmal and SR phospholamban (activation of the calcium pump and relaxation).

It is thus quite probable that the portion of ATP produced in glycolysis is used by the calcium pump ATPase to ensure relaxation. These facts of course do not exhaust the problem, but they are in line with the hypothesis that the comparatively small amount of glycolytic ATP is a unique, indispensable source of energy for a certain vital physiological operation, namely for the relaxation process.^{103,104}

This issue has been confirmed and developed in later studies. The most demonstrative result has been obtained by Bricknell et al.,¹⁰⁵ who studied the role of glycolysis in the energy provision for membrane Ca²⁺ transport with a model of cardiac contracture developing

in prolonged myocardial ischemic hypoxia. This contracture, being an energy-deficient muscle-relaxation disorder, results not from simple lack of ATP in the contractile apparatus, but precisely from the ATP-deficient interruption of the membrane pump responsible for Ca^{2+} removal from the myofibrillar troponin complexes.^{106,107} To collate the roles of the mitochondrial and the glycolytic ATP in the calcium pump function, the authors have studied the development of contracture (1) under blocked glycolysis and intact oxidative phosphorylation and mitochondrial ATP transport, and (2) under operative glycolysis and blocked mitochondrial ATP transport. The scheme in Figure 12 shows that in the first series glycolysis was switched off by removing glucose from the perfusing solution and adding glycolytic inhibitors — monooiodoacetate or deoxyglucose; simultaneously, acetate was added as a substrate to maintain oxidative phosphorylation. In the second series the glucose concentration was normal, glycolysis proceeded freely, oxidative phosphorylation also was not specially blocked, but translocation of mitochondrial ATP to the perimyofibrillar space was inhibited with atracyloside (Figure 12B).

In both series the isolated heart was first allowed to function aerobically, then it was stopped with KCl, and myocardial ischemia was created by reducing the perfusing flow to 1 to 0.5 ml/min. The oxidative phosphorylation which is the main source of total myocardial ATP under such conditions was similarly attenuated in both series, and accordingly the ATP level was also approximately the same. However, despite the same ATP concentration the state of relaxation in ischemia proved quite different. With blocked glycolysis and intact oxidative phosphorylation, in 100 min after the onset of ischemia a pronounced contracture developed, with the indices of tension at rest approximating those of systolic tension in aerobic conditions prior to cardiac arrest. With intact glycolysis in the cardiac muscle, no appreciable contracture developed. These data fit into the hypothesis of the prime importance of the glycolytic ATP for the relaxation process. They allow one to think that the decline in myocardial glycogen reserve in poststress animals observed in our studies and the attending disturbances in glycolysis and glycogen resynthesis may be involved in the stress disorders of myocardial contraction and relaxation, and first of all in the above phenomenon of myocardial stress rigidity and its decreased resistance to hypoxia and excess calcium. To test this suggestion, we made a study of the effects of high glucose concentrations and of a cofactor of glycogen resynthesis, uridine, on the extensibility and contractility of the myocardium from animals subjected to a 6-h EPS.

In Figure 13, the curves on the left reflect the stress-induced defects in extensibility and contractile function described earlier, and on the right the effects of a fourfold increase (from 2.5 to 10 mM) of the glucose concentration in the perfusing solution. Analysis of the curves demonstrates that such a rise in glucose practically restores the stress-impaired extensibility and contraction force of the auricle. A similar effect is observed with the left ventricular papillary muscle. In considering this protective effect of glucose on the stress damage to the cardiac contractile functions it should be borne in mind that administration of glucose into the coronary bed enhances the glycogen content, aerobic and anaerobic glycolysis, creatine kinase activity, and macroergic phosphate content in the ischemic myocardium.¹⁰⁸ Concurrently, the loss of myocardial calcium is diminished and the ischemic disturbances in the cardiomyocyte electric activity (shortening and step-down of the action potential, arrhythmias) are prevented.¹⁰⁹ In our experiments the excess of glucose could also normalize the glycolytic process, restore the flux of glycolytic ATP to the membrane calcium pump, and thereby abolish the excess of myofibrillar Ca^{2+} and myocardial stress rigidity.

The fact that the declining glycogen content and its impaired resynthesis in stress are associated with relaxation and contraction defects substantiates the use of some cofactor of glycogen resynthesis to restore its content and metabolism in cardiomyocytes and to restore therewith myocardial relaxation. In our experiments use was made for this purpose of the nucleoside uridine. The first step in glycogen synthesis is known to be impossible without

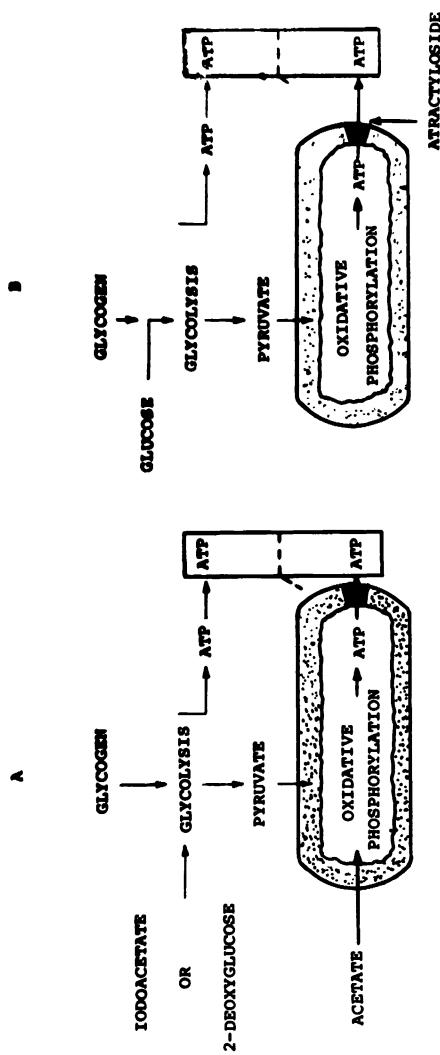


FIGURE 12. Scheme for comparative assessment of the effect of blocking (A) glycolysis and (B) mitochondrial ATP translocation on the development of ischemic contracture. For explanations see text. (After Bricknell, O. L., Daries, P. S., and Opie, L. H., *J. Mol. Cell. Cardiol.*, 13, 941, 1981. With permission.)

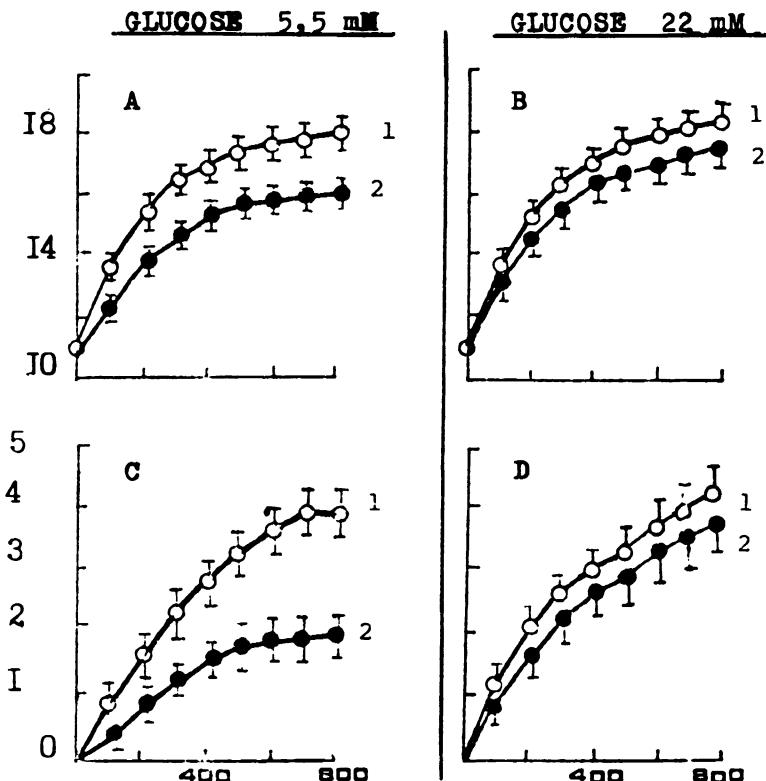


FIGURE 13. Effect of increased glucose concentration on the (A,B) extensibility and (C,D) contractile function of auricles from rats subjected to EPS. The abscissa is the load at rest in mg; the ordinates are (A,B) atrial length in mm, (C,D) contraction force in mN. 1, Control; 2, EPS.

uridine phosphorylated to uridine triphosphate, which is used to convert glucose 1-phosphate to UDP-glucose, the direct substrate for glycogen synthesis by glycogen synthase. In full conformity with this, uridine has been proved to enhance glucose uptake, glycogen content, and renewal of the ATP microstores in the aerobic myocardium, and to restore the cardiac function, glycogen, and adenine nucleotide contents under hypoxia.¹¹⁰

Basing on this, we have studied the effects of uridine added to the perfusing solution on the stress defects of myocardial extensibility and contractility and on its resistance to hypoxia and excess Ca^{2+} . The experiments were carried out with isolated auricles at 5.5 mM glucose. Half of both control and poststress auricles were supplemented with uridine at $2 \times 10^{-5} M$. Figure 14 shows that in our experiments uridine acted identically to quadrupled glucose: it did not affect the atrial extensibility and contractility in controls, but completely abolished the defects in extensibility and most of the depression of contractile function after stress.

Figure 15 depicts the effects of uridine on atrial hypercalcium contracture. It can be seen that a threefold rise in Ca^{2+} elicited a significant increase in the tension of rest, i.e., contracture which was reliably more pronounced in poststress than in control auricles. In the presence of uridine the contracture was much smaller: the resistance of the myocardial relaxation process to excess Ca^{2+} was markedly enhanced by the cofactor of glycogen resynthesis. As this took place, an apparently paradoxical phenomenon was observed: in the poststress auricles the protective effect of uridine was greater than in controls. Indeed, the hypercalcium contracture assayed as the absolute tension in these experiments was quite reliably reduced by uridine in the poststress auricles, whereas with the controls its effect did not reach statistical significance. As a result, the contracture in the presence of uridine

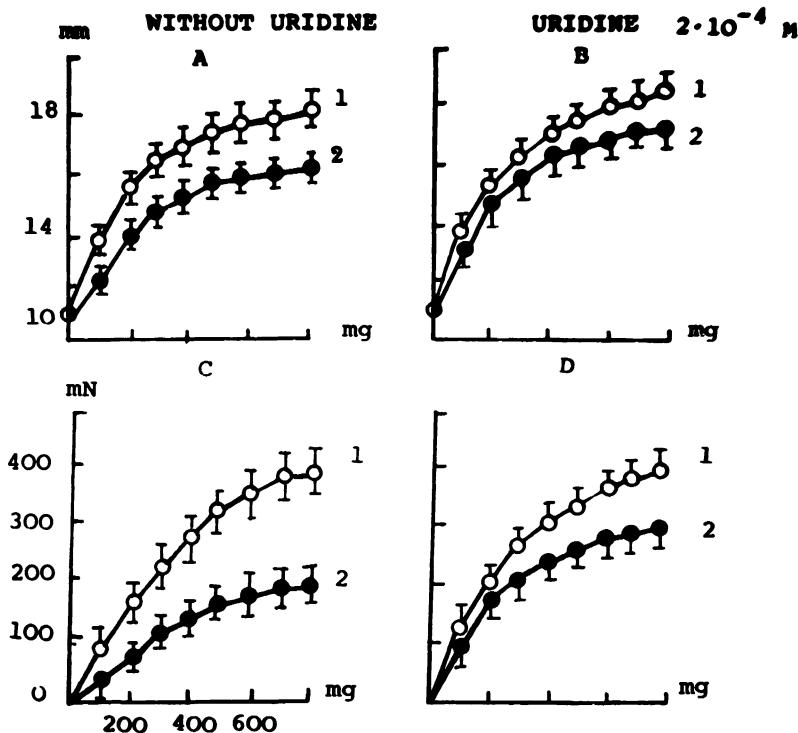


FIGURE 14. Effect of uridine on the (A,B) extensibility and (C,D) contractile function of auricles from rats subjected to EPS. The abscissa is the load at rest in mg; the ordinates are (A,B) atrial length in mm, (C,D) contraction force in mN. 1, Control; 2, EPS.

proved to be more pronounced in the control than in the poststress auricles. Such potentiation of the uridine effect by stress may be because the glycogen deficit caused by stress enhances the sensitivity of the glycogen-synthesizing system to excess uridine. A certain role may also be played by an increased uridine permeation through stress-damaged cardiomyocyte membranes.

On the whole, the above gives grounds for thinking that stress induces quite pronounced defects in the creatine kinase and glycolytic systems which may be involved in the stress disturbances to the electric stability and contractile function of the heart, most likely through upsetting the energy provision for the cardiomyocyte calcium pump.

In this context note should be made of some points of interest for clinical cardiology. The first one is that the stress-induced hindrance to myocardial extensibility is not only a marker of impaired relaxation, but may also interfere with cardiac filling and pump function, i.e., decrease its stroke volume and output. It cannot be excluded that such phenomena contribute to the evolution of collaptoid stress conditions.

The second point is that stress-induced glycolytic defects revealed in the myocardium and the ensuing functional alterations must be most pronounced in those parts of the heart whose energy supplies are most dependent on glycolysis. Undoubtedly such a part is the cardiac conductive system whose cells have much fewer mitochondria than the cells of contractile myocardium. This system must to the largest extent suffer from repeated stress situations inevitably encountered in one's life. In full conformity with this suggestion is the fact that right and then left bundle-branch blockade of the bundle of His may develop very early in practically healthy persons and is often regarded as a hallmark of age wearing of the heart.

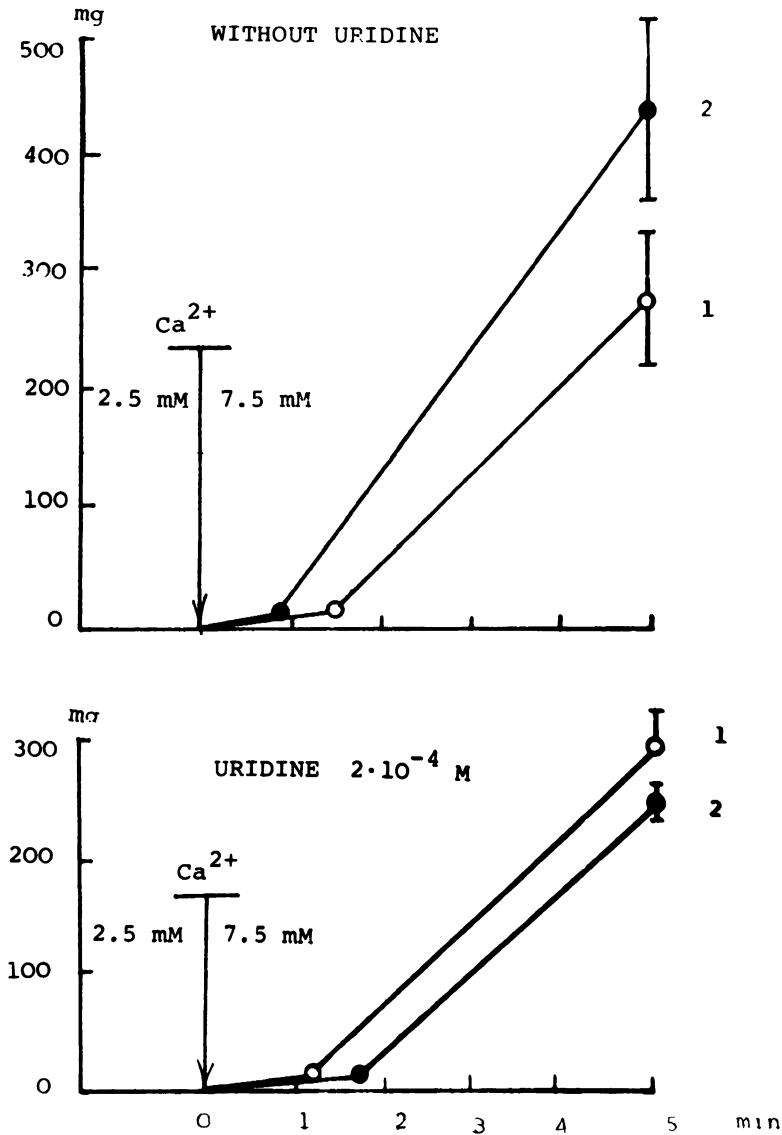


FIGURE 15. Effect of uridine on the calcium contracture in auricles from rats subjected to EPS. The abscissa is time in min; the ordinate is the increment in tension of rest in mg. 1, Control; 2, EPS.

Finally, the third point is that the stress alterations in the myocardial cell are not at all limited to the disturbances in the contractile function and energy metabolism considered above, but are much more deep. This is evidenced by our finding, of some years ago, of the stress damage and subsequent repair of nuclear DNA in the cardiac muscle, and structural damage to the myocardium.

V. MYOCARDIAL DNA INJURY AND OUTBURST OF ITS REPARATIVE SYNTHESIS IN STRESS

Activation of lipid peroxidation (LPO), lipases, phospholipases, and consequent membrane damage considered above must naturally lead to the injury to main cell structures in the cardiac muscle. Indeed, such a transitory injury to proteins and nucleic acids has been found to occur in a 6-h EPS.¹¹¹

It has been found that stress substantially decreased the half-life of ribosomal RNA and increased the percentage of RNA replaced within 1 d in the internal organs. This complex of changes directly testifying to enhanced breakdown of RNA was clearly pronounced in the cardiac muscle: the half-life was decreased by about one fourth and the portion of replaced RNA increased by one third. Further, the half-life of the total myocardial protein after stress proved to be decreased by more than one third. Thus, stress brings about significant acceleration of RNA and protein breakdown.

Taking this into account, we studied the influence of stress on the apparatus which is central for the cell formation and at the same time the most conservative and protected from deterioration, namely on the DNA of the cell nuclei.

We reasoned from the fact that, e.g., in radiation sickness the accumulation of free-radical LPO products in the cell is accompanied by injury to the DNA helix. A similar phenomenon could well happen in LPO activation by stress. On this basis we¹¹² conducted the experiments aimed at evaluating the polymeric state and reparative synthesis of DNA in cardiac muscle cells after EPS.

Stress causes partial depolymerization of myocardial DNA, most probably because of single-strand nicks. In most of the myocardial cells that continue functioning after the exposure to stress, these DNA nicks are rapidly repaired.

Further studies demonstrated that such changes —DNA damage and subsequent repair — upon stress are found not only in the heart, but also in kidneys, liver, brain, and other organs.¹¹³

Considering the biological significance of this phenomenon and its role as a marker of organ stress damage, we further made a more comprehensive study of it by comparing the effects of stress on DNA replication and repair in nuclei and mitochondria of heart and liver, and by elucidating the dependence of the stress injury to DNA (as judged from the outburst of its reparative synthesis) on the cardiac function.¹¹⁴

First, the effect of a 6-h EPS was compared on the replicative and reparative synthesis of DNA in the heart and the liver. In the control the replication rate was approximately twice lower in cardiac than in hepatic nuclei. EPS greatly accelerates nuclear DNA replication in the heart cells; this increase is observable 4 to 6 h after the end of stress and reaches its maximum in 10 to 12 h, with the replication rate exceeding the control by a factor of 3.5. After 22 to 24 h and later the replication in cardiac cells returns to the initial level. In the hepatic nuclei the pattern is quite different, with EPS decreasing the replication rate about by half for 48 h after stress and still by some 20% after 72 h.

The next series concerned reparative DNA synthesis in cardiac and hepatic nuclei on the background of hydroxyurea, which is known to inhibit nuclear DNA replication, but to have almost no effect on its reparative synthesis.^{115,116} In our experiments hydroxyurea suppressed DNA synthesis by 88% in the cardiac and by 89% in the hepatic nuclei. These results are comparable with the data obtained for other cells.^{117,118} DNA synthesis taking place in the presence of hydroxyurea apparently reflects the reparative synthesis.

As can be seen in Figure 16, the rate of DNA synthesis in both cardiac and hepatic cells is significantly increased upon EPS — the outburst of DNA repair (testifying to its damage) is realized to the most. Notably, the reparative synthesis is activated in cardiac cells on the background of enhanced, while in hepatic cells, of suppressed replication.

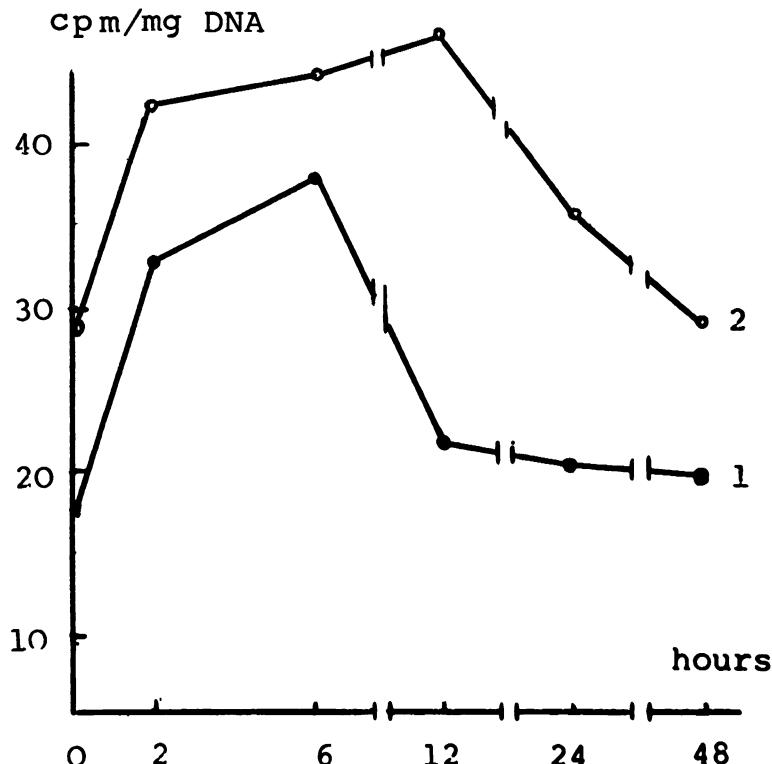


FIGURE 16. Effect of EPS on reparative DNA synthesis in (1) cardiac and (2) hepatic nuclei. The abscissa is time after EPS in hours, the ordinate is ^3H -thymidine incorporation in cpm/mg DNA.

Then DNA synthesis was studied in cardiac and hepatic mitochondria. In agreement with the known data,¹¹⁹ the specific radioactivity of pulse-labeled mitochondrial DNA exceeded that of nuclear DNA. The high rate of precursor incorporation into mitochondrial DNA is held to reflect its replicative synthesis.¹²⁰ Hydroxyurea, the inhibitor of nuclear DNA replication, proved to have no effect on the mitochondrial process. EPS does not affect mitochondrial DNA replication in the heart and substantially suppresses it in the liver, as shown in Figure 17.¹¹⁴

Thus, under the influence of EPS the two different systems of DNA synthesis in two vital organs — the heart and the liver — change in different ways. In the heart the rates of nuclear DNA replication and repair increase, while those of mitochondrial DNA synthesis remain the same. In the liver the rates of nuclear and mitochondrial DNA replication decrease, while the nuclear repair rate increases as well.

It is essential that *by the criterion of suppressed DNA replication the liver proved to be a more stress-sensitive organ than the heart*, and we shall further see that this is not a stray occurrence, since many organs are damaged by stress not at all less than the cardiac muscle.

In studying the second question of interest, and namely how the myocardial function affects the DNA resistance to stress injury, i.e., the extent of its repair, we made use of operative (surgical) stress.

Preliminary experiments showed that operative stress caused by laparotomy entails an outburst of reparative synthesis in the myocardial nuclei as consistently as EPS. However, if the operation ends in coarctation of aorta with resulting compensatory heart hyperfunction, there is no outburst of DNA synthesis in the myocardium. This prompted an idea that the enhanced function somehow stabilizes the DNA structure, preventing its injury and correspondingly its repair.¹²¹

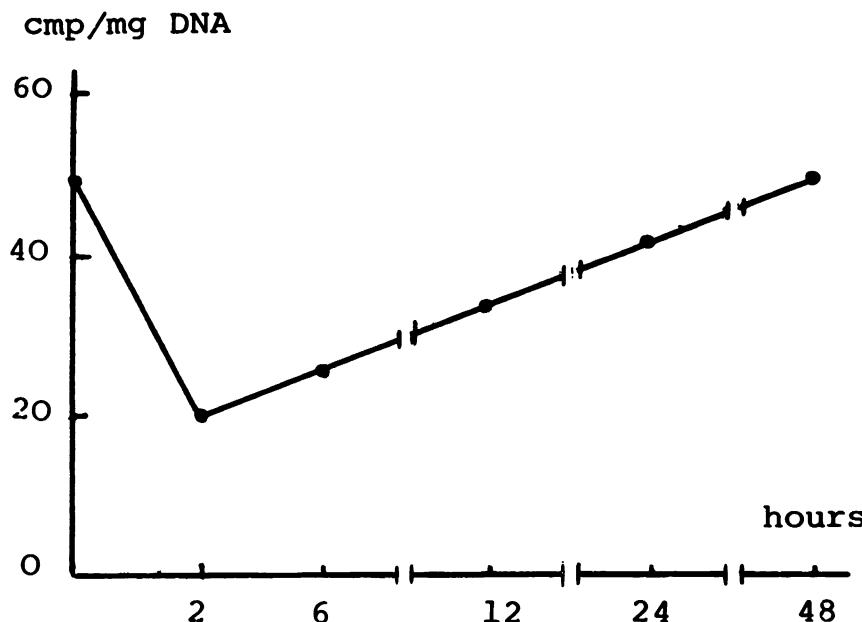


FIGURE 17. Effect of EPS on DNA synthesis in liver mitochondria. The abscissa is time after EPS in hours, the ordinate is ^{3}H -thymidine incorporation in cpm/mg DNA.

To check this suggestion, it was important to find out whether the "stabilizing effect" of myocardial hyperfunction is realized only locally in the cardiac cell nuclei or if it also encompasses other internal organs where DNA repair is also enhanced in stress.^{122,123} To this end, the influence of operation without coarctation of aorta and of a similar operation ending in coarctation of aorta and compensatory heart hyperfunction was studied on the DNA repair rate in the cell nuclei of the heart, the liver, and the brain.¹¹⁴

The data presented in Table 10 allow a comparison of the rates of reparative nuclear DNA synthesis in hepatic, cardiac, and brain cells both at rest and after operative stress. It can be seen that the greatest reparative ability is found in the liver and the smallest in the brain, with the heart being intermediate in this respect. Both kinds of operation, without and with aortic coarctation, cause an outburst of DNA repair in hepatic and brain cells. Another thing is found in the heart. The operation without coarctation, i.e., operative stress, causes an outburst of DNA repair in the heart like in other internal organs. However, upon aortic coarctation with ensuing myocardial hyperfunction, there is no outburst of repair. *Hence, the DNA injury by operative stress does not occur under compensatory hyperfunction of the cardiac muscle. This protective effect is local and does not expand on other organs.*

In eukaryotic cells the DNA injuries can be repaired by two means: excision and postreplicative repair. The latter is inhibited by hydroxyurea.¹²⁴ Therefore, *in our experiments we were only dealing with excision repair*. In mammals there are two ways of this, the short and the long one. In the first, three to seven nucleotides are eliminated from DNA and the process is completed within an hour. In the second, several dozen nucleotides are removed and the process lasts 20 to 24 h and longer.¹²⁵

After operative stress, the enhancement of reparative synthesis in cardiac, hepatic, and brain cells is quite definite after 24 h. Thus, under the given conditions the excision repair proceeds via the long way.

A principal question is why no acceleration of DNA reparative synthesis is observed upon operative stress in the cardiac muscle performing compensatory hyperfunction. In discussing this question, one should keep in mind the following. Organ hyperfunction

TABLE 10
Compensatory Hyperfunction Prevents the Outburst of DNA
Reparative Synthesis in Cardiac Nuclei in Stress

	³ H-Thymidine incorporation (dpm/mg DNA)		
	Liver	Brain	Heart
Control	3050 ± 25	1000 ± 15	1500 ± 80
Operative stress	4300 ± 80	1315 ± 40	2900 ± 50
Operative stress with cardiac hyperfunction	4600 ± 70	1350 ± 20	1400 ± 20

Note: Each figure is the mean from six to ten animals.

markedly alters its metabolism, increasing the rate of RNA and protein synthesis and decreasing protein breakdown.¹²⁴

Further, at the onset of hyperfunction protein factors appear that initiate organ hypertrophy¹²⁶ and accelerate transcription.¹²⁷ At the same time, proteins regulating translation are capable of binding to internal DNA regions.¹²⁸ Further, there are a number of reports that the spectrum of newly formed soluble cytoplasmic proteins is altered in myocardial hyperfunction.^{124,129} Thus, the data available allow a suggestion that under enhanced function in myocardial cells some proteins accumulate to stabilize the DNA structure and prevent it from the damaging action of stress.

By and large, *there is no doubt that the metabolic effects of myocardial hyperfunction prevent the stress-induced damage to cardiac DNA. The stabilizing effect of cardiac hyperfunction on the DNA template is local and does not cover other internal organs.*

VI. EXTRACARDIAC DAMAGES IN STRESS AND THEIR POSSIBLE CONTRIBUTION TO IMPAIRMENT OF CARDIAC FUNCTION

The stress reaction is a generalized phenomenon, and accordingly not only the heart, but the whole organism may become the target of excessive stress reaction. In the process, damage to the respiratory apparatus controlling blood oxygen tension, to the liver predetermining the cholesterol level, to the pancreas that may release trypsin, can in a most direct way tell on the status of the heart and indirectly contribute to its damage by stress. Here we shall consider such possibilities as applied to the respiratory system, where in our opinion there are sufficient grounds for introducing the term "stress lung", and more briefly as applied to the liver and the pancreas.

It is known that the respiratory system has vast functional reserves and, by adjusting the pulmonary ventilation and oxygen transport and utilization, ensures the energy balance in various ambient conditions. Further, it has been shown that exposure to stress is accompanied by mobilization of the respiratory apparatus.¹³⁰⁻¹³² The resistance of the respiratory system to heavy stress has not been studied until recently. Yet the stress damage to the respiratory system may be essential for the impairment of its function in most various conditions and diseases caused by stress situations.

In accordance with this, we studied the influence of severe immobilization stress on pulmonary ventilation, oxygen diffusion from air to blood, blood transport of respiratory gases, and O₂ consumption by tissues.

The control animals were first anesthetized and then fixed by four limbs in the supine position, whereas the "stressed" animals were kept in this fixed position for 6 h before anesthesia. Then a standard acute test was performed in both groups under anesthesia. As

a result, all the disturbances described below were observed only in animals subjected to immobilization without anesthesia, i.e., indeed subjected to stress. This important experimental detail indicates that it is not immobilization as such and not the procedures of the acute test carried out under anesthesia, but just the stress reaction evoked by immobilization that damages the organism in whole and the respiratory system in particular.

The data presented in Table 11 show that a 6-h immobilization stress substantially alters the basic parameters of oxygen transport in the organism and circulation. Thus the alveolar ventilation in animals subjected to stress proved to be decreased by half, yet the oxygen tension in the alveolar air remained at the normal level. Such a relation of these parameters is usually due to attenuated O_2 consumption by the organism.¹³³ However, a study of the P_{O_2} cascade revealed that at the same partial oxygen pressure in the alveolar gas mixture in stress and control, the O_2 tension in arterial blood running off the lungs in stressed animals is lowered by 26.5 hPa (20 mmHg column). Such stress-induced arterial hypoxemia may result from hindered oxygen diffusion from the alveoli to the lung capillaries and some other factors to be considered below.

Notably, such hypoxemia is not attended with lowered mean P_{O_2} in the blood of tissue capillaries and mixed venous blood. This at first glance paradoxical fact should be due to decreased oxygen consumption by tissues.

Furthermore, Table 11 shows that in animals that have undergone immobilization stress the minute blood volume is decreased by 45%.

Taken together, these data mean that *the drop in arterial O_2 tension and in the minute volume in stress diminish the O_2 supply to tissues by a factor of about 2.5. However, this does not decrease the O_2 tension in the tissue capillaries and in the mixed venous blood, since the O_2 consumption by tissues and the whole organism is diminished to the same extent.*

Despite the maintained balance between oxygen supply to and consumption in tissues, Table 12 demonstrates that the lactate content in blood is almost twice as high in poststress animals, and the pH is lowered by 0.25 in the arterial and mixed venous blood with an attending fall in bicarbonates and aggravated deficit of bases at a practically normal CO_2 tension in the arterial blood. In other words, the given stress exposure elicits uncompensated metabolic acidosis in rats. To appreciate this phenomenon, one should take account of an important index — the ratio of supplied to consumed oxygen which usually varies between 3 and 4 in the control and regularly decreases in various types of hypoxia. The secondary tissue hypoxia, due to insufficient oxygen supply to the cells and attended with lactate accumulation and metabolic acidosis, has been shown¹³⁴ to set in only after this index falls to 2.0 to 1.5. In the present study there was no reliable decrease in this index upon immobilization stress (see Table 11), hence there was no deficit of O_2 due to its inadequate supply to the cells. Therefore, in stress the excess of lactic acid and acidosis are not the result of secondary tissue hypoxia. The most plausible cause of these shifts is the depressed tissue respiration and oxygen utilization by mitochondria and marked activation of glycolysis with associated mobilization of the glycogen reserves in liver, heart, and other organs.¹³⁵ Under suppressed mitochondrial respiration the surplus pyruvate formed in glycolysis cannot be used by the respiratory chain, is converted to lactate, and goes into the blood. Such an interpretation is supported by the fact that the plasma from animals subjected to immobilization stress shows a threefold elevation of malonic dialdehyde, an intermediate product of lipid peroxidation; the latter is known to be activated in stress and to play an important role in cell membrane damage.

It is essential that as a result of stress damage to tissue respiration the overall energy consumption by the organism (calculated from oxygen consumption, respiratory quotient, lactate content, and coefficient of Margaria) decreases by 50%, with the portion of energy derived from anaerobic processes involving lactate accumulation increasing from 6 to 20%.

TABLE 11
Effect of Immobilization Stress on the Main Parameters of Oxygen Transport in the Organism

Indices	Control	Stress
Alveolar ventilation \dot{V}_A , ml/min·100 g	47.3 ± 3.5	23.2 ± 2.3^a
Oxygen tension (mmHg/in.)		
Alveolar air, P_{AO_2}	105 ± 3	104 ± 2
Arterial blood, P_{aO_2}	87.1 ± 1.5	67.1 ± 5.6^b
Mixed venous blood, P_{vO_2}	37.5 ± 1.4	34.1 ± 3.2
Tissue capillary blood, P_{tO_2}	50.3 ± 1.1	46.0 ± 3.4
Hemoglobin, C_{Hb} , g/l	126 ± 3	115 ± 4^c
Circulation minute volume \dot{Q} , ml/min·100 g	48.8 ± 5.4	26.7 ± 5.0^b
Oxygen transport rate by		
Arterial blood, \dot{V}_{aO_2} , ml/min·100 g	8.21 ± 0.71	3.33 ± 0.57^a
Oxygen consumption rate \dot{V}_{O_2} , ml/min·100 g	2.57 ± 0.29	1.13 ± 0.11^a
Ratio of oxygen arterial transport and consumption $\dot{V}_{aO_2} / \dot{V}_{O_2}$	3.21 ± 0.18	2.80 ± 0.26

^a $p < 0.001$.^b $p < 0.01$.^c $p < 0.05$.

TABLE 12
Effect of Immobilization Stress on the Acid-Base Equilibrium, Lactic Acid, and Malonic Dialdehyde Content in Blood

Indices	Control	Stress
Arterial blood pH	7.39 ± 0.01	7.14 ± 0.02^a
Mixed venous blood pH	7.35 ± 0.01	7.10 ± 0.02^a
CO_2 tension in arterial blood, P_{aCO_2} (mmHg)	36.0 ± 2.7	34.8 ± 3.3
True arterial bicarbonate AB (mmol/l)	21.4 ± 1.6	11.6 ± 1.2^a
Buffer bases of arterial blood BB (mmol/l)	44.8 ± 1.5	30.8 ± 1.2^a
Normal buffer bases NBB (mmol/l)	47.0 ± 0.3	46.5 ± 0.2
Buffer base shift in arterial blood, BE (mmol/l)	-2.5 ± 1.3	-15.7 ± 1.2^a
Lactic acid, C_L (mmol/l)	2.8 ± 0.5	5.0 ± 0.3^a
Malonic dialdehyde, C_M (mmol/l)	1.26 ± 0.06	4.28 ± 1.28^b

^a $p < 0.001$.^b $p < 0.01$.

In the aggregate, the above provides evidence that *stress damage elicits a peculiar hypoxic condition having features of hypoxic (arterial hypoxemia), hemic (decreased hemoglobin concentration), circulatory (smaller minute volume), and primary tissue (impaired tissue consumption of O_2 and activated glycolysis) hypoxia*.

The above data per se do not yet point to the damage to respiratory organs and circulation and may create an illusion that the observed depression of these functions results from lowered basal metabolism. Actually the situation is different. Cardiac and vascular disorders in stress are now well known, while stress damage to the respiratory apparatus and the lungs proper have been reported only in a few works.^{130,131} We have obtained unambiguous evidence to such disturbances in breathing either air or a hypoxic mixture enriched in carbon

dioxide: upon stress, despite the low blood P_{O_2} and marked acidosis, the respiration rate and volume are both decreased.

The disorders in external respiration and in its response to changing oxygen parameters and carbon dioxide tension in blood are usually explained by brain hypoxia.¹³⁰ However, in the present case the respiration regulatory disorders cannot be simply attributed to a decreased oxygen supply to the brain. As already mentioned, the secondary tissue hypoxia (which develops when the oxygen delivery is inadequate to the tissue demand) is only weakly pronounced upon stress. Moreover, the comparison of the extent of arterial hypoxemia (decreased supply of O_2 to the tissue capillaries) found in this study with the well-known hypoxic hypoxia caused by inspiring oxygen-depleted mixtures¹³⁴ demonstrates that a drop in arterial oxygen tension to 80 to 90 hPa cannot by itself depress respiration. The attenuated O_2 delivery due to the lesser blood flow also does not appreciably change the P_{O_2} in the tissue capillaries of the organs well supplied with oxygen, which include the brain (see Table 11). All this prompts a suggestion that immobilization stress elicits defects of respiratory regulation which are not directly associated with the stress hypoxia.

Our study of the effect of stress on external respiration revealed that the respiration rate is lowered mainly at the expense of a substantially prolonged expiratory phase, which may indirectly point to decreased excitability of the respiratory center.

In elucidating the mechanism of the described hypoxemia we proceeded from the accepted concept that it may chiefly be due to three factors: increased diffusion resistance of the alveolo-capillary membranes, inadequacy of blood flow and ventilation in certain pulmonary regions, and finally anatomic bypassing.¹³⁶ All these factors result in a part of mixed venous blood going into the arterial bed without oxygenation, i.e., being shunted. It is therefore convenient to evaluate their role in arterial hypoxemia by an equivalent amount of blood discharged into the arterial bed per unit time, assuming that the P_{O_2} in this shunted flow is constant and equal to the oxygen tension in mixed venous blood. This approach has been first used by Piiper et al.,¹³⁷ who introduced the notion of an equivalent shunt in describing oxygen diffusion in the lungs. We have somewhat improved this method,¹³⁸ taking into account the nonlinearity of the hemoglobin dissociation curve, pH shifts, and a number of other factors, which allowed its use not only with hypoxic hypoxia, but with other conditions including stress.

Analysis of the data in Table 13 shows that the growth of overall pulmonary shunting and consequently arterial hypoxemia is mainly due to a more irregular distribution of ventilation and blood flow and to a greater anatomic bypassing. Thus, not only are ventilation and blood flow decreased, but their coordination in many regions of the pulmonary tissue is hampered in stress.

Another fact attracting attention in Table 13 is a marked loss in pulmonary diffusion capacity for oxygen. In our experiments, in the resting state its contribution was small; but under real conditions of active life, or in stress combined with hypoxic hypoxia or physical load, this damage may have fatal consequences.

It further follows from Table 13 that the pulmonary diffusion capacity is decreased mainly because of the membrane component, i.e., because the stress impairs the diffusion properties of the membrane separating the alveolar space from the blood. This is especially important as an indication of direct stress damage to the tissue of the pulmonary aerohematic barrier.

Thus, the complex of the stress-induced disturbances in external respiration, its regulation and biomechanics, as well as the depression of pulmonary gas exchange, among other things, are due to lung damage as such. The "stress lung" condition implies at least three components: upset coordination between local blood flow and ventilation; increased anatomic bypassing, i.e., direct flux of mixed venous blood into the arterial bed; and, finally, decreased pulmonary diffusion capacity, in all probability caused by direct damage to the alveolar and capillary cell membranes.

TABLE 13
Effect of Immobilization Stress on the Parameters of
Blood Oxygenation in Lungs

Indices	Control	Stress
Lung diffusion capacity		
D_L ($\mu\text{mol}/\text{min}\cdot\text{kPa}\cdot 100 \text{ g}$)	221 ± 27	93 ± 27^a
Membrane component of D_L		
D_M ($\mu\text{mol}/\text{min}\cdot\text{kPa}\cdot 100 \text{ g}$)	647 ± 30	141 ± 20^b
Blood component of D_L		
V_c ($\mu\text{mol}/\text{min}\cdot\text{kPa}\cdot 100 \text{ g}$)	335 ± 48	269 ± 40
Equivalent shunts (% of blood flow)		
Total, E_T	7.02 ± 0.61	31.20 ± 5.80^b
Diffusion, E_D	1.05 ± 0.02	2.13 ± 0.04^b
Anatomical, E_A	3.00 ± 0.32	8.00 ± 0.71^b
Due to lung inhomogeneity, E_I	2.97 ± 0.95	21.07 ± 1.60^b

^a $p < 0.01$.

^b $p < 0.001$.

We have assessed this last component by direct electron-microscopic examination of lungs from animals that had undergone stress.¹³⁸

Immobilization stress has been found to evoke drastic ultrastructural alterations in the lungs, most pronounced in the aerohematic barrier (AHB).

In the electron microscope, lung tissues from stress animals reveal substantial seepage of blood liquid and erythrocytes into the alveolar lumen, i.e., development of an edematous-hemorrhagic syndrome (Figure 18A).

Simultaneously, in the alveolar epithelium stress induces regions of total edema of type I pneumocytes with velum-like protuberances (Figure 18B), testifying to increased hydration of the lung tissue. Rather often seen were regions of complete destruction of the alveolar epithelium (Figure 18C). The interstitial layer also displayed regions of thickening, which indicated enhanced liquid accumulation (Figure 18D).

Significantly altered was also the lung capillary endothelium, where regions of subendothelial edema were not infrequently found as pockets peeling off the endothelium (Figure 19A).

Another electron-microscopically revealed manifestation of the reaction to stress damage is the marked enhancement of pinocytosis, especially pronounced in the lung capillary endothelium (Figure 19B). In occasional regions of cytoplasmic veils, particularly of endothelial cells, there was "excessive vesiculation"¹³⁹ with the number of vesicles per 1 μm along the barrier amounting to 35 and more (compared to the normal 5 to 10) (Figure 19B). The micropinocytotic vesicles were from 0.06 to 0.15 μm in diameter. In some cases they fused to form larger vacuole-like structures. Such pinocytotic activation probably reflects enhanced transport of structural and energetic provisions from the circulatory bed and cell endoplasmic reticula to the injured alveolar epithelium with a view to subsequent repair.

The mentioned alterations in the alveolar apparatus are accompanied by substantial thickening of the AHB at the expense of all its layers: lung capillary endothelium, interstitial layer, and alveolar epithelium. In sum, the AHB thickness is augmented about threefold. Such pronounced thickening of the AHB has not been reported in pathological conditions studied heretofore, including hypoxic cell injury.¹⁴⁰ We are inclined to explain this by excessive hydration of the AHB structures. Thus, stress elicits pulmonary tissue edema which in some regions results in intra-alveolar edema.

It is interesting that the AHB edema is accompanied by formation of peculiar "clefts" in both endothelial and epithelial layers comparable in size to and sometimes exceeding the

barrier thickness (Figure 19C). The barrier itself in such localities becomes thinner, which may to some extent facilitate O₂ transfer from air to blood. Such a structural response is considered typical of hypoxic exposure.¹⁴¹

A fact worth attention is the practically complete lack of both intracellular (reserve) and extracellular (active) surfactant in the lungs of stress animals, which indicates depression of the pulmonary surfactant system. This appears to be only natural in view of intimate interrelation between the number and activity of pulmonary β-adrenoreceptors on the type II pneumocytes, and pulmonary surfactant synthesis and secretion.¹⁴² Since stress (including immobilization) decreases the number of β-adrenoreceptors in a number of organs and tissues,^{143,144} and supposedly in the lungs as well, the loss of total surfactant becomes more explicable.

One should also note an appreciable increase in the amount of primary and secondary lysosomes in pulmonary cells (Figure 19D).

In whole, the ultrastructural alterations that we have observed in lungs after a single heavy stress exposure comprise edema and marked thickening of the AHB in all its components, edematous-hemorrhagic syndrome and injury to the alveolar epithelium, and defects in the pulmonary surfactant system. This complex of alterations combines with the pulmonary functional disorders described above, which in whole we designate as "stress lung", is very likely to hinder oxygen transport from the alveoli to the pulmonary capillaries. It is therefore plausible that the stress damage can substantially disturb the external respiration and cause hypoxemia, which in its turn can promote cardiac damage or handicap its function. However, in our version of stress — the immobilization "stress of capitulation" — this element should not be overestimated, since such impeded oxygen supply in animals that had undergone stress was concurrent with another quite important event, namely a direct decrease in the oxygen consumption by the organism, which could to some extent limit the immediate hypoxic injury to tissues and specifically to the myocardium. More important is the observed lowering of the blood pH, which may depress the cardiac contractile function mainly because of calcium displacement from the sarcolemmal and myofibrillar binding sites. Finally, the greatest danger in our opinion comes from possible infection of the lungs profoundly damaged by stress and formation of a sort of vicious circle in the cardiorespiratory system.

Stress damages to the liver and prevention thereof will be further considered in detail in connection with the role of stress in evolvement of atherogenic dyslipidemia and coronary atherosclerosis (see Chapter 2). Here it should be noted that stress (and in particular the EPS used by us) consistently causes damage to hepatic cells manifesting itself as a drastic rise in the plasma level of the liver-specific enzyme fructose 1-phosphate aldolase. This damage results in great measure from activation of hepatic free-radical peroxidation as manifest by a markedly increased content of malonic dialdehyde and inactivation of superoxide dismutase. The most essential thing is that the stress damage encompasses the cytochrome P₄₅₀ system and, affecting its important member cholesterol 7-α-hydroxylase, hinders cholesterol oxidation to bile acids.¹⁴⁵

This results in a higher plasma content of low-density beta-lipoproteins and a lower content of alpha-lipoproteins. The atherogenicity index is substantially enhanced.¹⁴⁶ The significance of stress atherogenic dyslipoproteinemia in coronary atherosclerosis and ensuing ischemic heart damage at present raises no doubts and will be considered in Chapter 2 devoted to the place of stress in the evolvement of ischemic disease. Here it is essential that hypercholesterolemia, caused by various and in particular alimentary factors, directly and detrimentally affects the myocardium: it decreases the specific activity of the SR Ca ATPase and the efficiency of the calcium pump, causes cholesterol accumulation in the SR membranes where it is usually absent, with a simultaneous fivefold to tenfold increase in the LPO products; all this can impair the intracellular calcium transport which is important for contraction and relaxation.¹⁴⁷ Thus, the stress-induced atherogenic dyslipidemia may apparently not only contribute to coronary atherosclerosis, but directly harm the myocardial metabolism.

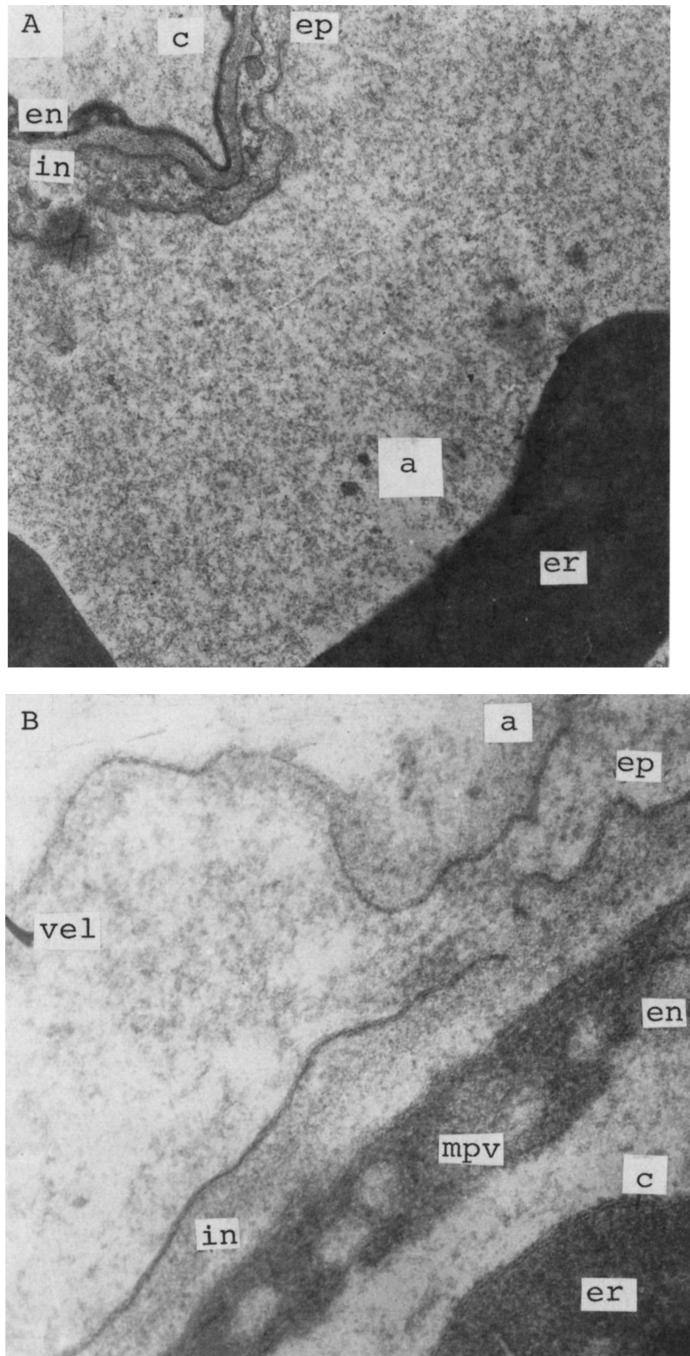


FIGURE 18. Ultrastructural alterations of the aerohematic barrier in the lungs of rats subjected to stress. For explanations see text. (a) Alveola, (c) capillary, (d) destructive alterations, (en) endothelial layer, (ep) epithelial layer, (er) erythrocyte, (in) interstitial layer, (le) local edema, (lys) lysosome, (mpv) micropinocytotic vesicles, (vac) vacuole-like formations, (vel) velum-like protuberances. (Magnification: A, $\times 22,500$; B, $\times 103,000$; C and D, $\times 6,400$.)

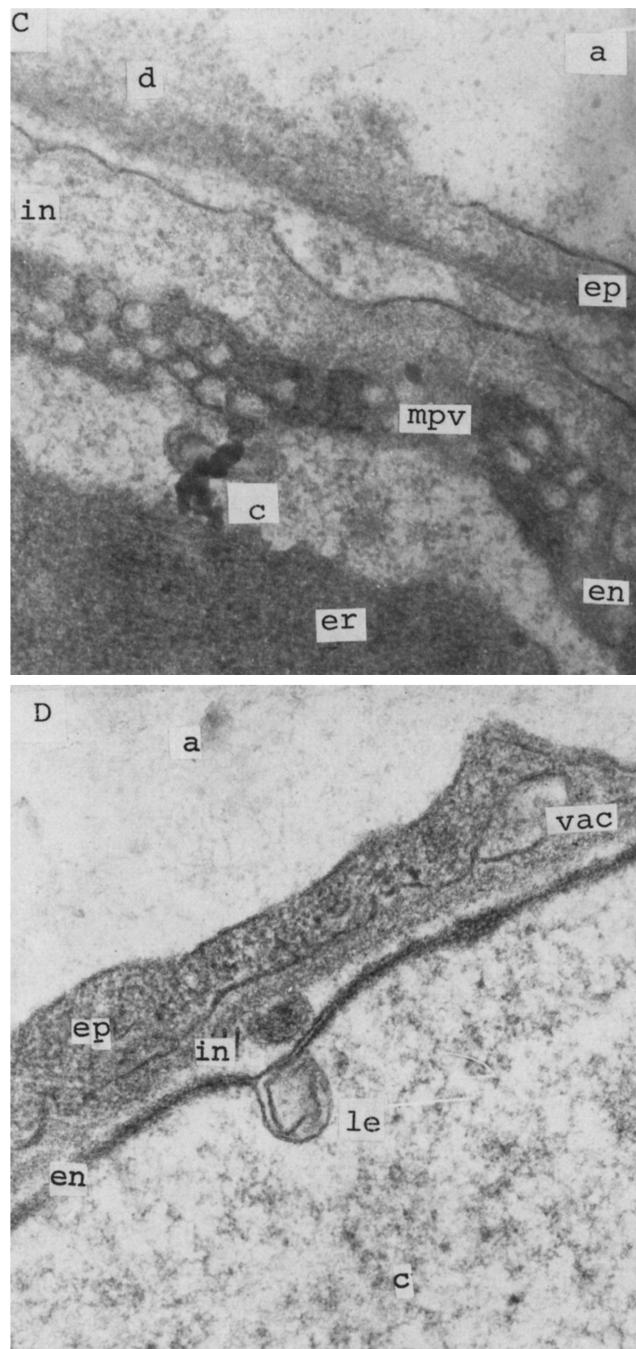


FIGURE 18C and D.

Another example of extracardiac effects of stress mediating heart damage is the stress injury to the pancreas. Initially, together with Saulya and Gudumak we found ¹⁴⁸ that with increasing duration of immobilization stress the activity of the main proteolytic enzyme, trypsin, increases in the plasma. In this connection a special study was undertaken to correlate the plasma activities of trypsin and α_1 -antitrypsin with the myocardial content of sialic acid.

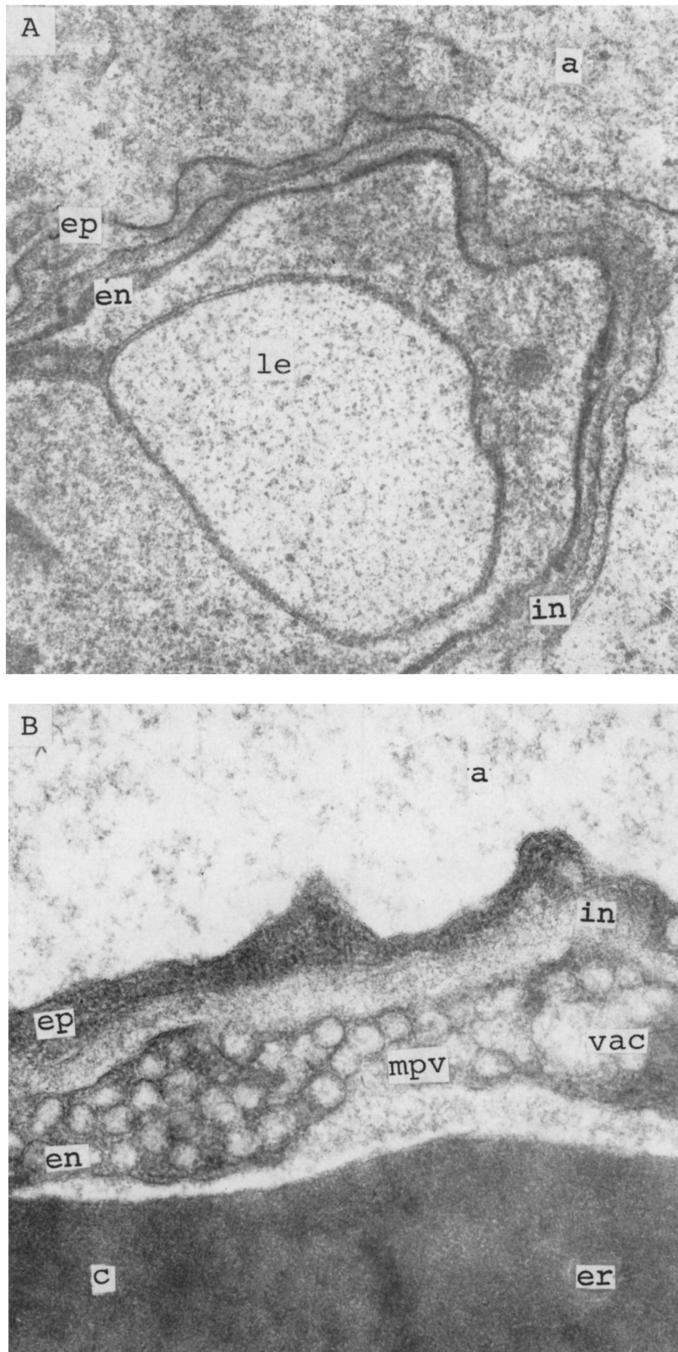


FIGURE 19. Ultrastructural alterations in the pulmonary aerohematic barrier. For explanations see text. Designations are as in Figure 18. (Magnification \times 22,500.)

The rationale stemmed from the concept of Langer¹⁴⁹ that the sarcolemmal lipid bilayer and the glycocalyx covering it carry a large number of fixed negative charges which are the binding sites for calcium ions. The ionized calcium binds predominantly to the anionic regions of the molecules of sialic acid entering into the composition of cell membrane

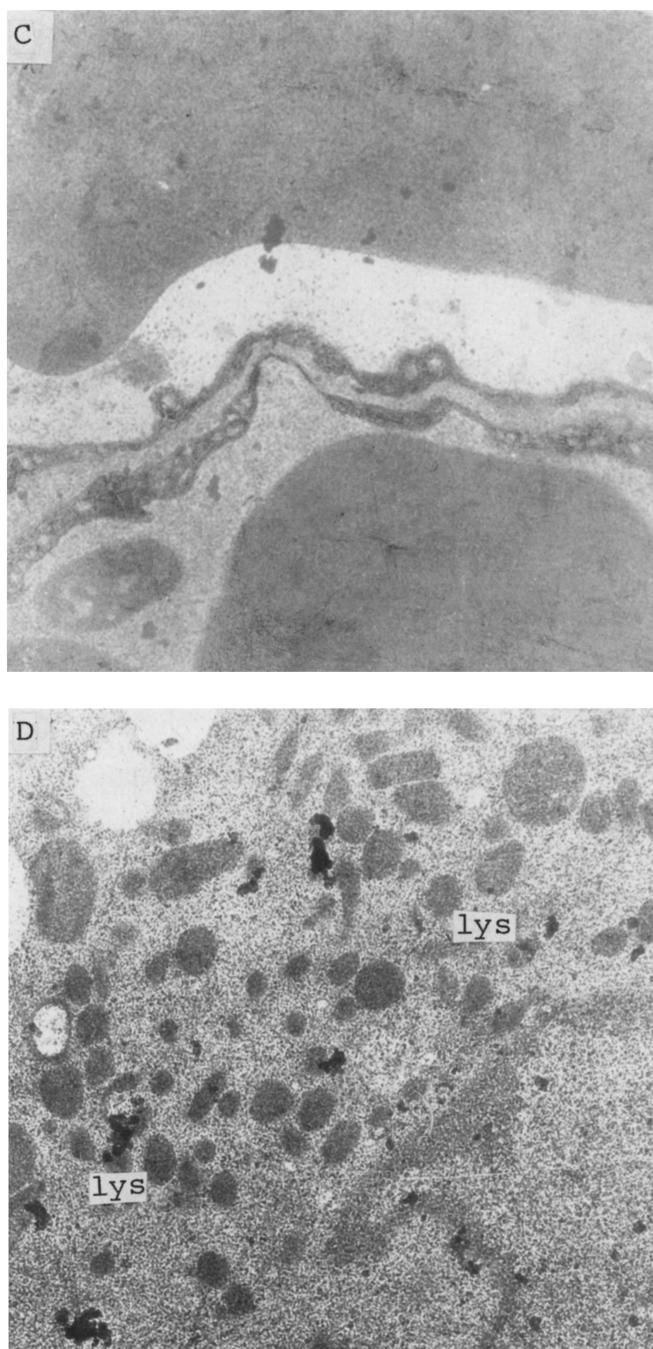


FIGURE 19C and D.

glycoproteins and glycolipids.¹⁵⁰ It seemed probable that glycoprotein hydrolysis by excess trypsin in stress could decrease the myocardial content of sialic acid and thereby diminish calcium binding in the zone immediately adjacent to the sarcolemma.

The biochemical indices were assayed 2 h after the end of stress exposure. *N*-Acetyl neuraminic (sialic) acid was determined in plasma and myocardial homogenates from control

TABLE 14
Effect of Prolonged Immobilization Stress on Sialic Acid, Trypsin, and Antitrypsin Levels in Myocardium and Serum

Indices	Control (n = 12)	Immobilization		
		3 h (n = 10)	6 h (n = 11)	9 h (n = 12)
Sialic acid				
Myocardial (nmol/mg wet wt)	1.27 ± 0.05	1.06 ± 0.05 ^a	0.96 ± 0.05 ^a	0.77 ± 0.06 ^a
Serum, total (mmol/l)	3.72 ± 0.17	3.82 ± 0.17	3.77 ± 0.16	5.12 ± 0.26 ^a
Serum, free (mmol/l)	0.05 ± 0.002	0.05 ± 0.001	0.05 ± 0.001	0.10 ± 0.007 ^a
Trypsin activity				
Serum (μmol/l·s)	52.09 ± 5.73	75.28 ± 6.01 ^a	94.78 ± 6.73 ^a	141.85 ± 12.7 ^a
α₁-Antitrypsin				
Serum (g/l)	1.74 ± 0.05	1.56 ± 0.05 ^a	1.48 ± 0.05 ^a	1.20 ± 0.06 ^a

^a p <0.05.

and stress animals by a modified thiobarbituric method of Horgan.¹⁵¹ Trypsin, α₁-antitrypsin, and α₂-macroglobulin were measured by agarose gel electrophoresis according to German et al.¹⁵⁰

Table 14 shows that sialic acids in plasma are elevated only after a 9-h immobilization stress with the total content and the free fraction exceeding the control by 37 and 80%, respectively. Meanwhile, in the myocardium the sialic acid content falls proportionately to stress duration, declining by 39% after 9 h of immobilization.

The trypsin activity in plasma exceeds the control level by 44, 82, and 173% after 3, 6, and 9 h of stress, respectively. It is noteworthy that the increased proteolytic activity is accompanied by a decline in the major trypsin inhibitor α₁-antitrypsin responsible for 90% of the total antitryptic activity in serum.

On the whole, the data in this Section serve to emphasize that the pathogenesis of cardiac stress damage is not reducible to direct cardiomyocyte injury by various stress hormones and secondary ischemic injury resulting from stress shifts and coronary thrombosis. Disorders caused by stress in other organs through a variety of pathways also lead to heart damage.

However, the multiplicity of the pathogenetic mechanisms of cardiac stress damage does not change the basic fact that the ultimate target of all these mechanisms is the cardiomyocyte. It is essential in this context that many of the phenomena described above (myocardial stress rigidity, enhanced cardiac sensitivity to external Ca²⁺, enzyme leakage from isolated stress heart, destruction of glycocalix, and finally the focal contractual injury to cardiomyocytes) provide unequivocal evidence to the impairment of cardiomyocyte membranes and systems of ionic, first of all calcium transport.

VII. ACTIVATED LIPID PEROXIDATION AND CARDIOMYOCYTE MEMBRANE INJURY IN STRESS

Cardiomyocyte membrane injury by the excessive lipotropic effect of stress, as already pointed out, is determined by the lipid triad, namely overactivation of phospholipases and lipases, direct damage by lysophosphatides and acyl carnitine, and overactivation of free-radical lipid peroxidation. The first two factors have already been the subject of comprehensive reviews.^{153,154} Studies of the disturbances to the free-radical homeostasis in cardiac damage, and in stress in particular, have only recently been initiated.¹⁵⁵

In 1979 we demonstrated that EPS is accompanied by enhanced chemoluminescence of lipids isolated from the cardiac muscle.¹⁵⁶ This fact per se indicated that stress may activate free-radical oxidation in the myocardium. Then we determined the primary (hydroperoxides

of polyenic lipids) and final LPO products (fluorescing Schiff bases) in the heart, skeletal muscle, and brain of rats that had undergone EPS,¹⁵⁷ and found that they are all reliably increased already in 2 h after stress and for the next 2 to 3 d.

Thus, exposure to stress entails activation of lipid peroxidation, which is most pronounced in the heart and maintained for a long period.

A medically important question is to what extent this issue, proved experimentally in animals, is applicable to man. It is known that LPO activation in animals, through decomposition of lipid hydroperoxides and β -cleavage of the alkoxy radicals, yields lower hydrocarbons. These gases are released with the exhaled air and their content is a specific and sensitive criterion of the intensity of LPO processes. Basing on this, Prilipko et al.¹⁵⁸ measured the gaseous LPO products in the exhaled air to elucidate whether or not LPO is activated in humans under stress caused by intense intellectual work under time shortage.

The stress exposure consisted in operator work requiring consecutive solution of seven complicated enough mental tasks in certainly too little time; the overall duration of such load was 2 h. The amounts of gaseous LPO products in the exhaled air and of catecholamines in the blood were assayed 24 h and 15 min before stress, during stress, and up to 5 d after. In the quantitative analysis of hydrocarbons in the exhaled air we reasoned from the fact that the preferred LPO substrate are the ω -6-unsaturated fatty acid residues and correspondingly the predominant gaseous LPO product would be pentane. Therefore, pentane was measured to ensure maximal sensitivity. Of the 20 healthy persons studied, 13 were subjected to stress.

Figure 20 depicts the typical chromatograms of exhaled air before (1) and after (2) stress. The first peak on the left of each chromatogram corresponds to pentane: one can see that the pentane content is appreciably higher after stress. Before stress the pentane content in the exhaled air was 1.71 ± 0.34 nmol/l and the epinephrine and norepinephrine contents in the blood were 0.70 ± 0.05 and 0.8 ± 0.05 $\mu\text{g/l}$, respectively. Upon stress there was an immediate but relatively short elevation of blood catecholamines and a slower but prolonged elevation of exhaled pentane (Figure 21); this reflects the excitation of the adrenergic system typical of stress that precedes maximal LPO activation.

The results obtained show that in humans, as well as in animals, an emotiogenic stress influence (not associated with direct damage to the organism from exogenous physical or chemical factors) elicits substantial activation of peroxidation as manifest by increased amounts of exhaled gaseous LPO products.

It is essential that in the cell membranes LPO activation is not isolated, but inseparably linked with activation of lipases and phospholipases, detergent action of lysophosphatides and acyl carnitine, and excessive entry of Ca^{2+} into the cell. One of the manifestations of cell membrane damage in this case is the "stress enzymemia", i.e., release of both cytoplasmic and lysosomal enzymes into the blood.

Indeed, the activities of aspartate transaminase, alanine transaminase, lactate dehydrogenase, and malate dehydrogenase have been shown to increase twofold in 2 h after EPS.¹ Acid cathepsins decrease by 25% in the myocardial lysosomal-mitochondrial fraction and increase by 45% in the supernatant. Their activity in plasma is elevated more than twice. This leakage of cytosolic and lysosomal enzymes is completely abolished by the β -blocker propranolol as well as by the antioxidant ionol (butylated hydroxytoluene), i.e., results in great measure from catecholamine-induced LPO activation.¹⁵⁹ The mechanism of LPO activation in stress will be considered in Chapter 5, which also presents data on LPO activation in ischemia and on antioxidant protection of the heart. Here it is important that, first, the β -adrenergic membrane stress damage linked with LPO activation can be documented for the heart, and second, that it can be blocked with propranolol. *A priori*, one can envisage that LPO activation, lysosomal labilization, and membrane injury in any case affect such membrane-bound enzymic systems central to cardiac function as the Na,K and Ca pumps.

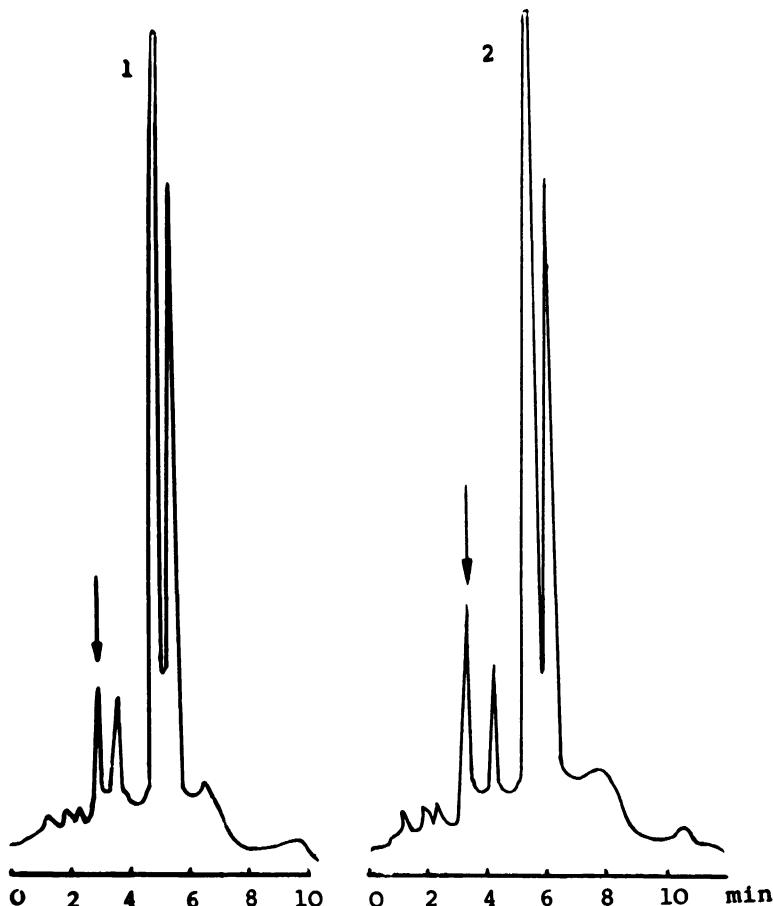


FIGURE 20. Chromatograms of exhaled air before (1) and after (2) stress exposure. The first peak on the left reflecting the pentane content (arrow) is appreciably enhanced upon stress.

VIII. IMPAIRED ACTIVITY OF CARDIOMYOCYTE Na,K AND Ca MEMBRANE PUMPS IN STRESS

The sarcolemmal Na,K pump is known to play a decisive part in maintaining the transmembrane cation gradient, the $\text{Na}^+/\text{Ca}^{2+}$ exchange on the sarcolemma, normal cell calcium concentration, and thereby the physiological values of rest potentials, generation and conduction of action potentials, and the electric stability of the heart as a whole.

We have studied the effect of EPS on the Na,K ATPase activity in cardiac muscle and the action of a β -blocker in this system.¹⁶⁰ The Na,K ATPase activity in the myocardium of rats subjected to EPS proved to decrease by 25%, and this could be prevented by prior administration of propranolol.

Further, this study was reproduced in greater detail. ATPase activity was assayed in the sarcolemmal fraction by P_i accumulation with and without ouabain.¹⁶¹ Protein was determined according to Lowry. Heat denaturation was carried out at 50 to 60°C. The thermodenaturation kinetics was determined and the thermodynamic parameters calculated as described.¹⁶²

The diagram in Figure 22 shows that upon stress, the sarcolemmal Na,K ATPase activity decreased by 28%, and this could be prevented with the β -blocker propranolol or the antioxidant ionol, i.e., the decrease resulted from β -induced LPO activation. Interestingly,

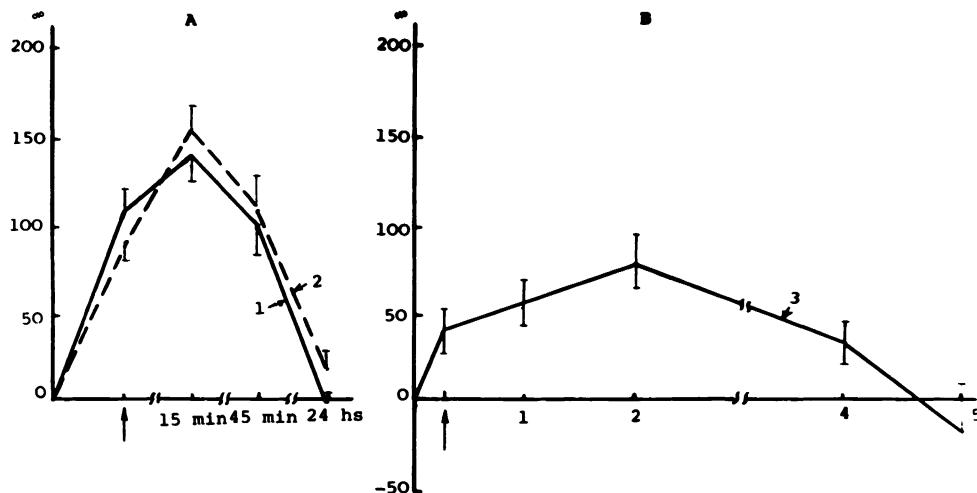


FIGURE 21. Dynamics of (A) catecholamines in blood and (B) pentane in exhaled air of subjects in and after stress. 1, Epinephrine; 2, norepinephrine; 3, pentane. The abscissa is time after the end of stress (arrows denote the values obtained during stress exposure); the ordinate is the percent excess over initial levels.

the ATPase inactivation could be reproduced *in vitro* by inducing LPO with the Fe-ascorbate system, and also prevented with ionol.

Taken together, these data furnish evidence that the mechanism “ β -adrenergic LPO activation \rightarrow Na,K ATPase inactivation” is actually operative in stress. The nature of damage to the sarcolemmal Na,K ATPase system was elucidated by studying the effects of stress and antioxidant on the dynamics of ATPase inactivation during heat denaturation.

Figure 23 presents the results of studies with rat myocardial sarcolemmal membrane preparations. One can see that upon EPS the enzyme loses activity much more rapidly, so that the half-inactivation time decreases by half (Figure 23A). Stress increases the denaturation rate constant for the Na,K ATPase, as shown in Figure 23B where the shaded zone quantitatively reflects this effect. It is essential that administration of ionol to animals (50 mg/kg body weight) prior to stress completely prevents such stress alterations.

The thermodynamic parameters of Na,K ATPase heat inactivation in Table 15 reveal two basic facts: the lower activation energy and a smaller change in entropy during denaturation of the enzyme from stress animals. In essence they both mean that *the stress itself brings the Na,K ATPase protein-lipid complex closer to the denatured state; these changes are prevented by prior suppression of free-radical lipid oxidation*.

Considering the decisive role of the Na,K ATPase in maintaining the rest potential (RP), one could suppose that impaired activity of this enzymic system in stress would in some way affect the ability of cardiomyocytes to preserve this principal parameter of electric stability.

In our experiments the RP was measured in isolated hearts perfused with a Krebs-Henseleit solution (pH 7.4, oxygenated with 98% O₂/2% CO₂) at a constant hydrostatic head of 100 mm to sustain the electric and contractile activity. The heart was perfused at 36°C and the RP was recorded within ± 1 mV with a glass electrode from the left ventricular surface. Then the heart was chilled to 4°C by perfusing it with a cold solution, and the RP dropped. Then the temperature of the perfusing solution was returned to 36°C and the dynamics of RP restoration was monitored.

Figure 24 shows that in the control group (curve 1) the cooling decreases the RP from -80 to -45 mV; a subsequent rise from 4 to 36°C entails restoration and overshoot of the RP to a maximum of -100 ± 5 mV in 2 to 2.5 min. The restoration rate at the half-

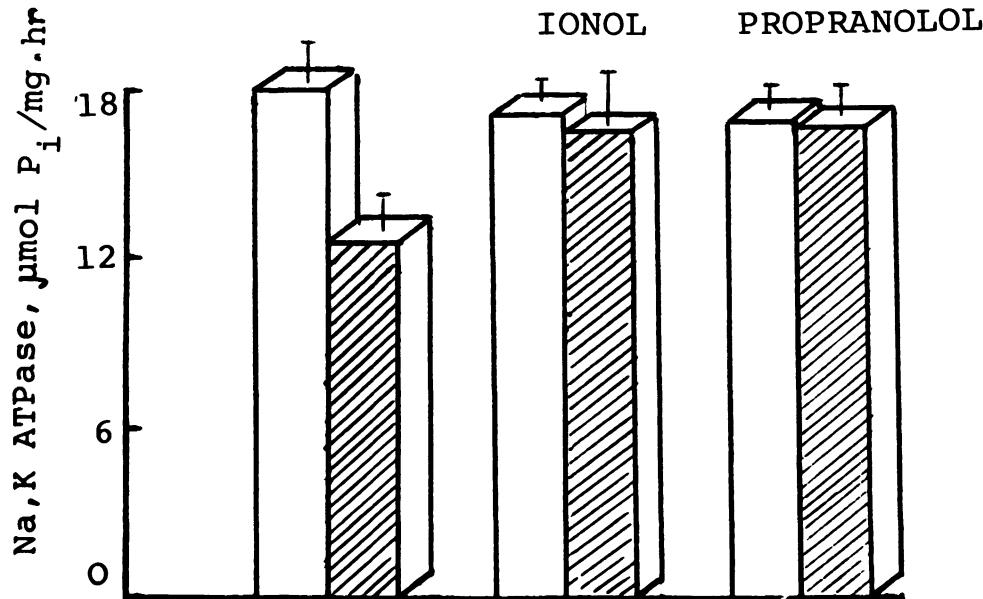


FIGURE 22. Prevention of the stress-induced inhibition of the myocardial sarcolemmal Na,K ATPase with the antioxidant ionol (butylated hydroxytoluene) and the β -blocker propranolol. Empty blocks, control; shaded blocks, stress.

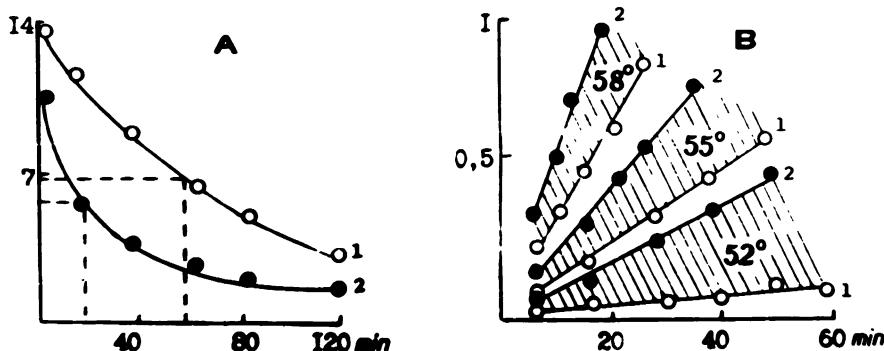


FIGURE 23. Heat inactivation of rat cardiac sarcolemmal Na,K ATPase. (A), Enzyme activity at 52°C in the control (1) and after EPS (2). The abscissa is time in min; the ordinate is activity in $\mu\text{mol/h per mg protein}$. (B), Changes in the denaturation rate constant ($K = 2.303 \frac{\text{tga}}{\text{A}_0 - \text{A}_t}$) with temperature (52°C, 55°C, 58°C). The abscissa is time in min; the ordinate is $\lg \frac{\text{A}_0}{\text{A}_t}$, where A_0 is the initial and A_t the current activity.

maximal point was about 0.7 mV/s. In animals subjected to EPS (curve 2) the RP was initially diminished by 10 mV; upon cooling it fell to -35 ± 2 mV; in the course of restoration there was no hyperpolarization and the restoration rate did not exceed 0.2 mV/s, i.e., less than one third of the control. Prior administration of ionol to animals (50 mg/kg daily for 3 d before the experiment) prevented the stress-induced impairment of RP restoration. Curve 3 in Figure 24 is close to the control one, showing that the restoration rate and hyperpolarization are retained despite the experienced stress. Thus, *under certain conditions the β -induced LPO activation can be involved in the damage to the Na,K ATPase system and electric stability of cardiomyocytes*.

TABLE 15
Parameters of Heat Denaturation of Rat Myocardial Sarcolemmal Na,K
ATPase, Effect of Stress and Antioxidant

Indices	Control (n = 26)	Stress (n = 26)	Ionol (n = 20)	Ionol + stress (n = 20)
E _a	75.6 ± 2.3	60.7 ± 2.0 ^a	72.5 ± 2.8	72.1 ± 3.3
ΔH*	75.0 ± 2.3	60.1 ± 2.0 ^a	71.9 ± 2.8	71.5 ± 3.3
ΔS*	156.8 ± 0.6	111.3 ± 3.6 ^a	149.3 ± 2.4	146.7 ± 2.6
ΔF*	24.4 ± 1.5	23.7 ± 1.9	24.3 ± 1.6	23.9 ± 1.6

Note: E_a, Activation energy (kcal/mol); ΔH*, enthalpy change (kcal/mol); ΔS*, entropy change (kcal/mol. degree); ΔF*, free energy change.

* Difference from control significant at p < 0.001.

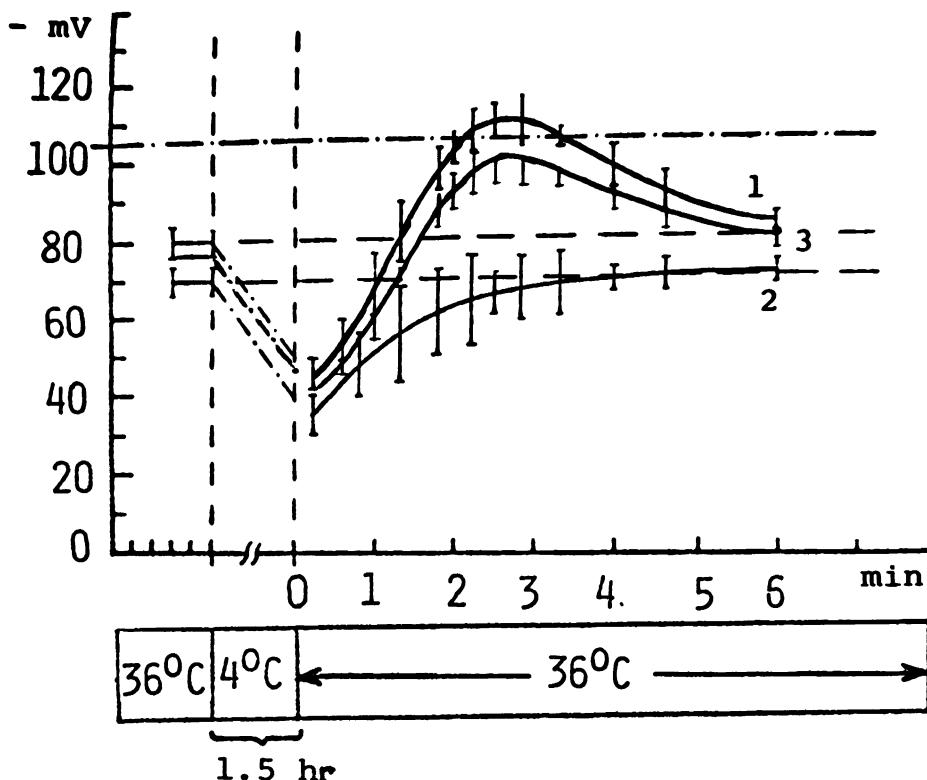


FIGURE 24. Effect of cooling and subsequent warming of isolated rat heart on the rest potential of left ventricular cardiomyocytes. The abscissa is time before and after cooling in min; the ordinate is the RP in mV. 1, Control; 2, stress; 3, ionol plus stress.

It should be borne in mind that the role of such disturbances is quite well known in modern arrhythmology. In the healthy heart the impulse from the sinus node having the highest automatism depolarizes any latent pacemakers before they can depolarize themselves. However, higher automatism and timely depolarization are not the sole mechanism by which the sinus node suppresses latent pacemakers. An important part is played by the phenomenon known as overdrive suppression. Stimulation of the cells of the conducting system and working myocardium at a frequency exceeding their own results in enhanced Na^+ entry into the cells, with ensuing marked activation of the Na,K pump; since sodium extrusion is

TABLE 16
Effect of EPS on the Enzymic System of Ca^{2+} Transport in the
SR Vesicles

Indices	Control	Stress
Ca ATPase (nmol P/min per mg protein)	502 \pm 68 (n = 12)	359 \pm 127 ^a (n = 10)
Mg ATPase (nmol P/min per mg protein)	694 \pm 113 (n = 12)	642 \pm 153 (n = 10)
Calcium uptake rate (nmol Ca^{2+} /min per mg protein)	37.2 \pm 4.0 (n = 10)	23.1 \pm 4.2 ^b (n = 8)

^a $p < 0.01$.

^b $p < 0.001$.

increased much more than potassium entry, the resulting repolarizing efflux augments the RP and thereby suppresses spontaneous depolarization of the latent pacemaker cells. Thus, the sinus rhythm is imposed not only because of its higher automatism, but also owing to the overdrive suppression in the lower regions of the conducting system, which is provided for by the mobilized capacity of the sarcolemmal Na,K pump.¹⁶³ The stress damage to the sarcolemmal Na,K pump found in our studies makes probable that the overdrive suppression can be hindered and thus ectopic foci can develop even despite the high automatism of the sinus node; for example, in sinus tachycardia, under adrenergic effect, in myocardial infarction inevitably combined with stress, etc. This is still more probable in bradycardia when the numerous groups of damaged myocardial cells become the ectopic foci of excitation and, as will be seen, the source of multiple ventricular extrasystoles.

The other and no less important cation pump that determines the cardiac rhythmic activity, namely the Ca pump, is functionally linked with the Na,K ATPase, since any decrease in the activity of the latter means diminished calcium removal from the cell through the $\text{Na}^+/\text{Ca}^{2+}$ exchange and enhanced load on the sarcolemmal and SR Ca ATPases. This is the more important that the lipid triad of membrane damage in stress may as well affect the performance of both sodium and calcium pumps. Therefore we studied the effect of stress on some parameters of membrane calcium transport in the cardiac muscle.¹⁶⁴

The myocardial microsomal fraction was isolated by standard differential centrifugation 2 h after stress. By the data of enzymic analysis, it consisted mainly of the SR. Calcium uptake in the presence of oxalate was monitored by ^{45}Ca radioactivity. ATPase activities were measured pH-metrically as acidification of the medium due to ATP hydrolysis.

The results presented in Table 16 show that upon EPS the Ca ATPase activity decreases almost by 30%, whereas the Mg ATPase is not affected. The rate of Ca^{2+} uptake in the presence of oxalate declines by 38%. This significantly attenuated performance of the SR Ca pump appears to be due not only to the lower activity of the calcium transporting enzyme, but also to a greater calcium permeability of damaged SR membranes.

For a more precise evaluation of the role that depressed calcium pumping activity may play in the damage to cardiac function and electric stability in stress, together with Sazontova and Vovk we undertook a study of calcium uptake by SR vesicles from control animals and those subjected to 1- to 2-h and 6-h immobilization stress of capitulation. Since it has been shown earlier that a 2-h stress of this type diminishes cardiac resistance to the contractural and arrhythmogenic action of calcium, these experiments allowed a quantitative comparison of the stress damage to cardiac calcium resistance and to the SR calcium pump function.

The rate of calcium uptake by the SR vesicles in the presence of oxalate was determined with a Ca-selective electrode and an Orion® EA 940 ion meter. Mitochondrial uptake was prevented with NaN_3 . The uptake was monitored for 5 min with 50 to 200 μl homogenate

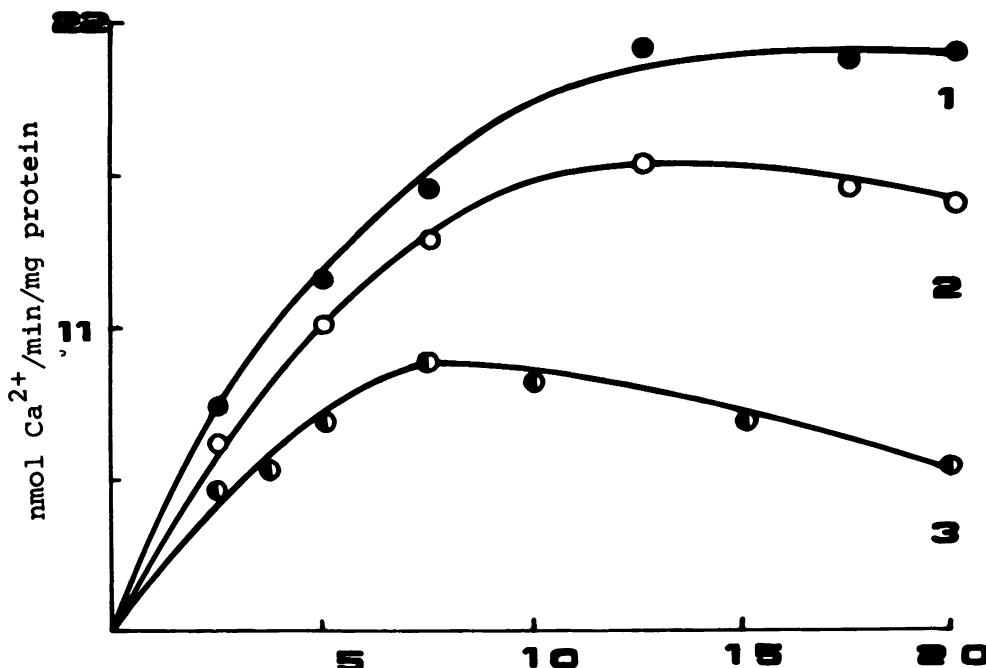


FIGURE 25. Effect of immobilization stress on the Ca^{2+} transport by rat myocardial SR vesicles. The abscissa is $\mu\text{M Ca}^{2+}$; the ordinate is nmol Ca^{2+} /min per mg protein. 1, Control; 2, 6-h stress; 3, 1-h stress.

added to 5 ml of 100 mM KCl, 15 mM potassium oxalate, 20 mM Hepes pH 7.0, 4 mM MgCl_2 , 5 mM NaN_3 , 4 mM ATP, and 1 to 20 $\mu\text{M CaCl}_2$. The rates were then determined by drawing tangents to the reaction curves 1.5 min after the onset and quantitated using calibration curves.

Figure 25 demonstrates the dependence of the calcium uptake rate on its concentration in the medium. It can be seen that in control SR the uptake rate increases with the ion concentration, reaching a plateau in the region 10 to $20 \cdot 10^{-6} \text{ M}$.

After a 1-h stress, the maximal rate is attained already at $7.5 \cdot 10^{-6} \text{ M}$ and is only 9.4 nmol/min/mg protein as compared to 16.2 in the control. Further rise in the external concentration of Ca^{2+} inhibits its transport; at 20 μM it decreases by more than one third of the maximum and proves to be 3.5 times lower than the corresponding control value.

Curve 2 in Figure 25 shows that a 6-h stress causes qualitatively similar, but much less pronounced alterations; thus, instead of the depression of uptake at high concentrations, here one can only see a tendency to this. At the highest calcium concentration tested (20 μM) the uptake rate after 6 h of stress is 25% lower.

Thus there is no doubt that the immobilization stress of capitulation impairs the calcium-uptake ability of the SR. It is also obvious that this is not just a test-tube artifact, since (see the end of Section II) it is the immobilization stress that markedly aggravates the propensity of the isolated heart to respond by contracture to elevated calcium in the perfusing fluid. Furthermore, the physiological and the biochemical results concur in one quite important feature: a short capitulation stress is much more effective than a long one both in impairing the calcium uptake by the SR and in aggravating the calcium-induced contracture. Such an unexpected and ostensibly paradoxical dependence of the extent of damage on the duration of the capitulation stress is probably due to the appearance of some stabilizing factor already in the course of the stress reaction, and deserves becoming the subject of special study. Here we must emphasize that the stress damage to calcium transport proved above is essential

for understanding the pathogenesis of cardiac stress damage as well as of ischemic and reperfusion damage that usually accompany stress.

In all these cases there is an excess of calcium in cardiomyocytes, which is either adrenergic or caused by the lack of energy or direct sarcolemmal injury, and which combined with the impaired SR ability to remove calcium can engender a peculiar vicious circle and focal contractural damage to the cardiac muscle.

In this context it should be borne in mind that the cardiac muscle is marked by pronounced bioelectric heterogeneity.¹⁶⁵ Impulse propagation through specialized intercellular contacts is more rapid in the anterograde than in the retrograde¹⁶⁶ and in the longitudinal than in the transverse¹⁶⁷ directions. All factors increasing calcium concentration have been proved to impair the cell contacts and excitation spreading.¹⁶⁸ This becomes a plausible cause of enhanced cardiac heterogeneity and evolvement of functional conduction blocks with consequent reentry and various arrhythmias and fibrillation.

Generally speaking, the combined disturbances to the performance of the sarcolemmal Na,K and the SR Ca pumps results in the sarcoplasmic cation accumulation and thereby is the most likely reason of decreased rest potentials observed in our studies with isolated heart (see Figure 24). It is of interest that this has been confirmed by Sudakov et al.¹⁶⁹ who have found that in animal cardiomyocytes upon emotional stress the RP is reduced from 83 to 69 mV and the action potential is shortened. Those myocardial regions where such alterations of bioelectric activity occur have a higher probability of spontaneous or evoked depolarization, and become potential foci of ectopic excitation.

It is noteworthy that the stress-induced decrease in the RP is in line with the observations of Wit and Cranefield¹⁷⁰ that *in vitro* norepinephrine can not only lower the RP by diastolic depolarization, but also trigger the automatism of a quiescent myocardial cell. It is not hard to envisage that in damaged cells with reduced RP the appearance of such adrenergically originating foci of automatism is rather probable, and they may be actuated by additional adrenergic influences, owing to the weaker inhibitory control from the sinus node, or by impulses arriving in the relative-refractory period.

On the whole, the above testifies that the lipid triad and in particular enhanced lipid peroxidation naturally found in stress damages the sarcolemmal membrane and its Na,K pump as well as the Ca pump of the SR. These defects are the key link in the pathogenetic chain of cardiac stress damage and may play a weighty part in the impairment of cardiac electric stability and evolvement of arrhythmias.

IX. STRESS DAMAGES TO CARDIAC STRUCTURES

Stress damages to cardiac function, electric stability, cation pumps, energy metabolism, and even DNA, all had a distinguishing feature which we maybe failed to clearly outline, namely their pronounced reversibility in the overwhelming majority of the myocardial cells. This is in conformity with our starting concept that the stress reaction evolved as a link of organismic adaptation to the environment, and causes damage only when excessively enhanced. Nevertheless, at the climax of the stress reaction and in certain groups of cells the stress damage finds its morphological equivalent which we made a point of study.

Our electron-microscopic investigations together with Frolov have shown that stress mainly affects the cell and intracellular membranes. The sarcolemma loses its distinct outline, becomes blurred, and in some places completely disintegrates. Normal myocardial nuclei possess a clear double-bordered nucleolemma, whereas in EPS the nucleolemma loses its integrity, and chromatin can be seen to escape from the nucleus through the ruptures. The myocardium of intact rats has few lysosomes, all of them with clearly defined membranes. In stress their number increases and their membranes become less distinct or altogether destroyed. A typical feature of the stress myocardium is the alterations in its capillaries: severe edema of endothelial cells, pronounced pinocytosis in the capillary walls in EPS.

In whole, these data correlate well with the biochemical evidence to the stress damage to membranes, nuclear DNA, and lysosomes, emphasizing thus the importance of studying further the vascular component of the stress damage.

The second step of this research was prompted by the biochemical data on the stress depression of the SR calcium pump and the physiological data on the attenuated resistance of the stress heart to excess calcium. This work⁶⁶ was an attempt to assess the amount and distribution of calcium in the cardiomyocytes in stress by combining electron-microscopic and histochemical techniques.

Left ventricular posterior papillary muscle was obtained from animals 2 and 48 h after exposure to stress and fixed with 4% paraformaldehyde in 0.6 M phosphate (pH 7.2). To locate calcium, the material was incubated with 2% potassium pyroantimonate,¹⁷¹ 1% Os O₄, pH 7.0 to 7.1 for 1 h at 4°C, and then embedded in Araldite-Epon. Sections were poststained with lead hydroxide and uranyl acetate. Preparations incubated with 5 mM EGTA for 1 h prior to fixation served as control for the specificity of histochemical staining.

In control papillary muscle pretreated with EGTA (Figure 26A) there are no deposits of calcium pyroantimonate, and the cells have normal ultrastructure. Figure 26B demonstrates the localization of calcium in control myocardial cells: the deposits are seen in the SR terminal cisternae, the N strips of myofibrillar I discs, and are lacking or negligible in the subsarcolemmal space and mitochondria. (Occasional granular deposits in mitochondria appear to be nonspecific and not calcium pyroantimonate. A similar result was obtained in mitochondrial studies¹⁷¹ and is in line with the biochemical data that indicates the calcium concentration in papillary muscle mitochondria of normal rats is relatively low.¹⁷²

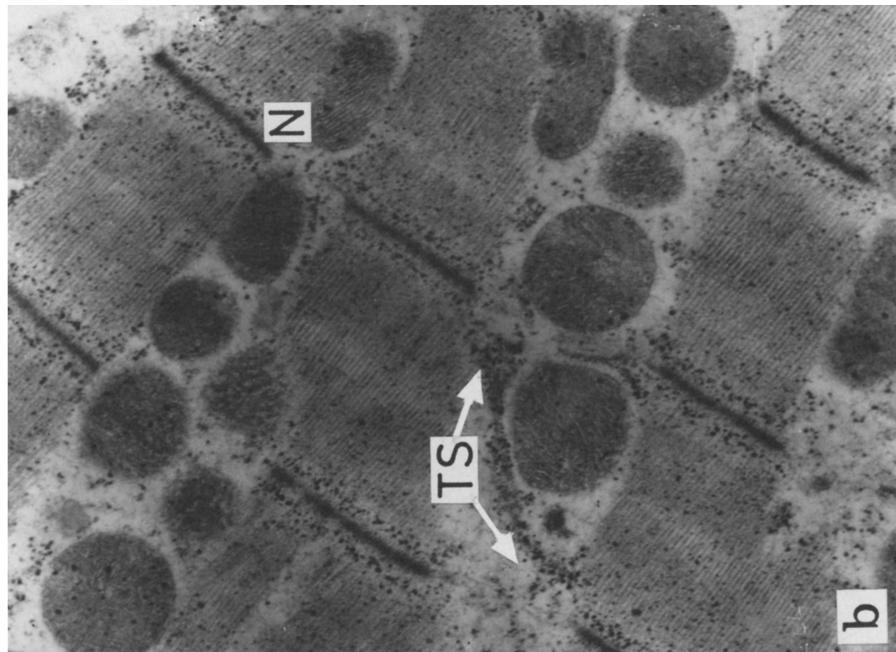
The micrographs in Figure 26C and 26D depict the myocardial ultrastructure 2 h after stress. One can see (Figure 26C: calcium-depleted material, no deposits) that shortly after stress there are moderate ultrastructural changes such as swelling of T-system elements and sarcomere contraction manifest as strips of contraction in place of the Z lines. Simultaneously the localization of intracellular calcium is somewhat altered: deposits are enhanced in the subsarcolemmal space, in the sarcoplasm among the myofibrils, and around mitochondria (Figure 26D). Increased amounts of disperse deposits are also observable in the myofibrils proper, especially in the strips of contraction.

The most demonstrative results were obtained 48 h after stress. Figure 26E (no calcium deposits) shows a significantly greater number of dilated elements of the T system and SR in most cardiomyocytes; there is also peculiar shrinkage of mitochondria with condensed matrix and enhanced osmophilicity of mitochondrial membranes. Simultaneously the calcium pyroantimonate deposits are markedly increased in the subsarcolemmal space, sarcoplasm, myofibrils, and especially around mitochondria (Figure 26F).

Such enhanced sarcoplasmic calcium content as we have observed in stress constitutes, according to the modern data, a common pathogenetic link of the most various forms of myocardial damage — from hereditary cardiopathies to the failure of hypertrophied heart — and becomes the cause of calcium cardiotoxicity and, in particular, of the myocardial stress rigidity described above.⁸⁷

The next step of our research¹⁷³ combining light transmission and polarization microscopy, yielded the most essential morphological result that the membrane injury and ensuing calcium triad of cell damage are not at all uniform through the stress myocardium, but, on the contrary, reach their maximum in certain spatially separated groups of cardiomyocytes, i.e., become the basis of profound but focal damage and subsequent noncoronarogenic cardiosclerosis.

These morphological studies have determined that soon after stress the myocardium develops focal alterations which gradually lead to complete muscle cell contracture in 39 to 45 h. In some cases the contracture is attended with cell death followed by their resorption and formation of fibroplastic granuloma; in other cases the contracture regresses and the cell structures are restored.



b



a

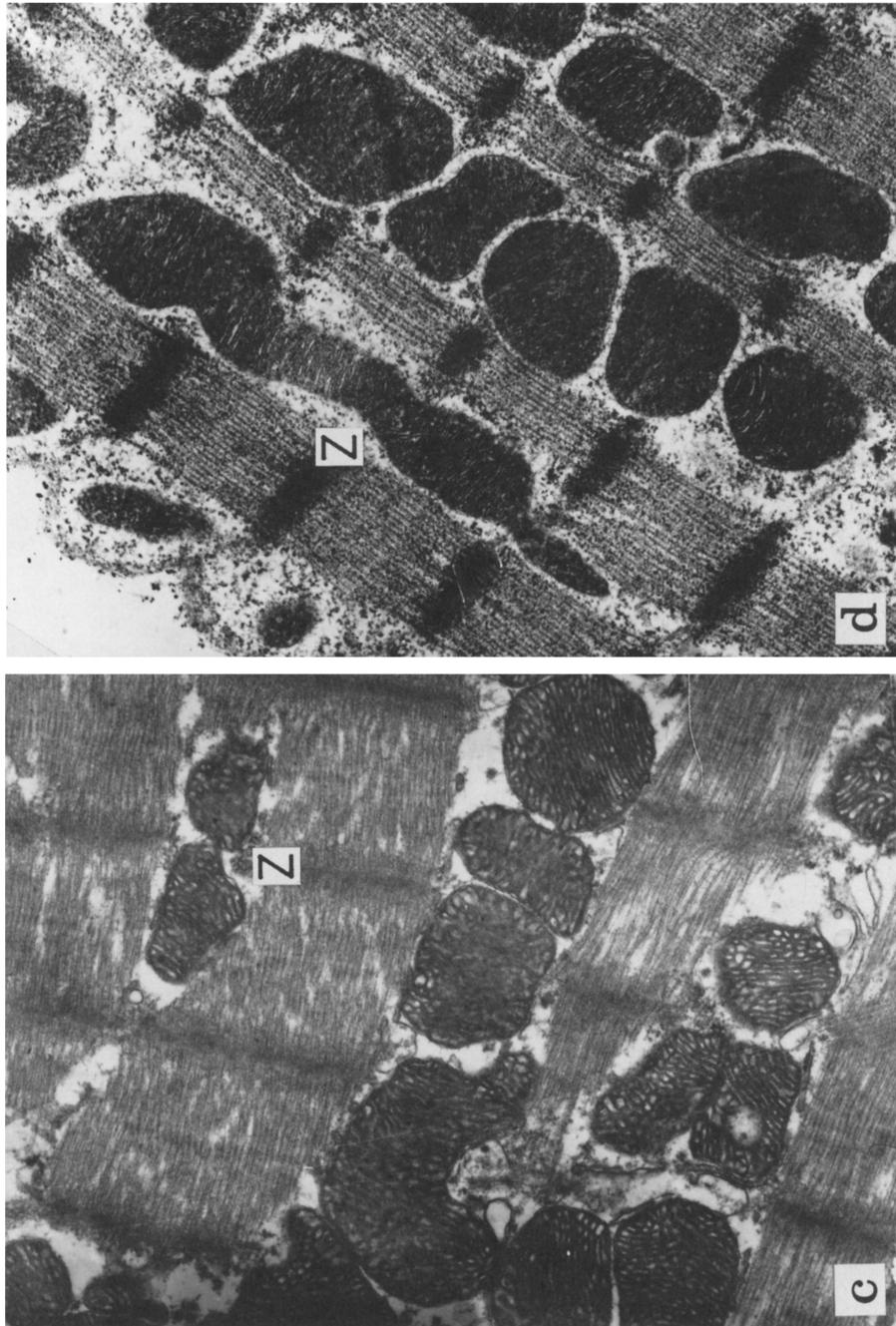


FIGURE 26. Influence of EPS on cardiomyocyte ultrastructure and calcium localization. (a) and (b), Intact myocardium; (c) and (d), 2 h after EPS; (e) and (f), 48 h after EPS. (M), Mitochondria; (N), the N strips of the I discs; (TC), terminal cisternae of the SR; (Z), the Z lines; arrows indicate dilated elements of the T system. Calcium localization is revealed in deposition of calcium pyroantimonate. (a), (c), and (e), Controls for the reaction specificity, pretreated with EGTA to remove calcium.

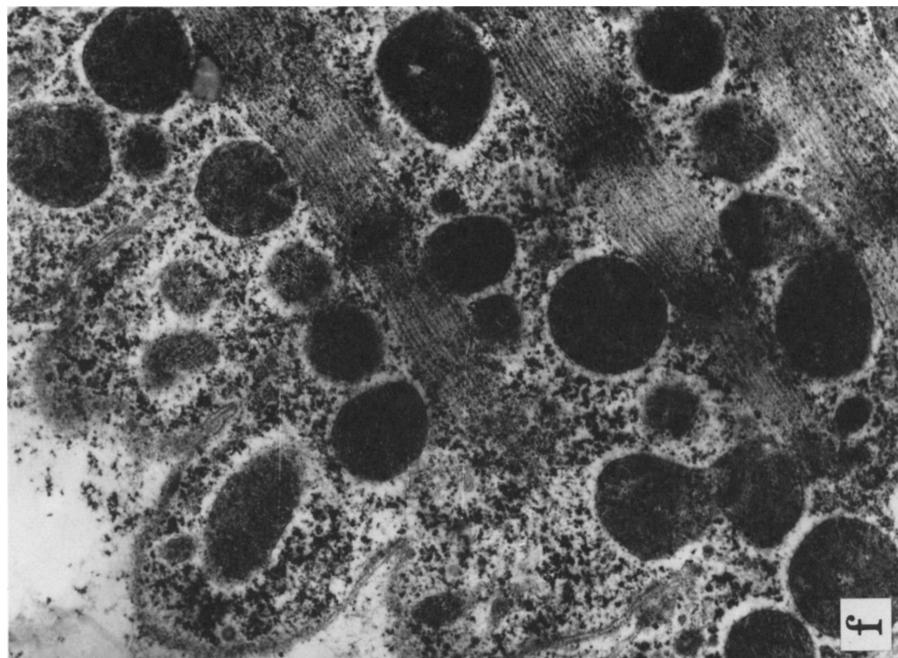


FIGURE 26E and F.

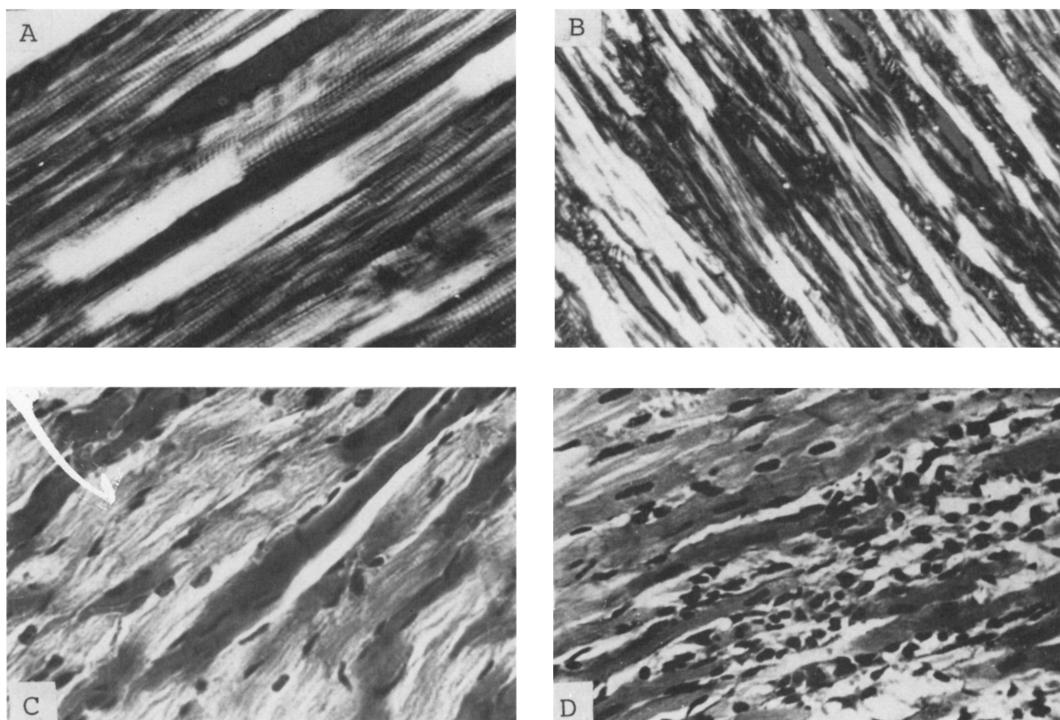


FIGURE 27. Rat myocardial damage 45 h after stress termination. (a) and (b), polarized light microscopy; (c), Pearls' reaction for iron; (d), staining with hematoxylin-eosin. (Magnification: a, $\times 197$; b, $\times 49$; c, $\times 126$; d, $\times 49$.) For explanations see text.

Figure 27 shows that 45 h after stress (i.e., in the phase of maximal morphological alterations) there is deep myocardial contracture with the merging of the A discs of the myofibrils, here and there forming anisotropic conglomerates (Figure 27A); a large number of such contracturally altered muscle fibers with pronounced anisotropy are shown at lower magnification (Figure 27B). Figure 27C shows the staining for iron and Figure 27D demonstrates formation of cell infiltrate around necrotic muscle fibers, with pyknotically altered nuclei and fiber homogenization in the necrotic locus.

Again it should be emphasized that these irreversible changes are microfocal, affect only a minority of cardiomyocytes, and just for this reason, on the one hand, may not tell on the myocardial function some time after a single stress, but, on the other, have at least two unfavorable consequences. First, they aggravate myocardial heterogeneity which, combined with impaired ionic transport, may to a large extent precondition arrhythmogenesis. Second, such damages accumulating in inevitable repeated stress exposures may underlie gradually progressive noncoronary cardiosclerosis.

Finally, our electron-microscopic study of the sinus node and downstream regions of the conducting nervous system was prompted by the widely observed and typical feature of the stress heart in the whole organism: disparity between normal parameters of contractility and markedly upset electric stability. In animals subjected to stress or receiving β -agonists, such disorders as ectopic arrhythmias or AV block occur only on the background of pronounced vagal bradycardia, which in its turn results from diminished resistance of sinus automatism to the negatively chronotropic action of the vagus nerve.

In accordance with this we¹⁷⁴ aimed our work at elucidating the ultrastructural changes elicited by a 6-h immobilization stress in the conducting system and contractile myocardium,

and correlating the stress alterations in the sinus node with its resistance to the negatively chronotropic vagal effect.

In controls, the sinus node was characterized by exclusive location of colloid lanthanum (added during specimen fixation to assess membrane permeability),¹⁷⁵ in the intercellular space along the sarcolemma, and by typical small mitochondria with few cristae (Figure 28A). In the sinus nodes from stress animals the lanthanum particles clearly penetrated not only into the sarcoplasm, but also into the mitochondrial matrix of specific node cells, and the number of cristae was markedly reduced in mitochondria, some of which appeared "empty" (Figure 28B). The sinus nodes in stress specimens were also marked by disappearance of the normally present glycogen, formation of lipid droplets in the sarcoplasm, foci of myofibrillar contracture.

Such alterations were much less pronounced in the atrioventricular node. Both in control (Figure 28C) and stress (Figure 28D) animals the colloid lanthanum is mainly distributed along the sarcolemma in the intercellular space, and only occasionally penetrates into the sarcoplasm in stress. However, in the atrioventricular node region as well as in the contractile myocardium stress gave rise to a large number of contractual cells with distinct myofibrillar overcontracture (Figures 28E and 28F).

We failed to reveal any stress-induced ultrastructural changes in the subendocardial Purkinje fibers. On the whole, a qualitative analysis of electron micrographs suggests a decreasing downstream gradient of stress damage to the cardiac conducting system, alterations being maximal in the sinus node and minimal in the Purkinje fibers. A quantitative analysis shows that the number of mitochondrial cristae per unit area decreases in stress by 35% in the sinus node, by 19% in the atrioventricular node, and by 15% in the contractile myocardium of the interventricular septum.

Taken together, these data point to the preferential damage to the conducting system and first of all to the sinus node as compared to the contractile myocardium. All this is in line with the concept that stress is a weighty arrhythmogenic factor, and various damages to the sinus node upset the resistance of its automatism to the impeding action of the vagus nerve and may cause the known sinus node dysfunction syndrome¹⁷⁶⁻¹⁷⁸ and diverse arrhythmias. Similarly, preferential or selective injury to the upstream regions of the conducting system, exemplified in Figure 28, may contribute to the evolution of various functional and stable blocks, reentry, and grave arrhythmias. In particular, we have observed auricular and AV blocks, atrioventricular dissociation in conditions of pronounced vagal bradycardia in animals with adrenergically injured heart; these disorders were apparently determined by (1) sinus node dysfunction and profound depression of its automatism and (2) impairment of the atrioventricular node and other parts of the conducting system.

By and large, the body of morphological evidence fits into the hypothesis of selective or preferential stress damage to the cardiac conducting system, and makes expedient further morphological studies that would allow a comparison of the state of the contractile myocardium and of the different regions of the conducting system in stress.

X. THE PATHOGENETIC CHAIN OF CARDIAC STRESS DAMAGE

The scheme in Figure 29 is an attempt at summarizing the data on cardiomyocyte stress damage. It shows that in stress situations the excitation of the higher vegetative centers determining the stress reaction constitutes the first link of the process, exposing the heart to greatly increased concentrations of catecholamines, parathyroid hormones, probably vasopressin, histamine, and other agonists of the Ca-mobilizing receptors. This results in activation of two regulatory systems — adenylate cyclase and IP₃-DAG (the second link). The ensuing third link comprises the lipid triad (activation of LPO; lipases and phospholi-

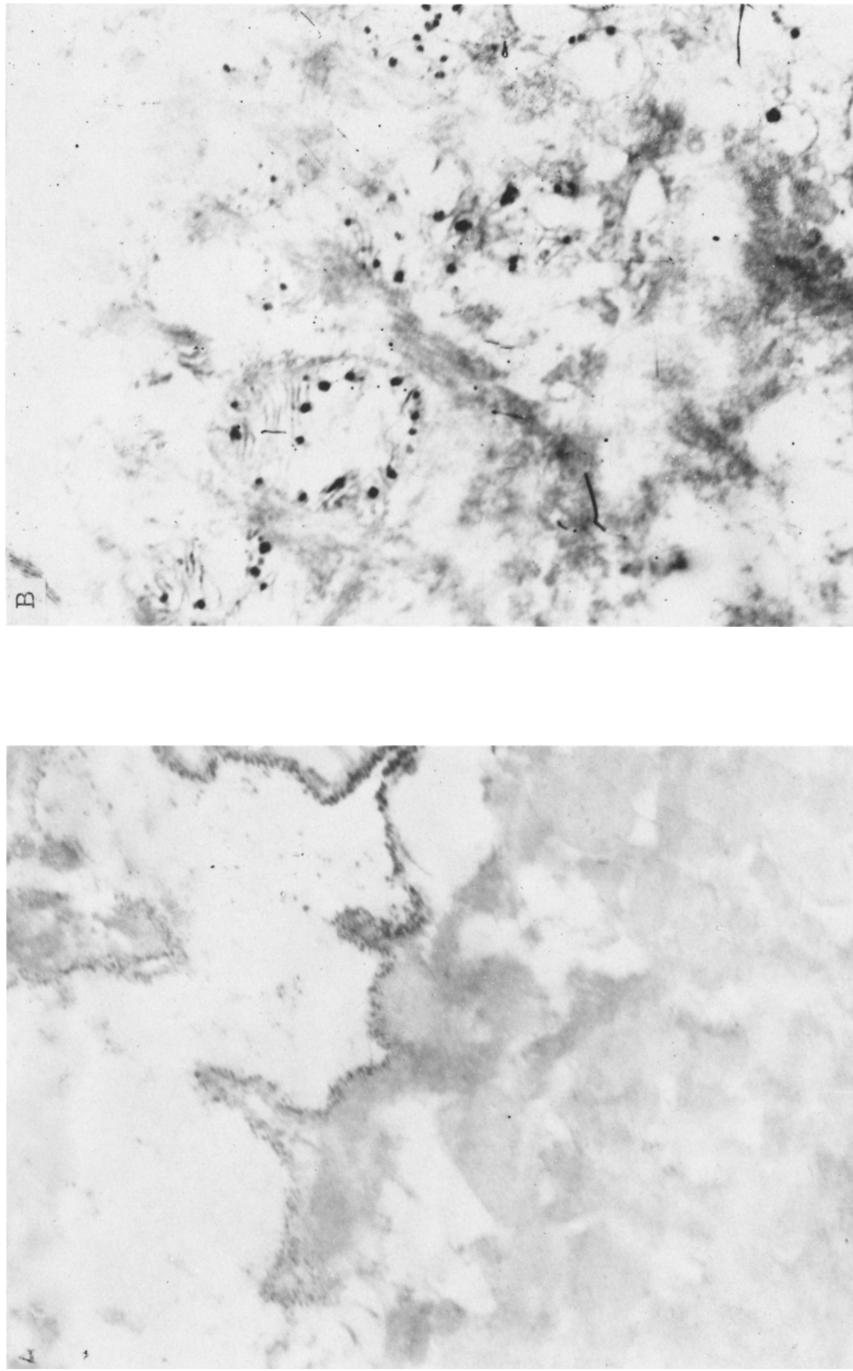


FIGURE 28. Ultrastructure of the conducting system and myocardium in stress. (A), Control, sinus node. Small mitochondria with few cristae are distributed in the sarcoplasm. Colloid lanthanum particles are seen in the intercellular space and do not penetrate through the sarcolemma. (Magnification $\times 5700$.) (B), Stress, sinus node. Colloid lanthanum particles are found in the sarcoplasm of specific cells, on mitochondrial membranes, and in the mitochondrial matrix. (Magnification $\times 5700$.)

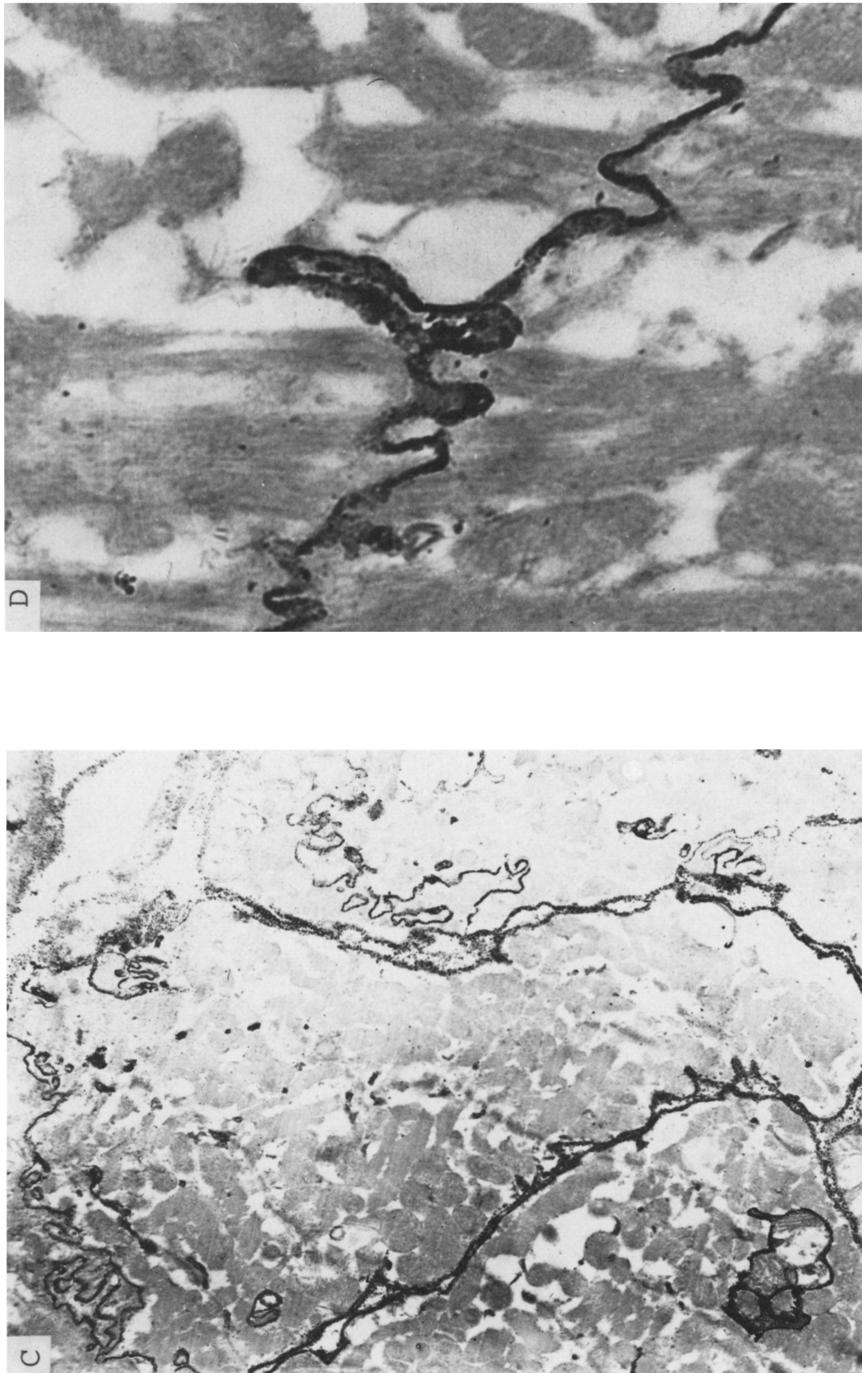


FIGURE 28. (C,D). (C), Control, atrioventricular node. Colloid lanthanum stains the intercalated discs. (Magnification $\times 5700$.) (D), Stress, atrioventricular node. Colloid lanthanum particles are distributed in the intercellular space and on the outer surface of the sarcolemma, staining the intercalated discs of two specific conducting cells (center and top), penetrate into the cell (bottom right), and line the mitochondrial membranes without entering the matrix. (Magnification $\times 3200$.)

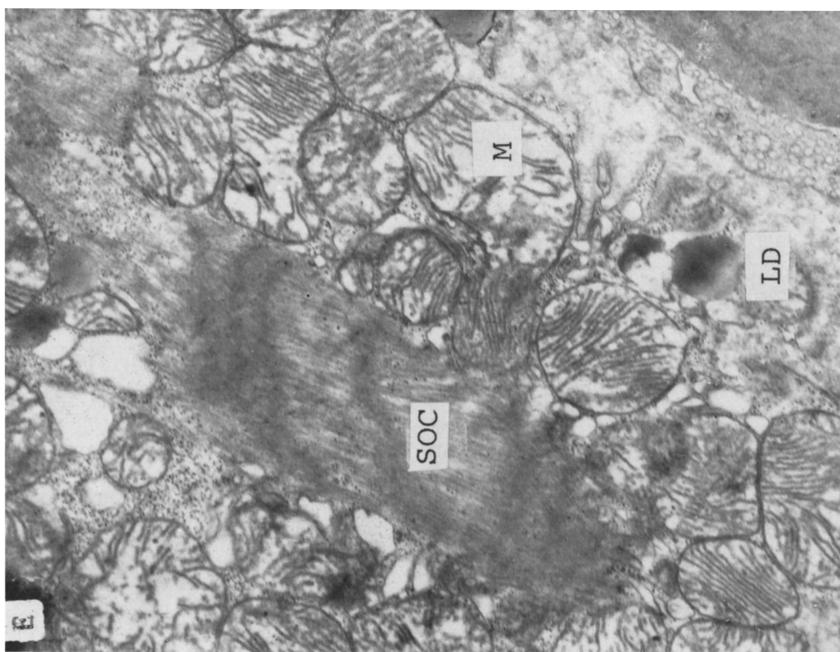
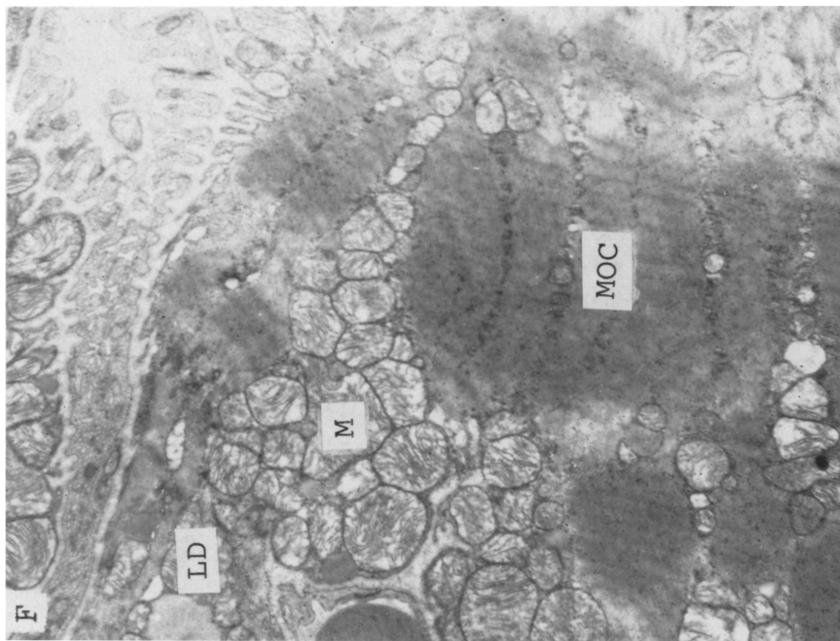


FIGURE 28. (E,F). (E), Stress, atrioventricular node. (LD), Lipid droplet; (M), mitochondria; (SOC), strips of overcontraction. (Magnification $\times 7200$.) (F), Stress, interventricular septal myocardium. (LD), Lipid droplet; (M), mitochondria; (MOC), myofibrillar overcontraction encompassing more than 20 sarcomeres. (Magnification $\times 5700$.).

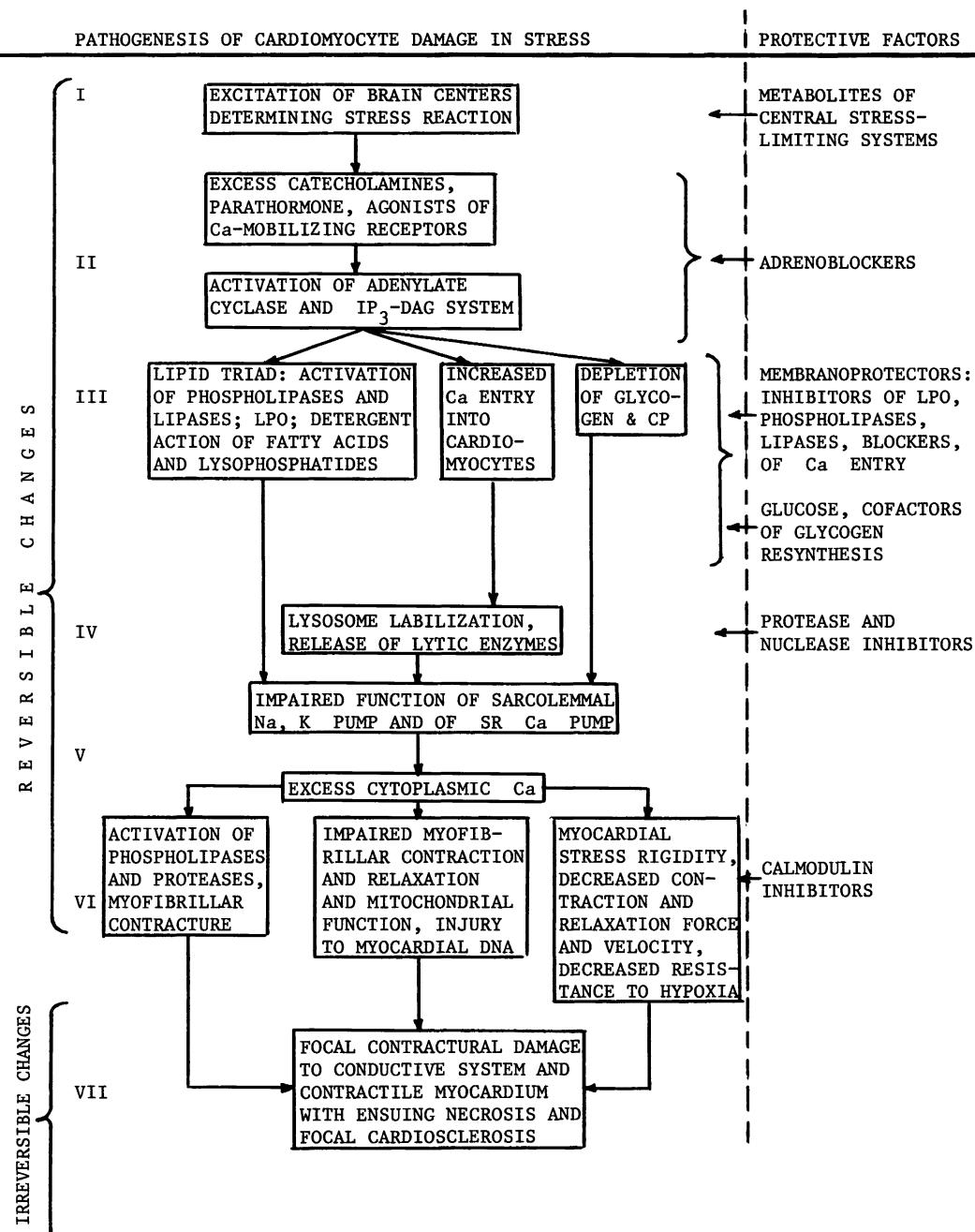


FIGURE 29. Pathogenetic scheme of stress damage to the cardiomyocyte. For explanations see text.

pases; and detergent action of lysophosphatides, acyl carnitine, and long-chain fatty acids), enhanced calcium entry, and mobilization of glycogen and creatine phosphate stores. Subsequent injury to lysosomal membranes releases lytic enzymes (the fourth link), which, together with the lipid triad and glycolytic defects, cause damage to the sarcolemmal and SR membranes responsible for calcium transport, and impair the performance of the sarcolemmal Na,K pump and the SR Ca pump (the fifth link). It is important that, among other

consequences, there is a common outcome of the impairment of these two cation pumps. Depressed function of the Na,K pump increases the intracellular Na⁺ content, thereby hindering Ca²⁺ removal from the cells and thus contributing to its increasing level, similar to the action of cardiac glycosides. Depressed function of the SR Ca pump diminishes calcium uptake into the SR and also contributes to its increasing cytoplasmic content. Combined with the enhanced calcium entry, all this gives rise to a real excess of intracellular calcium. This serious alteration has at least two sequelae. First, it can activate the set of processes constituting the lipid triad and thereby close the vicious circle aggravating myocardial membrane damage. Second, it is itself directly detrimental to the cells, causing the calcium triad composed of myofibrillar contracture, impaired function of calcium-overloaded mitochondria, and activation of myofibrillar proteases and mitochondrial phospholipases; this makes the damage still worse (the sixth link).

In most cardiomyocytes this chain is stopped at some point owing to an "automatic blocking" system inherent in the mechanism of the stress reaction and to the specialized stress-limiting systems (see Chapters 4 and 5); the alterations undergo regression, and at the level of the whole heart it goes no further than transient disturbances of its contractile function and electric stability, which will be dealt with in Chapter 2. Yet, in some cardiomyocytes, irreversible myofibrillar contracture always develops with ensuing necrobiosis and microfocal noncoronarogenic cardiosclerosis (the seventh link).

Of course, the scheme covers only the direct stress damage to the cardiomyocyte, leaving out such pathogenetic mechanisms as stress coronary spasm, thrombosis, stress-imposed load on the heart, or liver-mediated coronary sclerosis. In all these cases the stress may provoke absolute or relative ischemia, which becomes the immediate detrimental factor. Accordingly, they will be considered in the section devoted to the place of stress in the ischemic disease (Chapter 2). Here, in considering the primary stress (i.e., mainly adrenergic) damage to the cardiomyocyte, let us deal with some issues of substantial clinical import.

First, the above discussed disturbances of metabolism, structure, and function were observed after the cessation of stress; hence they are not just a reaction to stress, but relatively stable sequelae of a damage inflicted during stress. This fact, together with the vast clinical data, give grounds for believing that it is such metabolic and functional disturbances, persisting after the stress reaction has passed, that accumulate from stress episode to episode and may be involved in primary noncoronarogenic cardiosclerosis and chronic heart failure, which not infrequently develop in persons who previously had no circulatory disorders.

Second, adrenergic and parasympathetic regulation are known to be most widely represented in the cardiac conducting system,¹⁷⁹ which is also the most dependent for its energy provision on glycolysis profoundly upset in stress.¹⁸⁰ This may apparently be the cause of preferential stress damage to the conducting system, for example, the right-branch block of the bundle of His occurring early in practically healthy persons, or more grave blocks and arrhythmias in the advanced age.

Third, the right part of the scheme in Figure 29 presents some possible means of protection against the stress damage stemming from the concept of its pathogenesis. Further on, the reader will have an opportunity to see that most of these possibilities have been realized in our experiments either through preliminary adaptation which mobilizes the protective stress-limiting systems of the organism, or administering the metabolites of these systems and other chemical factors.

Fourth and finally, the data presented on the disturbances to the energy metabolism and membrane ionic transport have been obtained upon a single stress not very rich in emotiogenic moments. The observed injury to the conducting system (sinus node) and the working myocardium (focal damage) drastically increase the heterogeneity of the heart and, as we shall see, upset its electric stability. However, repeated, diverse, and — most importantly — emotionally tense stress situations abounding in the real life of animals and people would

cause greater stress reactions and accordingly much greater damage. This is in our opinion the angle from which to analyse not only the electric disorders and fatal arrhythmias in animals, but also the idiopathic, often neurogenous, arrhythmias and sudden death in man, which is now known to occur without any alterations in the coronary bed (see Chapter 2). These extremely important stress pathologies of the heart will be the subject of further consideration.

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Chapter 2

THE PLACE OF PRIMARY CARDIAC STRESS DAMAGE IN THE PATHOGENESIS OF ARRHYTHMIAS, ISCHEMIC DISEASE, AND SUDDEN CARDIAC DEATH

I. INTRODUCTION

The conventional idea of the place of stress in the pathogenesis of arrhythmias and sudden death is that man, as compared to other species, is most open to the influence of mainly biosocial stressful situations which he either enters himself or is put in by social circumstances. In our opinion, however, the problem is not so straightforward since intelligence, which is an exclusive property of *Homo sapiens*, allows the person to clearly understand many of the ambient events and thereby obviates groundless fear and stress reaction. Indeed, Laughlin¹ has shown that in subjects aware of the particulars of the centrifugal test there was no or minor increase in the blood catecholamines. Accordingly, the most thorough clinical examination, and in some cases post-mortem studies upon incidental death, failed to reveal any damage to the cardiac muscle. On the contrary, for animals the centrifugation proved to be a drastic stress factor: the blood catecholamine concentration rose up to fiftyfold with ensuing grave cardiac damage manifest in subendocardial hemorrhages, myofibrillar contractures evolving into micronecroses, etc. In other words, the mental mechanisms often certainly avert the stress reaction hazardous to the organism and, in particular, detrimental to the heart. With all this it should be borne in mind that just the same mechanisms and linked associative activity may cause reiteration of stress situations that either have really occurred or are quite probable. That is to say, a person going through multiple brief or prolonged stress episodes may not only ignore them by virtue of the mental mechanisms, but, vice versa, amplify and aggravate them through the same mechanisms.

Taking into account these important peculiarities of the psychics and cardiac neural regulation, in this chapter we shall first consider the experimental data on the role of cardiac stress damage in the pathogenesis of arrhythmias and sudden death, and then the materials directly pertaining to man.

II. ROLE OF STRESS DAMAGE TO THE HEART IN IMPAIRMENT OF ITS ELECTRIC STABILITY AND DEVELOPMENT OF ARRHYTHMIAS IN THE EXPERIMENT

Researchers concerned with the place of stress in arrhythmias, fibrillation, and cardiac arrest never fail to mention that the literary heritage provides us with numerous examples of sudden cardiac death of apparently healthy people brought about by acute stressful situations.

Abiding by the tradition, I can cite A. K. Tolstoi's famous drama, *Ivan the Terrible*, where the Tsar receives a prophecy that he will die on one particular day, namely St. Philip's Day; one of his officers, having thoroughly consulted the physician, and assured that emotions are all important in the Tsar's illness, kills his sovereign with a single, perfectly timed remark, "St. Philip's Day is not yet over, Sire."

Getting on to the gist of the matter, it must be recalled that as far back as early in this century it has been found that direct introduction into the brain of drugs or chemicals such as nicotine, barium chloride, caffeine, epinephrine, acetylcholine, or strophanthin causes ventricular arrhythmias and fibrillation. Further studies using more precise techniques of stereotaxic electrode introduction have shown that ventricular arrhythmias are elicited upon stimulation of the frontal, orbital, motor, and premotor cortical zones, anterior temporal

zone, island, and convolution of the cingula. Arrhythmias are relatively easy to evoke by stimulation of subcortical regions such as posterior hypothalamus and lamina quadrigemina (for review see Korteweg et al.).² Similarly, the probability of arrhythmias and susceptibility to ventricular fibrillation increase upon electric stimulation of peripheral neural structures such as cardiac sympathetic nerves and stellate nodes. These artificial pathological phenomena are of interest as indications of the possible central genesis of arrhythmias, but of course do not themselves prove their stress origin. Important in this context are the experiments of Lown et al.,³⁻⁵ who reproduced the emotional pain stress (EPS) by placing dogs in a stanchion according to Pavlov and delivering moderate electric pain stimulation. This procedure was repeated for 3 d, after which the stress reaction as manifest by blood catecholamine elevation developed without electric pain stimulation, as a conditioned response to the experimental setting. In the process, the heart was stimulated by electric current through an electrode implanted in the apex cordis. It turned out that the stress situation substantially lowered the threshold current, causing extrasystoles and cardiac fibrillation, i.e., substantially increased their probability.³⁻⁵

Further studies of Lown's group⁶ showed that the decisive part in the arrhythmogenic action of stress on the heart is played by inordinately intense and protracted excitation of adrenergic regulation.

In our experiments we used two main approaches to assessing the effect of stress on cardiac electric stability: determining the cardiac fibrillation threshold and evaluating the resistance of cardiac automatism and its ectopic activity upon stimulation of the distal end of the dissected vagus nerve.

Figure 1 illustrates the technique used to determine the fibrillation threshold. A premature single impulse delivered to the apex cordis from a stimulator triggered by the ECG R wave is seen to evoke transitory fibrillation attended by a drop in arterial pressure. The fibrillation threshold is defined as the current sufficient to cause fibrillation; it is an index of the probability of cardiac fibrillation and death of fibrillation.

Figure 2 presents another approach to evaluating the electric stability of the heart: the normal automatism of the sinus node is hindered by stimulating the vagus nerve. On the background of the resulting vagal bradycardia, extrasystoles are very rare in control animals, but quite common in experimental infarction and postinfarction cardiosclerosis.

Before considering the data obtained with the use of these approaches, it must be noted that the results of all experiments on the effects of stress on cardiac electric stability differed basically from what had been found for the contractile function. Indeed, the contractile function had to be studied with isolated heart preparations, since in the whole organism it was only little or not at all altered even upon appreciable exposure to stress. On the contrary, the electric stability parameters, and first of all the cardiac fibrillation threshold, changed markedly in the intact organism. A similar dissociation between the preserved contractile function and the impaired electric stability was observed by us in cardiac damage with the selective β -agonist isoproterenol. Moreover, such dissociation is now demonstrable with 24-h monitoring in both diseased and practically healthy persons; many of them develop more or less pronounced arrhythmias with quite intact pump and contractile functions of the heart.^{7,8} This essential dissociation is one of the causes of often sudden cardiac death; its mechanism is of great interest, and we shall return to it again.

At the first stage we studied the effect of stress on the cardiac fibrillation threshold and reactivity to the inhibitory action of the vagus nerve.⁹ Immobilization stress was created in male Wistar rats by fixing them in the supine position for 10 h. It turned out that 2 h after the end of the stress exposure the fibrillation threshold fell more than twofold and remained decreased for the next 2 d, with a minor tendency to restitution by the end of this period.

Simultaneously, the stress substantially enhanced the sensitivity of the sinus pacemaker to vagal inhibition. This was only pronounced enough 12 and 24 h after stress, but regressed by the end of the second day (Figure 3).

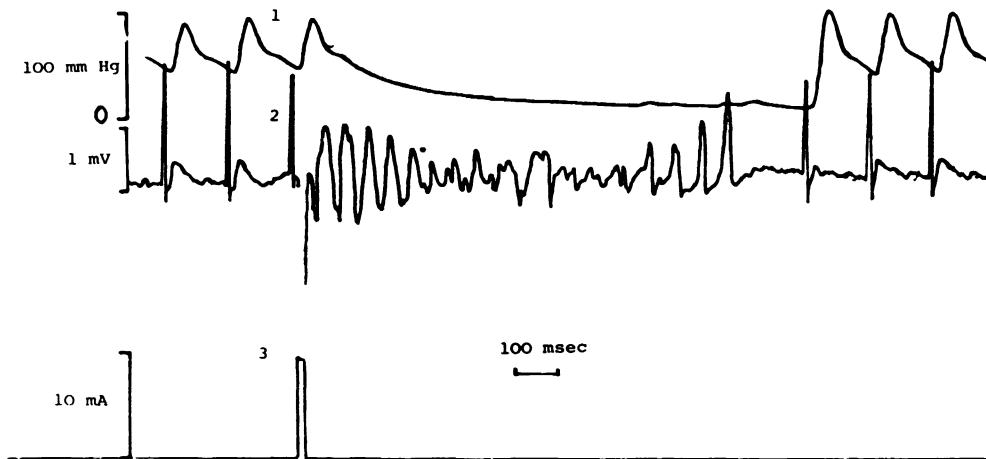


FIGURE 1. Determination of the electric threshold of cardiac fibrillation. (1) Pressure in the carotid artery, (2) ECG, (3) recording of the stimulating impulse.

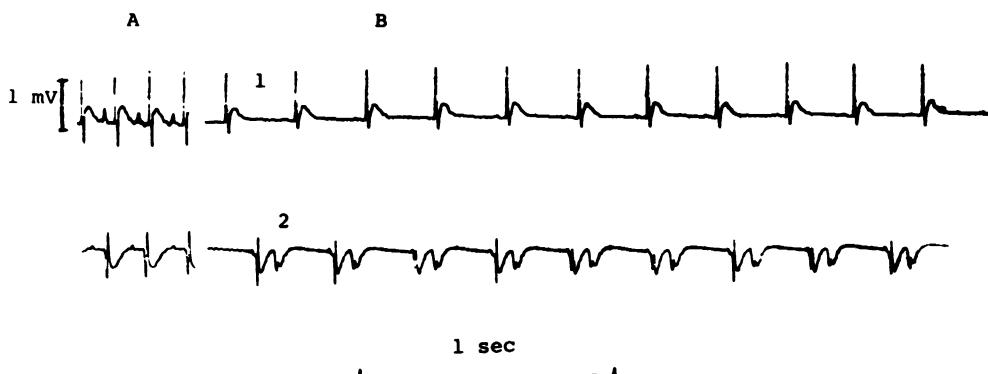


FIGURE 2. Ectopic activity of the heart in vagal stimulation. (A) ECG before stimulation, (B) ECG during stimulation. (1) Control: bradycardia caused by stimulation of the vagal periphery; (2) postinfarction cardiosclerosis: bradycardia attended with bigeminy.

Obviously, in the whole organism the lower cardiac fibrillation threshold, together with the lesser ability of the sinus node to withstand vagal inhibition, significantly increase the probability of appearance of heterogenous foci of excitation and consequently of arrhythmias and fibrillation.

It is quite important that all these electric stability disorders, as in other studies, could be completely prevented with prior administration of a β -blocker. The data in Table 1 show that the β -blocker itself raises the fibrillation threshold in control animals, while the α -blocker has no such effect.

When administered before a 6-h immobilization stress, the β -blocker totally prevented the stress-induced decline in the electric fibrillation threshold, while the α -blocker exhibited no effect. It thus seems likely that the observed stress impairment of contractile function and electric stability is in essence a β -injury. The studies of recent years with whole organisms in behavioral stress situations fit in with this idea.

Thus, Verrier and Lown¹⁰ studied the changes in cardiac susceptibility to fibrillation, and in so doing tried to assess the mechanism through which the adrenergic action on the heart affects its electric stability. It turned out that various stress conditions evoked by pain,

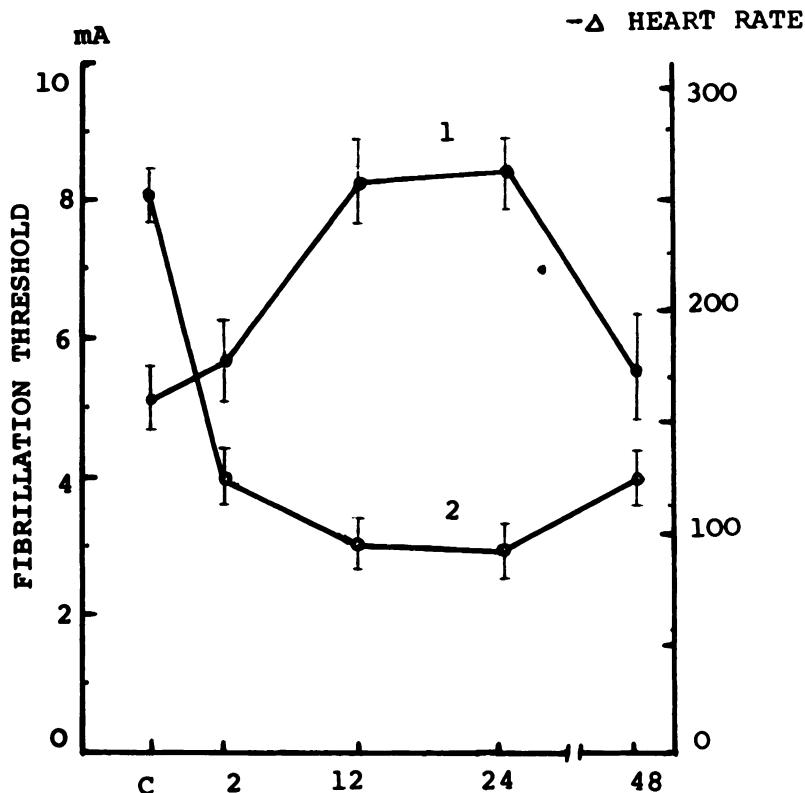


FIGURE 3. Dynamics of the fibrillation threshold and the negative chronotropic vagal effect on the heart after stress. The abscissa is time after the end of stress; C denotes the control values. (1, right ordinate) Drop in the heart rate upon vagal stimulation; (2, left ordinate) fibrillation threshold in milliamperes.

TABLE 1
Effect of α - and β -Adrenoblockers on the Cardiac
Fibrillation Threshold in Control Animals and in Animals
Subjected to Stress

Conditions	Fibrillation threshold (mA)*
Control (n = 12)	6.5 \pm 0.63
Propranolol (β -blocker) (n = 10)	11.0 \pm 1.50 ^b
Prazosin (α -blocker) (n = 10)	6.1 \pm 0.54
Stress (n = 19)	2.9 \pm 0.26 ^b
Propranolol + stress (n = 17) ^c	6.6 \pm 0.44
Prazosin + stress (n = 17) ^c	3.1 \pm 0.40 ^b

* The threshold was determined 1 h after the end of a 6-h immobilization stress.

^b $p < 0.05$ as compared to the control.

^c The adrenoblockers were administered at 1 mg/kg body wt 30 min before stress.

fear, or rage caused by taking away food significantly enhance the myocardial blood flow, i.e., in the healthy animal behavioral stress elicits not ischemia, but hyperemia of the myocardium. However, the myocardial oxygen consumption greatly increases therewith;¹¹ therefore in stress hyperemia there still can be inadequate supply of oxygen to the myocardium, i.e., relative hypoxia. In this connection the authors made use of the calcium antagonist nifedipine, which causes coronary vasodilation and at the same time prevents the stress-induced increase in myocardial oxygen consumption. With nifedipine, stress can evoke neither ischemia nor hypoxia of the myocardium, and yet nifedipine does not affect the drop in fibrillation threshold in stress. On the other hand, the β -blocker methoprolol does not change the myocardial oxygen consumption, but averts the stress decline in the fibrillation threshold.

We can see that the propensity of the normal heart to ventricular fibrillation in behavioral stress (decline in the fibrillation threshold) is due to direct catecholamine action on the myocardial β -adrenoreceptors, and takes place at the background of enhanced coronary blood flow. Essentially similar data have been obtained by Vatner and Hintze¹² in continuous measurement of coronary blood flow in primates. The diurnal variations can be fivefold, with minimal flow during the night sleep and maximal in the behavioral emotional stress, when the blood supply to the heart proves quite adequate to its metabolic demands. In other words, we can conclude that acute stress situations engender not myocardial ischemia, but, on the contrary, hyperemia, which is nevertheless attended by a strong β -adrenergic effect on the heart and ensuing upset of its electric stability.

This can be stated to conform to the present day concepts of the differential reaction of the cross-striated cardiac muscle and of the smooth muscle of coronary arteries to catecholamines. While the β -adrenergic effect in the cross-striated cardiac muscle via enhanced cAMP formation and calcium entry into the sarcoplasm (see the scheme in Figure 29, Chapter 1) results in enhanced contraction force typical of stress, the β -adrenergic stimulation of the coronary vessels conversely leads to hyperpolarization of the smooth-muscle cell membranes, greater cAMP formation promotes calcium removal by the ion pumps, and the smooth muscle relaxes providing for enhanced coronary blood flow, i.e., myocardial hyperemia.¹³ Catecholamines acting on the α -receptors of the vascular wall set in motion another chain of events including depolarization of the vascular smooth-muscle cells and increased calcium permeability of the cell membranes; elevated cytoplasmic calcium activates the contractile proteins with ensuing smooth-muscle cell contraction.

The above data on the cardiac effects of stress give grounds for believing that when an initially healthy organism encounters a heavy enough stress situation, the β -adrenergic effect of stress on the heart certainly dominates over the α -adrenergic one and, accordingly, the discovered complex of rather stable alterations in the function and electric stability retained after the end of the stress is the result of the stress β -adrenergic damage to the heart. This complex, completely preventable with β -blockers, has been shown to comprise myocardial stress rigidity; depressed force and velocity of contraction and velocity of relaxation; greatly increased myocardial response to calcium, sodium, and hydrogen ion shifts; and, finally, lower cardiac fibrillation threshold and lower resistance of the sinus node automatism to the negatively chronotropic vagal action.

The question arising in this connection is how a healthy human or animal heart in the absence of ischemia, only under adrenergic influence, develops not just arrhythmia, but pronounced fibrillation. To answer this question it is essential to recall the already mentioned concept of the electric heterogeneity of the healthy heart as well as the fact that it may be increased by the marked hypercalcium effect of stress. With this in mind it can be envisaged that upon stimulation of the apex cordis in the works of Lown et al.³ and Verrier and Lown,¹⁰ or in our experiments, an impulse evoked in the vulnerable period and slowly propagating in the retrograde direction may even in a healthy myocardium encounter zones of increased

resistance. Circumventing them and spreading further to the base, it can concurrently propagate anterograde, returning to the recently blocked zone which now becomes patent. This results in cyclic excitation which becomes the basis of cardiac fibrillation.¹⁴

It is essential that *the probability of reentry caused by retrograde and anterograde impulse propagation is much higher in the stress than in the healthy heart, since excessive stress damages the cell membranes and nexuses and markedly increases the number of zones with decreased RP and conductivity which can give rise to reentry.* As a result, for the first fixed reentry to appear in stress animals the excitation should spread over a comparatively small region of the myocardium and the stimulation need be much weaker than in the norm, as the electric threshold of extrasystole is lowered. Accordingly, much weaker current is required for multiple nonfixed reentries which trigger and replace each other to encompass the heart, i.e., become the basis of ventricular fibrillation. The decreased cardiac resistance to arrhythmias and fibrillation is due not only to the presence of one-way conduction blocks and potential pacemakers, but also to the impulse getting through a zone of partial blockade (where the rapid response linked with sodium entry is mostly inhibited) which turns out to be deformed. It has a flat excitation front and diminished amplitude, i.e., it is transformed into the slow response provided mainly by the calcium influx. Hence there is a significantly higher probability that this impulse would be subsequently blocked and would participate in formation of reentry.

These considerations are important not only for explaining the drop in the electric threshold of fibrillation in stress, but also for understanding some weighty realities of the cardiological clinics. The matter is that at the background of microfocal cardiac stress damage and markedly enhanced bioelectric heterogeneity, an ectopic impulse arising in the zone of a long-existing organic injury (e.g., in the subendocardial cells of the postinfarct scar zone) or in the region of partial or complete ischemia is much more likely to entail arrhythmias and cardiac fibrillation than in the absence of stress damage.

It should be emphasized that the essentially β -adrenergic damage to the heart not associated with ischemia was discovered and described in detail 30 years ago in excellent studies of Rona, et al.,¹⁵ who were the first to find that large doses of the synthetic norepinephrine analog, β -agonist isoproterenol, act through the adenylate cyclase of the heart, adipose tissue, and other organs to cause enhanced calcium entry into myocardial cells; this, together with excess fatty acids, uncouples oxidative phosphorylation in mitochondria and decreases the cell contents of ATP and CP; simultaneously, excess calcium induces myofibrillar contracture. These mostly microfocal lesions develop at the background of cardiac hypertrophy, and at moderate doses of the agonist may not impair the contractile function, whereas at large doses they depress it. The outcome, if not lethal, is focal necrobiotic damage with ensuing focal cardiosclerosis.

Later, Waldenström et al.,¹⁶ basically following up the works of Rona et al., replaced isoproterenol with norepinephrine and ran experiments with the isolated heart excluding the changes in coronary flow. In this case, certainly ruling out coronary spasm and ischemia, increasing dosage of norepinephrine consistently caused myocardial damage as manifest by declining ATP level and leakage of creatine kinase and transaminases into the perfusate. These alterations were proportionate to the acting catecholamine concentration in the perfusing solution and duration of the exposure, and were attended with depression of the cardiac contractile function. Pathological changes such as myofibrillar contracture developed in the myocardial cells. These alterations could in great measure be prevented with β -blockers or verapamil, which is known to block calcium entry into the cells.

Thus, *β -adrenergic nonischemic damage to the myocardium, caused not by a synthetic, but by the natural catecholamine, taking place both in the whole organism and in the isolated heart and attended with marked impairment of the contractile function and — most importantly — electric stability of the heart, is an undebatable reality and is very probably involved in arrhythmias and sudden death.*

The data available on the molecular bases of β -adrenergic damage in stress have been considered in Chapter 1. Here it must be pointed out that the direct β -adrenergic injury to the contractile myocardium and to the conducting system of the heart is not the sole pathway through which the stress damage can be realized.

The contractile myocardium, the conducting system, and, what is most important, the coronary arteries, are rich in α_1 -adrenoreceptors which, under the action of catecholamines, activate Ca^{2+} entry into muscle cells through slow channels¹⁷ and probably its release from the sarcoplasmic reticulum (SR) mediated by inositol triphosphate.^{18,19} Unlike the β -adrenergic effect, this is not accompanied by cAMP formation or increase in contraction and relaxation velocities and, notably, is not blocked by endogenous adenosine.²⁰ Accordingly, the α -adrenergic effect, if inordinately amplified, may be directly arrhythmogenic as well as cause coronary spasm. The arrhythmogenic action of α_1 -adrenoreceptors has been demonstrated by Thandroyen et al.²¹ administration of the α -agonist methoxamine results in a pronounced inotropic effect and a 2.5-fold decrease in the fibrillation threshold.

Both these shifts could be prevented with the α -blocker prazosin, the slow-channel blocker nizoldipine, or rianodine blocking Ca^{2+} release from the SR. The authors arrive at a conclusion that both the positive inotropic and the arrhythmogenic effects of overactivation of α -adrenoreceptors are actualized through excess calcium coming both from the extracellular space and from the SR.

The vasoconstrictive effect of α -receptor activation has been demonstrated in experiments with intact animals as well as in clinicophysiological studies on human subjects. Thus Murray and Vatner²² used a flowmeter to measure the animal coronary blood flow under physical load. The flow increased according to the metabolic demands of the myocardium; however, the α -blocker phentolamine caused additional increase in the blood flow, indicating the existence of a constant tonic vasoconstrictive α -adrenergic influence on the coronary vessels.

The clinicophysiological studies of Mudge et al.²³ show that in coronary disease this tonic α -adrenergic influence may increase and result in coronary spasm. Thus, immersion of the hand into cold water proved to elevate the ST segment and cause pectoral pains in patients with coronary disease; this was preventable with the α -blocker phentolamine.

Of principal importance in this context is the fact that all effects of α -adrenoreceptor stimulation are consistently enhanced upon blocking the β -receptors with propranolol^{12,24} or their desensitization by sustained high catecholamine levels in the blood.²⁵

The latter is most essential for us as it suggests that desensitization of the β -adrenoreceptors in prolonged or chronic stress may result in coronary spasms. Indeed, Verrier and Haegeston¹¹ justly recall that a primary decrease in the coronary flow independent of shifts in the systemic hemodynamics has been observed not only in variant stenocardia,²⁶ but also in classical and unstable angina pectoris.²⁷⁻²⁹ Deanfield et al.²⁷ reported recurrent episodes of ST segment depression during ambulatory monitoring of patients with stable angina; they were not associated with changes in the cardiac pace and appeared to indicate transitory myocardial ischemia. Further, it was found that coronary spasm could be brought about by a stress situation²⁹ and in particular by conditioned verbal stimuli.³⁰ In the context of our discourse it is important that in all these cases the coronary spasm is apparently realized on the basis of β -receptor desensitization and is due to the fact that the α -adrenergic effect involving calcium release from the SR cannot be rectified by such a physiological factor as excess adenosine. These data also give grounds for thinking that in chronic stress situations attended with β -desensitization, coronary spasms damaging the myocardium may be elicited by other agonists of the calcium-mobilizing receptors: vasopressin, histamine, etc. Such spasms develop in impaired function of the central stress-limiting systems, one of which is the parasympathetic system restricting the stress adrenergic effects on the heart.

This stress-limiting cardioprotective action is manifest in that at a high adrenergic tonus and lowered fibrillation threshold, stimulation of the vagus nerve raises the threshold, re-

turning it to normal; administration of atropine, conversely, decreases the threshold still further and is conducive to fibrillation.³¹ In line with this, atropine has been shown to act on muscarine receptors to suppress norepinephrine release from adrenergic terminals³² and to restrain the adrenoreceptor response to norepinephrine and the cyclic nucleotide formation.²³ However, such cardioprotective vagal influence can also become detrimental. In prolonged, heavy enough stress, when catecholamine consumption outstrips its resynthesis and the catecholamine levels decline not only in the nervous centers,³⁴ but also in the working organs including the heart,³⁵ the vagus nerve starts to predominate in cardiac regulation. This is initially expressed as bradycardia, but may accrue with more grave sequelae. Hazards of this type prove common in stress situations when escape or avoidance are precluded and reactions of capitulation and immotility prevail.

Indeed, a number of researchers who had studied susceptible animals in drastic stress situations observed not only vagal bradycardia, but asystolia and cardiac arrest. Engel³⁶ has systematized these works to substantiate his concept of the role of vasovagal cardiac arrest in the pathogenesis of sudden death. Of the most interesting in this respect are the studies of Corley et al.,³⁷⁻³⁹ who put forward an idea of sudden parasympathetic activation or cessation of sympathetic influences that occur when the stress becomes excessive and the animal capitulates. Similar conclusions were arrived at by other researchers.^{40,41}

In this connection we believe that *at least three factors evolving in prolonged stress may participate in the switchover of cardiac regulation from sympathetic to parasympathetic domination.*

The first factor appears to consist in profound alteration of the functioning of the higher divisions of the nervous system when, in the face of insurmountable difficulties, the stress of struggle (attack or flight) changes over to the stress of capitulation (resignation and standstill) with attending significant activation of the parasympathetic system. Engel³⁶ has shown that in humans this phenomenon is realized as a vasodepressive syndrome in which vagal asystolia is combined with neurogenic vasodilation and a deep drop of arterial pressure. This syndrome may result in more or less heavy syncopes as well as sudden death. Again, the capitulation stress has been shown (Chapter 1) to cause rather pronounced and rapid cardiac damage and to upset its arrhythmogenic resistance.

The second factor is the desensitization of cardiac adrenoreceptors by high catecholamine concentrations, or impairment of their function because of membrane damage by the lipid triad. Such decreased adrenoresponsiveness of the cardiac muscle has been observed in our experiments with rats subjected to a 6-h EPS.⁴²

The third factor is that the preceding adrenergic action on the heart may damage the sinus node and other parts of the conduction system, with the resultant decreased resistance of the cardiac automatism to the negatively chronotropic vagal influence and loss of sinus node control over the downstream ectopic pacemakers. This is in line with the direct electron-microscopic observations of sinus node lesions and the data on the greater vagal effect on the automatism of the sinus node in stress (Chapter 1, Section IX, and above).

Notably, the β-adrenergic damage to the sinus node and other parts of the conduction system may under certain conditions cause pronounced disturbances of the cardiac rhythm. In our studies with Belkina and Kolarova this effect was achieved by administration to animals of 10 mg/kg of the selective β-agonist isoproterenol.

Rats subjected to such treatment were tested for (1) the contractile function of the heart at physiological rest and during aortic clamping and (2) electric threshold of cardiac fibrillation and the effect of increasing stimulation of the vagus nerve. The tests were run 1 d after isoproterenol administration. It turned out that isoproterenol, like EPS, had no effect on the contractile parameters in the whole organism.

At the same time, the ventricular fibrillation threshold fell on average from 7.5 ± 0.55 to 3.1 ± 0.94 mA, i.e., 2.5-fold. This upset of the electric stability was accompanied by

Heart rate

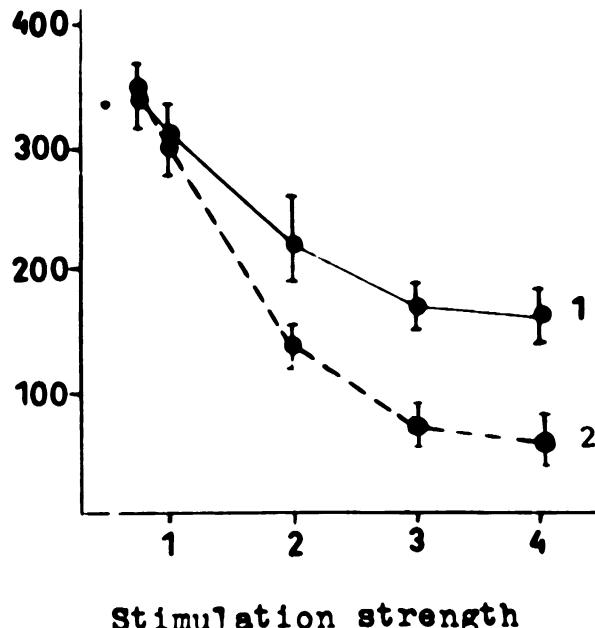


FIGURE 4. Effect of vagal stimulation on the heart rate in control animals (1) and 24 h after isoproterenol administration (10 mg/kg) (2). The abscissa is the strength of vagal stimulation, multiples of the threshold current.

a decrease in the resistance of sinus node automatism to the negatively chronotropic vagal influence. Figure 4 shows that stimulation of the vagus nerve depresses the sinus node automatism to a much greater extent in animals with isoproterenol damage than in controls: at the same strength of stimulation the heart rate decreased to 68 beats per minute vs. 168 beats per minute in the control.

The data in Table 2 demonstrate that in animals with β -adrenergic damage such vagal bradycardia was attended with rhythmic disorders not seen in controls; arrhythmias were observed in seven out of nine animals.

Figure 5 displays the main types of these ectopically originating arrhythmias: unlike the controls (1) where vagus stimulation only elicits sinus bradycardia, animals with cardiac β -adrenodamage exhibit (2) atrioventricular (AV) rhythm with ventricles being excited prior to auricles, (3) idioventricular rhythm intermittent with sinus and AV rhythms, and (4) I-degree AV block with regular atrial complexes, but every second ventricular complex omitted.

On the whole the results of this work are in accord with the concept that *cardiac β -adrenergic damage at the early stage of reaction associated with struggle is conducive to dangerous vagal arrhythmias and cardiac arrest at the late stage of reaction, in exhaustion of adrenergic regulation, and behavioral capitulation. The experimental data appear to agree with the clinical situations considered further in which stress arrhythmias, fibrillation, and sudden cardiac death may occur. For the initially healthy heart without organic alterations we can discern several situations of this kind.*

TABLE 2
Cardiac Rhythm Disturbances in Vagal Bradycardia 1 d
After Isoproterenol Administration to Animals

	Control (n = 9)	Isoproterenol (n = 9)
Heart rate (min^{-1})	344 ± 42	353 ± 13
Vagal stimulation threshold (V)	0.17 ± 0.03	0.20 ± 0.04
Number of cases of:		
Arrhythmias	0	7
AV block	0	2
Idioventricular rhythm	0	4
Bradyarrhythmia	0	2

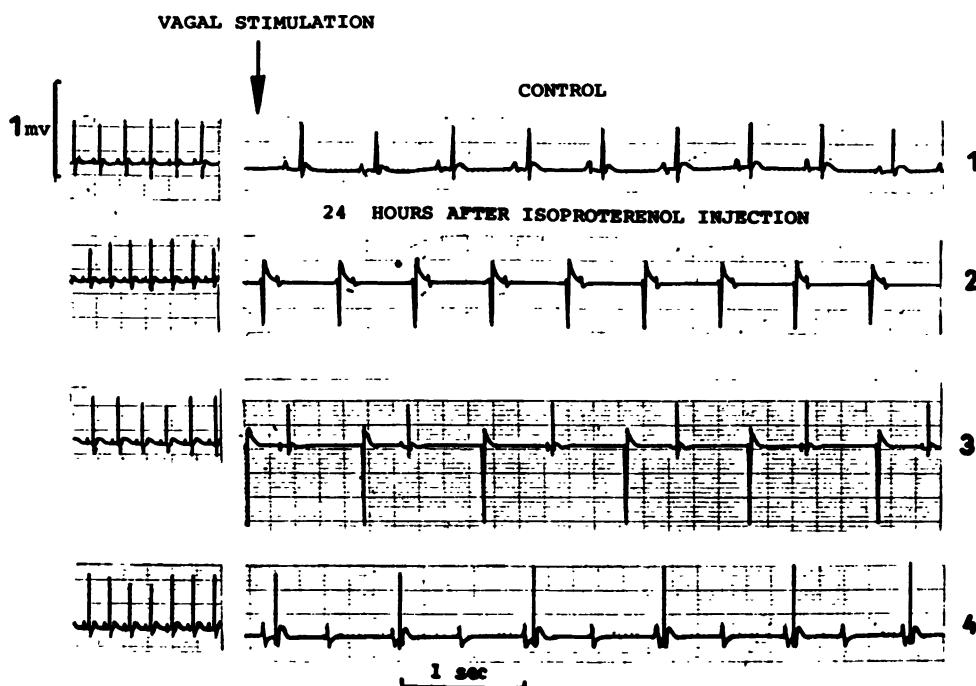


FIGURE 5. Effect of isoproterenol damage on the cardiac rhythm upon vagal stimulation. Stimulation of the vagus nerve in the control elicits sinus bradycardia (1), while after isoproterenol administration it gives rise to atrioventricular (AV) rhythm (2), idioventricular rhythm (3), and first-degree AV block (4).

1. A stress situation engendering reactions of anxiety, rage, or struggle exerts a strong β -adrenergic effect on the heart, resulting in cardiac damage that is not ischemic, but takes place in coronary dilation and manifests itself first of all as impairment of the electric stability of the heart, and also as upset contractile function. This acute β -adrenergic damage is the most plausible cause of sudden cardiac death directly in the stress episode with no observable organic lesions in the heart.
2. Prolonged action of the same stress situation also prolongs the adrenergic effect on the heart. Owing to desensitization of the β -adrenergic receptors, it may transform into a mainly α -adrenergic effect and potentiate the action of other agonists of the calcium-mobilizing receptors — vasopressin, histamine, etc. Activation of all such receptors in the coronary arteries is realized as constriction and can result in coronary spasms with more or less pronounced ischemic injury to the myocardium. This may

- explain some cases of myocardial infarction in aging persons with intact or moderately atherosclerotic coronary arteries, or in patients with vague invariant angina.
3. Still further persisting stress situation, and especially stress of capitulation, engenders a fearful behavioral reaction of "freezing" reflected, in particular, in depressed circulation and respiration and in vagal bradycardia. With exhausted adrenergic regulation and preexisting β -adrenergic lesions of the heart and its conduction system, this may lead to dangerous bradycardias and vagal cardiac arrest. The latter is most probable in the Pokkuri disease and in epidemics of sudden nocturnal deaths among contingents of people finding themselves in alien and often hopeless circumstances.

In assessing these possible variants it is easy to see that *their pivot is the strong β -adrenergic effect on the heart. In the final analysis it is this factor that causes initial injury, preconditions the α -adrenergic effect, and through the damage to the conduction system and contractile myocardium makes the heart highly vulnerable to the vagally elicited ectopic arrhythmias and functional disturbances.*

Now it seems expedient to elucidate to what extent this experimentally substantiated notion of the role of stress in the pathogenesis of arrhythmias can be applied to man.

III. ROLE OF PRIMARY STRESS DAMAGE IN ARRHYTHMIAS AND SUDDEN CARDIAC DEATH IN MAN

Since any normal person goes through multiple, more or less serious, stress episodes, and we could see that stress induces disturbances of cardiac electric stability, a reasonable conclusion is that stress and adrenergic effects on the heart may rather often entail transient and therefore nonhazardous arrhythmias.

Indeed, this suggestion is favored by the now commonly known fact that with continuous monitoring various disturbances of cardiac rhythm and conduction are found no less frequently than the inevitable stress situations of everyday life. Thus cardiac arrhythmias with variation of intervals between contractions up to 8% were recorded at least once in 86% of subjects while 50% showed pronounced sinus arrhythmias with interval variation of 100% and more.⁷

The data of Bjerregaard⁸ testify that a large part of those examined reveal episodes of bradycardia with pauses between contractions reaching 1500 to 1750 ms and sometimes 2000 ms.

Such episodes of transitory sinus bradycardia, caused in all probability by enhanced negative chronotropic influence of the vagus nerve, are not infrequently associated with sinoauricular block and are designated as a secondary sinus node dysfunction syndrome.⁴³ It can be easily envisaged that damage to the sinus node (e.g., from previous adrenergic stress attack) and decreased vagal resistance of its automatism can aggravate this phenomenon to the extent of blocked sinus rhythm and activation of the downstream ectopic foci of automatism. The data of continuous monitoring⁸ demonstrate that atrial extrasystoles are found in more than half of uncomplaining subjects examined. Ventricular extrasystoles are observed with approximately the same incidence, and their daily number in practically healthy persons may exceed 1800; the proportion of people with such extrasystoles steadily increases with age, hence it is a progressing condition. It is noteworthy that multifocal and multiple extrasystoles in the form of bigeminy and trigeminy, and in 4 cases out of 260 even ventricular tachycardia, have been revealed in uncomplaining subjects.

In discussing the question of whether or not people with such extrasystoles can be considered completely healthy, it should be borne in mind that besides extrasystoles, 24-h monitoring also reveals conduction defects, albeit much more seldom. Thus in several cases, especially during sleep, unstable I- and II-degree AV blocks⁴⁴⁻⁴⁶ as well as intermittent

elongation of the Q-R interval have been reported. In a 6-h monitoring by Von Dietz and Kirchhoff,⁴⁷ three of nine uncomplaining subjects displayed a transitory block with Wenckebach's periods. Clearly the combination of ectopic impulses with transitory conduction defects is conducive to formation of reentry and heavy arrhythmias. In general, even a brief analysis of the results of continuous monitoring of the so-called healthy subjects allows one to point out at least two moments.

First, many of these transitory arrhythmias in "healthy" people verge on pathology, progress with age, and therefore make probable a gradual evolution of a grave enough "arrhythmic heart disease" in the absence of recorded organic alterations.

Second, some of these at first glance causeless, idiopathic arrhythmias are most likely the sequelae of influenzal or other toxic infectious myocarditis, hereditary abnormalities, etc. Along with this, the transient or, more exactly, recurrent character of these arrhythmias suggests that many of them result from transitional processes in the cardiac neural regulation provoked by either exogenous or endogenous (emotional) stress.

This point of view is supported by numerous clinicophysiological studies on healthy subjects and patients.

It has been shown that among certain contingents whose occupation involves repeated stress situations, cardiac disturbances of nonischemic origin may develop that are characterized by more or less pronounced arrhythmias despite high tolerance to physical load. In other words, there is the same dissociation between impaired electric stability and intact pump function of the heart that we have described in detail in the stress damage to animal heart. Thus in our work⁴⁸ we correlated the cardiac resistance to physical and stress loads in men of such indisputably stressful occupation as international airline pilots. These people have to pass systematic examinations and are not admitted to work if showing an ischemic response to standard physical load.

As the first step, we analyzed the test results for 807 pilots that had passed the examination, and found that in the younger age group (30 to 39 years) ischemic responses were never observed, but 3% (14 cases) showed disturbances of automatism mainly in the form of atrial rhythm, and 4 cases exhibited supraventricular extrasystoles.

In the elder age group (40 to 52) rhythm disorders were found in 17% (58 cases), with equal extent of both ventricular and atrial extrasystole in 17 cases and atrial rhythm in others.

Notably, the overwhelming majority of the arrhythmic responses to physical load were observed not at peak load, but in relief, i.e., were not ischemic. *This phenomenon, not infrequently encountered in any functional diagnosis unit, is in all probability explained by the fact that when the load is relieved the rapidly increasingly vagal tonicity suppresses the sinus node automatism while the adrenergic regulation is still excited and the potential pacemakers being depolarized by excess blood catecholamines become real sources of ectopic excitation and extrasystoles.* It is essential that under conditions of such nonischemic arrhythmias there may evolve a more striking and in a sense paradoxical phenomenon when a physical load or an orthostatic test abolishes the arrhythmia steadily observed without load.

At the next stage of our work,⁴⁹ 54 pilots of the highest class aged 35 to 53 were compared in a standard test with veloergometric load (VEL) and in solving professional tasks in a flight simulator (takeoff, landing, failures, etc.). Arrhythmias turned out to occur only in 5 pilots under VEL, but in 34, i.e., more than 60%, in the simulator. In all cases extrasystoles in the simulator proved only occasional and mostly appeared during "breakdown in flight" and "flight continued after breakdown". Despite the small number of extrasystoles during flight simulation, further studies with the use of ECG monitoring showed these results to be really significant. Figure 6 displays the results of monitoring a group of pilots who either had (seven individuals) or did not have (four individuals) extrasystoles during flight simulation. It can be seen that in persons who had rhythmic disturbances in simulation the number of atrial extrasystoles is several times greater than in those who had

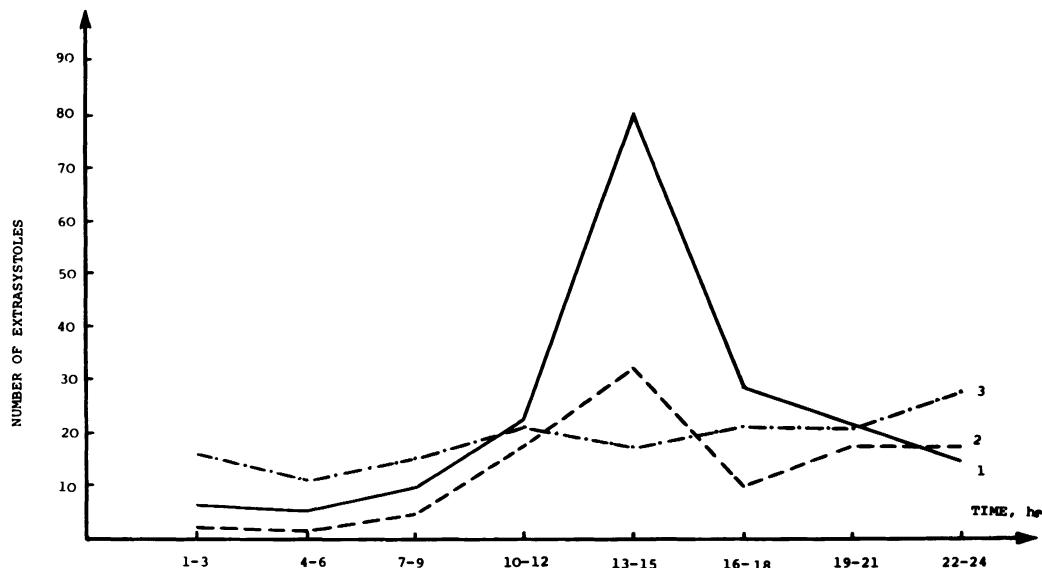


FIGURE 6. Dynamics of extrasystoles in pilots during continuous ECG monitoring. (1) Atrial extrasystoles in pilots with rhythm disturbances observed in flight simulation; (2) ventricular extrasystoles in pilots with rhythm disturbances in flight simulation; (3) atrial extrasystoles in pilots without rhythm disturbances in flight simulation.

none. Ventricular extrasystoles were completely absent from the latter group, but observable throughout the day in the former.

Much more vivid stress disturbances of cardiac rhythm and conduction in some of these people were brought on not by simulation or real flight, but by a qualitatively different stress situation — apprehension of the results of examination which could deprive them of their job.

An interesting example in this respect is pilot Z, 40, whose ECGs are presented in Figure 7. Appreciable ventricular extrasystole is seen during examination in the recuperative period after VEL and in clinical monitoring, but is totally absent at maximal physical load and during real flight including takeoff and landing. In other words, the arrhythmogenic effect is highly pronounced under stress of examination and wholly lacking in normal occupational activity, albeit involving certain risk of life. Thorough analysis revealed two more similar cases. In the aggregate these data unequivocally testify to stress-induced rhythmic disorders in apparently healthy persons (without ischemic disease). Notably, it is the more socially significant factor that proves to cause greater stress and dangerous arrhythmogenic effect.

The fact that some situations can provoke arrhythmias in practically healthy persons highly tolerant to physical load is in line with the idea that many arrhythmias may be of stress origin.

This is supported by, among other things, the very existence of a peculiar condition that had been called cardiac neurosis,⁵⁰ cardiac form of neurocirculatory dystonia,^{51,52} and in recent years has been referred to in both Russian and English literature as idiopathic arrhythmias, i.e., arrhythmias the cause of which is unknown.^{53,54} Of course this term can cover arrhythmias of toxic/infectious origin or those resulting from congenital defects. Notwithstanding, there are several circumstances favoring the neurogenous or simply stress origin of like arrhythmias.

First, many patients with such idiopathic arrhythmias exhibit neurotization in the form of hypochondriac, anxiety depressive, cardiophobic, and less frequently hysterical syndromes

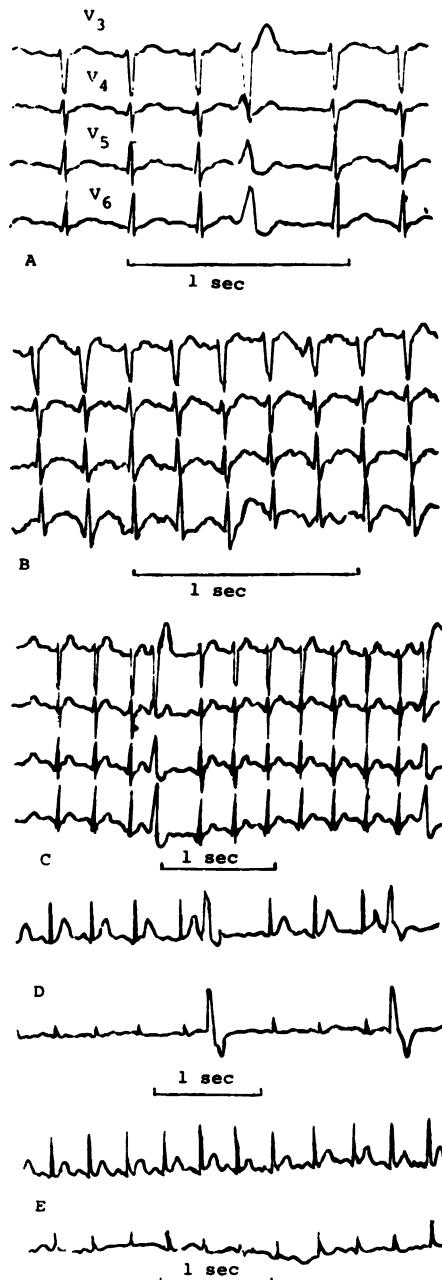


FIGURE 7. Electrocardiograms of pilot Z, age 40. (A) During examination before VEL; (B) at maximal VEL; (C) immediately upon termination of VEL; (D) continuous monitoring in the clinic during examination; (E) during real flight.

of varying degrees of severity; and arrhythmias are often proportionate to the neurotic syndrome and may disappear as the syndrome is eliminated by successful pharmacotherapy.

Second, along with the complete absence of myocardial ischemia and rather high tolerance to physical loads in such arrhythmias there often are defects in specific stress-limiting systems which will be specially considered later. Thus Skibitskii⁵² has shown that in 40

patients with neurocirculatory dystonia suffering from attacks of paroxysmal supraventricular tachycardia, a GABA derivative phenibut (acting like the known GABA-receptor activator baclophen) decreased the number of attacks by a factor of 5.5 and prolonged the fitless period to 6 months. In half of 32 patients with ventricular extrasystole, this activator of the GABA-ergic system completely abolished or decreased the number of extrasystoles by 75% or more. The antiarrhythmic effect was observed not only in neurocirculatory dystonia, but also in IHD, and was accompanied by a positive psychotropic effect. It is known that the GABA-ergic system can be activated via the benzodiazepine receptors; accordingly, benzodiazepine receptor agonists have been also successfully used as antiarrhythmics.

The abolishment of arrhythmias by activation of the GABA-ergic system is in itself an indication of the important role of this central stress-limiting system (Chapter 4) in the so-called idiopathic arrhythmias.

The studies of Smetnev et al.⁵⁵ demonstrate the involvement of defects in a cellular stress-limiting system, the prostaglandin one, in arrhythmogenesis. In patients with idiopathic paroxysmal supraventricular rhythm disorders the attacks of tachycardia develop with the blood flow in both coronary arteries enhanced proportionally to the heart rate owing to decreased coronary bed resistance. In IHD patients similar attacks are not accompanied by adequately enhanced coronary flow and may in some cases lead to coronary insufficiency. Further studies have shown that persistence of idiopathic nonischemic arrhythmias and increasing frequency of spontaneous paroxysms are attended by progressive impairment of the regulatory system of prostaglandins (PG) and cyclic nucleotides: decreasing PGF/PGF_{2α} ratio in coronary sinus blood, and increasing thromboxane/PG and decreasing cAMP/cGMP in the blood flowing to the heart.

Thus, a preexisting or evolving deficiency of the systems limiting the adrenergic effects on the heart may play a substantial part in development of arrhythmias having no organic basis in the heart, and this can be evidence for the stress origin of such arrhythmias.

The third argument in favor of idiopathic arrhythmias caused by stress is that myocardial needle biopsy in such patients often reveals focal alterations and fibrosis resembling those described in experimental stress damage to animal hearts. Neglia et al.⁵⁶ studied 44 patients without ischemic disease or cardiac failure, but who complained of palpitation, heart pains and faints, and had complex ventricular arrhythmias or conduction defects. In up to 73% of these patients bidimensional ECG revealed impaired contractility of the left and/or the right ventricle. Myocardial biopsy performed on 13 of such patients demonstrated hypertrophy of muscle fibers and focal interstitial fibrosis of both ventricles in 7 and of only the left ventricle in 5 cases.

Finally, the most weighty evidence for the role of stress in evolution of idiopathic arrhythmias has been obtained in clinical use of psychoemotional or stress loads (SL). Such techniques have been rather popular in recent years.⁵⁷⁻⁶¹ Of special interest are the studies where the inducibility of arrhythmias with SL has been correlated in IHD patients and those with neurocirculatory dystonia or idiopathic arrhythmias with pronounced neurasthenic syndrome. According to Rizhinashvili et al.,⁶² among the whole contingent of cardiological patients with potentially possible cardiac rhythm disorders the SL test elicited arrhythmias in 18% of cases; whereas, in patients with idiopathic arrhythmias and anxiety depressive syndrome, the respective portion was 42 to 46%. These arrhythmias could be abolished with a benzodiazepine receptor agonist, seduxen, which is known to activate the brain stress-limiting GABA-ergic system. Thus, even such moderate, standard clinical tests can evoke arrhythmias in a large proportion of people with certain disturbances of cardiac neural regulation.

Together with Khalfen and Lyamina we studied the adrenergic and cardiac responses to physical and stress load in 98 healthy male subjects (mean age 35) and in 48 male patients of the same age with neurocirculatory dystonia. Since this approach was further used for patients with coronary disease, it seems expedient to consider the technique in more detail.

Stress was created by an operator type of psychoemotional load associated with time shortage for solving information tasks.⁶³ There were three steps of increasing complexity, each of 5-min duration.

At step 1, the subject had to respond to words heard from a tape recorder by choosing a pencil of corresponding color and striking out from a form the word denoting this color. At step 2, he had to strike out two words: one heard from the tape recorder and the other corresponding to the color of a word projected on a screen. At step 3, he had to strike out three words: one heard, one read, and one corresponding to the color of the projected word.

The performance was evaluated by a qualified observer who responded to the subject's errors (missed word, wrong word, or wrong pencil) by actuating a sharp and unpleasant audio signal. Besides, regardless of the quality of work the testing was accompanied by standard comments, "Your work is poor", "Too many mistakes", "All men before you were better".

The subjects were tested with a stepwise-increasing VEL (initial load 25 W with 25-W increments, each step of 3-min duration), 2 d before the stress load.

The cardiovascular response was assessed by arterial pressure (AP), heart rate (HR), the index $HR \times APs/100$, and myocardial contractility indices.

The response of the adrenergic system was assayed by catecholamine excretion in urine collected from 9 to 11 a.m. at the day of testing and the day before when there were no examinations or procedures.

As follows from Table 3, in healthy subjects the SL enhanced cardiac function by about one third as judged by the index of Katz ($HR \times APs/100$), which characterizes myocardial O₂ consumption; the contraction and relaxation velocities increased approximately to the same extent so that the contraction and relaxation indices remained unchanged.

The VEL produced a much more pronounced mobilization of the cardiac contractile function: the Katz index increased 1.7-fold and contraction and relaxation velocities 1.3 to 1.4-fold; this resulted in a small decrease in the contraction and relaxation indices. On the whole, the heart is much more responsive to physical than to stress loads.

Another situation was found for catecholamine excretion with urine, which in response to the SL increased by more than 80% for epinephrine, 24% for norepinephrine, 87% for dopa, and 27% for dopamine. Under physical load the elevation in all catecholamines except norepinephrine was significantly smaller (only 18% for epinephrine or 12% for dopamine).

Despite the somewhat greater norepinephrine excretion under VEL, the overall adrenergic response (i.e., the sum increment in norepinephrine and epinephrine excretion) at moderate standard SL exceeds by half that at maximal VEL. Since catecholamine excretion depends on its concentration in the blood, this means that the blood catecholamine levels directly acting on the heart were higher in SL than in VEL. Accordingly, the antidiuretic effect proved to be twice as great in SL.

The same studies carried out on patients with the cardiac form of neurocirculatory dystonia (Table 4) show that at rest the excretion of epinephrine is 50% higher than in healthy subjects, and the relative response to SL is the same. *Norepinephrine excretion, unchanged at rest, drastically increases in SL (the increment is over threefold that in healthy subjects). In sum, this signifies that the adrenergic response in patients with neurocirculatory dystonia is greater than in healthy people.*

Further, it can be seen that neurocirculatory dystonia moderately decreases dopamine excretion at rest; the significance of this shift will be considered later in connection with the effect of SL in IHD.

The standard VEL revealed no ischemic or arrhythmic reactions in any of the patients examined. At the same time, upon SL arrhythmic reactions in the form of supraventricular and ventricular extrasystoles were observed in 12 patients (i.e., 25% of those examined). In other words, in dystonic patients the SL called for much lesser enhancement of cardiac

TABLE 3
Responses to Stress Load and Maximal Physical Load in Healthy Subjects

Indices	Stress load			Physical load		
	Before	After	Δ, %	Before	After	Δ, %
Heart rate (beats/min)	73	85	16	73	146	100
APs (mmHg)	123	144	17	121	160	32
APd (mmHg)	80	94	13	83	92	15
Katz index (HR × APs/100)	90	122	36	88	234	166
Diastolic relaxation velocity (cm/s)	3.56	4.76	34	3.58	8.46	136
Systolic contraction velocity (cm/s)	3.80	5.13	35	3.71	8.98	142
Relaxation index	4.22	4.20	-0.5	4.22	3.84	-9
Contraction index	4.00	3.90	-2.5	4.01	3.61	-10
Urine excretion (ml)	105	82	-22	105	96	-9
Epinephrine excretion (ng/min)	3.60	6.50	81	3.60	4.25	18
Norepinephrine excretion (ng/min)	6.25	7.73	24	6.25	9.06	45
Dopa excretion (ng/min)	5.10	9.85	87	5.10	5.46	7
Dopamine excretion (ng/min)	292	372	27	292	327	12

TABLE 4
Effect of Neurocirculatory Dystonia (NCD) on the Adrenergic Response to Stress Load (SL) (Excretion, ng/min)

Indices	Healthy subjects (n = 21)		NCD patients (n = 19)	
	Before SL	After SL	Before SL	After SL
Epinephrine (E)	3.60 ± 0.80	6.50 ± 0.81	5.40 ± 0.82*	10.40 ± 0.90
Norepinephrine (NE)	6.25 ± 0.81	7.77 ± 0.83	6.02 ± 0.78	11.60 ± 0.91*
E/NE	0.57 ± 0.06	0.84 ± 0.09	0.89 ± 0.06*	0.89 ± 0.06
Dopamine	292 ± 18	372 ± 22	260 ± 21	315 ± 31
Dopamine/E + NE	29.6 ± 1.81	26.0 ± 1.61	22.8 ± 1.00	18.8 ± 1.00*

* Difference with respective healthy controls significant at $p < 0.05$.

function than VEL, as in healthy subjects, but therewith caused much greater activation of the adrenergic system and in 25% of cases elicited potential arrhythmias. It is easily conceivable that in such patients with increased adrenergic reactivity the multitudinous biosocial stress episodes can cause and gradually aggravate adrenergic damage to the heart, and this process underlies the pathogenesis of the peculiar arrhythmic stress disease.

Another weighty argument in favor of the actual existence of the stress disease associated with attenuated organismic and first of all cardiac resistance to adrenergic injury is, in our opinion, the data on fibrillation and sudden cardiac death in families with marked stress intolerance. Thus Green et al.,⁶⁴ reported on sudden death in three generations, which included a 14-year-old girl who fell dead after being told that her 15-year-old brother had died immediately after a running competition. In a family studied by McRae et al.,⁶⁵ one son suddenly died at the age of 17, having been frightened by his pal, another at 12 while swimming, the third also at the age of 12 was startled by a spider and fell dead while running to tell his comrades. Their mother had about 25 syncopal episodes in the period between age 15 and 25 which had always been preceded by emotional stress causing fear and anger. Her fourth son showed no symptoms, but at 11 was found to have a shortened P-R interval and a pronounced U wave; his first syncopal episode occurred at the age of 12½ when he got angry during a game of baseball. In the clinic no rhythm disorders were observed when the patient was left alone and quiet, but any diagnostic procedures from venipuncture to application of anesthesia and heart catheterization caused great anxiety and, as a consequence,

sinus tachycardia, multifocal ventricular extrasystoles, and sometimes ventricular tachycardia developing into ventricular fibrillation.

In other words, in all these cases cardiac fibrillation and sudden cardiac death were predetermined by genetically attenuated resistance of these individuals to stress exposures. As will be shown further, such decreased resistance to the arrhythmogenic effect of stress not only can be explained by insufficiency of stress-limiting systems, but also can be rectified by adaptive activation of these systems or introduction of their metabolites.

Generally speaking, sudden cardiac death is the most grievous, but at the same time the most significant, natural phenomenon that can be used to evaluate the role of stress as a primary heart-damaging factor.

It is noteworthy that pathomorphological examination of people that have died of cardiac fibrillation reveals cases and whole groups of cases with no alterations indicative of atherosclerosis or ischemia.^{66,67} A typical example is the case reported by Davies et al.⁶⁸ A 20-year-old girl suddenly fainted during her wedding ceremony and, despite intensive medical care, died of ventricular fibrillation. The autopsy revealed a nonhypertrophied, macroscopically and microscopically normal heart, without coronary atherosclerosis, focal, or other myocardial alterations. The medical history gave no indications of heart pathology; she had never complained about her heart and did not take medicinal drugs. No pathology could be found in other organs and systems. In evaluating this quite representative case, attention should be paid to the fact that fibrillation occurred during an event of great psychological significance for the young girl; this stress element was the only meaningful finding.

There are also studies covering selected groups of cases where stress appears to be the direct cause of sudden cardiac death. Thus, Cebelin and Hirsh⁶⁹ carried out a comparative pathomorphological examination of 15 persons who had died of sudden cardiac death immediately upon heavy stress, 15 who had been killed in traffic accidents, and 15 who had died of stenosing coronary atherosclerosis.

The fatal stress situations included conflicts with parents or neighbors, homicidal assaults, etc. Pathoanatomical examination of these cases revealed groups of cardiomyocytes with contractual injuries and dispersion of myofibrils, and the interstitial connective tissue had homogeneous regions alternating with zones of granulation.

Such microfocal alterations bear much resemblance to those demonstrated in the myocardium by us in EPS (Chapter 1) or by Rona et al.¹⁵ upon administration of isoproterenol, and the authors⁶⁹ justly attributed these lesions to the action of catecholamines and calcium. As already said, these alterations substantially increase the heterogeneity of the myocardium and may give rise to ectopic foci of recurrent excitation, i.e., seriously upset the cardiac electric stability. The above data testify that in a certain proportion of cases by the moment of sudden death the heart had no irreversible organic alterations that could be regarded as the cause of death.

In accordance with this, Lown,⁶⁶ Wolf,⁷⁰ Meerson and Pshennikova,⁷¹ and many others searched for a cause of fibrillation and sudden cardiac death beyond the heart, and pointed to biosocial stress as the most likely environmental factor. Indeed, there is a considerable body of evidence that stress may lead to fibrillation and sudden death not only through aggravation of atherosclerosis or ischemia, but directly through neurogenic or hormonal action on undamaged myocardium.

There is thus a case of a 22-year-old girl who suffered her first attack of cardiac fibrillation and clinical death in September 1983 on her first day in medical school. After resuscitation she had a defibrillator implanted; further on (in 1984 and 1985) fibrillation was sure to happen on the first day of studies, and only actuation of the defibrillator prevented sudden death.⁷² Quite similar cases have been reported by Wellens et al.⁷³ and Lown.⁶⁶ *It is important that such "conditioned" attacks of cardiac fibrillation were not accompanied by any coronary or valvular damage or other organic alterations upon thorough clinicophysiological examinations.*

This unambiguously proves the basic possibility of primary stress fibrillation and sudden death, but gives no idea of their incidence. Of special interest therefore are descriptions of thoroughly examined patients in whom cardiac fibrillation and clinical death with subsequent resuscitation were not attended with any alterations in the coronary arteries (i.e., were primary).

The first work along this line appears to have been undertaken by Lown,⁶⁶ who, upon a complex cardiological and psychiatric examination of 117 patients revived after sudden death, discerned 25 with the least pronounced pathological alterations in the heart, but the most clear triggering of dangerous arrhythmias by external stress.

Two more groups of similar patients have recently been described in different clinics.^{74,75}

Further, account should be taken of the reports of peculiar "epidemics" of sudden cardiac death among young people without coronary atherosclerosis, such as the one among Malay and Cambodian immigrants in the U.S.⁷⁶ The overwhelming majority of those who suddenly died had no or minimal coronary sclerosis, but differed from others of the same nationality in poorer prospects of social adaptation, i.e., an element of desolation. These people usually died at night, practically instantly, as apparently healthy young Japanese males die of the "Pokkuri disease" which has now been shown to result from ventricular flutter.⁷⁷

Stress not involving direct physical action also can definitely affect people without IHD and adapted into society. Thus Helsing and Szklo⁷⁸ and Helsing et al.⁷⁹ have shown that among young adults sudden cardiac mortality is increased after bereavement.

In an interesting study by Rabkin et al.,⁸⁰ 3983 cases of sudden cardiac death in men were analyzed with respect to the day of the week. Among men with no manifestations of IHD, increased mortality fell on Monday. It is essential that such increased mortality associated with going back to work after the weekend was not observed either in cancer patients or in those with myocardial infarction; hence its mechanism differed from that in IHD.

In the aggregate, the available clinical evidence (apparently healthy persons with arrhythmias revealed with the wide use of continuous monitoring, patients with neurocirculatory dystonia, and cases of sudden cardiac death in clear absence of coronary atherosclerosis) throws doubt on the validity of the term "idiopathic arrhythmias" and at the same time seems to outline the dynamics of a certain progressive stress disease of the heart. The main role in its pathogenesis is played not at all by ischemic, coronarogenic cardiac injuries, but by direct adrenergic damage to the conduction system and contractile myocardium.

As individuals with decreased resistance to stress diseases (i.e., with defects in the stress-limiting systems considered in detail in Chapters 4 and 5), like all others, encounter numerous stress situations, the stress injuries inflicted every time may accumulate to cause considerable damage to the myocardium and especially to its conduction system, which has the most dense adrenergic and cholinergic innervation.^{81,82}

Of considerable interest in this respect are the results of autopsies performed from 1963 till 1981 on 200 patients with electrocardiographically proved complete AV block. These summarized data presented in an excellent book of Davies et al.⁶⁸ testify that only in 17% of cases the pathoanatomical examination allows one to put down ischemic disease as the cause of impaired conduction. Even leaving out calcification of the node, congenital cardiomyopathy, and other causes, we still have 38% of cases where the condition is underlain by a process of unknown etiology, i.e., idiopathic bilateral His bundle-branch fibrosis. We believe that the possible part of adrenergic stress injury in the development of a chronic process leading to this rather frequent finding deserves special study.

Another morphological phenomenon often observed in autopsies for sudden cardiac death — pronounced myocardial hypertrophy — may also be caused by prolonged action of high catecholamine concentrations⁸³ and, combined with focal cardiosclerosis, is a plausible factor impairing the electric stability of the heart. Finally, there are reports of myocardial

infarction with angiographically normal coronary arteries. The number of cases is not large, e.g., 10 out of 528 infarction patients examined by Salem et al.⁸⁴ Still, the very existence of this phenomenon justifies further studies concerned with the possibility of large adrenergic myocardial necroses under natural heavy stress. At present it can be thought that hypertrophy, fibrotic AV block, and necrobiotic foci can also be of stress origin.

By and large, it can be said that stress damage is really involved in the pathogenesis of arrhythmias, fibrillation, and sudden death, setting in through noncoronary impairment of metabolism and electric stability with only minor lesions in myocardial structures, yet under certain conditions resulting in pronounced injury to the cardiac muscle. On the other hand, it should not be forgotten that in many cases the stress acts not as a self-contained factor, but combines with ischemia brought on by stenosing coronary sclerosis. This combination stands out quite definitely in the analysis of the pathogenesis of grave arrhythmias and sudden cardiac death.

Accordingly, we shall first consider the role of stress in the pathogenesis of ischemic disease, and then the relation of stress and ischemia in arrhythmias and sudden cardiac death.

IV. ROLE OF STRESS REACTION IN ISCHEMIC DISEASE

Obviously, to assess the contribution of stress cardiac damage to the pathogenesis of ischemic heart disease it is necessary to analyze first the pathogenetic chain of the ischemic damage itself. Our analysis will be only brief, as the vast material on this problem has been comprehensively covered in the proceedings of international symposia,⁸⁵⁻⁸⁸ fundamental reviews, and handbooks⁸⁹⁻⁹¹ of the last decade.

These works demonstrate that, on the one hand, the ischemic injury to the myocardium constitutes the basis of the most common disease of the century — IHD and myocardial infarction — and on the other the very concept of its pathogenesis is far from being complete and final, and in many aspects is obscure or rapidly changing. In the framework of this book a description of this pathogenetic chain is not an end in itself, and for the sake of clarity we shall use the body of evidence available to discern four basic stages of the development of ischemic damage,⁹² mostly with a view to evaluating the contribution of stress usually associating with ischemia.

A general overview in Figure 8 shows that at first the two major etiological factors of IHD — excess cholesterol in food and genetically determined defects in hepatic uptake and elimination of cholesterol — combine to cause atherogenic dyslipidemia and ensuing stenosing coronary atherosclerosis. At the same time the environmental stress situations combined with defects in the stress-limiting systems cause hepatic damage (to be considered) and thereby aggravate the dyslipidemia. It can further be seen that the role of stress reactions in IHD is not restricted to this, as excessive stress entails primary cardiac stress damage, coronary spasm, and alterations in blood rheological properties conducive to coronary thrombosis. At this first stage the process is reversible and can be stopped by various factors suppressing the stress reaction, causing coronary dilation, and preventing blood clotting, either through medical intervention or by endogenous means (like metabolites of the stress-limiting systems).

However, under certain conditions when ischemia evokes endogenous stress of pain and fear that potentiates the stress reaction, the process goes into the second stage: interruption of the mitochondrial respiratory chain, despite transient activation of glycolysis, leads to an energy-deficient inhibition of the Na,K and Ca pumps of the sarcolemma and of the Ca pump of the SR. Simultaneously, excess catecholamines and other stress hormones act through cAMP and inositol triphosphate and diacyl glycerol (IP₃-DAG) to cause enhanced entry of calcium and loss of potassium in the cardiomyocytes. The resulting excess of calcium

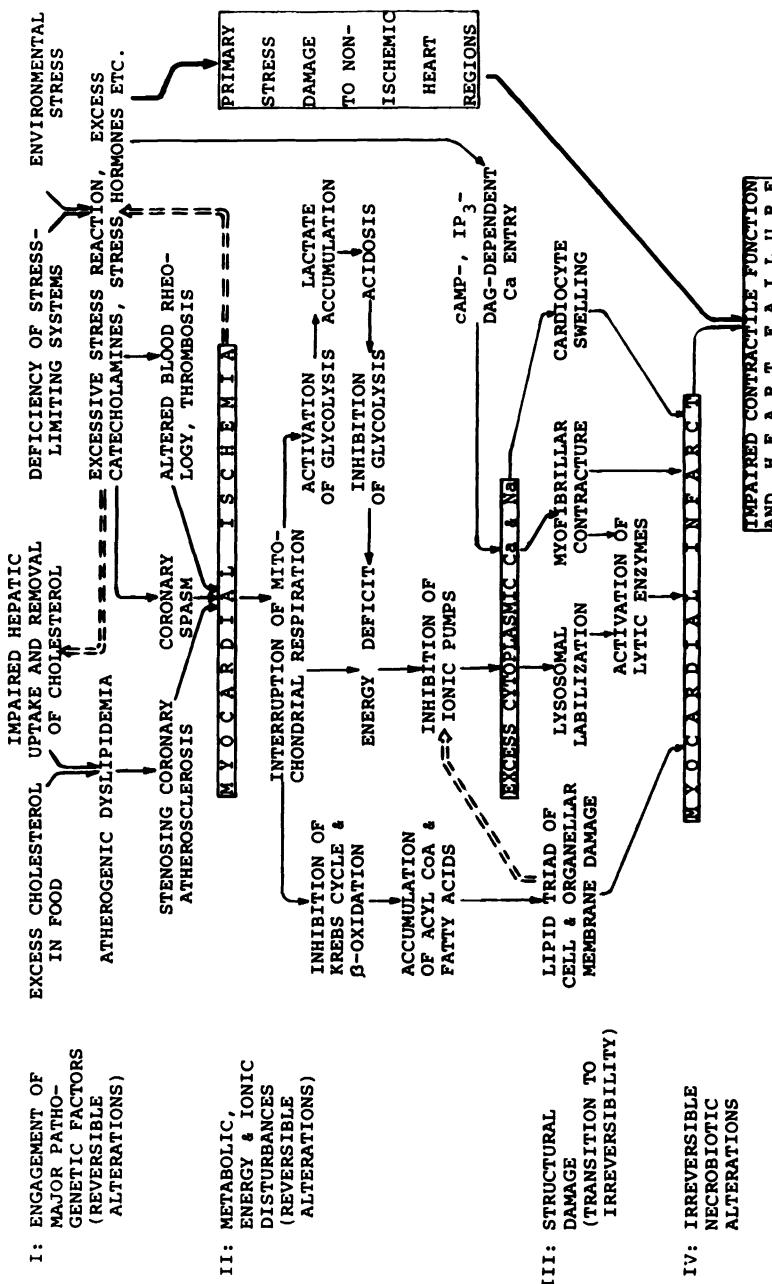


FIGURE 8. Role of stress reaction in the pathogenesis of ischemic disease. For explanations see text.

and sodium in the cells of the contractile myocardium and the conduction system, with the consequent decrease in the rest potential underlie the profound impairment of the cardiac electric stability. In principle this stage is also reversible since the endogenous mechanisms considered further can abolish ischemia by, e.g., opening the collaterals or suppressing the stress reaction through activation of the stress-limiting systems. Yet in the combination of heavy ischemia and intense stress reaction the upset of cationic transport at all levels of the conduction system or contractile myocardium can deeply impair the electric stability of the heart and cause grave early arrhythmias, fibrillation, and cardiac arrest.

If this does not happen, but both detrimental factors — ischemia and stress — persist, the process develops into its third stage when stress hormones and excess calcium and fatty acids activate the lipid triad (lypolytic enzymes, lipid peroxidation, detergent action of lysophosphatides); concurrently the cardiotoxic action of calcium, cardiomyocyte swelling from excess sodium, release of lysosomal enzymes lead to deterioration of the sarcolemma, myofibrils, mitochondria, and nuclei. With the growing deficiency of ATP all these alterations become irreversible, evolving into the fourth stage — necrobiosis that underlies myocardial infarction. The depression of cardiac contractility preceding and accompanying the infarction may be complicated with upset electric stability and late arrhythmias, which have been dealt with comprehensively.^{14,93}

Here I would like to underline the main feature characteristic of ischemic damage to the heart: in a conscious organism ischemic injury is always attended with a marked stress reaction; the pathogenetic stages presented on the scheme allow a statement that the damage is not purely ischemic, but its dynamics and outcome are determined by the combined action of stress and ischemia. We shall therefore further consider the concrete mechanisms through which stress contributes to ischemic damage.

Regardless of whether the stress reaction is caused by an exogenous or an endogenous factor, there are several mechanisms by which it can cause or be conducive to the development of IHD.

The first pathogenetic mechanism of this kind is stress hypercholesterolemia and hyperlipidemia resulting from overenhancement of the initially adaptive lipotropic effect of stress.

There is experimental evidence that neurogenous, essentially stress-induced atherosclerosis can be created in animals by frequent changes of conditional-reflex stereotypes, or by emotional excitation caused by intermittent starvation or prolonged electric stimulation of the ventromedial nucleus.⁹⁴⁻⁹⁶ These data are consistent with the results of epidemiological studies. The incidence of coronary atherosclerosis, IHD, and IHD-linked myocardial infarction is especially high in people whose occupation involves continual emotional strain.^{97,98} An important development in this line of research is a recent work of Clarkson et al.⁹⁹ who correlated the biosocial position of primates in a stable group with plasma lipoprotein composition and found that in subordinate animals the ratio of total plasma cholesterol to high-density lipoproteins is more than half as great again compared to dominating animals; this was naturally accompanied by a fourfold greater percentage of coronary stenosis in subordinate females and an almost twofold increase in subordinate males than in respective dominating specimens. Thus, a protracted biosocial stress is a real cause of atherogenic shifts and progressive coronary atherosclerosis. We have further demonstrated¹⁰⁰ the atherogenic effect of a single emotional pain stress on rat blood lipoprotein and cholesterol levels, and were the first to prove the involvement of hepatic stress damage in this phenomenon. In this work the effects of prolonged stress according to Desiderato et al.¹⁰¹ were studied in respect of serum lipoproteins on the one hand, and indices of hepatic function and metabolism on the other.

The correlation with atherosclerotic damage to coronary vessels in humans is known to be negative for high-density lipoproteins (HDL)^{102,103} and positive for low-density and very

low-density lipoproteins (LDLP).^{104,105} This has been confirmed in wide epidemiological and experimental studies and gave grounds for the concept of an antiatherogenic role of HDLP and the atherogenic role of LDLP as well as for the use of the atherogenicity index (AI) for prognosing the probability of coronary atherosclerosis:

$$AI = \frac{Ch_{tot} - Ch_{HDLP}}{Ch_{HDLP}}$$

where Ch_{tot} is total plasma cholesterol and Ch_{HDLP} is cholesterol in the HDLP fraction.

Table 5 shows that a 6-h EPS does not appreciably alter the total cholesterol content, but causes a substantial decrease in cholesterol contained in the fraction of antiatherogenic HDLP, which is clearly seen already 2 h after stress and reaches a maximum (over twofold) in 24 h. As a result the AI proves to be 1.5 times greater in 2 h and more than 4 times greater in 24 h after stress as compared to the control. Thus, a single long stress indeed gives rise to pronounced atherogenic dyslipidemia.

We further suggested that the decreased cholesterol content in the HDLP fraction may be due to decreased activity in the blood of the enzyme lecithin:cholesterol acyltransferase (LCAT) that catalyzes transfer of an unsaturated fatty acyl residue from position 2 in lecithin to free cholesterol, producing cholesterol esters and promoting thus formation of HDLP.¹⁰⁶⁻¹⁰⁸

The data in Table 6 demonstrate that upon exposure of rats to stress their overall serum esterifying capacity reliably decreased to 78% of the control value in 2 h and to 70% in 24 h after the end of a 6-h EPS. Still more marked was the decline in LCAT, which fell more than threefold in 2 h and then was restored to the initial level.

The observed alterations in lipid metabolism in EPS fit in with the suggestion that impaired esterification of cholesterol is essential to stress dyslipidemia and specifically to the decline in HDLP cholesterol. LCAT is a "short-lived" protein synthesized in the liver and then supplied to the blood where it participates in lipoprotein conversions.^{109,110} The short half-life and high rate of biosynthesis of this enzyme is the most plausible explanation for the marked but transient drop in its activity in serum upon stress impairment of hepatic metabolism.

In further experiments we evaluated the activation of hepatic lipid peroxidation (LPO) in stress by the level of its products, and hepatic damage by the blood level of a hepatocyte-specific enzyme such as fructose 1,6-diphosphate aldolase.

Table 7 shows that stress consistently causes LPO activation as manifest by a twofold increase in the hepatic content of malonic dialdehyde, a marked decrease in the hepatic activity of the antioxidant enzyme superoxide dismutase, and a 2.5-fold rise in the plasma fructose 1,6-diphosphate aldolase. These alterations are most pronounced 2 h after stress and abate to some extent in 1 d.

This supports the idea that stress acts through LPO activation to cause damage to the key organ of cholesterol metabolism, the liver, but leaves open the main question of how the hepatic stress damage interferes with cholesterol elimination and leads to atherogenic dyslipidemia. A reasonable suggestion was that it affects cholesterol 7- α -hydroxylase; to test this we studied the effect of EPS on hepatic cholesterol oxidation.¹¹¹

Hydroxylation of cholesterol (assayed by the amount of 3H_2O excreted with urine after administration of labeled cholesterol to rats) proved to be appreciably diminished upon EPS (Figure 9).

Thus, hepatic stress damage is associated with impaired activity of the cytochrome P₄₅₀ system and specifically of its important functional form cholesterol 7- α -hydroxylase that limits cholesterol conversion to bile acids and elimination from the organism.

In whole, these data prompt one to think that stabilization of the hepatic hydroxylase

TABLE 5
Effect of EPS on the Serum Lipid Content and the Atherogenicity Index

Series	Cholesterol		Triglycerides	Atherogenic index
	Total	HDLP*		
1. Control (n = 9)	70.8 ± 3.5	52.6 ± 2.8	52.8 ± 4.2	0.35
2. 2 h after EPS (n = 10)	66.4 ± 2.0	43.6 ± 2.1	46.3 ± 2.7	0.52
3. 24 h after EPS (n = 9)	58.6 ± 4.5	22.7 ± 1.7	40.8 ± 3.2	1.60
	$p_{1-3} > 0.05$	$p_{1-3} < 0.01$	$p_{1-3} < 0.05$	

* HDLP = High-density lipoproteins.

TABLE 6
Cholesterol Esterification in Rat Blood Serum After EPS

Time after EPS	Total cholesterol (mg/dl)	Cholesterol-esterifying activity (nmol/ml·h)	LCAT* activity (nmol/ml·h)
Control	65 ± 2.1	141 ± 13	23.4 ± 2.3
2 h	65 ± 1.3	110 ± 12	8.0 ± 1.5
24 h	62 ± 1.1	99 ± 13	21.1 ± 2.3
48 h	67 ± 0.9	133 ± 12	27.4 ± 1.5

* LCAT = Lecithin:cholesterol acyltransferase.

TABLE 7
Effect of EPS on the Content of Malonic Dialdehyde (MDA) and Superoxide Dismutase (SOD) Activity in the Liver, and Fructose 1,6-Diphosphate Aldolase (FDA) Activity in Rat Serum

Time after EPS	MDA (nmol mg protein ⁻¹)	SOD (U g protein ⁻¹)	FDA, ($\mu\text{mol ml}^{-1} \cdot \text{h}^{-1}$)
Control (n = 9)	0.30 ± 0.03	64.63 ± 7.31	0.124 ± 0.08
2 h (n = 8)	0.64 ± 0.05 $p_{1-2} < 0.001$	30.84 ± 2.08 $p_{1-2} < 0.001$	0.316 ± 0.02 $p_{1-2} < 0.001$
24 h (n = 8)	0.37 ± 0.02 $p_{1-3} > 0.05$	50.33 ± 3.89 $p_{1-3} > 0.05$	0.282 ± 0.02 $p_{1-3} < 0.001$

activity or induction of this enzyme is a promising way of preventing atherogenic dyslipidemias of various origins and may be important in designing new means of prophylaxis of atherosclerosis. Of course this in no way contradicts the concept of Brown and Goldstein¹¹² about the decisive role of hepatic receptors; on the contrary, there arises a rather interesting question of the effect of stress on these receptors.

There is thus unambiguous evidence that *stress causes deep alterations in the liver and impairs cholesterol elimination from the organism. Therefore, even when there is no excess of cholesterol in food, stress can be conducive to stenosing coronary sclerosis and plays an important part in the development of IHD.*

The second pathogenetic mechanism by which stress may aggravate the dangerous consequences of myocardial ischemia is the primary cardiac damage from stress arising endogenously in response to pain and fear caused by ischemia. At least two components of this aggravating effect should be distinguished: exaggeration of arrhythmias in ischemia, and impairment of the contractile function in nonischemic cardiac regions in infarction.

The arrhythmogenic effect of the neural factor in acute ischemia is well known. Thus

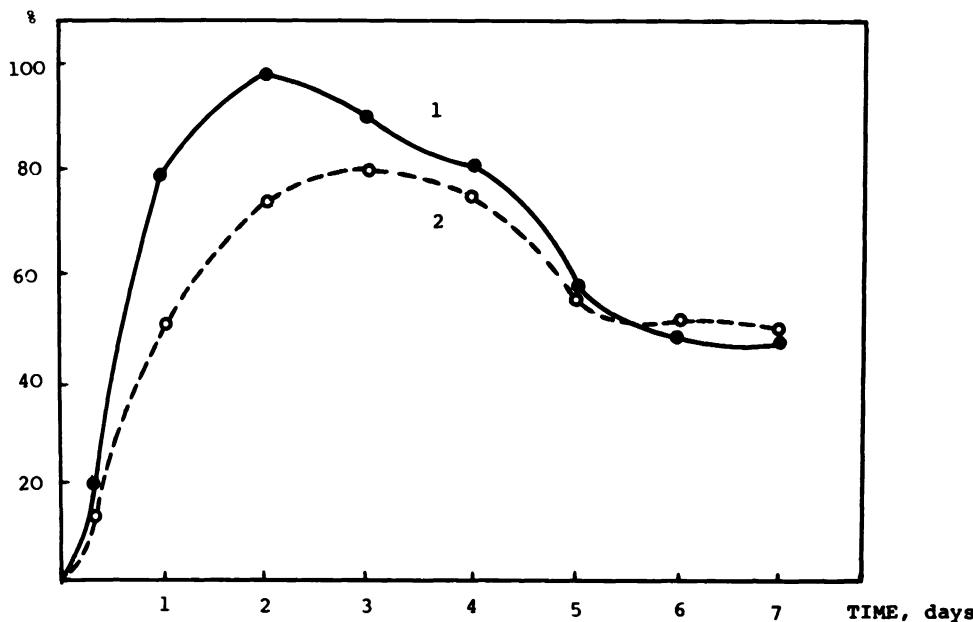


FIGURE 9. Effect of EPS on ^3H -cholesterol oxidation assayed by the amount of $^3\text{H}_2\text{O}$ in rat urine. (1) Control; (2) after EPS.

Satinsky et al.¹¹³ found out that under deep anesthesia ligation of the descending branch of the left coronary artery causes cardiac fibrillation only in 6.3% of cases, whereas upon stimulation of posterior hypothalamic centers that control the circulatory function arrhythmias are ten times more frequent. Similarly, stress situations increase threefold the incidence of fibrillation caused by coronary occlusion.¹¹⁴ Then again, the cardiac fibrillation threshold decreases in acute ischemia with the probability of fibrillation increasing twofold to threefold.¹¹

It is noteworthy that these arrhythmogenic effects of stress can be restricted or prevented by removal of local sympathetic nodes or administration of adrenoblockers.^{115,116}

At the same time, stimulation of cardiac sympathetic nerves and sympathetic nodes allows the arrhythmogenic effects of the midbrain to be reproduced even under conditions of stabilized blood pressure and heart rate.¹¹⁷ Numerous clinical studies testify to the excitation of the sympathoadrenal section of the nervous system during attacks of arrhythmia in humans, with enhanced catecholamine excretion as well as increased cAMP and decreased cGMP production.¹¹⁸

There is thus no doubt that excitation of the sympathetic nervous centers and the adrenergic effect on the heart are essential to the pathogenesis of arrhythmias caused by neurodynamic shifts in the higher divisions of the brain.

In studying these shifts it was observed that some known antiarrhythmics such as noricisine (ethmazin) and dysopiramide (norpace) not only suppress the activity of ectopic foci and abolish cardiac arrhythmias, but also affect the bioelectric activity of the frontal lobe cortex in animals and humans, decreasing the amplitude of slow potentials, and these two effects are in good quantitative correlation.^{118,119} Skinner and Jingling¹²⁰ and Skinner¹²¹ recorded in parallel the bioelectric activity of the heart and of the frontal cortex in arrhythmias caused by stress or acute ischemia, and came to the conclusion that stress evoked by ischemia excites a certain zone in the frontal lobe cortex, and in both cases the further chain of events leading to cardiac fibrillation proves to be cortically determined.

The most essential experimental result obtained by Skinner is that in occlusion of the coronary artery and acute myocardial ischemia in pigs, excitation of this zone is accompanied

by cardiac fibrillation, whereas cold blockade of the subcortical zone or tonsil as well as a corticotrunical blockade with propranolol prevent fibrillation, cardiac arrest, and animal death despite myocardial ischemia. In other words, *myocardial ischemia is a mere result of mechanically interrupted blood flow, while the reaction to ischemia in the form of fibrillation and cardiac arrest is a result of complex intercentral connections in the brain.*

It is important that these connections may be primarily determined by an environmental stress situation which gives rise to a pathological dominant¹²² or a pathological system of excited centers¹²³ that in its turn determines the damaging adrenergic effect of ischemia on the heart.

The mechanism through which the adrenergic effect leads to cardiac arrhythmias can be envisaged on the basis of the experimental data from our laboratory presented earlier and pertaining to the complex of stress damages upon EPS. This complex is indicative of the impairment of microstructures and function of the cardiac membrane apparatus responsible for generation and conduction of excitation. Such damage can play a prominent part in the formation of two essential pathogenetic links of arrhythmias and fibrillation, namely ectopic foci of premature excitation impulses and foci of functional conduction blocks.^{124,125}

In infarction, acute ischemia, and microfocal stress injury, a premature impulse generated in such ectopic foci may encounter a functional conduction block arising again from microfocal stress-induced (i.e., adrenergic) injury to nonischemic regions of the myocardium. In this case the premature impulse will not go through the blocked zone, but bypass it and return later when the conduction block will have passed, forming thus a reciprocal excitation wave and the well-known phenomenon of reentry which is the generally recognized cause of ventricular tachycardia and cardiac fibrillation.^{125,126}

The second component of aggravation of ischemic damage by stress is the impairment of the contractile function of nonischemic heart regions in infarction. This was the subject of our special study in which we assessed the changes in a certainly nonischemic part of the heart — right auricle in experimental infarction of the left ventricle. The auricles were taken from animals 1 d after ligating the left coronary arterial descending branch according to Selye, and their contractile function was studied under isometric conditions as described earlier.¹²⁷

The auricles obtained from animals that had undergone myocardial infarction did not differ from the controls in their mass and initial length, but had significantly decreased extensibility (Figure 10A) and accordingly responded to stretching with smaller increments in developed tension (Figure 10B), i.e., with a smaller Starling effect.

These data fit in with the concept that stress caused by the infarction affects the nonischemic regions of the myocardium. The resulting arrhythmias and depressed contractile function of these uninjured sections can be fatal for the myocardial infarction patient, and this justifies the wide use of β -adrenoblockers and agonists of GABA and benzodiazepine receptors in the acute infarction period.

Obviously, if stress arrhythmias and impairment of contractility were realized to the full in every infarction, then without timely drug protection most afflicted people would die a sudden death. Yet most of the myocardial infarction cases do not have such a fatal outcome, and it will be later shown that this is due to activation of the central stress-limiting systems (opioidergic, GABA-ergic, serotonergic) that opportunely restrain the stress reaction and thereby prevent death from stress that complicates myocardial ischemia.

The third pathogenetic mechanism consists in that the strong adrenergic component of the stress reaction may cause spasm of the smooth muscle of anatomically intact coronary arteries, and this rather stable spasm results in secondary ischemic damage to the myocardium.

It has already been said (see Section II) that the vascular smooth muscle responds by contraction to α -adrenoreceptor and by relaxation to β -adrenoreceptor stimulation by catecholamines. Since the β -adrenergic effect, as we have shown, prevails in stress situations,

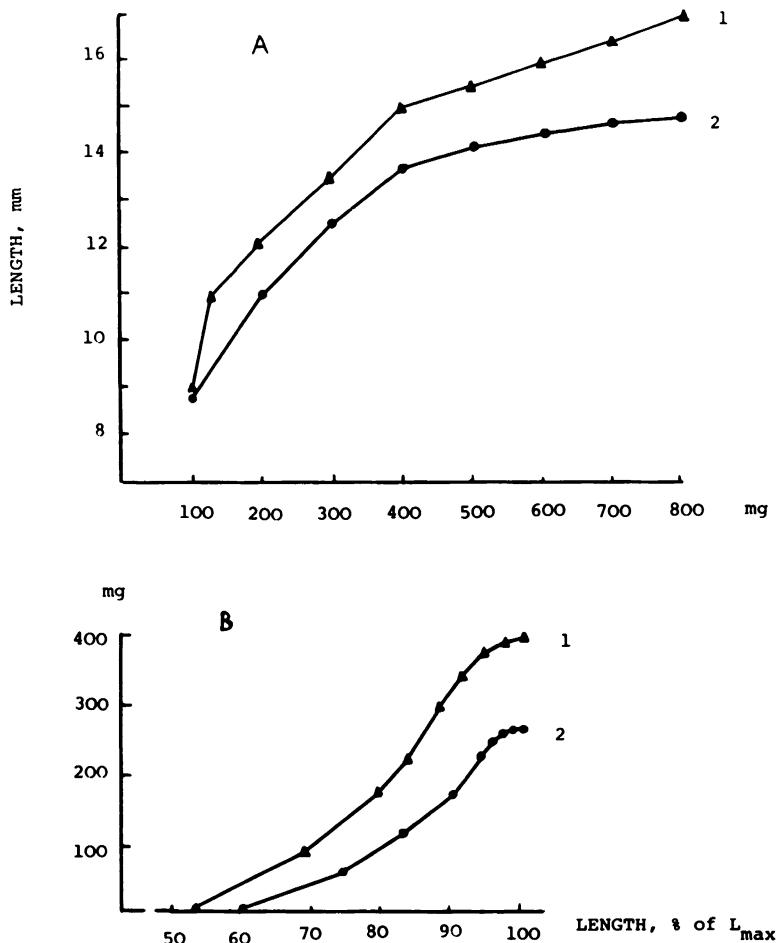


FIGURE 10. Depression of (A) extensibility and (B) developed tension of the right auricle in experimental infarction of the left ventricle. (1) Control, (2) infarction. (A) Abscissa, applied external load, mg; ordinate, atrial length, mm. (B) Abscissa, atrial length, percent of L_{\max} ; ordinate, developed tension, milligrams.

this design of the neural apparatus of the coronary vessels should, and really does, ensure their dilation and enhancement of coronary blood flow during stress, physical work, etc. Still, recent studies demonstrate that this does not at all exclude a stress-induced coronary spasm. The matter is that in the healthy organism, when the resistive coronary vessels are dilated owing to activation of "β-receptor-cAMP" system and action of local metabolites, there is still a certain tonic vasoconstrictive influence from the α-receptors. Thus the high coronary flow observed under large physical load is still further augmented upon blocking the α-adrenoreceptors with phentolamine.²² In the healthy organism this tonic influence of sympathetic nerves on postsynaptic α-receptors of the coronary vessels serves to prevent the wasteful excessive coronary flow under physical load. On the other hand, this means that any naturally occurring or pharmacologically induced blockade of the β-adrenoreceptors may lead to coronary spasm. In particular, propranolol has been found to cause coronary constriction,¹²⁹ i.e., to demask the sympathetic vasoconstrictive action of α-receptors by blocking the β-receptors. Under natural conditions a decline in the coronarodilatory effect of β-receptors can be due to their lower (as compared to α-receptors) resistance to desensitization in protracted stress or prolonged elevation of blood catecholamines¹³⁰ in cardiac

failure. This desensitization by high catecholamine concentrations proves to develop rather quickly. Thus, Simons and Downing¹³¹ have found that during continuous intravenous infusion of norepinephrine the coronary blood flow first naturally increases, then returns to the initial level, and by the 40th min of infusion the coronary resistance is almost twice higher, the coronary flow declines, and 48 h later the myocardium displays the usual set of ischemic lesions. No coronary spasm or myocardial damage is observable in animals who beforehand received phentolamine. Hence the spasm and myocardial damage are indeed likely to result from the "breakdown" of the β -receptors and predominance of the vasoconstrictive action of the α -receptors. Similarly, the α -adrenergic effect and coronary spasm can take over in prolonged administration of cardiac glycosides, since the inhibition of the Na,K pump abolishes the prevalence of the β -effect.

Furthermore, the ischemia brought on by coronary spasm causes a release of nickel from the myocardium, while exogenous Ni ions in quite comparable amounts are known to induce coronary constriction.¹³² We can thus envisage a vicious circle in which ischemia caused by spasm, atherogenic coronary stenosis, or thrombosis entails alterations which in their turn stabilize the spasm, turning it into contracture, and all this results in protracted ischemia leading to coronary necrosis.

On the other hand, the adrenergic effect of stress accompanying ischemia is responded to by activated endothelial synthesis of PGs, and first of all prostacyclin, which is an endogenous vasodilator and antiaggregative agent; this can prevent the formation of the above vicious circle and the ischemic damage. Another natural vasodilatory and consequently anti-ischemic agent is the endothelial relaxing factor.¹³⁴ Interestingly, this appears to be the mechanism of the vasodilatory effect of such a major anti-ischemic pharmacological factor as nitrates, as nitroglycerin was found to induce PG biosynthesis.¹³⁵

The fourth pathogenetic mechanism by which stress aggravates ischemic damage involves such a component of the stress reaction as hyperventilation, which naturally leads to increased blood oxygen tension and hypocapnic alkalosis. These shifts in their turn attenuate the coronary blood flow¹³⁶ and augment the tonicity of coronary arteries (through enhanced access of calcium to the binding sites on myofibrillar troponin in the vascular muscle cells) followed by their contracture.¹³⁷ Clinicophysiological studies demonstrate that hyperventilation and attending hypocapnic alkalosis in IHD patients entail a 15% decrease in coronary flow¹³⁸ as well as attacks of angina¹³⁹ that can be abolished with a calcium blocker diltiazem, which does not change the coronary blood gas composition. This again points to the central role of excess calcium in the mechanisms of damage.

The fifth pathogenetic mechanism is that catecholamines promote blood coagulation and formation of thrombi,¹⁴⁰ whereby the blood platelets release potent vasoactive compounds, and in particular thromboxane A₂,¹⁴¹ and 5-hydroxytryptamine (serotonin) and histamine, which augment the spasm dangerously combining with thrombosis. This is most likely to happen in those coronary arterial regions where there are initial atherosclerotic alterations.¹⁴²

Shepherd and Vanhoutte¹³ have shown that normally the intact endothelium releases prostacyclin to inhibit platelet aggregation and monoamine oxidase to decompose serotonin and other monoamines. Thus the action of the PG system is not only vasodilatory, but also antithrombotic, which is definitely seen in experimental studies of the blood flow through stenosed coronary arteries. The partial narrowing of the coronary artery may result in gradual cyclic attenuation of coronary flow in dogs, these oscillations being due to platelet aggregation and disaggregation.¹⁴³ This only happens when coronary constriction is accompanied by elevation in blood thromboxane, while in other cases it can only be elicited by large doses of indomethacin, which inhibits PG synthesis.¹⁴⁴ Conversely, administration of prostacyclin in all cases abolishes the flow oscillations in the constricted coronary artery and thereby prevents thrombosis. So the question of whether or not there is risk of persistent spasm, thrombosis, and finally myocardial infarction is determined by the state of such a "regional" stress-limiting system as PGs.

The sixth pathogenetic mechanism has been recently elucidated and consists in that protracted stress reaction lowers the resistance to hypoxia and reoxygenation.

Our experiments together with Belkina and Matsievskii were aimed at evaluating the cardiac contractile function, its work, and coronary bed resistance during normoxia, ischemia, and reoxygenation in control animals and in those that had undergone EPS 2 h prior to testing. The left ventricular and the carotid pressure were recorded in parallel, and the stroke volume and cardiac output were determined with the use of an ultrasonic flow sensor.¹⁴⁵

Table 8 demonstrates that the stress itself did not cause appreciable changes in the contractile function or in the total peripheral resistance. Substantial differences between control and poststress animals were found in reoxygenation: the developed ventricular pressure, the mean carotid pressure, and the cardiac output at the 30th min of reoxygenation proved to be significantly lower upon stress, so that the external cardiac work was threefold less. There is thus no doubt that EPS markedly aggravates the impairment of the cardiac contractile function in reoxygenation.

The seventh mechanism stems from the adrenergic mobilization of the cardiac function in stress which, combined with the regulatory increased vascular resistance, causes heart overloading and may aggravate the ischemic damage not only in spasm or thrombosis, but in simple atherosclerotic stenosis.

At first glance this seems to be contradictory with the coronary vasodilation due to the main β -adrenergic effect of stress and to activation of the local adenosinergic and PG systems. Mudge et al.²³ explained that in persistent stenosis of a large coronary artery a surplus load cannot be compensated for by regulatory dilation of the downstream region of the coronary bed because of the lack of a sufficient volume of incoming blood to distend the vessels. Such an interpretation, albeit not exhaustive, is nevertheless in full agreement with the data of Verrier et al.¹⁴⁶ concerning the effect of coronary artery stenosis on the behavioral stress in dogs. When the dogs were enraged by taking away food, in the absence of coronary stenosis this enhanced the coronary arterial flow by 147% and lowered the coronary resistance by 38%; in mechanical stenosis with a special cuff there was no appreciable change in the coronary flow, whereas the coronary vascular resistance increased by 33%, indicating a stress-induced vasoconstriction.

Notwithstanding all the danger of ischemia brought about by stenosing atherosclerosis, spasm, thrombosis, or a stress-induced combination of these factors, it should be borne in mind that the organism possesses both immediate and long-term mechanisms for limiting the cardiac overload and ischemia. An immediate response of this kind is attenuation of the circulatory function in adjustment to the lower capabilities of the damaged heart,¹⁴⁷ when cardiovascular reflexes (1) decrease the arterial bed resistance and relieve the cardiac load, and (2) cause partial storing of blood. This of course can only be effective at rest and diminished basal metabolism. Long-term compensation for ischemia is ensured by proliferation of intracardiac and extracardiac collaterals¹⁴⁸ involving enhanced nucleic acid and protein synthesis.¹⁴⁹ In essence *the course of ischemic disease in great measure reflects the dynamic balance between the stenosing atheroma formation and the growth of collaterals. In many cases this latter compensating factor proves no less effective than aortic-coronary shunting and ensures clinical compensation or subcompensation.*

It is noteworthy that, besides medicinal agents causing adenosine accumulation (dipyridamole, etc.), the development of the coronary bed and specifically collaterals can be efficiently induced without drugs, and first and foremost with adaptation to intermittent hypoxia, which will be further dealt with in detail.

The eighth and quite important mechanism by which the stress reaction (caused exogenously or by myocardial infarction) can upset the circulation and entail not only cardiac, but also brain ischemia is the profound impairment of vascular contractile function and tonicity. We made a study of two aspects of such disturbances: (1) impaired contractility of

TABLE 8
Effect of EPS on the Cardiac Contractile Function in Transient Ischemia and Subsequent Reperfusion

Indices	Series	Before ischemia	Ischemia		Reperfusion	
			1 min	30 min	1 min	30 min
Left ventricular developed pressure (mmHg)	Control	116 ± 4.4	87 ± 4.1	83 ± 2.5	81 ± 3.5	72 ± 5.6
	EPS	107 ± 6.3	73 ± 6.4	64 ± 5.2	63 ± 5.5	49 ± 6.3
Heart rate (min ⁻¹)	Control	388 ± 15	383 ± 17	379 ± 17	368 ± 14	349 ± 18
	EPS	415 ± 16	300 ± 15	418 ± 18	384 ± 19	310 ± 18
Mean carotid pressure (mmHg)	Control	77 ± 4.7	60 ± 4.2	54 ± 3.9	51 ± 4.1	45 ± 4.0
	EPS	67 ± 6.8	54 ± 5.1	37 ± 1.3	38 ± 3.1	31 ± 3.0
Cardiac output (ml/min)	Control	34 ± 2.9	41 ± 3.4	33 ± 2.9	27 ± 2.5	20 ± 2.7
	EPS	37 ± 3.2	43 ± 3.0	35 ± 3.9	25 ± 3.2	11 ± 1.3
Total peripheral resistance (dynes·cm ⁻³)	Control	3174 ± 309	1950 ± 181	2181 ± 214	2517 ± 230	3286 ± 340
	EPS	2717 ± 432	1674 ± 180	1599 ± 190	2025 ± 304	3799 ± 234
Cardiac external work (J/min)	Control	0.35 ± 0.03	0.33 ± 0.02	0.23 ± 0.01	0.18 ± 0.02	0.12 ± 0.02
	EPS	0.33 ± 0.03	0.31 ± 0.03	0.18 ± 0.02	0.14 ± 0.02	0.04 ± 0.01

Note: Control, n = 10; EPS, n = 7.

the capacity vessels attending stress or infarction and leading to excessive storing of blood and diminished blood inflow to the heart, minute volume, and arterial pressure,¹⁵⁰ and (2) impaired endothelium-dependent contraction and relaxation of aorta which, though being a conducting vessel, nevertheless roughly reflects the ability of the coronary vessels to respond by contraction to catecholamines and by relaxation to the endothelium-derived factor.¹⁵¹

The first part of work was carried out on the portal vein representing one of the main reservoirs for blood in the organism. It was found that normally the rate of spontaneous contractions of the portal vein were $4.4 \pm 0.4 \text{ min}^{-1}$, developed tension $122 \pm 24 \text{ mg}$, IFS $192 \pm 15 \text{ mg/min per mg wt}$ ("intensity of the functioning of structures" calculated as the product of developed tension by contraction rate divided by vein mass), tension buildup velocity $48 \pm 7.7 \text{ mg/s}$, and relaxation velocity $67 \pm 17 \text{ mg/s}$. Upon EPS all contractile parameters were markedly lowered: developed tension 8-fold, tension buildup and relaxation velocities 4.4- and 6-fold, respectively, and IFS 6-fold. Thus the depression of the portal vein contractile activity (exemplified in Figure 11) in EPS was much more pronounced than that of the heart.¹⁵²

Further, it was found that the dependence of developed tension on the stretching load is not appreciably altered, suggesting that the relation between actin and myosin is not impaired in stress. This, together with the high stability of the myofibrillar proteins even in prolonged ischemia,¹⁵³ argues against the stress damage to the vascular smooth-muscle contractile machinery as such and makes it most plausible that the observed depression of contractility of the portal vein is due to hindered access of calcium or energy provision.

This is supported by the greater sensitivity of the isolated portal vein preparations from poststress animals to a lack of calcium or glucose. Indeed, 7 min after a calcium stepdown in the supplied fluid the IFS decreased by 36% as compared to only 13% in the control; this is likely to result from impaired ability of the sarcolemmal and SR membranes in the portal vein myocytes to take up and retain calcium. When glucose was removed from the solution, the decreases in the rate and tension of contractions in poststress preparations were also more pronounced than in controls; this can be explained by the activation of glycolysis and glycogenolysis by excess catecholamines in stress which exhausts the glycogen stores in the portal vein.

The most dramatic differences in the reaction of the portal vein preparations from control and poststress animals were observed when the temperature was changed in the range 28 to

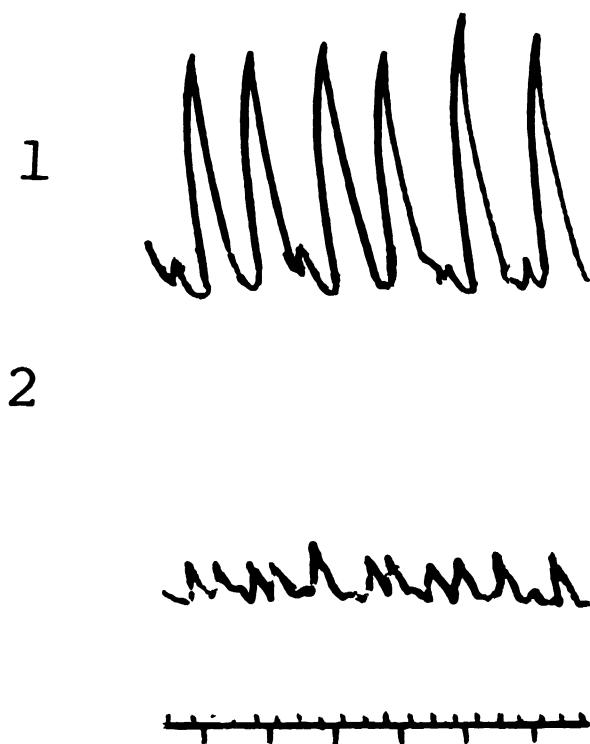


FIGURE 11. Effect of EPS on portal vein contractility. Spontaneous phasic contractions of preparations from (1) a control rat and (2) a rat subjected to EPS.

35°C. Figure 12A shows that with increasing temperature the contraction rate reflecting the automatism of the natural vein pacemakers¹⁵⁴ clearly increases in the control, but tends to decrease upon stress; the IFS (Figure 12B) only slightly declined in the control, but fell fivefold upon stress. It should be emphasized that all experiments had to be carried out at subphysiological temperatures because at 37.7°C the poststress preparations, unlike the controls, display no spontaneous contractile activity.

The tonic response of the portal vein preparations to 6×10^{-7} g/ml norepinephrine upon EPS proved to be ten times less. Hence the lesser ability of norepinephrine to cause tonic contraction and thereby to decrease the volume of the portal bed would affect the efficiency of sympathoadrenal mobilization of the blood stored in the portal vessels.

In the aggregate the above demonstrates that *EPS affects the portal vein muscle in such a way as to diminish its contractile activity and adrenoreactivity; this may be involved in overfilling of the portal bed, arterial hypovolemia, and collaptoid effects in severe stress or myocardial infarction.*

In the second part of our work, studying the effects of stress and infarction on contraction and relaxation of the isolated ring of the thoracic aorta, we took account of the fact that the intact endothelium of arterial vessels is essential for regulation of their reactivity.¹⁵⁴ Endothelial damage in various conditions such as atherosclerosis,¹⁵⁵ hypertension,¹⁵⁶ toxic catecholamine concentrations,¹⁵⁷ or anoxia¹⁵⁸ can weaken or even invert the process of the endothelium-dependent vascular relaxation, which may result in enhanced peripheral resistance to the blood flow and coronary spasm.¹⁵⁹

On the other hand, it is known that acute myocardial infarction is often attended by a marked drop in vascular tone and arterial pressure, up to collapse and cardiogenic shock. We therefore tried to find out whether or not these clinically important events, besides the neurohumoral factors, might involve augmented endothelium-dependent relaxation.

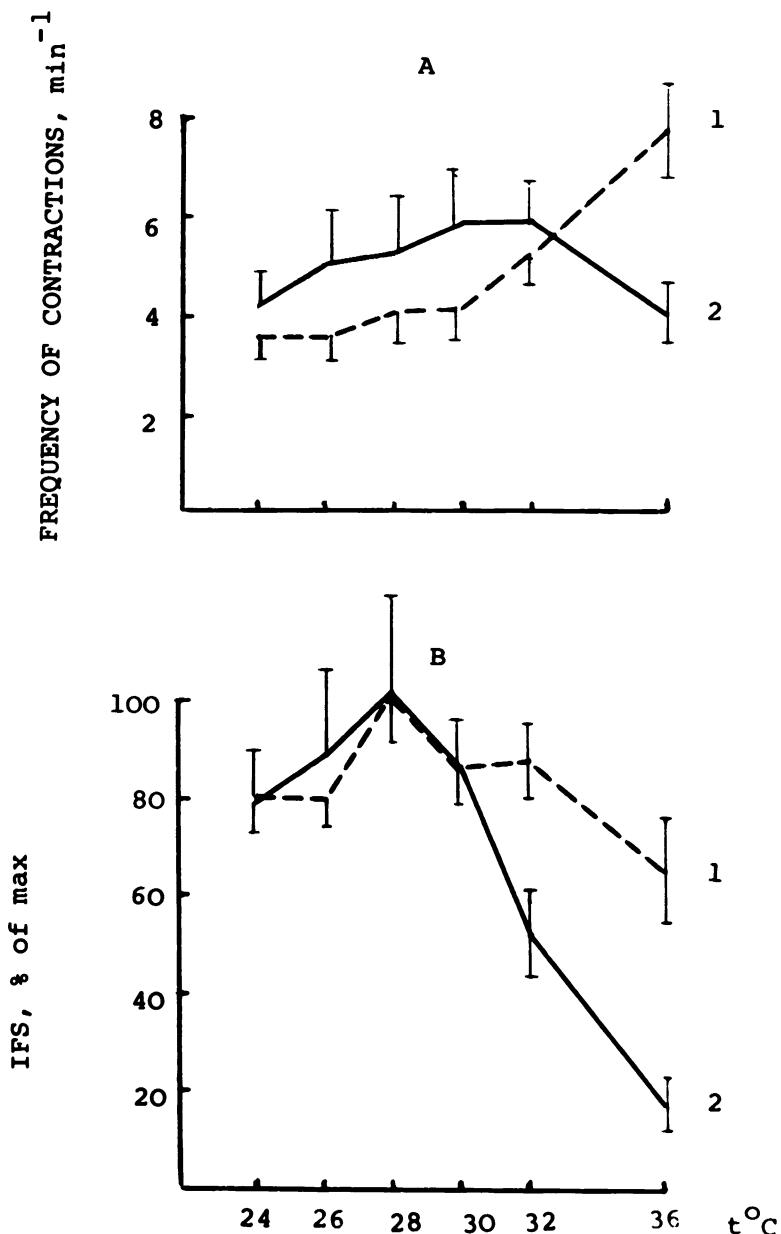


FIGURE 12. Dependence of the contraction rate (A) and intensity of functioning of the structures (B) of the portal vein on the temperature of the perfusing solution. IFS is expressed as percentage of that at 28°C . (1) Control, (2) EPS.

The force of contraction developed by the aorta with intact endothelium in response to different norepinephrine concentrations was not appreciably altered by stress or myocardial infarction. Removal of the endothelium, as could have been expected, resulted in increased response to norepinephrine which was much more pronounced after EPS and infarction (Figure 13).

A similar pattern is observed in the effects of EPS and infarction on the velocity of aortic contraction. The contractile reaction to the α -adrenergic agonists has two components:

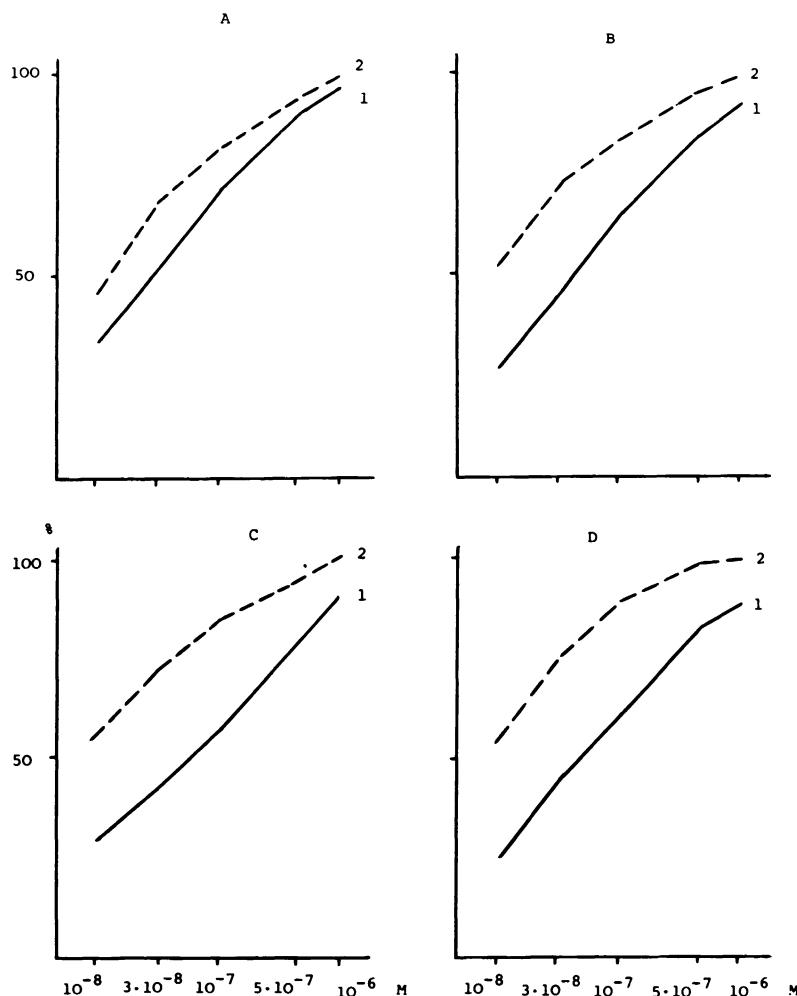


FIGURE 13. Effect of EPS and experimental myocardial infarction on the norepinephrine-induced contraction of isolated rat aorta. (A) Control, (B) sham operation, (C) EPS, (D) myocardial infarction. (1) Intact preparation, (2) preparation with removed endothelium. Abscissa, norepinephrine concentration, M ; ordinate, contraction force, percent (100% assigned to contraction of the de-endothelialized preparation at the highest norepinephrine concentration).

a rapid phasic and a slow tonic one.^{160,161} The velocity of the tonic response remained practically the same in all series and at all acting concentrations, and also was not affected by removal of the endothelium. As to the phasic response, its velocity upon stress and infarction is decreased by 15 to 20% in preparations with intact endothelium; upon removal of the endothelium, the velocity of response to norepinephrine increased much more after stress and infarction: e.g., by 74 and 88%, respectively, at $10^{-7} M$ norepinephrine, as compared to 28% in the control.

These facts agree with the idea that the endothelium produces an inhibitory effect on the velocity and force of the catecholamine-induced aortic contraction.¹⁶² Furthermore, our data suggest that this inhibitory influence of the endothelium is augmented in infarction and EPS.

To test this we studied the effects of stress and myocardial infarction on the acetylcholine-

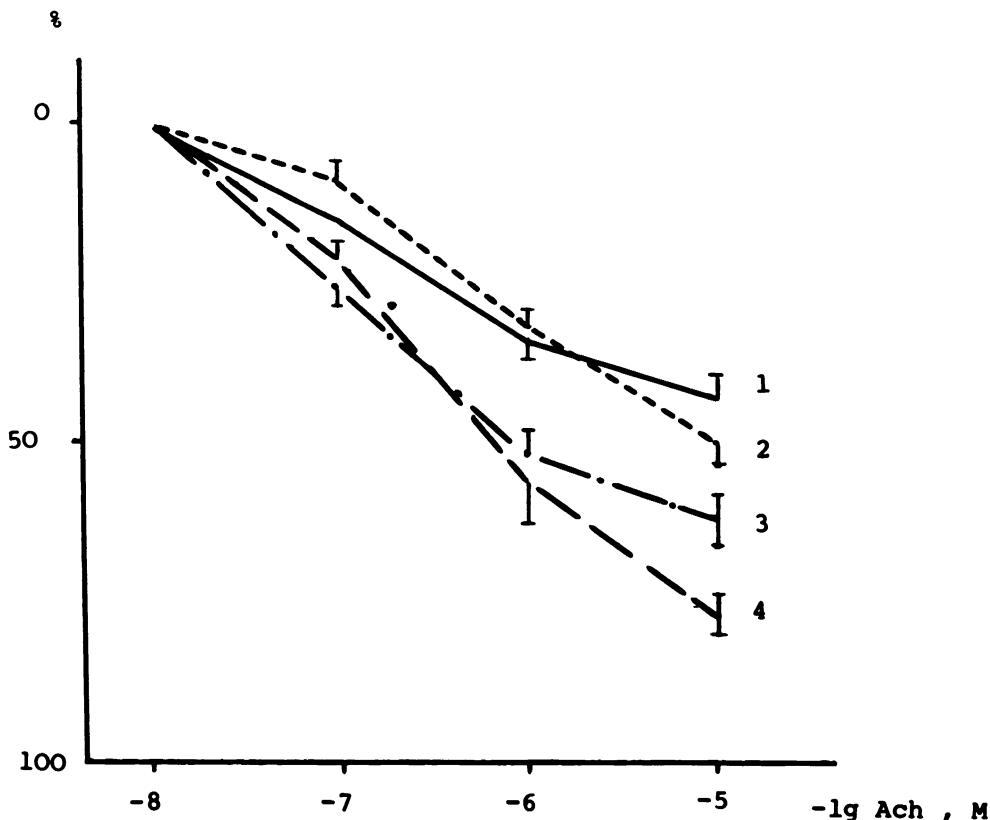


FIGURE 14. Effect of EPS and experimental myocardial infarction on the endothelium-dependent relaxation of isolated rat aorta caused by acetylcholine. (1) Control, (2) sham operation, (3) EPS, (4) myocardial infarction. Abscissa, acetylcholine concentration, M ; ordinate, extent of relaxation, percent of contraction caused by $5 \cdot 10^{-7} M$ norepinephrine.

induced aortic relaxation which directly characterizes the function of the intact endothelium.¹⁶² Figure 14 shows that aortic preparations from animals that had undergone infarction and EPS were relaxed by acetylcholine in a significantly greater degree than those from control or sham-operated animals. Preparations without endothelium did not relax in response to acetylcholine.

Thus, *experimental myocardial infarction and EPS potentiate the relaxing influence of the endothelium on the vascular smooth muscle, which is manifest in attenuation of norepinephrine induced contraction as well as in acetylcholine-induced relaxation.*

It is essential that the alterations brought on by experimental myocardial infarction are similar to those after EPS, which points to the stress origin of such shifts. This is also supported by the fact that thoracotomy — surgical stress — although not enhancing the endothelium-dependent relaxation, still causes similar, albeit much smaller, changes in the contractile parameters (Figure 14). Again, Webb et al.¹⁶³ have recently shown that psychosocial stress in mice augments the endothelium-dependent vascular relaxation.

Further, Table 9 demonstrates that in animals with experimental myocardial infarction the drop in arterial pressure coincides in time with the enhancement of the endothelium-dependent relaxation, and vice versa, the restitution of blood pressure is simultaneous with the reduction of the endothelial function; again, one can see that in all groups there is a high negative correlation between these indices. Hence the enhancement of the endothelium-dependent vascular smooth muscle relaxation that we have found in myocardial infarction

TABLE 9
Correlation Between Arterial Pressure and Endothelium-Dependent Relaxation

Conditions	Arterial pressure (mmHg)	Relaxation (% of contraction force)	Correlation coefficient
Control	109 ± 1.0	49 ± 4.6	-0.87
Infarction, 3 h	73 ± 1.4*	71 ± 0.7*	-0.93
Infarction, 24 h	99 ± 6.5	49 ± 8.2	-0.78
Sham operation, 3 h	112 ± 2.3	50 ± 3.2	-0.78
Sham operation, 24 h	108 ± 1.6	39 ± 3.9	-0.56

* $p < 0.01$ as compared to the control.

and EPS can, among other factors, be involved in the drop in blood pressure typical of infarction and heavy stress.

In assessing the probable mechanism of the enhanced endothelial influence on the vascular smooth muscle, it should be recalled that stress entails activation of LPO and phospholipases, and increases the blood levels of free fatty acids.¹⁵² A number of free-radical oxidation products induce the release of the endothelium-derived relaxing factor (EDRF) or potentiate its release by endothelium-linked vasodilators.¹⁶⁴ The free fatty acids amplify the endothelium-dependent relaxation; conversely, the latter is hindered by phospholipase inhibitors.¹⁶⁵ The metabolism of arachidonic acid, which is considered an EDRF precursor,^{165,166} is increased by catecholamines.¹⁶⁷ However, the significance of each of these factors for the enhancement of relaxation in stress is yet to be elucidated.

Regardless of its mechanism, the EDRF-mediated relaxation is indisputably important in the regulation of vascular tone. Until recently, impairment of this endothelial function has only been regarded as a possible cause of increased vascular resistance and coronary spasm.¹⁵⁹ In our experiments the situation is quite the opposite: activation of the endothelium-dependent relaxation overcomes the constrictive action of catecholamines on the aortal smooth muscle with an ensuing arterial-pressure drop in EPS and infarction. *This may be involved in collaptoid conditions and cardiogenic shock developing in infarction and other stressful situations;* this suggestion, of course, requires thorough testing, in particular, of the behavior of arterioles which mainly determine the peripheral resistance.

All these data once again emphasize the danger brought by the combination of ischemia and stress. On the other hand, it cannot be overlooked that these disturbances to the vascular walls of the venous and arterial bed are but transient and disappear rather soon (within 1 to 3 d). This reversibility, which we have seen to be typical of the most various stress damages, apparently arises from activation of the central stress-limiting systems that restrict the very stress reaction in duration, as well as of the local stress-limiting systems: the adenosinergic one which blocks calcium entry into the vascular smooth-muscle cells,^{168,169} the antioxidant one which suppresses lipid peroxidation, etc.

All the above-considered mechanisms through which an excessive or protracted stress reaction is intertwined with the pathogenesis of ischemic heart disease are summarized by the scheme in Figure 15. One can see that the detrimental factors (in essence, the manifold effects of the stress reaction) can as well be actualized in exogenously evoked stress and in endogenous stress secondary to and caused by ischemic pain in the heart and fear of death.

The gloomy side of the concept is that the fatal vicious circle ought to close both in severe exogenous stress causing ischemia and in ischemia causing a strong and protracted stress reaction. This is, however, not really so. Many millions of people go through trying stresses and do not respond with ischemia, and millions of people with ischemia for many years remain active members of the society, and not infrequently sustain considerable stress

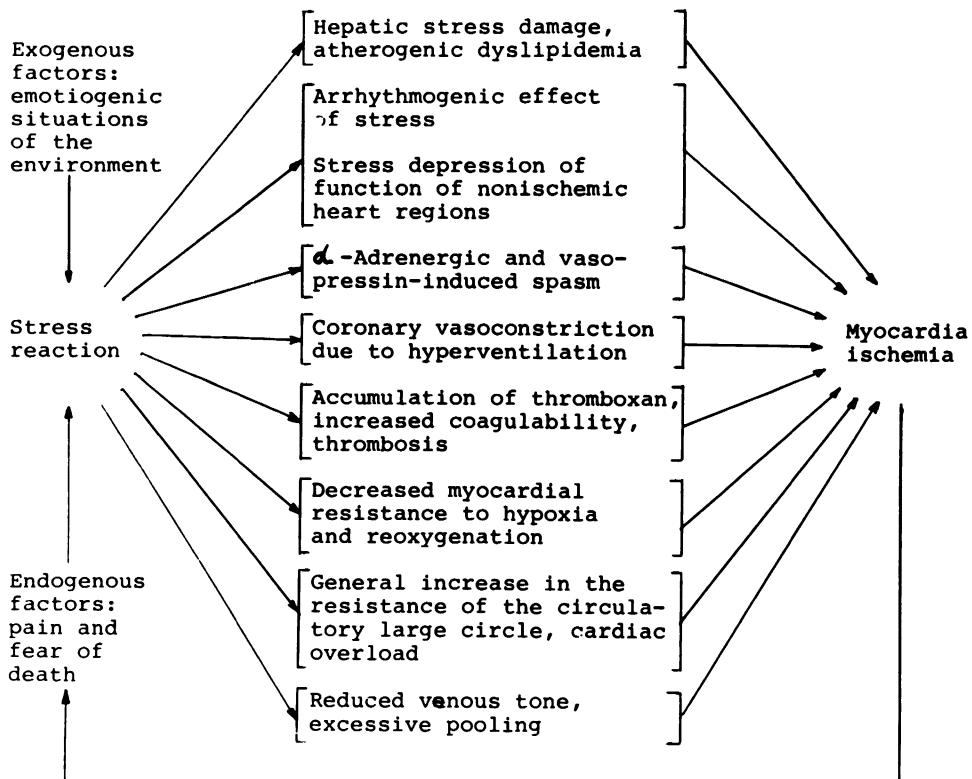


FIGURE 15. Role of stress in ischemic myocardial damage. For explanations see text.

loads, though not without unfavorable consequences. This paradox is especially vivid with the environmental stress reactions. Indeed, the role of biosocial stress in the pathogenesis of mental disorders, duodenal and gastric ulcers, grave bile-duct dyskinesia, skin diseases, malignant tumors, and finally ischemic heart disease dealt with here, is quite well known. Moreover, the pathological role of the stress reaction is such a popular idea as prevents many researchers from appreciating the paramount circumstance that most people and animals encountering the so-called hopeless situations do not die, but acquire a certain resistance to them.

We shall concentrate on the mechanism of this admirable resistance later when considering the stress-limiting systems. Here it must again be emphasized that *the stress reaction is really quite important for the onset, course, and outcome of the ischemic disease*. To understand the principles of its therapy it is essential to clearly realize the relation of ischemic and stress factors at every stage of its development. This is especially significant in recognizing the mechanisms of arrhythmias, sudden death, and *in approaching correctly the major question of the differentiation between the primary ischemic disease, which evolves as the direct result of stenosing coronary atherosclerosis, and the stress arrhythmic heart disease, which evolves first of all as the result of primary stress damage*.

V. RELATION BETWEEN STRESS REACTION AND ISCHEMIA IN THE PATHOGENESIS OF ARRHYTHMIAS AND SUDDEN CARDIAC DEATH

The modern definition of sudden cardiac death is the death of an apparently healthy normal person ensuing from a cardiac attack within an hour from its onset. In point of fact,

various epidemiological studies demonstrate that in half of the cases or more the death comes in the first 10 to 15 min from the beginning of the attack. The ECG, when recorded, in most cases reveals cardiac fibrillation.¹⁷⁰ In the U.S. the annual toll of sudden death was estimated at 220,000 to 280,000 in the early 1980s;¹⁷¹ it is no less common in other developed countries and on the whole constitutes one of the main causes of mortality and a major health problem in the modern world.

Since coronary atherosclerosis is quite widespread, and according to many epidemiological studies^{172,173} the sudden death and the coronary disease share the risk factors, a rather common viewpoint is that sudden death is the direct result of the progression of coronary arterial atherosclerosis. However, the vast experience of modern resuscitation shows that in many cases the timely use of defibrillation and other proper means restores the rhythmic activity of the heart and preserves life without recurrence of fibrillation for quite some time.¹⁷⁰

On the other hand, quite a number of researchers maintain that in most people who die a sudden cardiac death, despite more or less pronounced coronary sclerosis, there is no thrombosis of the coronary arteries, myocardial necrosis, or other organic damage of the cardiac muscle that could itself be the cause of death.^{170,174}

It is such contradictory results that have made most topical the question of to what extent atherosclerosis as such can cause fibrillation and sudden death, and what part therein can be played by other aggravating factors such as stress.

A quantitative analysis of Thomas et al.¹⁷⁵ shows that pronounced coronary stenosis (75% in one or two arteries) is found with the same frequency in a group of IHD and sudden death and in a control group of those who died of unrelated causes, so that *even advanced atherosclerosis can hardly be regarded as the cause of fibrillation and sudden cardiac death*. Furthermore, about half of the cases of sudden death display none of such reliable hallmarks of ischemia as thrombosis of the coronary vessels or myocardial infarction. Indeed, a comparative study of 868 autopsies carried out in 5 research centers in the U.S. demonstrates that in sudden cardiac death the incidence of a fresh thrombus obstructing a large coronary vessel is from 17 to 46%.¹⁷⁶ In full conformity with this, it has been shown that in many cases fibrillation and sudden cardiac death are not associated with myocardial infarction, and that acute myocardial infarction is reliably found only in 13 to 40% of cases of sudden cardiac death within 6 h from the onset of attack.^{177,178}

Nevertheless, there is a standpoint that the myocardial infarction is not found in sudden cardiac death only because the pathoanatomist is dealing with the early stage of ischemic alterations that are not revealed with routine methods. Indeed, obvious macroscopic alterations typical of myocardial infarction take 18 to 24 h to develop, whereas with histological techniques it can be diagnosed in 8 to 12 h of myocardial ischemia. A shorter ischemia preceding death is most difficult to discern,¹⁷⁶ yet a macroscopic test with nitro tetrazolium blue detects an early stage of infarction in 52% of cases of sudden cardiac death,¹⁷⁹ while the microscopic test detects ischemic damage in 46%.¹⁸⁰ These indices are unlikely to change much in the future. In fact, patients who develop an infarction after resuscitation from sudden cardiac death are now known to differ paradoxically in prognosis from those who have no infarction. Recurrence of fibrillation in resuscitated patients who "have got their infarction" is far rarer than in those who have none (2% vs. 22%, respectively). Hence a large necrotic region in the myocardium is associated with a much smaller probability of arrhythmias.¹⁷⁶ To complicate the matters still more, we can recall the rather old but none the less significant work of Enos et al.,¹⁸¹ who found pronounced atherosclerosis of coronary vessels among U.S. soldiers who had fought in Korea, i.e., in healthy young men. Of those killed in action (mean age 22.1) 10% had coronary artery stenosis exceeding 70%. *All this means that marked atherosclerosis or even ischemic damage in the form of infarction by themselves are often not at all sufficient to cause fibrillation and sudden cardiac death.*

On the other hand, we have seen that there are many cases of sudden cardiac death in the absence of any atherosclerotic or ischemic alterations. In other words, *people may live with pronounced atherosclerosis, and people may suddenly die without any atherosclerosis. Hence, besides stenosing atherosclerosis and attending ischemia there must some other etiological, triggering factor.*

Stress can be such a trigger, as demonstrated by Myers and Dewar,¹⁸² who analyzed the circumstances preceding sudden death in 100 men under 70 with coronary atherosclerosis proved at necropsy. It was found that 23% of the deceased had experienced severe emotional stress within 30 min before death, 40% within the last day, and 22 to 23% had been under stress for 6 months or more.

At present there is extensive epidemiological evidence available that shows that biosocial stress in great measure predetermines the mortality from heart fibrillation and sudden cardiac death in masked and overt coronary disease, whereas alleviation of this "stress pressure" has an opposite effect. Thus a study of 17,500 Whitehall employees having coronary atherosclerosis (age 40 to 64) showed that in the group occupying the lowest positions the cardiac mortality was 3.6 times higher than among those of the same age, but close to the top.¹⁸³ This result could have been simply and plausibly explained by the effect of stress on the development of coronary atherosclerosis. However, the most impressive point of this work is that age, blood pressure, smoking, height-weight ratio, glucose tolerance, and plasma cholesterol could only account for a minor portion of this drastically increased cardiac mortality. *The major determinant of mortality was the low social status and attending chronic biosocial stress pressure.*

These facts put in the forefront the question by what mechanisms the stress can provoke arrhythmic and ischemic cardiac responses in IHD, and how much it contributes to these often fatal events. To get some insight into this problem, in our already mentioned study with Khalfen and Lyamina we compared the response of adrenergic regulation and cardiac function to the stress load and to the standard VEL in healthy subjects and in patients with IHD of different severity.

As follows from Table 10, epinephrine excretion at rest in IHD with functional class I-II angina is 40% higher as compared to the healthy subjects, and under stress its relative increase is about the same; thus the blood epinephrine concentration is higher in IHD patients.

Norepinephrine concentration in IHD declines by 22% at rest, but drastically increases under stress (by 90 to 102% vs. 24% in healthy subjects, $p < 0.001$). It is noteworthy that in IHD with class I-II angina the epinephrine/norepinephrine ratio at rest is already elevated to 0.89 to 0.91, i.e., actually to or above the stress level, and does not appreciably change under stress.

Table 10 further shows that dopamine excretion in IHD is decreased significantly and proportionately to the severity of the disease. Indeed, it is a 31% decrease in class I-II angina, 37% in class III-IV angina, and 50% in IHD with previous myocardial infarction. Accordingly, the physiologically important ratio dopamine/(epinephrine + norepinephrine) is 30 to 40% lower both at rest and under stress.

It should be borne in mind that first, dopamine is the precursor of epinephrine and norepinephrine in catecholamine synthesis, and may accumulate in stress owing to reduced activity of the limiting enzyme β -dopamine hydroxylase,¹⁸⁴ and second, dopamine is the mediator of a separate dopaminergic system whose neurons are widely represented in the brain and the spinal cord.^{185,186} Dopamine restricts the adrenergic activity at the central level,¹⁸⁷ and accordingly the density of dopamine receptors in the brain is higher in rats that are more resistant to stress.¹⁸⁸ Activation of dopamine receptors by dopamine agonists has also been shown to prevent ulceration of the gastric mucosa in stress.¹⁸⁹

The dopamine deficiency in IHD patients at rest and under stress, and progression of this deficiency with progressing IHD severity are in our opinion one of the explanations of the enhanced vulnerability of such patients to stress situations.

TABLE 10
Adrenergic Responses (ng/min) to Stress Load in Healthy Subjects and IHD Patients

Group	Epinephrine		Norepinephrine		E/NE		Dopamine		Dopamine/E + NE	
	Before load		After load		Before load		Before load		Before load	
	Before load	After load	Before load	After load	Before load	After load	Before load	After load	Before load	After load
Healthy (n = 21)	3.60 ± 0.70	6.50 ± 0.81	6.30 ± 0.81	7.71 ± 0.83	0.57 ± 0.06	0.74 ± 0.09	292 ± 18	372 ± 22	29.6 ± 1.8	26.0 ± 1.6
IHD (n = 72)	4.06 ± 0.91	6.82 ± 0.92	4.46 ± 0.81	8.22 ± 0.82	0.91 ± 0.08	0.88 ± 0.08	194 ± 18	276 ± 19	22.7 ± 1.2	19.6 ± 1.1
Angina pectoris class I-II (n = 26)	5.04 ± 0.90	9.01 ± 0.92	4.99 ± 0.83	9.79 ± 0.86	1.01 ± 0.06	0.92 ± 0.08	202 ± 21	254 ± 30	20.1 ± 1.1	18.8 ± 1.1
Angina pectoris class III-IV (n = 25)	3.80 ± 0.75	6.08 ± 0.84	4.57 ± 0.73	7.69 ± 0.61	0.83 ± 0.05	0.79 ± 0.05	186 ± 18	245 ± 21	22.2 ± 1.2	17.6 ± 1.1
IHD with previous myocardial infarction (n = 21)	3.20 ± 0.63	4.99 ± 0.73	3.72 ± 0.68	6.93 ± 0.69	0.85 ± 0.05	0.72 ± 0.06	148 ± 16	216 ± 18	21.3 ± 1.2	18.1 ± 1.2

Under physical load the minor shifts in dopamine excretion did not reach statistical significance. Epinephrine excretion in all forms of IHD except those with myocardial infarction increased 1.5- to 2-fold, while norepinephrine excretion decreased to the same extent; therefore under physical load the change in the epinephrine/norepinephrine ratio was similar to though smaller than that under stress load.

In the aggregate these data demonstrate that IHD is attended with such potentially arrhythmogenic alterations in the adrenergic regulation as elevated epinephrine/norepinephrine ratio and lowered dopamine/epinephrine + norepinephrine ratio, which are more pronounced under stress than under physical load.

The physical tolerance was lowered in IHD patients(maximal load 109 ± 11 W as compared to 201 ± 15 W for healthy subjects). At the same time, increases in the Katz index ($HR \times APs/100$) under SL and VEL were about the same in IHD patients and healthy controls. This decreased efficiency of cardiac performance has been demonstrated in a number of works. Further, the increases in the contraction and relaxation velocities are two to three times less in IHD patients, so that under both types of load the IHD patients display depression of the contraction and relaxation indices.

The use of the relaxation index in this form has been first proposed in the mid-1970s,¹⁹⁰ and its pronounced decrease under load or high catecholamine concentrations reflects the impairment of membrane calcium transporting mechanisms which have been demonstrated in both stress-induced and ischemic cardiac damage. It is of interest in this context that in IHD patients under stress both indices were depressed proportionately to increased excretion (and therefore blood concentration) of epinephrine.

On the whole, this means that the enhanced adrenergic effect, along with alterations in the cardiac muscle itself, is involved in the depression of contraction and relaxation indices in IHD patients under stress load.

Table 11 demonstrates the detectability of ischemic and arrhythmic reactions in IHD with the use of stress and physical loads. The ischemic reaction is revealed in 64% of cases with VEL and in only 29.5% with SL; however, out of 27 patients with an ischemic response to SL only 8 responded to VEL. Hence the SL revealed 19 additional cases of ischemic response, and the total percentage of patients with documented myocardial ischemia increased to 84.8%.

The practical significance of this fact is obvious, and makes expedient a combination of both tests for a fuller diagnosis of IHD. The theoretical aspect is that the pathogenesis of ischemic attacks may differ among coronary patients: in some of them the attacks are provoked by enhanced cardiac function, whereas in others by the adrenergic effect on the pathologically altered heart.

Table 11 further shows that the physical load elicited potential arrhythmias only in 8% of IHD patients, while the SL was effective in 22%. Moreover, of 7 patients responding arrhythmically to VEL, 5 also responded to SL. In sum, the detectability of rhythm and conduction disorders with the two tests was about 24%.

The fact that the stress load is a more efficient means of revealing arrhythmias in IHD, besides being practically important, also unambiguously testifies that enhanced adrenergic action on the heart is more likely to provoke arrhythmias in IHD patients than surplus physical load.

In full conformity with this, the SL eliciting arrhythmias in IHD in most cases increases the Katz index only by 30 to 40% whereas the maximal VEL, which increases this index twofold to threefold, quite rarely causes such disturbances. In essence this means that in stress the elevated catecholamine concentration evokes arrhythmias not through increased cardiac function (which is not large enough to cause relative hypoxia), but directly affecting the electric stability of the heart.

Interestingly, the ischemic responses to SL, unlike those to VEL, were also independent

TABLE 11
Detection of Ischemic and Arrhythmic Reactions with Physical Load (VEL) and Stress Load (SL) in IHD Patients (n = 92)

	Detectability (%)
Ischemic response to:	
VEL	64
SL	29.5
VEL and/or SL	84.8
Arrhythmic response to:	
VEL	8
SL	22
VEL and/or SL	23.9

of increased cardiac function. At maximal physical load the various forms of ischemic reaction in 91% of all IHD cases were observed after the Katz index (mainly reflecting the cardiac oxygen consumption) had increased by more than 75%; under the SL 89% of ischemic reactions took place before a 75% increase in the Katz index.

Thus, the greater cardiac vulnerability to the arrhythmogenic and ischemic effects of stress observed in this work may be due to the enhanced adrenergic component of the stress reaction, to the lower efficiency of the stress-limiting systems, and of course to profound alterations in the heart itself. As a result, in IHD patients a moderate stress is more likely to cause an arrhythmogenic effect than the maximal tolerable physical load; this effect appears to be realized not through enhanced cardiac function, but by direct action of catecholamines and other stress hormones on the heart, as has been postulated earlier.

Taken together with the data on primary stress cardiac damage (Chapter 1) and primary stress arrhythmias (the beginning of this chapter), the above signifies that in cardiac disease the detrimental factors — ischemic caused by stenosing atherosclerosis, and excessive stress reaction caused by defects in stress-limiting systems — may result in arrhythmias and cardiac fibrillation in three ways:

1. Primary ischemic cardiac damage and its arrhythmogenic effect
2. Primary stress cardiac damage and its arrhythmogenic effect in the absence of stenosing coronary sclerosis and ischemia
3. Combined arrhythmogenic effects of ischemia and stress

The first variant is conventional, well studied, and comprehensively presented in the literature. The second one is substantiated by the above experimental and clinical data. Finally, the third one appears to occur at the later stages of stenosing coronary sclerosis, and can only be understood on the basis of clear notion of the first two. Therefore, in summing up this chapter it is reasonable to first compare the ischemic and the stress damage to the heart and only then to consider how their combination can bring on arrhythmias, fibrillation, and sudden cardiac death. The data available on the primary stress damage to the heart show that this phenomenon is profoundly different in its etiology, pathogenesis, and clinical manifestations from what is usually implied by the term "ischemic disease". Indeed, stenosing coronary sclerosis causing myocardial ischemia is now well known to be itself the sequela of genetical defects in or alimentary overloading of the hepatic mechanisms for metabolic elimination of cholesterol. This idea has been supported by the studies of Anichkov¹⁹¹ on experimental alimentary atherosclerosis and by the works of Brown and Goldstein,¹¹² who found a hepatic system of receptors removing LDLP from the blood, and convincingly proved by dietary and other measures for preventing coronary disease.

TABLE 12
Differentiation Between Ischemic Heart Disease and Stress Arrhythmic Heart Disease

	Ischemic heart disease	Stress arrhythmic heart disease
1. Etiology	Defects in cholesterol-eliminating systems	Defects in stress-limiting systems
2. Key pathogenetic link	Senescent coronary sclerosis → myocardial ischemia	Excessive stress reaction → stress damage to the heart
3. Ischemic response to physical load	Marked "ischemic" ECG changes and cardiac pains	Absent
4. Arrhythmic response to physical load	Rare at peak load	Absent or occurs after load
5. Ischemic response to stress load	Occurs in about one third of cases	Absent
6. Arrhythmic response to stress load	Rare (about 10% of cases)	Frequent
7. Arrhythmias at rest	Secondary, ischemia-linked, abolishable with vasodilators	Primary, without ischemia, vasodilators poorly effective
8. Myocardial infarcts	Vast, early	Microfocal, late
9. Therapy with sedatives, neuroleptics, tranquilizers, adrenoblockers	Partly effective, restricts secondary stress reaction to ischemia	Quite effective, restricts primary stress reaction
10. Prophylaxis by adaptation to hypoxia, physical load, etc.	Partly effective	Quite effective

As to the stress damage to the heart, it is a result of exceedingly long and strong stress reactions, and can be more or less completely prevented by adaptation to short stress exposures which activate the modulatory stress-limiting systems of the organism. These systems — opioidergic, GABA-ergic, serotonergic, as well as antioxidant, adenosinergic, and PG ones — have already been briefly mentioned and will be dealt with in detail in Chapters 4 and 5. Notably, it is the genetic or acquired deficiency of the central and the cellular systems of protection from stress, and not secondary ischemia caused by stress spasm and thrombosis, that is in many cases the basis of stress arrhythmias, blocks, and cardiopathies.

The set of distinctions between the ischemic heart disease and the stress arrhythmic heart disease listed in Table 12 is proposed as a working hypothesis with a clear understanding that at a certain stage of the process stress and ischemia are deeply intertwined. The main justification of this concept is not only the profound and to my mind undebatable differences in the etiology and the pathogenesis of these two conditions, but also the fact that their prognosis, prophylaxis, and therapy are also different in many aspects of clinical importance.

While differentiating the etiology, pathogenesis, and clinics of these two processes, we should at the same time bear in mind their pronounced mutual potentiation, for which three basic mechanisms can be discerned.

First, the stress by direct noncoronarogenic damage to the conduction system and contractile myocardium can give rise to multiple zones of depolarization and conduction defects. This stress-induced enhancement of the electric heterogeneity of the heart is aggravated by interruption of intercellular contacts by excess calcium and adds to the depolarization foci and numerous conduction blocks caused by ischemia. As a result, the cardiac electric stability, which we have seen to be only relative even in apparently healthy people, turns out to be deeply impaired and brings on grave arrhythmias, fibrillation, and cardiac arrest.

The second and more conventional mechanism is that under certain conditions the stress can be conducive to further development of coronary sclerosis, coronary spasm, and coronary thrombosis, thereby deepening the ischemia and leading to acute myocardial infarction which in its turn is complicated with arrhythmias and cardiac fibrillation.

The third mechanism is that the ischemic focus or the infarct causes pain and fear, i.e., gives rise to a stress reaction which, as we have seen, damages the nonischemic myocardial regions, increases the load on the heart, and may directly aggravate the ischemia.

The major issue from these three possibilities is that *prevention, restriction, or abolishment of a stress attack on the heart can be highly effective in protecting the heart not only from arrhythmias, but also from ischemia induced or aggravated by stress*. On the basis of this general concept we shall in subsequent chapters turn to the main subject of this book, that is, to nondrug protection of the heart from stress and ischemia with the use of adaptation to environmental factors which causes a stable enhancement of the power and efficiency of the stress-limiting and antihypoxic systems of the organism.

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Chapter 3

ADAPTATION TO STRESS AND ITS CARDIOPROTECTIVE EFFECT IN STRESS, ISCHEMIC, AND REPERFUSION DAMAGE

I. INTRODUCTION

Adaptation to stress is a state achieved as a result of repeated exposure to moderate stress, and manifests itself in that a hopeless emotiogenic situation or other strong stress factors (that before impaired the animal or human behavior and caused real damage to the organism) lose their destructive effects. In this chapter we shall consecutively consider the very phenomenon of adaptation and its neuroendocrine mechanisms, the adaptive protection from cardiac stress damage, and finally, the chief place will be occupied by the cardioprotective influence of adaptation in ischemic and reperfusion damages, infarction, and postinfarction cardiosclerosis, with the main attention being paid to its antiarrhythmic effect.

II. ADAPTATION TO STRESS AND ITS MAIN NEUROHUMORAL MECHANISMS

The process of adaptation to stress situations has for millenia been empirically used in training for military and other occupations requiring stability in extreme situations, i.e., maintaining an expedient behavioral stereotype despite imminent hazards. Yet as a scientific problem adaptation to stress became a subject of study only about 25 to 30 years ago.

To date, reiteration of brief nondamaging stress exposures has been shown to enhance the organismic resistance to severe stress exposures and to prevent stress damage to the heart and other organs. Thus, prior adaptation of rats to stress efficiently prevents or restricts the disturbances caused by heavy stress to myocardial structure, contractile function, metabolism,¹⁻³ and cardiac electric stability, as well as the arrhythmogenic effect of stress.^{4,5} It also prevents gastric stress ulceration, impairment of portal vein contractility,⁶ stress anorexia,⁷ the oncogenic effect of stress,⁸ and mortality from severe electric-pain exposures.⁹ Adaptation to stress has also proved to enhance the organismic resistance not only to stress, but also to a broad spectrum of other detrimental factors. It prevents or restricts the ischemic and reperfusion damage to cardiac contractility and electric stability (i.e., it is an antiarrhythmic factor which we shall further consider in more detail) as well as direct chemical injury to internal organs¹⁰ and radiation injury,¹¹ enhances cold tolerance,¹² etc.

It can be thought that the protective action of adaptation is underlain by neurohormonal and cellular regulatory changes developing in the organism with repeated exposures to stress, and (1) restricting the intensity and duration of the stress reaction itself and (2) enhancing the resistance of the target organs to stress hormones and mediators.

Indeed, in response to heavy stress the adapted animals develop either no or only minor stress reaction. This has been convincingly proved for the major links of the stress reaction: the adrenergic and the hypophysial-adrenal ones.^{3,13,14} In rats adapted to brief emotional pain stress (EPS), a severe 6-h EPS did not change the corticosterone levels in the adrenal glands and plasma, whereas in nonadapted animals they increased twofold to threefold.³

Besides, such adaptation significantly attenuated the catecholamine “discharge” into the blood and completely prevented the decline of epinephrine in the adrenal glands and norepinephrine in the heart consistently observed under stress in nonadapted animals.^{3,15}

Adaptation to stress also restricts the duration of the stress reaction as evidenced by the shorter poststress eosinopenia,³ which is directly associated with the blood level of corticosteroids.

Thus, adaptation to stress results in abatement of the stress reaction. What are the mechanisms of this phenomenon? A natural suggestion is that in the course of adaptation the stress-effecting systems become less powerful, whereby the stress reaction to the detrimental factor proves weaker. Diverse experimental studies have shown that this is not so. On the contrary, adaptation turned out to enhance the potential capacity of the stress-effecting systems. This first of all applies to the leading stress-effecting system, the sympathoadrenal one, and has been convincingly proved for catecholamine biosynthesis in the adrenal glands in adaptation to immobilization stress, in the classical work of Kvetnansky et al.¹⁶ During such adaptation, enhanced adrenal secretion of catecholamines was accompanied by a more than threefold increase in adrenal tyrosine hydroxylase activity within the first week of daily immobilization episodes. Further on, repeated stress exposures for 6 weeks did not alter this index: a high plateau of enzyme activity and catecholamine synthesis has been established, ensuring stable adrenal hyperfunction. After the end of adaptation the enzyme activity returned to normal within 2 weeks. It is essential that the increased tyrosine hydroxylase activity corresponds to the enhanced catecholamine content in the adrenal glands. Therefore, adaptation augments the physiological resources of this important stress-effecting organ. It is also noteworthy that such adaptation is associated with increased potential ability of the brain adrenergic centers to synthesize and release catecholamines.¹⁷ Thus, adaptation to stress is attended by generalized enhancement of the physiological power of the stress-effecting adrenergic system.

It further turned out that the lack of blood corticosteroid elevation in response to heavy stress in adapted animals, i.e., "fading" of the adrenocortical link of the stress reaction, also does not result from exhaustion of the functional resources of the adrenal cortex. On the opposite, injected ACTH causes a much greater elevation of blood corticosterone in stress-adapted than in nonadapted animals.¹⁸ This is important evidence that adaptation involves (1) increased power of the adrenocortical link of the stress-effecting sympathoadrenal system and (2) altered organismic reaction to stress at the central level, i.e., at the level of releasing factors and ACTH. It is at this level that some mechanisms are actuated by adaptation and, despite the stress, hinder the output of releasing factors and tropic hormones, and prevent increased secretion of stress hormones and mediators (in this case corticosterone).

Study of the adaptation to stress also revealed that the abatement of the stress reaction in adapted animals combines with a peculiar decline in the sensitivity of the target organs to stress mediators and hormones: a phenomenon of desensitization. In recent years this has been demonstrated as a decreased number of β -adrenoreceptors and lower catecholamine sensitivity of the adenylate cyclase system in the brain and peripheral tissues,⁵ and as a decreased number of cytoplasmic receptors for corticosteroids in cardiomyocytes.¹⁹ The decreased number and efficiency of adrenoreceptors appears to be an essential element of enhanced resistance of executive organs to stress damage.

On the whole, *there are three main features characteristic of adaptation to stress: (1) increased power of the stress-effecting systems; (2) smaller extent of their activation, i.e., fading of the stress reaction; (3) enhanced resistance of executive organs and tissues to stress hormones and mediators, an important place in which is occupied by receptor desensitization.* Hence adaptation gives rise to a paradoxical situation: despite the increased potential of the stress-effecting systems, the stress reaction either does not occur at all or is substantially attenuated.

There are further two essential particulars of adaptation to stress situations. First, the process takes place under conditions when no way out of the situation can be found by external behavioral adaptation, and represents a kind of internal adaptation to an apparently hopeless situation — a probable physiological equivalent of what in common parlance is called patience or endurance. Second, checked excitation of the stress-effecting systems

(i.e., reduced concentrations of catecholamines acting on the target organs) combines with desensitization to diminish the stress damage to internal organs. A concept thus emerges that some mechanisms inhibiting the stress-effecting systems in repeated or protracted stress situations are the mechanisms of endurance and of natural prophylaxis of stress damage.

Several years ago we put forward a hypothesis²⁰ that an *essential element of adaptation to environmental stress is activation of central regulatory mechanisms that, during the action of emotional pain or like stimuli, hinder the output of releasing factors and thereby of catecholamines and corticosterone. In the brain, certain neuronal systems synthesize and release mediators: GABA, dopamine, serotonin, glycine, opioid, and other peptides, which interact with the stress-effecting systems to modulate their activity; they can be thought to restrict the stress reaction. No less important regulatory systems — adenine nucleotides, prostaglandins, and antioxidants — operate at the periphery to control the effects of catecholamines and other stress factors and thereby to prevent stress damage.*

We have designated these central and peripheral mechanisms as stress-limiting modulatory systems.

The function of the stress-limiting systems can be supposed to have become coupled in the course of evolution to the function of the stress-effecting systems, and accordingly their functional capacity should be augmented in the repetitive exposures to stress. This restricts the stress reaction and provides for the protective effects of adaptation to stress, which can as well include protection from diverse environmental factors whose action can be blocked by the metabolites of the stress-limiting systems.

This concept was the basis of using adaptation to brief stress exposures, or metabolites of the stress-limiting systems, for prevention and experimental therapy of not only stress damages, but also diseases in which stress is pathogenetically important.

To assess the validity of this approach and of the very concept of the stress-limiting systems, we shall further consider some facts characterizing the state of these systems in the stress reaction and their adaptation role.

1. *The stress reaction is consistently coupled with activation of biosynthesis and release into the blood of the metabolites of central and peripheral stress-limiting systems.* Thus, in acute stress beta-endorphin and ACTH are discharged into the blood from the pituitary body in equimolar amounts.^{21,22} It is essential that such coupling between the stress-effecting and the opioidergic systems is quite tight, since it is genetically determined by ACTH and beta-endorphin being synthesized in the pituitary cells as a common polypeptide precursor, the pro-opiocortin comprising, besides ACTH, alpha-, beta-, and gamma-melanotropins as well as beta-lipotropin. As a result, in response to the hypothalamic corticotropin-releasing factor there is a concomitant release of ACTH and beta-endorphin from the anterior pituitary into the blood. The second stage of coupling takes place at the regulatory level and consists in that corticosterone produced in response to ACTH suppresses, through the negative feedback mechanism, the pituitary synthesis of ACTH and endorphins.²¹⁻²³

The significance of the coupled mobilization of the opioid peptide system in activation of the stress-effecting systems will be later considered in fuller detail. Here it would suffice to say that opioid peptides are intimately linked functionally both with the stress-effecting adrenergic system and with the stress-limiting serotonergic and dopaminergic systems, and modulate them all: specifically, opioid peptides restrict the effects of adrenergic activation and potentiate the effects of the serotonergic system.

Activation of the peripheral stress-limiting systems is also coupled to the stress reaction; this has been most comprehensively studied for prostaglandins which control the adrenergic effect at the level of target organs (see Chapter 5).

Thus, the coupling of the stress-effecting and the stress-limiting systems is indeed a general mechanism of the timely restriction of the stress reaction.

2. *Adaptation to repeated stress episodes is attended by enhanced biosynthesis and accumulation of the stress-limiting metabolites in certain brain regions and on the periphery.*

Thus, in adaptation to short immobilization episodes the content of leu- and met-enkephalins and beta-endorphin in the structures of the striate body and hypothalamus rises by 70 to 100%, and the adrenal content of leu- and met-enkephalins increases 2 to 2.5 times on average.²⁴ Similarly, the hypothalamic content of met-enkephalin in the course of electroconvulsive treatment rises by about 60%, the maximum coinciding in time with the evolution of the antidepressive effect of electroconvulsive therapy.²⁵

At present, data are available that show that adaptation to repeated stress is accompanied by enhanced levels of and synthetic capacity for serotonin and dopamine in some structures of the hypothalamus, the midbrain, and the medulla oblongata.^{26,27}

It is yet difficult to fully appreciate the significance of such adaptive shifts since the specific interlinks between the regulatory systems are not yet clear enough. However, some facets are already conceivable. In particular, elevated dopamine appears to be important for the protective effect of adaptation, since stimulation of the presynaptic dopaminergic receptors on the sympathetic terminals suppresses norepinephrine release and thereby restricts the adrenergic effects of the stress reaction, e.g., prevents stress gastric ulceration and causes hypotension and bradycardia.²⁸ It is also known that at the brain level norepinephrine and dopamine act as a stimulator and an inhibitor, respectively, of ACTH secretion, especially in the amygdaloid central nucleus and anterior and lateral hypothalamus.²⁹ This favors a suggestion that elevated dopamine should restrict such a component of the stress reaction as the output of the corticotropin-releasing factor.

On the whole, a picture gradually emerging is one of *coordinated steady-state activation of the central stress-limiting systems in adaptation to stress with a resulting protective effect*.

3. *Adaptation to stressful situations has been shown to involve increased activity of the stress-limiting systems directly protecting the cell membranes from stress-induced and other damages.* An example is the increase in the activity of antioxidant enzymes, catalase and superoxide dismutase (SOD), in the myocardium by 38 and 16%, respectively.³⁰ Accordingly, the resistance of spontaneously contracting isolated auricles to the arrhythmogenic action of a chemical inducer of free-radical oxidation, H₂O₂, proves to be twofold to threefold greater in adapted animals.³¹ Likewise, adaptation to a permanent minimal restraint gradually, in 7 d, results in a threefold activation of prostaglandin synthesis in the fundus of the rat stomach. Thereupon, concentrated solutions of ethanol, mannitol, or hydrochloric acid, which, on direct contact used to injure about 95% of the mucosal surface, practically lose their detrimental effect. This protective action of adaptation is completely abolished by an inhibitor of prostaglandin synthesis, indomethacin.¹⁰

Thus, there are grounds for speaking about the adaptive enhancement of the efficiency of direct cytoprotective action of the stress-limiting systems operating at the level of executive organs, which are highly likely to attenuate the adrenoreactivity and responsiveness to corticosteroids and to be thus involved in the desensitization mechanism.

4. *Metabolites and activators of the stress-limiting systems, viz regulatory peptides, GHBA, prostaglandins, antioxidants, as well as synthetic analogs of these compounds, successfully prevent stress damage to internal organs, i.e., act like adaptation to stress.*

Studies in our laboratory have shown that administration of beta-endorphin, GHBA, delta-peptide and its cyclic derivative, and natural and synthetic antioxidants consistently prevent stress damage to the heart, stomach, and brain, as well as stress depression of some important links of antitumor immunity.³² In other words, the listed chemical agents act similarly to prior adaptation to repeated stress situations.

This implies that studying the central and peripheral mechanisms limiting the stress reaction and involved in adaptation to stress is not only theoretically important, but also allows the stress-limiting metabolites and their synthetic analogs to be used to prevent and abolish stress disorders as well as noninfectious diseases in which stress plays a pathogenetic role.

Accordingly, we shall first consider the cardioprotective effects of adaptation to stress, and then the opportunities of pharmacological heart protection with metabolites of the stress-limiting systems.

III. ADAPTIVE PROTECTION OF THE HEART FROM STRESS DAMAGE AND ATHEROGENIC DYSLIPIDEMIA

Our first work on prevention of stress damage to the myocardial contractile function³³ has shown that the disturbances of right atrial extensibility and contractility upon emotional pain stress (described in detail in Chapter 1) are preventable with a course of adaptation to brief immobilization stresses imposed by fixing the animals in the supine position for 15 min to 1 h daily.

Curves in Figure 1 demonstrate the dynamics of contractility during atrial stretching. It can be seen that by itself the adaptation achieved with not very numerous spaced stress episodes does not at all depress the myocardial contractility parameters; on the contrary, for adapted animals the curves of extensibility, developed tension, and the Starling curves lie somewhat higher than the control ones.

As in previous studies,³⁴ stress causes depression of myocardial extensibility, its peculiar rigidity (Figure 1A), depression of developed tension (Figure 1B), and marked depression of the Starling curve (Figure 1C). Prior adaptation counteracted this complex of disturbances to the atrial contractile function (its effect is denoted by the shaded zone in the figures), as well as the stress-induced decline in the integrative index of the myocardial contractile function — IFS (i.e., specific work per unit myocardial mass).

Thus, a rationally dosed adaptation to stress allows complete prevention of the depression of myocardial contractility in prolonged stress.

Further studies³⁵ showed that the same holds true for the ventricles of the isolated heart. Thus, a work with isometrically contracting hearts from control, adapted, and stressed animals was carried out according to Fallen et al.³⁶ and included, besides measuring the left ventricular developed and diastolic pressure, determination of the diastolic defect — an integrative index of impairment of diastolic relaxation (calculated as the integral diastolic pressure over the time when it exceeded the zero line.)

As known from our previous experiments,²⁰ the diastolic defect increases with increasing Ca^{2+} concentration in the perfusing liquid, increasing contraction frequency, and decreasing temperature which hinders the action of the cation pumps. In other words, this index in great measure depends on the membrane mechanisms of calcium transport.

Table 1 demonstrates that in hearts isolated from animals that had undergone immobilization stress, at stimulation frequencies of 120 and 300 min^{-1} there was a reduction in developed pressure (43 and 50 mmHg vs. respectively 80 and 97 mmHg in the controls). To the imposed rate of 300 min^{-1} these hearts responded by incomplete relaxation — the diastolic defect not observed in the control. The poststress hearts could not adopt a higher contraction rate.

Adaptation to repeated brief stress exposures did not itself affect the contractile function, but prevented the above defects in the hearts of animals subjected to stress after adaptation.

Thus, adaptation to brief stress episodes prevents the stress-induced depression of ventricular contractility and the diastolic defect which is a manifestation of impaired mechanisms of calcium homeostasis in the cardiac muscle.

This latter fact was the first to lead us to a suggestion confirmed in further studies that adaptation to stress somehow enhances the function of the cell mechanisms for calcium transport in the heart (see Chapter 6).

Hence it becomes understandable that prior adaptation to stress also prevented the main stress disturbances of the cardiac electric stability such as lowering of the fibrillation threshold

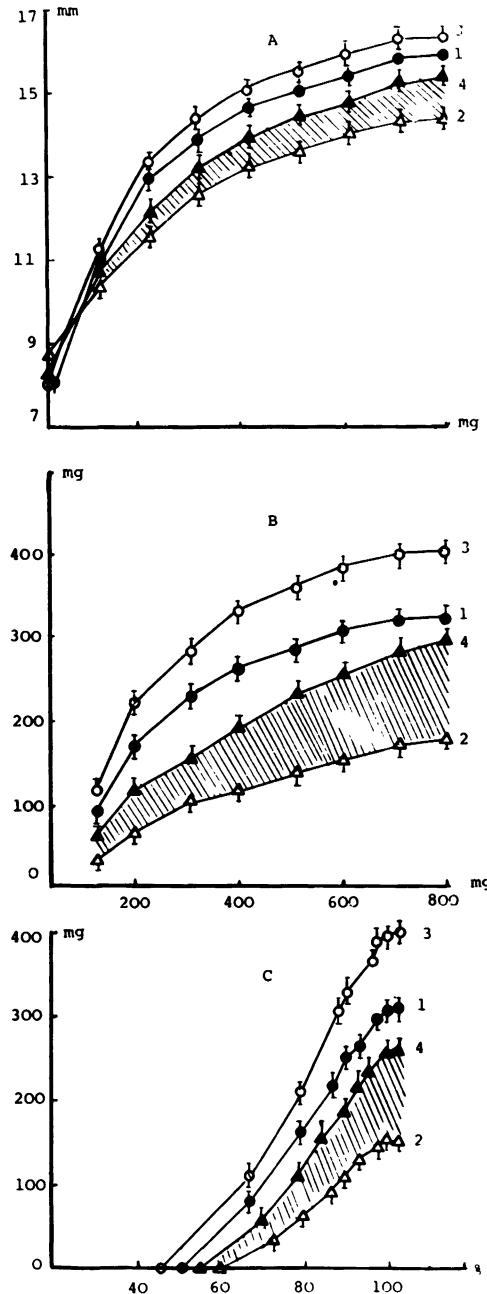


FIGURE 1. Effect of prior adaptation to short stresses on (A) extensibility, (B) developed tension, and (C) Starling curve of rat right auricle in prolonged stress. The abscissae are (A,B) load at rest, mg; (C) atrial length, percent of L_{max} . The ordinates are (A) atrial length, mm; (B,C) developed tension, mg. (1) Control, (2) stress, (3) adaptation, (4) adaptation plus stress.

and decreasing resistance of the sinus node to the impeding influence of the vagus nerve. The data presented in Table 2 and Figure 2 depict the results of these experiments. Table 2 shows that the marked fall in the fibrillation threshold caused by a 10-h immobilization stress is completely prevented with prior adaptation. The protective effect of adaptation is also clearly observed in respect of the vagal inhibition of the heart automatism (Figure 2).

TABLE 1
Impairment of Myocardial Contractile Function After Immobilization Stress at Different Imposed Contraction Frequencies

Indices	Series	Contraction frequency (min^{-1})			
		120	300	400	500
Developed pressure	Control	80 ± 2.5	97 ± 3.8	115 ± 4.5	106 ± 3.9
	Stress	43 ± 1.6	50 ± 2.5	—	—
	Adaptation	85 ± 3.1	105 ± 4.8	98 ± 4.0	90 ± 3.2
	Adaptation + stress	78 ± 2.6	108 ± 4.5	103 ± 4.2	73 ± 1.9
	p_{1-2}	<0.001	<0.001		
	p_{2-4}	<0.001	<0.001		
Diastolic defect	Control	0	0	26.5 ± 2.3	53.2 ± 3.7
	Stress	0	18.6 ± 1.9	—	—
	Adaptation	0	0	31.2 ± 3.0	59.6 ± 4.0
	Adaptation + stress	0	0	36.0 ± 2.8	63.8 ± 5.2
Diastolic pressure	Control	4.3 ± 0.40	±	6.9 ± 0.53	12.2 ± 1.03
	Stress	4.8 ± 0.32	8.1 ± 0.55	—	—
	Adaptation	6.1 ± 0.55	4.5 ± 0.33	11.7 ± 0.87	19.3 ± 1.07
	Adaptation + stress	5.8 ± 0.44	3.9 ± 0.24	11.1 ± 0.75	26.8 ± 1.56
	p_{1-2}	>0.05	<0.001		
	p_{2-4}	>0.05	<0.001		

TABLE 2
Effect of Prior Adaptation to Short Stress Exposures on the Fibrillation Threshold After Prolonged Stress

Series	Heart rate (min^{-1})	Ventricular fibrillation threshold (mA)
Control (n = 12)	445 ± 7	7.4 ± 0.3
Stress (n = 10)	446 ± 8	3.8 ± 0.2*
Adaptation (n = 12)	436 ± 8	7.9 ± 0.7
Adaptation + stress (n = 12)	443 ± 8	7.9 ± 0.5

* $p < 0.01$ compared to the control.

Thus, upon stimulation of the vagus nerve with a triple-threshold current the heart rate in control animals drops over twofold. The negative chronotropic effect of the vagus nerve is significantly enhanced upon prolonged stress: 12 h after, the contraction rate drops by a factor of 3.3. Adaptation itself markedly attenuates the negative vagal effect, the heart rate decreasing only by 25%, and thereby fully compensates for its poststress enhancement so that the heart rate in adapted animals subjected to stress drops not more than in the control. Thus, *gradual and not very long adaptation to brief stress episodes preventing stress disturbances of the cardiac function also abolishes the poststress decline in the cardiac fibrillation threshold and counteracts the poststress decrease in the resistance of sinus node automatism to vagal inhibition*.

The cardioprotective effect of adaptation, initially proved for the stress disorders in the cardiac contractile function and electric stability, soon got a direct explanation in that adaptation was found to prevent the complex of metabolic and structural alterations described in detail in Chapter 1 of this book under the term of primary cardiac stress damage.

The data of our work³⁵ presented in Table 3 show the release of enzymes from the isolated hearts into the perfusate, and demonstrate that though the adaptation itself tends to increase somewhat the release of myocardial enzymes, nevertheless with a high degree of reliability it attenuates the marked (twofold to threefold) enhancement of their leakage

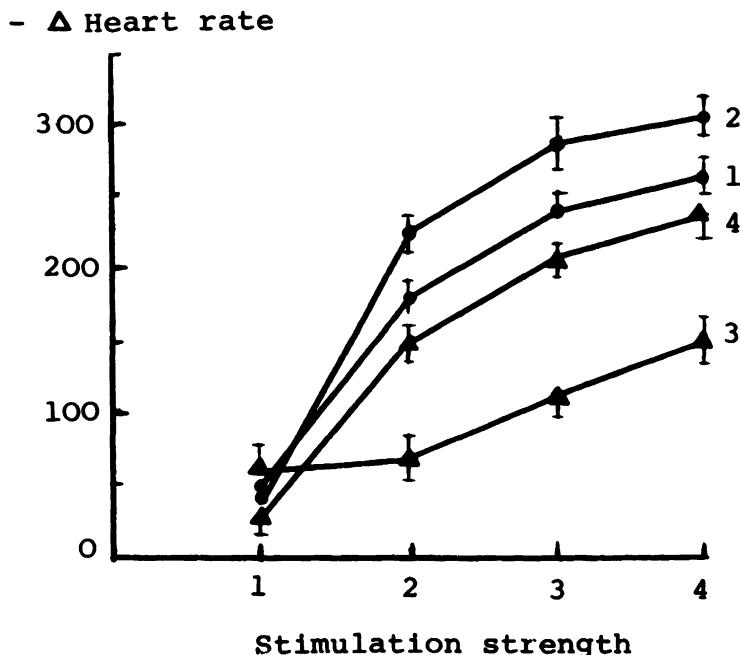


FIGURE 2. Effect of prior adaptation to short stress exposures on the negative chronotropic effect of vagal stimulation. The abscissa is strength of vagal stimulation, multiples of threshold current. The ordinate is the decrease in heart rate, min^{-1} . (1) Control, (2) stress, (3) adaptation, (4) adaptation plus stress.

TABLE 3
Effect of Stress and Adaptation on Enzyme Activity in the Perfusate of Isolated Rat Hearts (U/h·g myocardium)

Series	AST ^a	MDH ^b	LDH ^c	RNase
Control	18.9 ± 1.38	1.53 ± 0.12	10.2 ± 1.02	1279 ± 132
Stress	52.9 ± 4.97	4.32 ± 0.40	22.5 ± 1.78	4043 ± 312
Adaptation	25.7 ± 2.95	1.88 ± 0.20	12.5 ± 1.07	1582 ± 122
Adaptation + stress	35.2 ± 3.17	2.25 ± 0.23	14.7 ± 1.22	2182 ± 163
<i>P</i> ₁₋₂	<0.001	<0.001	<0.001	<0.001
<i>P</i> ₁₋₄	<0.001	<0.05	<0.05	<0.05
<i>P</i> ₂₋₄	<0.01	<0.001	<0.001	<0.001

^a Aspartate transaminase.

^b Malate dehydrogenase.

^c Lactate dehydrogenase.

induced by stress. This prompts an idea that adaptation somehow stabilizes the plasma membrane, diminishing its injury in stress. In full conformity with this are the data of Table 4, obtained with hearts instantly transferred into liquid nitrogen from adapted and nonadapted animals that had undergone stress.

Table 4 shows that the stress-induced activation of lipid peroxidation, described earlier and manifesting itself as a threefold rise in the myocardial content of hydroperoxides and Schiff bases, is largely prevented by adaptation; the same holds true for mobilization of glycogen store typical of stress.

Notably, the cardioprotective effect of adaptation turned out to be not an exclusive

TABLE 4
Effect of Immobilization Stress and Adaptation to Short Stress Exposures on Glycogen Content and Lipid Peroxidation in Rat Hearts

Series	Glycogen (mg/100 g tissue)	Lipid hydroperoxides (nmol/mg lipid)	Schiff bases (U/mg lipid)
Control	548 ± 53	5.1 ± 0.33	3.69 ± 0.19
Stress	317 ± 29	18.9 ± 1.22	9.72 ± 0.75
Adaptation	450 ± 36	6.7 ± 0.81	4.53 ± 0.40
Adaptation + stress	412 ± 32	7.4 ± 0.89	4.86 ± 0.33
<i>P</i> ₁₋₂	<0.001	<0.001	<0.001
<i>P</i> ₁₋₄	<0.05	<0.05	<0.02
<i>P</i> ₂₋₄	<0.02	<0.001	<0.001

feature of immobilization stress, but was also fully reproducible in adaptation to short emotional pain episodes, which gave protection from prolonged (6-h) EPS.

Thus in our study with Malyshев and Lifant'ev we adapted the animals to short episodes of EPS (the anxiety neurosis model according to Desiderato described in Chapter 1; 12 seances of 40 min each every other day) and then subjected the adapted and nonadapted animals to a 6-h EPS. As already indicated in Chapter 1, upon such stress exposure the maximal disturbances of the main parameters of myocardial energy metabolism develop 45 h later.

Table 5 unambiguously demonstrates that, as in earlier experiments, EPS deeply impaired mitochondrial oxidation and oxidative phosphorylation with the NAD-linked substrate glutamate. Adaptation completely prevented these alterations as well as the stress-induced decline in myocardial glycogen. In the same work it was also found that adaptation prevented the microfocal myofibrillar contractural injury typical of stress, and accumulation of technetium pyrophosphate usually observed in poststress myocardium.

Further studies demonstrated that besides the heart this adaptational protection encompasses other internal organs including the liver, whose stress-induced deterioration has been explicitly shown (Chapter 1) to be decisive in atherogenic dyslipidemia. Table 6 shows that gradual adaptation to brief immobilization stresses, while not affecting the overall plasma cholesterol content, at the same time prevents or substantially attenuates the stress-induced dyslipidemia: 2 h after stress the atherogenicity index in adapted animals remains at the control value, and after 1 d its rise is only half that in nonadapted animals. Hence the adaptation naturally coupled with activation of the organismic stress-limiting systems is really protective against stress atherogenic dyslipidemia.

In considering this pronounced protective effect of adaptation, it was important to elucidate which particular stress-limiting systems play the main part in it. Since activated lipid peroxidation in vital organs is known to be a chief pathogenetic link of stress damage (Chapter 1), we supposed that a prominent role in the antistress effect of adaptation is played by the antioxidant systems. Therefore, instead of adaptation we tried to prevent stress dyslipidemia with the antioxidant ionol (butylated hydroxytoluene), which was administered at 20 mg/kg daily for 3 d before stress. As evident in Table 6, prior administration of ionol produces approximately the same preventive effect as prior adaptation to stress.

The significance of these results is underscored by the fact that they are quite in line with the recent data³⁷ obtained in a survey of an organized contingent of young persons that had experienced a complex of such stressful influences as separation from the family, breaking the life stereotypes, entering a new community, and physical work. Therewith these people developed pronounced atherogenic dyslipidemia as manifest by increased low-density lipoprotein cholesterol and atherogenicity index; only 3 months later, upon prolonged adaptation to stress situations, these phenomena gradually wore off.

TABLE 5
Effect of Emotional Pain Stress (EPS) on Oxidative Phosphorylation of Cardiac Mitochondria and Myocardial Glycogen Content in Rats Not Adapted and Optimally Adapted to Stress

Series (n = 12 each)	Respiration and phosphorylation indices				Glycogen content (mM)
	O / t	RC	ADP / O	ADP / t	
Control	253 ± 23	2.86 ± 0.2	1.95 ± 0.1	522 ± 41	5.5 ± 0.26
Adaptation	269 ± 21	3.17 ± 0.3	1.97 ± 0.2	532 ± 45	6.1 ± 0.41
EPS, 45 h after	162 ± 14 ^a	1.70 ± 0.1 ^b	1.30 ± 0.2 ^c	276 ± 39 ^b	2.1 ± 0.15 ^b
Adaptation + EPS, 45 h after	254 ± 27	2.92 ± 0.3	1.94 ± 0.2	521 ± 42	6.0 ± 0.38

^a p < 0.01.^b P < 0.001.^c p < 0.05.

TABLE 6
Effect of Emotional Pain Stress, Adaptation to Stress, and Antioxidant on Serum Lipids and Atherogenicity Index

Series	Cholesterol			Atherogenicity index
	Total	HDL ^a	Triglycerides	
Control (n = 9)	70.8 ± 3.5	52.6 ± 2.8	52.8 ± 4.2	0.35
EPS, 2 h after (n = 10)	66.4 ± 2.0	43.6 ± 2.1	46.3 ± 2.7	0.52
EPS, 24 h after (n = 9)	58.6 ± 4.5	22.7 ± 1.7	40.8 ± 3.2	1.60
Adaptation (n = 9)	65.4 ± 6.8	48.4 ± 3.2	20.2 ± 3.4	0.35
Adaptation + EPS 2 h after (n = 8)	55.8 ± 6.2	42.7 ± 3.1	10.2 ± 1.4	0.30
Adaptation + EPS 24 h after (n = 8)	55.4 ± 5.6	32.7 ± 4.1	8.8 ± 1.0	0.70
Ionol (n = 8)	68.2 ± 5.8	50.2 ± 3.8	50.8 ± 6.9	0.36
Ionol + EPS, 2 h after (n = 9)	70.2 ± 4.0	50.4 ± 4.8	44.2 ± 6.1	0.39
Ionol + EPS, 24 h after (n = 8)	65.8 ± 1.3	34.7 ± 3.1	34.0 ± 5.6	0.80

^a HDL = High-density lipoproteins.

Thus, the stress dyslipidemia and its prevention with adaptation shown above are not just experimental observations, but natural events in humans, so the possibility of preventing such disorders with prior adaptation to stress or with synthetic antioxidants acquires much practical importance.

In full agreement with the above results, Table 7 shows that the changes characteristic of hepatic stress damage — accumulation of malonic dialdehyde (MDA), inactivation of SOD in the liver, and marked elevation of the specific hepatic enzyme fructose 1,6-diphosphate aldolase in the blood — are attenuated by prior adaptation to brief stresses or by ionol to about the same extent as the stress-induced dyslipidemia. It is interesting that by themselves (i.e., in nonstressed animals) both factors increase SOD activity in the liver and thereby create a certain “safety margin” against excessive lipid peroxidation and damage to the central organ of cholesterol metabolism. The latter fact is in line with our data obtained for peroxidation in liver homogenates induced with Fe²⁺/ascorbate (Figure 3): MDA accumulation was substantially greater in preparations from poststress nonadapted animals, whereas prior adaptation as such markedly attenuated MDA formation and largely counter-